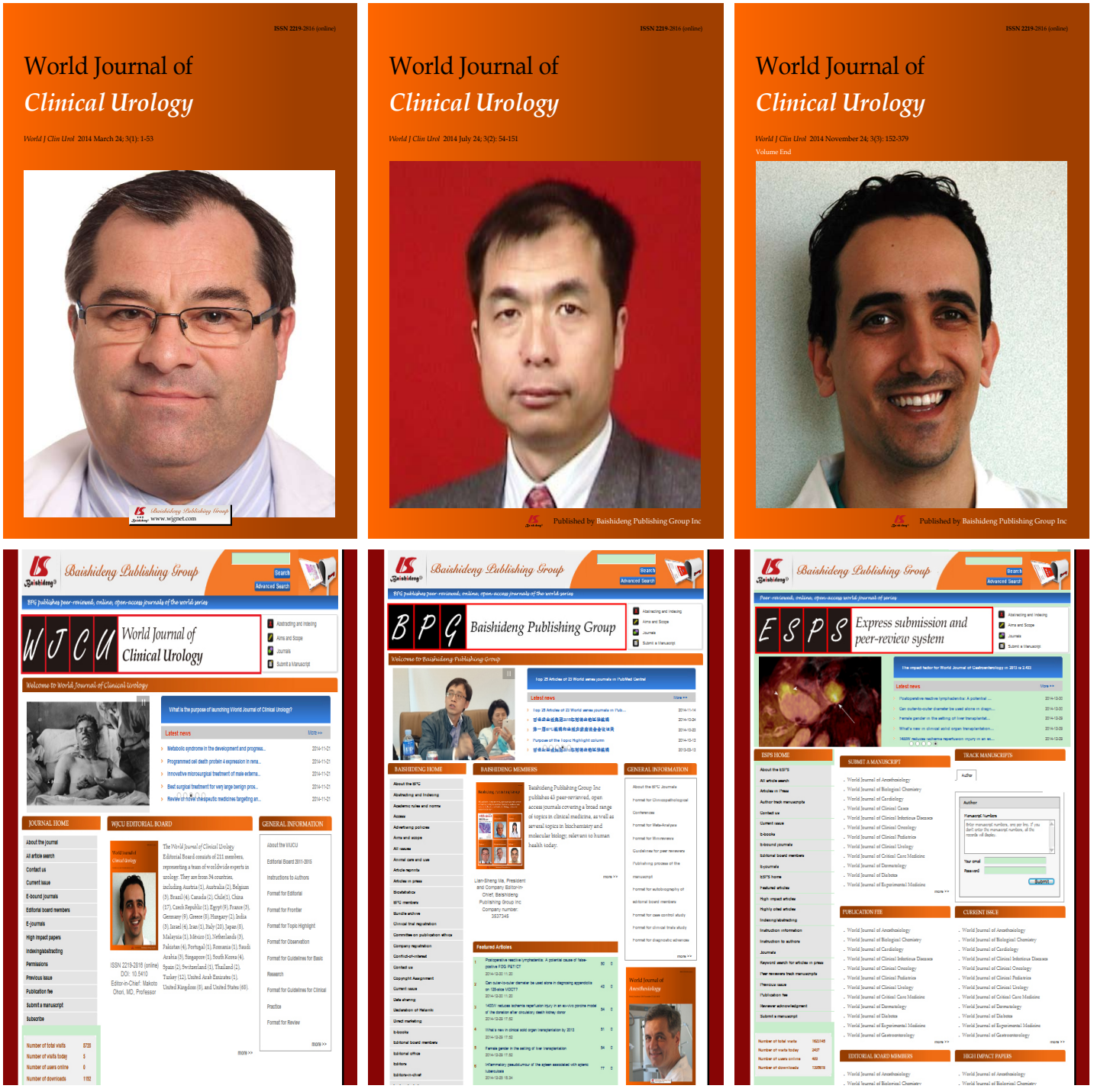
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REVIEW

Why is there a need for an interdisciplinary approach to assess erectile dysfunction?

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Abstract

Erectile dysfunction is a prevalent complaint among men. The majority of patients suffering erectile dysfunction exhibit various risk factors of vascular diseases. Erectile dysfunction used to be recognised as one of the consequences of vascular diseases in patients suffering heart attack or myocardial infarction. During the last decade, however, the role of endothelial dysfunction in the occurrence of erectile dysfunction has been signified, and it has been suggested that erectile dysfunction may not be simply a consequence of vascular diseases but an indicator of future vascular problems. Erectile dysfunction has been known as "the tip of iceberg" of a generalised vascular dysfunction, which typically happens before serious vascular problems. Considerable evidence shows a link between erectile dysfunction and vascular disorders. Several theories have been considered for the association between erectile dysfunction and vascular diseases. One of them is the "artery size" theory focusing on the differences between the diameter of the penile artery and other arteries. Another theory is based on "endothelial dysfunction", which highlights inappropriate vasoconstriction as a cause of erectile dysfunction and vascular diseases. "Age" has also been reported to have pivotal role in the development of vascular dysfunction resulting in erectile dysfunction and ultimately vascular diseases. Another theory explaining the pathophysiology of erectile dysfunction and its relationship with vascular diseases focuses on the formation of atherosclerosis plaques. This article endeavours to review the current literature and discuss why a multidisciplinary approach is needed while assessing erectile dysfunction.

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Key words: Coronary artery disease; Erectile dysfunction; Myocardial infarction; Stroke vascular disease

Core tip: Erectile dysfunction (ED) *per se* is not a life-threatening problem but it is a harbinger of more serious vascular impairments. Indeed, a strong correlation has been documented between the severity of ED and the severity of vascular diseases, myocardial infarction and stroke. The vascular symptoms are demonstrated 2-3 years on average after exhibition of the first symptoms of erectile dysfunction. This gap provides enough time to intervene and prevent subsequent vascular problems. Notwithstanding, the predictability of subsequent vascular disease may be missed as these patients are not simultaneously evaluated by an urologist and a cardiologist.

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INTRODUCTION

Erectile dysfunction (ED) is a prevalent complaint among men. It has been defined by the National Institute of Health Consensus Conference as "inability of the male to attain and maintain erection of the penis sufficient to permit satisfactory sexual intercourse"[1]. The prevalence of ED varies among different age groups.

Table 1 Some of the risk factors of vascular diseases

Diabetes mellitus	Older age
Psychological problem	Male
Psychiatric impairments	Drug and alcohol use
High blood pressure	Lack of nutrients
Hypercholesterolemia	Smoking
Hypertension	Overweight and obesity
Chronic systemic diseases	Lack of physical activity
Family history	Allergy

Table 2 Some of the risk factors of erectile dysfunction

Psychological disorders	Neurological problems
Various kinds of medicines	Parkinson's
Antihypertensive	Spinal cord injury
Cardiac drugs	Alzheimer's disease
Antidepressants	Local penile diseases
Anticholinergics	Smooth muscle dysfunction
Psychotropics	Smoking
Dopamine blockers	Overweight and obesity
Chronic systemic diseases	Injuries and trauma
Renal diseases	Alcohol use
Post-organ transplant	Older age
Cancer	Allergy
Vascular disease	
Endocrine disorders	
Diabetes mellitus	
Androgen deficiency/resistance	
Vascular disorders/insufficiency	
Atherosclerosis	
Venous leakage	
Arterial insufficiency	
Veno-occlusive diseases	

Research has shown that the prevalence of ED among 40 and 60 year old men is 8% and 40%, respectively[2]. It has also been reported in other research that five to ten percent of men who are in their 40 s experience ED, while 40%-60% of men who are 70 years of age or more suffer this disorder[3,4]. Research has shown that patients with vascular risk factors have greater risk of ED. In one study, 90.4% of men with ischemic heart disease reported moderate to severe degrees of ED[5]. In another study, 65% of men developed vascular ED after heart transplant[6]. The rate of severe ED was also reported to be 46.8% in men suffering ischemic heart disease, 59.1% of them had 3 or more cardiovascular risk factors[7].

A population of sixty-one million individuals in the United States suffer vascular diseases and it is still the most prevalent cause of death (40%) across the world. The risk of vascular diseases in men increases with age and becomes even worse in men suffering with ED[8].

Erectile dysfunction was primarily recognized as one of the consequences of vascular diseases in patients suffering heart attack or myocardial infarction. During the last decade, the introduction of sildenafil citrate to the market as a treatment for ED highlighted the mechanism of male arousal and signified the role of endothelial dysfunction in the occurrence of ED. It was then suggested that ED may not be simply a consequence of vascular diseases, but it can be an indicator of future vascular problems[9]. Although it has been well recognized that there is a relationship between the risk factors of vascular diseases and the progress of ED, there are still controversies over the issue of whether ED is a consequence of vascular diseases or a risk factor and predictor for the development of future vascular diseases.

Unlike neurogenic ED and ED resulting from pelvic trauma or surgical procedures, the severity of vasculogenic ED has been reported to be closely interrelated to the severity of vascular diseases[10]. Patients suffering ED exhibit various risk factors of vascular diseases, such as hypertension and older age[11], diabetes[12], smoking[13], dyslipidemia[14] and obesity[15] (Tables 1 and 2). In addition, men who experience erectile dysfunction suffer more undiagnosed ischemic heart diseases, myocardial infarction and other vascular diseases than men without ED[16].

Considering results of many research studies that focus on the relationship between ED and vascular diseases, this article endeavours to uncover the association between ED and vascular diseases. The paper also aims to highlight the importance of advanced medical assessment of patients suffering ED in order to prevent future vascular diseases. To this aim, the mechanism of male sexual arousal will be discussed first and factors affecting erectile function will be reviewed.

MECHANISM OF ERECTION AND FACTORS INDUCING ERECTILE DYSFUNCTION

The penis is comprised of three expandable erectile bodies, including a pair of corpora cavernosa and the corpus spongiosum. The corpus cavernosum consists of sinusoidal spaces and a bundle of smooth muscles which are responsible for the sponge-like tissue of the penis. The inside of the sinusoidal space is covered with endothelial tissue. The spaces are separated by connective tissue septa. There is also a complex network of arteries and nerve terminals within the tissues (Figure 1). All of these compartments turn the penis into an erectable body[17].

Penis blood supply is mainly provided by the paired internal pudendal artery which consecutively splits into three branches: the bulbourethral artery, dorsal artery and the cavernous artery (Figure 2). Although there are numerous small and large veins serving the glans penis, the deep dorsal vein provides the main venous drainage of the glans penis (Figure 3)[18].

Various psychogenic and sensory stimuli influence chemical, neurological and vascular cascades and trigger penile rigidity and erection. The stimuli in addition to spinal reflex intermediate the process and result in the relaxation of both corporeal and arterial wall smooth muscles, which in turn enhances arterial inflow. On the other hand, the venous wall smooth muscles contract in order to decrease the venous outflow to a minimum level and trap the blood in the penis, leading to penile

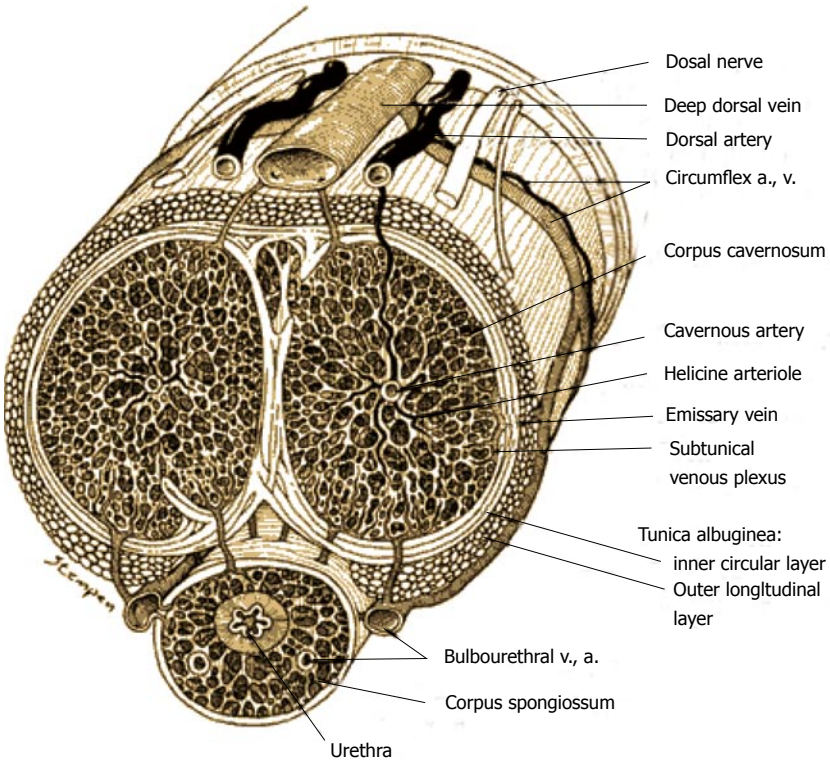


Figure 1 Cross section of penis. Tom Hubbard [Image]. (2012). Retrieved from http://penis-enlargement-manual.thundersplace.org/tom-hubbard/2001-01/anatomy.html.

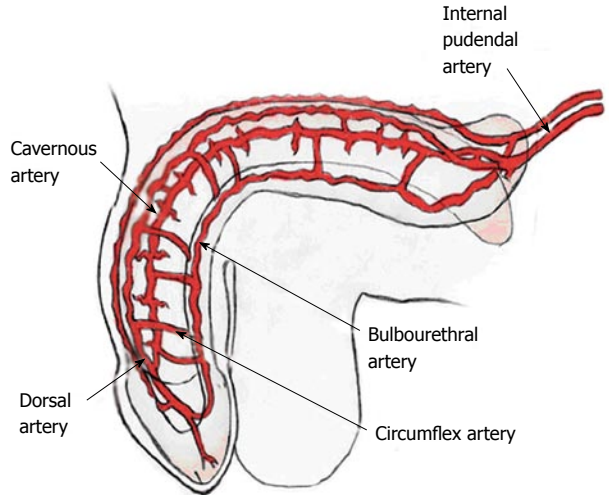


Figure 2 Arteries of penis. Pamela I Ellsworth [Image]. (2011). Retrieved from http://emedicine.medscape.com/article/1949325-overview#aw2aab6b3.

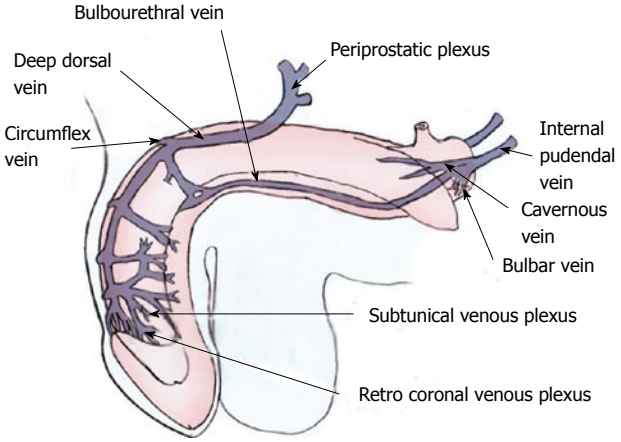


Figure 3 Veins of penis. Pamela I Ellsworth [Image]. (2011). Retrieved from http://emedicine.medscape.com/article/1949325-overview#aw2aab6b3.

erection. In fact, the penile tumescence highly depends on the balance between both contracting and relaxing factors which determine the arterial inflow and venous outflow[19]. It has been reported that 80-115 mL blood is necessary to keep a 7.5 cm penis fully rigid. At this stage, the intracorporeal pressure increases from 10-15 mmHg during flaccid state to 120 mmHg (suprasystolic pressure) during full rigidity when the penis is elevated to more than 90 degrees[20,21]. Subsequent to erection, several physical and physiological changes occur in the body, some of which are the increase of blood pressure, hyperventilation, sweating and generalized myotonia[21]. Penile tumescence can be affected by many factors creating various degrees of erectile dysfunction. A list of some of the influencing factors is presented in Table 1[21,22].

During arousal, nitric oxide (NO) is released into the penile corpus cavernosum[23] and stimulates the formation of cyclic guanosine monophosphate. This in turn results in the relaxation of smooth muscle of both blood vessels and corpus cavernosum followed by penile tumescence[24]. The relaxing effect of NO on vascular structure is also important to the health of the vascular system. Other functions of endothelial NO that contribute to the inhibition of atherosclerosis are that it decreases oxidation of LDL, reduces platelet aggregation to the endothelium, and prevents leukocyte adhesion and infiltration into the vessel wall. Therefore, it seems that any factor influencing the formation and activity of NO may be a risk factor for vascular diseases[25]. However,

DeBusk suggests that a lack of NO causes only ED in some men. Therefore, men who suffer ED but do not have the above-mentioned risk factors may not be at greater risk of vascular diseases[26].

ASSOCIATION BETWEEN ERECTILE DYSFUNCTION AND VASCULAR DISEASE

Although ED is a complex problem resulting from different kinds of factors, a link between ED and vascular disorders has been identified. Research has reported that vascular disorders/insufficiency is the most prevalent cause of ED[21] and suggested that "ED is an early symptom of generalised endothelial dysfunction"[22].

Considerable evidence shows the relationship between ED and vascular diseases. A strong correlation has been documented between the severity of ED and the severity of vascular diseases[27]. Patients who have vascular disorders, including peripheral vascular diseases, coronary diseases, cerebrovascular disorders and myocardial infarction, have a higher prevalence of ED[28]. Research has also demonstrated that more than half of the men who suffer coronary artery disease (CAD) experience ED[29].

Erectile dysfunction has been known as "the tip of iceberg" of a generalized vascular dysfunction which typically happens before serious vascular problems[29]. The predictability of CAD in ED patients was investigated in a population-based study conducted by Inman *et al*[4]. Results from 1402 men revealed that new ED developed in 6.4% in 2 years of follow-up, in an additional 5.4% at 6 years of follow-up, and in an additional 4.9% of men at 8 years of follow-up in each screening round. In addition, after the 10 year follow-up period, 11.1% of men developed coronary artery diseases and the incidence of coronary artery diseases was strongly associated with the increase of the patient's age. It was also reported that men suffering ED had a higher incidence of coronary artery diseases during the study period. Although the study by Corona *et al*[30] lacked information on other risk factors of coronary artery diseases such as dyslipidemia and lifestyle as reported in the literature, it showed that the risk of coronary artery diseases in men with ED was 80% more than that of men without ED. This finding was in accordance with the results of other studies in which the risk of coronary artery diseases in men suffering ED was 65%[31] and 45%[32]. Similarly, it was reported in another study by Vlachopoulos *et al*[33] that 19% of men who had ED and underwent angiographic evaluation had silent coronary artery diseases.

The development of new cases of coronary heart disease in patients with ED was investigated in another study[31]. A total of 2561 men were recruited in the research and were followed up for a period of 10 years. It was reported that the risk of coronary heart disease and stroke increased with an increase of age. While the risk of development of coronary heart disease after 10 years in men who had mild ED was 2.3%-11.2%, men with moderate/severe ED had a higher risk of coronary heart disease development (65%) during a 10 year follow-up period. Furthermore, the risk of stroke was higher among men with moderate/severe ED. These findings are compatible with those of the study by Blumentals *et al*[34] in which the predictability of acute myocardial infarction (AMI) in men with ED was investigated. Their cohort study consisted of 12825 men with ED and 12825 men without ED aged from 18 to 55 years. The results showed that there were no statistically significant differences between the two groups regarding smoking, obesity, the use of ACE inhibitors and the use of beta blockers and statins ($P > 0.05$).

Nevertheless, the risk of AMI among men who suffered ED was two times more than that of men without ED. The association between vascular disease and ED was also reported in an earlier study by Roumeguère *et al*[35] in which the risk of developing CAD during a ten year follow-up study was 34% higher among men with ED. Moreover, Blumentals *et al*[34] reported that the risk of AMI increased by the increase of participants' age, similar to the findings of the study by Inman *et al*[4]. A more recent study[36] supported these reports, indicating that ED is a significant marker for future stroke in men.

The link between hypertension and ED has also been documented in the research conducted by Solomon *et al*[16] in which the majority of patients who suffered ED and sought treatment for their problem had undiagnosed ischemic heart diseases and high blood pressure.

The relationship between ED and future vascular diseases was investigated in a study by Thompson *et al*[32]. In this study, 18882 men were recruited from the Prostate Cancer Prevention Trial and were allocated to either finasteride-receiving group or placebo-receiving group. Participants were then followed-up for a period of 10 years and were investigated for the development of vascular diseases. At the commencement of the study, 85% of men in the placebo arm had no vascular diseases and 53% of them did not have ED. After 5 and 6 years follow-up, 57% and after 7 years, 65% of men, respectively, developed erectile dysfunction. Compared with men who did not develop ED after 5 years, men who developed ED after 5 years were more likely to experience subsequent angina, myocardial infarction and stroke. It was also reported that this association was greater among men who had a history of hyperlipidemia or cigarette smoking. Although this study lacked information on the medications used by patients, findings of the study by Thompson *et al*[32] were similar to those of the study by Montorsi *et al*[37] in which 67% of patients who had acute chest pain and were diagnosed with CAD by the use of angiography had prior erectile dysfunction.

Diabetic patients have been reported to be at greater risk of both ED and vascular diseases[38,39]. In a cohort study by Ma *et al*[40], 2306 diabetic men were evaluated during a 4 year follow-up period. At baseline, vascular disease was not evident in any of the participants. However, 5.3% of diabetic men (123 subjects) developed

coronary heart disease during the follow-up period. In addition, the risk of ED was significantly associated with older age and longer duration of diabetes. The findings of the study showed that the prevalence of coronary heart disease was higher among those who had both ED and diabetes than in those who were only diabetic. It was also revealed that diabetic men with ED had 1.6 times greater risk of developing coronary heart disease. Although the study by Ma *et al*[40] suffered a bias selection and lacked the use of a standard questionnaire, their findings were in accordance with the results of other studies in which diabetic men who had ED were at increased risk of developing coronary heart disease[41-43]. Similarly, results of the study by Gazzaruso *et al*[44] showed that diabetic men with ED were at 14.8% greater risk of developing coronary heart disease. Gazzaruso *et al*[44] reported that ED was the most reliable indicator and a significant risk factor for future vascular diseases, which was supported by results of a recent study by Ryu *et al*[45].

In the study by Giuliano *et al*[42], diabetic and hypertensive patients were evaluated in order to investigate the prevalence of ED. To this aim, a population of 7689 patients were recruited and completed the study questionnaire. Analysis of the results uncovered that 66% of patients reported subjective feeling of having ED, which was in accordance with their scores obtained from the study questionnaire indicating that 67% of hypertensive patients had ED. The prevalence of ED increased with the longer duration of hypertension. The use of beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin Ⅱ receptor blockers was strongly associated with the decreased risk of ED. Moreover, 71% of diabetic patients suffered ED and the risk increased with the longer duration of diabetes mellitus. It was also revealed that 78% of patients who suffered both hypertension and diabetes were diagnosed with ED. Nevertheless, there is no conclusive evidence that shows whether hypertension and diabetes mellitus *per se* induce ED or if the medications used in these patients affect their sexual functioning[42].

THEORIES

Vascular disease and erectile dysfunction have been reported to share common risk factors[43]. It is suggested that early treatment of risk factors of ED may diminish the incidence of future cases of vascular diseases. Moreover, patients with ED need to be tested for hidden vascular diseases and should be followed-up for future vascular impairments[46,47].

Four theories have been developed to explicate the relationship between ED and vascular diseases. One of them is the "artery size" theory which focuses on the differences between the diameter of penile arteries (1-2 mm), coronary arteries (3-4 mm), carotid arteries (5-7 mm) and iliofemoral arteries (6-8 mm). This suggestion states that the small penile artery manifests obstruction sooner than the larger coronary and other vessels because an atherosclerotic plaque of the same size would cause significant obstruction sooner in a smaller vessel. Therefore, it could account for the earlier appearance of ED than of CAD[48].

Another theory highlights the existence of "endothelial dysfunction"[49]. Many men with ED exhibit endothelial cell inflammation and dysfunction. Endothelial dysfunction causes inappropriate vasoconstriction, impedes vasodilation and hinders the relaxation of smooth muscles of arterioles[50]. This theory suggests that the development of ED, CAD and atherosclerosis is based on similar endothelial dysfunction[34,51].

The third theory suggests that "age" has a pivotal role in the development of vascular dysfunction resulting in ED and ultimately vascular disease. The elastic lamella of vessels may suffer fatigue and be susceptible to fracture due to insistent and continuous pulsations during its life span. In addition, artery stiffness is likely to occur more in elderly men. These events result in the increase of systolic blood pressure and the fall of diastolic blood pressure. The arterial rigidity and systolic hypertension negatively affect the myocardium. It also puts the strain of cardiac pulsation on smaller arteries such as pudendal and penile arteries. The high blood pressure in penile arteries ultimately causes degeneration and ischemia of penile arteries and ends in ED[52].

Another theory explaining the pathophysiology of ED and its relationship with vascular disease emphasizes the importance of "atherosclerosis". This condition differs from the arterial stiffness due to old age. Atherosclerosis is recognised by the formation of plaques, especially lipid plaques, in the wall of blood vessels. The large-size plaques can lead to vessel occlusion. Larger plaques may obstruct the vessel or rupture. Considering the differences in diameters of penile, coronary, carotid and iliofemoral arteries, it is suggested that the penile artery may be the first place to be obstructed by atherosclerotic plaques. The occlusion of penile arteries may have different clinical presentations, one of which can be ED[4,53].

CONCLUSION

Talking about sexual performance has always been a taboo during different historical eras and men suffering ED have been reluctant to discuss the issue with professionals. Although recent breakthroughs in the science of sexology and campaigns promoting sexual health have decreased the social stigma relating to ED, the true rate of this disorder is underestimated because many men refuse to refer to a physician to treat their ED. Physicians are also reluctant to talk about it and would prefer not to bring up sexual matters during visits with patients[1]. On the other hand, the majority of cases of ED who refer to physicians are treated by general practitioners (GPs) but not specialists so the existence of vascular diseases or complex medical conditions in this group of men is typically not examined[54].

Erectile dysfunction *per se* is not a life-threatening problem but it is a harbinger of more serious vascular

impairments, such as vascular diseases, cardiac problems and cerebrovascular diseases. The Second Princeton Consensus Conference concluded that ED is an alarming signal of a hidden vascular disease. The consensus was that every man suffering different degrees of ED has to be regarded as a case of vascular disease unless proven otherwise[29,51].

It appears that vascular disease may be possible to predict in men, specifically those with various degrees of erectile dysfunction. Research has reported that vascular symptoms are demonstrated 2-3 years on average after exhibition of the first symptoms of ED[55]. This gap provides enough time to intervene and prevent subsequent vascular problems. Notwithstanding, the existing issue is that men with ED refer to general physicians or urologists but not cardiologists so the predictability of subsequent vascular disease may be missed. To resolve this problem, a multidisciplinary approach is suggested in which the cardiologists work closely with urologists and general physicians[56]. All men with ED need a vascular assessment and must be checked for high blood pressure, hyperlipidemia and hyperglycemia. The aim is not to detect ED and treat it by the cardiologist, but to actively develop a multidisciplinary management plan, which in turn may decrease morbidity and mortality of vascular events in the future[57].

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REVIEW

"Targeting" renal cell carcinoma patients with "targeted" agents: Are we there yet?

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Abstract

The rapid approval of several novel agents, targeting the vascular endothelial growth factor or mammalian target of rapamycin pathways (sunitinib, pazopanib, sorafenib, axitinib, bevacizumab, everolimus, temsirolimus) has given to metastatic renal cell carcinoma (mRCC) patients and their treating physicians many new and effective therapeutic options. The treatment paradigm for these patients is rapidly evolving, with future studies needed to define the optimal sequencing of these new agents. Despite progresses, no validated biomarkers able to predict clinical outcome or useful to guide patient selection for treatment are currently available. Recent studies have suggested that some biomarkers, including cytokines, circulating proangiogenic factors, markers of hypoxia or targets of signaling pathways are potentially promising prognostic or predictive factors in mRCC. We present an overview of the most recent developments in identifying biomarkers for targeted therapies in advanced RCC.

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Key words: Biomarkers; Renal cell carcinoma; Prognostic biomarker; Predictive biomarker; Vascular endothelial growth factor tyrosine kinase inhibitors; Mammalian target of rapamycin inhibitors

Core tip: To date, there are no fully validated biomarkers for daily clinical use in enal cell carcinoma (RCC) treatment and the therapeutic decisions still depend exclusively on morphological and clinical criteria. Predictive markers of response are not yet available and consequently, all patients continue to be exposed to potentially toxic therapies without certainty of clinical benefit. The identification of reliable biomarkers could represent the turning point of the personalized treatment in RCC. Therefore, the design of clinical trials based on a biomarkers-approach is highly desirable in order to minimize costs and risks of treatments and to maximize the benefit to the patient.

Maines F, Pilotto S, Milella M, Massari F, Vaccaro V, Felici A, Bria E, Tortora G. "Targeting" renal cell carcinoma patients with "targeted" agents: Are we there yet? *World J Clin Urol* 2014; 3(1): 9-19 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i1/9.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i1.9

INTRODUCTION

Advances in the understanding of the biology of metastatic renal cell carcinoma (mRCC) have led to the iden-

tification of vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) as key targets of signaling pathways involved in tumor growth and angiogenesis[1]. In recent years, several new drugs (sunitinib, pazopanib, sorafenib, axitinib, bevacizumab, everolimus, temsirolimus) targeting these pathways, have been approved by regulatory authorities for the treatment of mRCC on the basis of advantages in clinical outcomes demonstrated in phase Ⅲ clinical trials[2-9]. With the exception of axitinib, which was compared with sorafenib, these drugs were approved on the basis of comparison with either cytokine therapy or placebo.

The availability of many active drugs for the same disease setting, often with similar mechanisms of action, has certainly widened therapeutic perspectives for patients but, given the current lack of superiority, head to head, clinical trials, it has sometimes increased the difficulty of the physician in making the best clinical decision. Recently, data from COMPARZ trial showed that both sunitinib and pazopanib are suitable frontline treatments for patients with advanced RCC, considering the similar efficacy results (*i.e.*, non-inferiority)[10]. The trial confirmed the different toxicity profile of the two agents with more fatigue, mucositis and hand-foot syndrome seen in sunitinib-treated patients and more liver function abnormalities, weight loss and alopecia with pazopanib but clinical or molecular factors able to predict response to one rather than another are today lacking.

Inherited or sporadic von Hippel-Lindau (VHL) tumor suppressor gene inactivation (by mutation, promoter methylation or loss of heterozygosity) is involved in most cases of clear cell RCC, which is the most common histological subtype of renal cell carcinoma (RCC). VHL inactivation usually leads to increased levels of hypoxia-inducible factors (HIF) and consequently to an over-expression of HIF target genes such as VEGF, platelet-derived growth factor (PDGF), carbonic anhydrase IX (CAIX), transforming growth factor α (TGF-α), interleukin (IL)-8 and others, which notoriously promote angiogenesis, tumor growth and metastasis[11]. The comprehension of the critical role of VHL/HIF/VEGF axis in RCC pathogenesis has led to important advances in tumor treatment and consequently to relevant improvements in clinical outcomes. However, despite efficacy of the new drugs for mRCC, there are few complete responses and resistance eventually develops at a median of less than 12 mo[12].

For all these reasons, better comprehension of intrinsic or acquired resistance to targeted agents (TA) is an increasingly interesting objective for medical research in order to optimize personalized therapy and to reduce toxicities and costs. Considering the mechanism of action of VEGF-targeted agents (sunitinib, pazopanib, sorafenib and axitinib inhibit the tyrosine kinases of VEGF receptors 1-3, bevacizumab specifically targets the ligand VEGF), several elements of this signaling pathway have been recently investigated in multiple clinical trials of TA to identify predictive or prognostic biomarkers[13]. Biomarkers evaluated in phase Ⅲ or pivotal trials of targeted agents in advanced RCC are summarized in Table 1. The first results are encouraging but further confirming studies are needed to prospectively validate them for daily clinical practice. Blood biomarkers might have more advantages than analyses on tissue samples: in addition to being noninvasive, simple and relatively inexpensive, blood sample collections are potentially useful to monitor changes during treatment and disease progression.

SEARCH STRATEGY

The purpose of this paper is to review the most recent developments of biomarkers evaluation in TA-clinical trials for mRCC. Particularly, we analyzed biological or molecular factors potentially able to predict the clinical outcomes or potentially useful to identify the patients who are more likely to benefit from a given treatment. We included in our search observational, retrospective, phase Ⅱ and phase Ⅲ TA-mRCC studies evaluating at least one potential biomarker. Among these, we selected for our revision only studies, published from January 2007 to August 2013, with at least one biomarker evaluated in patients enrolled in phase Ⅱ or Ⅲ clinical trials and treated with tirosin kinase or mTOR inhibitors. The study selection process and reasons for exclusion are presented using a PRISMA study flow diagram (Figure 1). Full reports and trials' updates were gathered through Medline (PubMed), American Society of Clinical Oncology and European Society for Medical Oncology website searches. Published papers were obtained from the Pubmed database, using the subsequent MeSH (Medical Subject Heading) terms: "predictive" or "prognostic" or "biomarkers" each combined with "renal cell carcinoma". We also used combinations of the following words: "biomarker", "targeted agents", "tirosin-kinase inhibitors", "mTOR inhibitors", "kidney cancer", "metastatic RCC" and "phase Ⅲ clinical trial".

SORAFENIB

Sorafenib is an orally active multikinase inhibitor that blocks VEGF receptor (VEGFR)-2/-3, PDGF receptor (PDGFR)-β, RAF-1, Flt-3, RET and stem cell factor receptor, c-Kit. In the phase Ⅲ, placebo-controlled, Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET) study, sorafenib demonstrated to significantly improve progression-free survival (PFS) in mRCC patients, who experienced a treatment failure with a prior cytokine-based therapy (5.5 mo *vs* 2.8 mo; HR = 0.44; 95%CI: 0.35-0.55, $P < 0.000001$)[4]. A survival advantage was not observed because of the crossover of patients on placebo to sorafenib after the first-interim analysis results[14].

In RCC, increased serum VEGF concentrations have been observed not only in patients with higher tumor stage and grade but also in those with poor performance status (PS) and worse prognosis[15-17]. Baseline VEGF levels were analyzed also in the pivotal trial of

Table 1 Potential biomarkers evaluated in phase Ⅲ or pivotal trials of targeted agents in advanced renal cell carcinoma

Targeted agent	Authors	Biomarker		Outcomes			
				PFS		OS	
				HR (95%CI)	*P*	HR (95%CI)	*P*
Sorafenib	Escudier *et al*[14]; Peña *et al*[18]	Baseline VEGF	Low-VEGF (25th percentile)	0.92 (0.57-1.49)	0.001	NA	NA
			High-VEGF (25th percentile)	0.42 (0.31-0.56)		NA	NA
			Low-VEGF (75th percentile; ≤ 254 pg/mL)	0.58 (0.43-0.78)	0.02	NA	NA
			High-VEGF (75th percentile; > 254 pg/mL)	0.27 (0.15-0.46)		NA	NA
			Low-VEGF (median value; ≤ 131 pg/mL)	0.64 (0.49-0.83)	0.096	0.89 (0.63-1.27)	0.327
			High-VEGF (median value; > 131 pg/mL)	0.48 (0.38-0.62)		0.81 (0.60-1.09)	
	Peña *et al*[18]	sVEGFR-2	Low-sVEGFR-2 (median value)	0.56 (0.43-0.72)	0.981	0.90 (0.65-1.24)	0.598
			High-sVEGFR-2	0.55 (0.42-0.71)		0.82 (0.60-1.14)	
		CAIX	Low-CAIX (median value)	0.52 (0.26-1.03)	0.72	0.98 (0.40-2.42)	0.540
			High-CAIX	0.62 (0.33-1.16)		0.71 (0.35-1.45)	
		TIMP-1	Low-TIMP-1 (median value)	0.58 (0.26-1.30)	0.83	0.76 (0.26-2.19)	0.670
			High TIMP-1	0.54 (0.29-0.99)		0.58 (0.29-1.15)	
		Ras	Low-Ras (median value)	0.74 (0.37-1.52)	0.27	1.23 (0.50-3.04)	0.180
			High-Ras	0.41 (0.22-0.79)		0.53 (0.25-1.13)	
		VHL mutational status	Exon 1 only; WT	0.55 (0.34-0.89)	0.356	0.74 (0.39-1.40)	0.634
			Exon 1 only; mut	0.39 (0.15-1.04)		1.08 (0.32-3.59)	
			Exon 2 only; WT	0.45 (0.26-0.77)	0.363	0.56 (0.26-1.22)	0.536
			Exon 2 only; mut	0.89 (0.24-3.34)		0.33 (0.03-3.19)	
			Exon 3 only; WT	0.62 (0.38-1.00)	0.985	0.76 (0.40-1.44)	0.991
			Exon 3 only; mut	0.00 (0.00-NA)		0.00 (0.00-NA)	
			Exon 1, 2 or 3; WT	0.52 (0.23-1.19)	0.968	1.09 (0.36-3.27)	0.693
			Exon 2, 3 or 3; mut	0.49 (0.22-1.08)		0.88 (0.31-2.48)	
Pazopanib	Tran *et al*[25]	IL-6	Low IL-6	0.55 (0.38-0.81)	0.009	1.41 (0.65-3.05)	0.005
			High IL-6	0.31 (0.21-0.44)		0.42 (0.28-0.63)	
		IL-8	Low IL-8	0.41 (0.28-0.60)	0.472	1.49 (0.69-3.22)	0.002
			High IL-8	0.39 (0.27-0.56)		0.42 (0.28-0.63)	
		Osteopontin	Low Osteopontin	0.43 (0.29-0.64)	0.343	0.96 (0.50-1.86)	0.033
			High Osteopontin	0.35 (0.24-0.51)		0.41 (0.27-0.62)	
		VEGF	Low VEGF	0.47 (0.32-0.69)	0.376	1.2 (0.61-2.39)	0.006
			High VEGF	0.41 (0.28-0.60)		0.41 (0.27-0.62)	
		Hepatocyte growth factor	Low HGF	0.40 (0.27-0.58)	0.52	0.68 (0.36-1.27)	0.765
			High HGF	0.46 (0.32-0.67)		0.59 (0.39-0.90)	
		TIMP-1	Low TIMP-1	0.38 (0.26-0.55)	0.922	0.64 (0.38-1.10)	0.387
			High TIMP-1	0.40 (0.27-0.59)		0.5 (0.32-0.79)	
		E-selectin	Low E-selectin	0.49 (0.33-0.71)	0.219	0.55 (0.34-0.91)	0.872
			High E-selectin	0.36 (0.25-0.52)		0.59 (0.36-0.96)	
	Xu *et al*[27,29]	IL-8	Polymorphism 2767A > T		0.009		0.030
			AA				
			AT	1.3 (0.9-1.8)	0.1	NA	
			TT	1.8 (1.2-2.7)	0.009	2.2 (1.3-3.6)	0.030
			Polymorphism -251T > A		0.01	NA	NA
			TT				
			AT	1.4 (1.0-1.9)	0.08		
			AA	1.7 (1.1.-2.5)	0.01		
		HIF1A	Polymorphism 1790G > A		0.03	NA	NA
			GG				
			AG	1.8 (1.1.-3.1)	0.03		
		NR1/2	Polymorphism -25385C > T		0.06	NA	
			CC				
			CT	1.2 (0.9-1.6)	0.3		NA
			TT	1.5 (1.0-2.2)	0.07		0.020
		VEGFA	Polymorphism-2578A > C		0.7	NA	NA
			AA				
			AC	0.8 (0.6-1.2)	0.2		
			CC	0.9 (0.6-1.4)	0.7		
			Polymorphism -1498C > T		0.7	NA	NA
			CC				
			CT	0.8 (0.6-1.2)	0.2		
			TT	0.9 (0.6-1.4)	0.7		
			Polymorphism -634G > C		0.7	NA	NA
			GG				
			CG	0.9 (0.7-1.3)	0.7		
			CC	1.0 (0.6-1.7)	0.9		

	Xu *et al*[29]	FGFR2	Polymorphism IVS2 + 906C > T	NA	NA		0.02
			CC				
			CT			NA	NA
			TT			1.7 (1.1.-2.8)	0.02
Bevacizumab	Lambrechts *et al*[47]	VEGFR1	Polymorphisms rs7993418 or rs9554316	1.81 (1.08-3.05)	0.033	0.91 (0.45-1.82)	0.78
			TT				
			TC				
			CC				
			Polymorphism rs9513070	1.68 (1.12-2.52)	0.018	NA	NA
			AA				
			AG				
			GG				
	Escudier *et al*[46]	Baseline VEGF	Low-VEGF (median value; < 54 pg/mL)	NA	NA	0.75 (0.51-1.08)	NA
			High-VEGF (median value; > 54 pg/mL)	NA	NA	0.92 (0.66-1.28)	NA
Everolimus	Oudard *et al*[48]	Baseline VEGF-A	Low-VEGF-A	1.27 (1.03-1.57)	0.028	NA	NA
Temsirolimus	Figlin *et al*[50]	PTEN	Positive	0.66 (0.47-0.93)	NA	NA	NA
			Negative	0.99 (0.58-1.69)			
		HIF1α	Positive	0.98 (0.64-1.51)	NA	NA	NA
			Negative	0.72(0.51-1.00)			

NA: Not available; PFS: Progression-free survival; OS: Overall survival; HR: Hazard ratio; VEGF: Vascular endothelial growth factor; sVEGFR: Serum vascular endothelial growth factor receptor; CAIX: Carbonic anhydrase IX; TIMP: Tissue inhibitors of metalloproteinases; WT: Wild-type; mut: Mutated; HGF: Hepatocyte growth factor; IL: Interleukin.

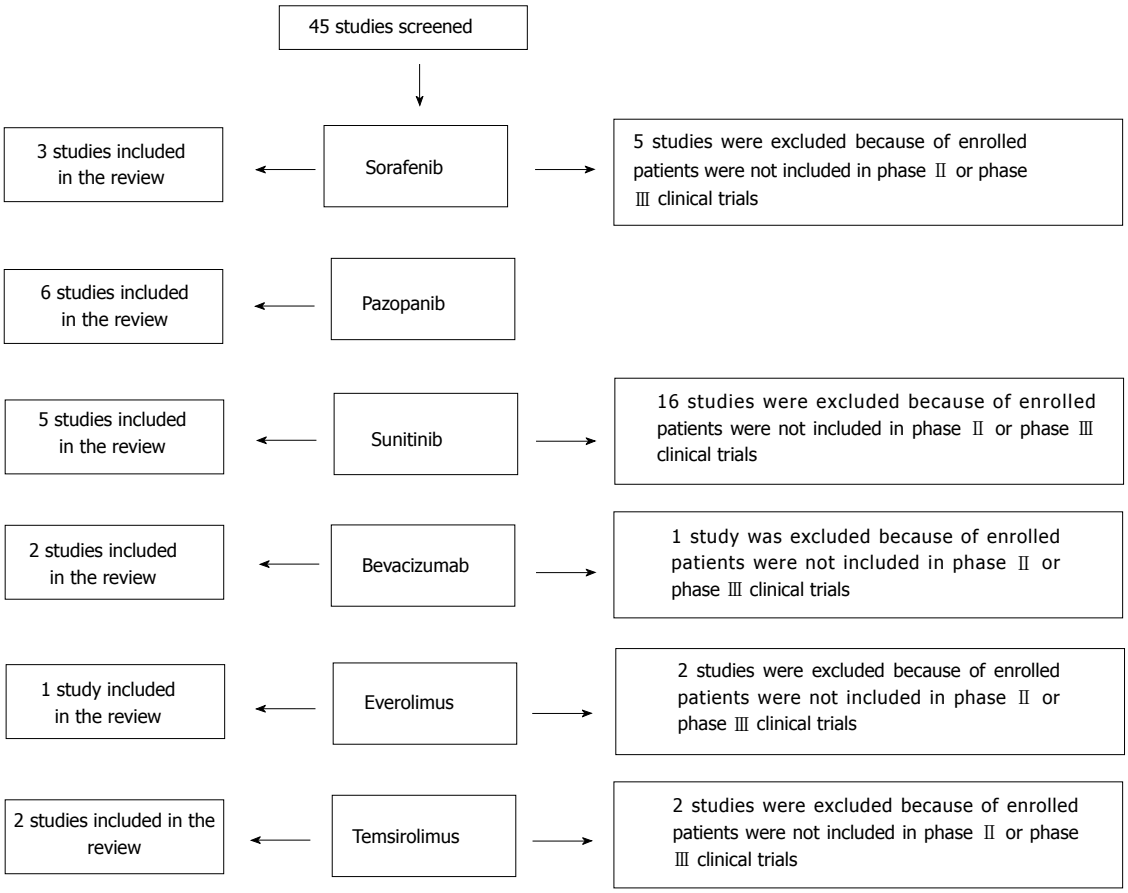


Figure 1 PRISMA study flow diagram.

sorafenib, confirming that serum VEGF concentrations are usually related to an aggressive tumor biology[14,18]. Patients with higher Memorial Sloan-Kettering Cancer Center (MSKCC) risk score or higher Eastern Oncology Cooperative Group (ECOG) PS and consequently with prognostically negative clinical indicators, had significantly higher pretreatment VEGF levels than those with a better prognosis according to MSKCC score ($P < 0.0001$) or with better ECOG PS ($P < 0.0001$)[14,18]. In the multivariate analysis that included also MSKCC score

and ECOG PS, baseline VEGF levels were independent prognostic factors both for PFS and overall survival (OS) in placebo-treated patients (P = 0.0231 and P = 0.0416, respectively) and only for OS in patients treated with sorafenib (P = 0.0145)[14]. With regard to sorafenib antitumor activity, Escudier and colleagues suggested that both patients with high and low baseline VEGF levels (above or below the median value) seem to benefit from sorafenib treatment, but those with higher VEGF levels (above the 75th percentile) may benefit more (in terms of PFS) (HR = 0.27; 95%CI: 0.15-0.46) than those in the low-VEGF group (HR = 0.58; 95%CI: 0.43-0.78)[14].

Further molecular analyses in plasma and tissue samples of patients enrolled in TARGET trial demonstrated that patients with an intermediate prognosis according to MSKCC score have also higher baseline concentrations of CAIX (P = 0.027) and tissue inhibitor of metalloproteinase 1 (TIMP-1) (P = 0.0001) than those with low MSKCC score. Moreover, a higher incidence of VHL mutations was most frequently observed in patients with poor ECOG PS (P = 0.008)[18]. Increased pretreatment levels of CAIX (P = 0.0034), TIMP-1 (P = 0.001) and Ras p21 (P = 0.016) were indicators of worse clinical outcome in the placebo cohort at univariable analysis; however, only TIMP-1 remained an independent prognostic factor for OS at multivariate analysis (P = 0.002)[18]. In this study, no correlation between VEGFR-2, CAIX, TIMP-1, Ras p21, VHL mutational status and sorafenib efficacy was observed. In the group of patients treated with sorafenib, the authors observed a significant increase of VEGF and a decrease of VEGFR-2 levels during treatment (P < 0.0001 and P < 0.0001, respectively), as already observed in preclinical models[19] and with other anti-angiogenic drugs[20,21].

The baseline analysis of more than 50 cytokines and angiogenic factors (CAFs) in blood samples from patients enrolled in a phase Ⅱ clinical trial comparing frontline sorafenib versus sorafenib plus interferon-α (IFN-α) in mRCC, has proven useful in identifying two prognostic groups of patients ("angiogenic" and "inflammatory" group) on the basis of a peculiar biomarker profile[22]. Moreover, a six-marker angiogenic signature seems to be useful to identify patients who obtained a greater PFS benefit from sorafenib alone than from sorafenib plus IFN. Particularly, higher osteopontin (OPN), VEGF, CAIX, collagen Ⅳ and VEGFR-2 levels were significantly associated with a shorter PFS in the combination arm while higher tumor necrosis factor-related apoptosis-inducing ligand concentrations showed an opposite effect on clinical outcome[22].

PAZOPANIB

Pazopanib is an oral multi-kinase inhibitor, targeting VEGFR-1/-2/-3, PDGFR-α/-β and c-Kit. As compared to placebo, pazopanib demonstrated to significantly improve PFS (9.2 mo *vs* 4.2 mo, P < 0.0001) in treatment-naïve or cytokine-pretreated advanced/metastatic RCC patients[3], without a significant difference in the intention to treat final analysis of OS, likely due to the extensive cross-over from placebo to pazopanib[23]. Recently, a phase Ⅲ, head-to-head clinical trial (COMPARZ trial), comparing pazopanib to sunitinib, two approved first-line treatment options, showed the non-inferiority of pazopanib versus sunitinib in terms of PFS (HR = 1.05, 95%CI: 0.90-1.22) and confirmed the different safety profile of the two drugs[10].

In a retrospective analysis of pretreatment plasma samples collected from two prospective cohorts of mRCC patients treated with pazopanib (one from a single-arm phase Ⅱ trial and one from a phase Ⅲ randomized placebo-controlled trial)[3,24], the authors showed that CAFs profiling could be useful to provide prognostic or predictive associations in RCC[25]. The authors initially screened blood CAFs in patients treated with pazopanib who had the greatest or least tumor shrinkage in a phase Ⅱ trial; then they confirmed the association of these biomarkers (and other from previous studies) with tumor response and PFS in the same cohort of patients. Finally, they validated confirmed CAFs in a group of patients (n = 344 samples, 79% of 435 enrolled patients) from a randomized placebo-controlled phase Ⅲ trial. The authors demonstrated that patients treated with pazopanib with higher (relative to median) pretreatment concentrations of IL-8 (P = 0.006), OPN (P = 0.0004), hepatocyte growth factor (HGF) (P = 0.01) and TIMP-1 (P = 0.006) had statistically significant shorter PFS than did those with lower concentrations[25]. In the placebo group, higher concentration of IL-6 (P < 0.0001), IL-8 (P = 0.002) and OPN (P < 0.0001) were prognostically related to a shorter PFS. In the study, only high IL-6 levels were predictive of PFS benefit with pazopanib (P = 0.009). Moreover, the study demonstrated that biomarkers such as IL-6, IL-8 and osteopontin have a stronger prognostic role than the standard clinical classifications such as ECOG, MSKCC and Heng risk grouping[25].

Single nucleotide polymorphisms (SNPs) are germline DNA variants involved in patient drug exposure with potential implications for drug selection, dosage optimization and toxicity management[26]. The potential association between SNPs and pazopanib efficacy or tolerance was recently evaluated on blood samples of patients enrolled in phase Ⅱ and phase Ⅲ RCC clinical trials with conflicting results[27-29]. Inherited genetic variants in angiogenesis- or exposure-related genes have shown a significant association with clinical outcomes (IL-8, FGFR2, NR1I2 and ABCB1 with OS, $P \leqslant 0.05$; IL-8 and HIF1A with PFS, $P \leqslant 0.05$; HIF1A, NR1/2 and VEGF-A with response rate, $P \leqslant 0.05$) in pazopanib-treated patients, suggesting that patients with genetic variations associated with angiogenesis or with increased clearance of pazopanib, may have a different clinical benefit from the treatment[27,29]. However, the accuracy of IL-8 polymorphisms to predict pazopanib efficacy has not been confirmed in COMPARZ trial (P = 0.30)[28].

Choueiri *et al*[30] have evaluated *VHL* gene status (mutation and/or methylation), HIF-1α/-2α immunochemistry staining and HIF-1α transcriptional signature

in tumor tissue samples of mRCC patients treated with pazopanib in a phase Ⅱ study and have not observed any correlation between these biomarkers and clinical outcomes, such as overall response rate (ORR) and PFS. Another study evaluating VHL inactivation and HIF-1α/-2α immunochemistry expression in tumor samples of mRCC patients who received first-line targeted therapy has confirmed that VHL gene status is not a predictive factor of efficacy for TA[31]. Moreover, Saez and colleagues showed a significant association between HIF-1α/HIF-2α expression and both PFS (both, $P = 0.04$) and OS (both, $P < 0.0001$) but using a different staining intensity score, compared to the abovementioned study of Choueiri *et al*[30]. HIF-1α was also identified as predictive factor for ORR ($P = 0.001$)[31].

Another retrospective study in patients treated with VEGF inhibitors (sunitinib, sorafenib, axitinib or bevacizumab) confirmed that VHL inactivation did not appear to affect ORR (41% *vs* 31% in wild-type VHL patients, $P = 0.34$) or PFS; however, it was observed that patients with severe/loss of function mutations of VHL (frameshift, nonsense, splice and in-frame deletions/insertions) show higher response rate than those with wild-type VHL (52% *vs* 31%, $P = 0.04$) but without a significant impact on PFS or OS[32].

Polymorphisms of VEGF-A/-C and VEGFR-1/-2/-3 seem to be also useful in first-line treatment decisions (between sunitinib and pazopanib) as recently suggest by Bianconi and colleagues[33]. Indeed, patients with polymorphisms of VEGF-A rs833061 (CC + CT *vs* TT) seem to significantly have better PFS and OS (both, $P < 0.0001$) if treated with sunitinib. Conversely, patients with CT + TT polymorphisms (*vs* CC) seem to have more benefit when treated with pazopanib ($P = 0.027$).

Recently, Hudes *et al*[34] demonstrated that patients affected by mRCC with specific tumor DNA copy number alterations (CNAs) are better responders to pazopanib. Particularly, the gain of chromosome 5q (5q+) seems to be related to a statistically significant longer PFS ($P = 0.026$). Moreover, the effect on PFS was even more relevant when 5q+ and no loss of chromosome 14 or 14q (HIF-1α locus) were both present[34].

SUNITINIB

Sunitinib is a small anti-angiogenic molecule that inhibits the tyrosine kinase activity of VEGFR, PDGFR, FLT3, c-Kit and RET. Sunitinib has become a therapeutic option for first-line therapy of good and intermediate prognosis patients with mRCC on the basis of significant advantages (in terms of both PFS and OS) over IFN-α in a phase Ⅲ trial[2]. Not all patients respond to the treatment and some develop significant toxicities resulting in dose delays, dose reduction or drug discontinuation. The identification of molecular factors able to identify the patients who will more likely benefit from the treatment or develop adverse events could help personalizing treatment, improving treatment outcomes and reducing toxic effects.

Molecular analysis performed on baseline blood samples of 52 sunitinib-treated patients enrolled in a first-line phase Ⅲ trial comparing sunitinib to IFN in mRCC, has demonstrated that higher pretreatment CAIX levels significantly correlated with shorter PFS and OS ($P < 0.007$ and $P < 0.0001$, respectively)[35]. In a multivariate analysis (including treatment arm, CAIX and MSKCC score) treatment with sunitinib (compared to IFN-α) and lower CAIX concentrations were significantly associated with longer PFS ($P = 0.006$ and $P = 0.04$, respectively) and better OS ($P = 0.037$ and $P < 0.0001$, respectively)[35]. In a phase Ⅱ study evaluating the efficacy and safety of sunitinib in mRCC patients previously treated with bevacizumab, the authors showed that baseline levels of VEGF-A and placental growth factor (PIGF) were significantly higher in patients who started sunitinib within ten weeks after bevacizumab discontinuation ($P < 0.0001$ and $P = 0.0008$, respectively), likely due to a residual bevacizumab effect[36]. Moreover, it was observed that sunitinib-responders had significantly lower pretreatment concentrations of VEGFR-3 and VEGF-C than those who did not respond ($P < 0.0318$)[36,37]. During treatment, the authors also observed an increase in VEGF-A and PlGF plasma levels and a decrease of VEGFR-3 and VEGF-C from baseline, without a correlation with PFS or ORR[37]. No clear associations between pre-treatment VEGF-A levels and sunitinib efficacy were observed, as also suggested by another study[38]. Conversely, Porta *et al*[39] reported a potential predictive role for baseline VEGF levels; patients who had a baseline VEGF titer above a given threshold seemed to have a higher risk of disease progression than those with values below the threshold. As for VEGF, the authors also observed that higher baseline levels of neutrophil gelatinase-associated lipocain (NGAL), a protein usually up-regulated in RCC tumorigenesis, were associated with a worse outcome in advanced RCC patients treated with sunitinib as first-line treatment[39]. In contrast to the results of biomarker analysis of Rini[37], Kontovinis and colleagues reported that the increase of VEGF-A levels was much higher in patients who progressed early during sunitinib treatment. Interestingly, the authors did not observed an increase of VEGF-A concentrations in patients who had a disease progression after an initial clinical benefit, suggesting an involvement of a different mechanism of resistance[39].

Recently, some authors prospectively identified a group of SNPs as predictors of efficacy and tolerability of sunitinib in previously untreated mRCC patients[40]. Particularly, two missense polymorphisms in VEGFR-3 (rs307826 and rs307821) were significantly associated with shorter PFS in sunitinib-treated patients ($P = 0.00049$ and $P = 0.014$, respectively). Moreover, the authors showed that a specific polymorphism in the *CYP3A5* (rs776746) gene, encoding for an enzyme that metabolizes sunitinib, was associated with a significantly increased risk of sunitinib dose reduction due to toxicity ($P = 0.022$)[40].

A retrospective study performed by Van der Veldt *et al*[41]

showed that other SNPs in *CYP3A5*, *NR1I3* and *ABCB1* genes, usually involved in sunitinib pharmacokinetics, were independent predictive factors for prolonged PFS (HR = 0.26, HR = 1.75 and HR = 0.55, respectively) at multivariate analysis, including current clinical prognostic factors such as MSKCC risk score, age and number of metastatic sites. Moreover, a genetic variant in the *VEGFR-2* gene (1718T/A) seems to predict a prolonged OS ($P < 0.05$), whereas a trend toward an improved OS was observed for patients with somatic genetic alterations in ABCB1 ($P = 0.078$)[41]. Conversely, Kim *et al*[42] did not observed any significant correlation between single VEGF or VEGFR polymorphisms and clinical outcomes, either PFS and OS, in 37 patients treated with sunitinib. However, a combination of VEGF SNP 936 and VEGFR-2 SNP 889 seems to predict OS ($P = 0.03$) during sunitinib treatment; this cohort of patients has a 3-fold greater risk of death than patients with other genotypes (HR = 3.18)[42]. SNPs in catechol-O-methyltransferase, an enzyme involved in catecholamine and estrogen metabolism, were associated with a significant difference in PFS and OS ($P = 0.0001$ and $P = 0.0001$, respectively) in sunitinib-treated patients[43].

Activation or up-regulation of alternative angiogenesis signaling pathways represents an important mechanism of resistance to drugs targeting VEGF/VEGFR pathway, underlying disease progression. IL-8-mediated angiogenesis seems to play a critical role in both acquired and intrinsic resistance to sunitinib; for this reason, IL-8 secretion could be a potential therapeutic target to overcome sunitinib resistance[44]. Recently, Xu *et al*[28] have demonstrated a significant association of IL-8 polymorphisms with both PFS ($P = 0.017$) and OS ($P = 0.0043$) in sunitinib-treated patients enrolled in the COMPARZ trial, suggesting a promising role of IL-8 in to treatment decision.

Other evidence suggest the potential role of HIF-1α/-2α levels to predict response to TA in mRCC, particularly to sunitinib; patients with higher levels of HIF-1α or HIF-2α are more likely to obtain a favorable tumor response according to RECIST criteria ($P = 0.003$ and $P = 0.001$, respectively) than those with lower or absent expression[45].

BEVACIZUMAB

Bevacizumab is a humanised monoclonal antibody that inhibits angiogenesis by targeting the VEGF protein. Two phase Ⅲ first-line clinical trials, CALGB 90206 and AVOREN, comparing the combination of bevacizumab plus IFN to IFN alone in mRCC have demonstrated a significantly longer PFS in the combination arm, as compared with IFN monotherapy[6,7]. Molecular analysis performed on plasma samples of patients enrolled in the AVOREN trial showed that the efficacy of bevacizumab, in terms of PFS, appears to be independent of baseline VEGF concentrations ($P = 0.15$)[46]. A recent evaluation of 138 SNPs in AVOREN patients receiving bevacizumab has demonstrated a significant correlation between polymorphisms in a locus in the tyrosine kinase domain of VEGFR-1, leading to an increase of VEGFR-1 expression, and shorter PFS ($P = 0.033$) and consequently with a unfavorable bevacizumab treatment outcome[47].

EVEROLIMUS

Everolimus is an orally administered selective mTOR inhibitor that has shown to improve PFS in mRCC patients progressing on VEGF-tyrosine kinase inhibitors, as compared to placebo (4.9 mo *vs* 1.9 mo; $P < 0.001$)[8]. In plasma samples of patients enrolled in the pivotal RECORD-1 (renal cell cancer treatment with Oral RAD001 given Daily) trial, Oudard *et al*[48] did not observe different baseline levels of VEGFR-2, VEGF-A and bFGF between everolimus-treated patients and those enrolled in the placebo arm. Moreover, the authors showed that everolimus significantly improved median PFS over placebo regardless of baseline biomarkers ($P < 0.001$), suggesting that these factors are not able to predict everolimus efficacy[48]. As yet suggested by other previously reported studies, VEGF-A baseline levels seem to have a prognostic role in mRCC; lower VEGF-A concentrations seem to be associated to a prolonged PFS ($P = 0.028$). Everolimus-treated patients experienced a significant reduction in bFGF ($P = 0.0095$) and VEGFR-2 levels ($P < 0.001$), as compared to baseline, during the treatment, confirming a critical role of everolimus in targeting these signaling pathways[48]. Recently, Pomerantz *et al*[49] demonstrated an association between inherited genetic variations across two critical genes in the mTOR pathway [phosphoinositide-3-kinase, catalytic, alpha polypeptide (PI3KCA) and mTOR itself] and clinical outcomes in 76 mRCC patients treated with everolimus or temsirolimus, after receiving a prior systemic therapy. Particularly, two PI3KCA SNPs (rs13082485 and rs2699905) were significantly associated with PFS ($P = 0.008$ and $P = 0.03$, respectively) and OS ($P = 0.007$ and $P = 0.006$, respectively) and a SNP in *mTOR* gene (rs12732063) was related to OS ($P = 0.01$). These associations were maintained when adjusted for age, gender and MSKCC risk score.

TEMSIROLIMUS

In the phase 3 global advanced renal cell carcinoma trial, temsirolimus demonstrated to improve PFS and OS in poor prognosis mRCC patients, compared to IFN-α or to the combination of temsirolimus plus IFN-α[9]. Temsirolimus is a selective inhibitor of mTOR, a serine/threonine kinase involved in several signal transduction pathways such as phosphoinositide 3-kinase (PI3K)/Akt pathway. This latter may be activated not only by a loss of function of the tumor suppressor gene *PTEN* but also by an amplification or mutation of PI3K or Akt. Once mTOR is activated, it phosphorylates ribosomal subunit S6 kinase which, in turn, phosphorilates the ribosomal S6 protein, leading to an increased expression of HIF1-α, HIF2-α, VEGF and other pro-angiogenic

proteins. Immunohistochemical analysis on tumor tissue samples of some patients enrolled in ACRR trial did not show any correlation between baseline PTEN or HIF-α expression and clinical efficacy outcomes, suggesting that these biomarkers may not predict response to temsirolimus[50]. Another phase Ⅱ study in 20 treatment-naïve mRCC patients receiving temsirolimus, confirmed that not only PTEN but also CAIX expression and VHL mutational status are not able to predict objective responses or clinical benefit to temsirolimus[51]. However, the authors demonstrated that higher tumor tissue expression of phospho-S6 might be useful to identify the patients who are more likely to experience an objective tumor response to the treatment ($P = 0.02$). A trend was observed also for phospho-Akt, although this did not reach statistical significance ($P = 0.07$). The authors observed also a greater median OS in patients with higher phospho-S6 expression than those with lower or intermediate S6 expression (17.3 mo *vs* 9.1 mo, $P = 0.02$), suggesting a potential prognostic role of this biomarker, worthy of further explorations[51].

Although biomarkers evaluation has been performed in patients enrolled in phase Ⅱ, phase Ⅲ or even pivotal clinical trials of targeted therapies in mRCC, the obtained data are mostly the result of secondary endpoints or retrospective evaluations. Moreover, the studies were not usually neither designed nor powered to show significant difference in molecular or biological factors and the tissue sample collection was in most cases desirable but not mandatory. Moreover, external validation of biomarkers results usually lacked, affecting accordingly accuracy and reproducibility of the data. Considering all these limits and consequently, the high attrition bias, the strength of the results and consequently the levels of evidence are doubtless low, suggesting that to date, biomarkers evaluation cannot be applied in clinical decision making.

CONCLUSION

In recent years, discovery of clear "driver" genetic alterations such as epidermal growth factor receptor mutation or anaplastic lymphoma kinase rearrangement in non-small cell lung cancer (NSCLC), human epidermal growth factor receptor 2 amplification in breast cancer or Ras mutation in colon-rectal cancer and the availability of new effective drugs, targeting these molecular aberrations has radically changed the natural history of these diseases, leading to a personalized therapeutic approach. Despite the better knowledge of genetic mechanisms underlying cancer growth and drug resistance, "driver" genetic aberrations have not yet been demonstrated in RCC.

Unlike to NSCLC in which acquired resistance to tyrosin-kinase inhibitors is in most cases related to new mutations in targeted genes, mechanisms of secondary resistance to TA in RCC seem to be more complex and not yet fully clarified. Cross-talk with other signaling pathways is doubtless involved in treatment failure but recent evidences suggest that acquired resistance to TA might be also adaptive and transitional. Indeed, phase Ⅲ clinical trials have recently demonstrated efficacy and clinical activity of sequential VEGF-targeting approaches after documented progression in RCC[5,52]. Moreover, retrospectives evidences supported sunitinib re-challenge after failure of prior therapy with the same drug, particularly in patients with increasing time-off prior VEGF-inhibiting therapy, suggesting the possibility of revert drug resistance[53].

Promising molecular markers of biological activity, treatment response and prognosis have been recently evaluated in RCC clinical trials but none of these seem to have an unequivocal role as predictive or prognostic factor because of conflicting results. For all these reasons, there are no fully validated biomarkers for daily clinical use in RCC treatment and therapeutic decisions still depend exclusively on clinical criteria (histology, prognostic stratification, previous treatments, patient-comorbidities and safety profiles).

Biomarkers might play a critical role also in the rational design of genomically driven, personalized clinical trials. Considering the time between the discovery of a new active molecule and its introduction in clinical practice and the escalating costs of drug development in "unselected" populations, especially in terms of human lives, the design of "biomarker-selected" clinical trials should be clearly advocated. A power analysis performed at our institution to assess how much a biomarker-based approach can affect the theoretical sample size of a planned trial with TA for RCC showed that a dramatic difference in the required accrual could be expected, selecting patients according to a bio-molecular predictor, with easy-to-understand future feasibility scenarios[54]. This would satisfy two purposes: improve the feasibility of the trial design, minimizing costs and risks and shorten the gap between clinical research and current practice, accelerating the drug approval process and the expected patient benefit. Altogether these considerations further encourage to intensively pursue the quest for reliable, reproducible and validated biologic predictors of efficacy of TA for the treatment of RCC.

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Role of β-microseminoprotein from prostate cancer initiation to recurrence: A mini-review

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Abstract

Medline/Pubmed articles relevant to this topic were considered using the search terms β-microseminoprotein, MSMB, prostate secretory protein of 94 amino acids and PSP94. Full articles were retrieved when the abstract was considered relevant. In addition, other data related to this topic including our own are discussed. Summary of findings-β-microseminoprotein (MSMB) is increasingly being considered as a marker for prostate cancer, as reduced levels have been associated with the disease. Here we review various aspects of this protein including its biological and physiological variants, binding proteins and immune modulation; its importance as a marker for biochemical recurrence of prostate cancer; prostate cancer related splice variants and its therapeutic utility. Two of the most important properties of MSMB are related to anticancer functions and immune modulation. Predominant expression of two (short and full-length) splice variants of MSMB has been observed from normal prostate and several other tissues. In benign prostate hyperplasia the short isoform is dominant, constituting 98% of this isoform, whereas in prostate cancer 96% constitute the full-length isoform. The MSMB promoter single nucleotide polymorphism rs10993994 with the C allele functions as an activated cyclic adenosine monophosphate response element binding protein binding site. This C variant of rs10993994 could be responsible for the production of splice variants under variable conditions. MSMB has binding motifs to a few known proteins including immunoglobulin G and several Cysteine-rich secretory proteins family proteins. MSMB bound to these proteins is considered as immune modulating. Use of MSMB as a urinary marker for detecting aggressive prostate cancers that could resist radiation and surgical treatments, seems possible, but needs further investigation. The ratio of MSMB splice variants could also be a possible approach in understanding prostate cancers, with higher ratios indicating severe disease.

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Key words: β-microseminoprotein; Anticancer properties; Immune modulation; Splice variants; Promoter single nucleotide polymorphism rs10993994; Biochemical recurrence

Core tip: Potential exists to use the ratio of MSMB full-

length and short splice variants as a predictor of prostate cancer recurrence particularly after radiation therapy or surgical procedure or both. Control of the level of full-length splice variant in proportion to the short isoform could also carry a therapeutic use in controlling biochemical recurrence of the disease.

Karunasinghe N, Bishop K, Murray P, Xu Y, Goudie M, Ng L, Zhu S, Han DY, Ferguson LR, Masters J, Benjamin B, Holmes M. Role of β-microseminoprotein from prostate cancer initiation to recurrence: A mini-review. *World J Clin Urol* 2014; 3(1): 20-30 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i1/20.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i1.20

INTRODUCTION

With the widespread use of prostate specific antigen (PSA) as a screening tool for prostate health, there is an associated prostate cancer stage shift in the diagnosed population and many patients carrying indolent disease are increasingly captured through these assessments[1]. For this and other reasons there is an immediate need to differentiate between indolent disease and aggressive forms of cancer (*i.e.*, that will proceed to biochemical and clinical recurrence after primary treatment). The utility of various markers for use in this differentiation have been widely discussed. These include clinical markers, bone markers, markers of proteomics, tissue DNA and protein/RNA microarray, identification of microRNA, tumor stem cells, and pathological markers including tumor immunohistochemistry[2-9]. Amongst protein markers with suggested utility for differentiating aggressive cancers, is prostate secretory protein of 94 amino acids (PSP94); also known as β-microseminoprotein. Here we review and discuss involvement and utility of this protein from prostate cancer initiation to recurrence after treatment.

RESEARCH

This review is based on Medline/Pubmed articles relevant to this topic searched through the terms β-microseminoprotein, MSMB, prostate secretory protein of 94 amino acids and PSP94. Full articles written in English were retrieved when the abstract was considered relevant. The bibliographies of retrieved papers were subsequently searched and relevant articles were considered to be presented in this review. In addition, other data related to this topic including our own are discussed.

CHARACTERISTICS OF β-MICROSEMINOPROTEIN

β-microseminoprotein (MSMB) coded by *MSMB* gene and is synthesized by epithelial cells in the prostate gland similar to that of PSA. It is not specific to the prostate gland as noted by Weiber *et al*[10]. MSMB produced in the prostate gland is secreted into the seminal plasma and is the second most abundant protein in seminal plasma after PSA[11]. Both PSA and MSMB are generally contained in prostatic ducts and thought to leak into the blood circulation system in detectable levels. However, abnormal levels in blood show that secretion of these proteins can be erratic[12]. Liang *et al*[13] have estimated the molecular weight of MSMB as 16 kDa, using sodium dodecyl sulphate polyacrylamide gel electrophoresis under reducing conditions and 27 kDa under non-reducing conditions. The elution profile of MSMB using gel-filtration chromatography has revealed that MSMB is a dimeric protein molecule under high pH and a monomer under low pH[14]. MSMB has been shown to have five disulphide bonds and the reduction of these bonds have shown changes in the secondary and tertiary structure of the molecule[15].

Table 1 A summary of recorded functions of β-microseminoprotein

Growth inhibition and apoptosis control of prostate epithelium	[16]
Decrease of tumor growth, hypercalcemia and skeletal metastases	[17]
Decrease tumor vessel density	[19]
Anti-vascular endothelial growth factor effects	[19]
Inhibit the vascular endothelial growth factor binding to tyrosine kinase receptor Flk-1/KDR expressed by endothelial cells	[19]
Inhibits the secretion of a matrix metalloproteinase	[20]
Controls anchorage-independent colony growth of prostate cancer	[21]
Interaction with IgG and modulating immune function	[57]
Interaction with CRISP-3 and modulating ion channel regulation	[43]
Interaction with CRISP-3 to inhibit effects on innate immunity	[43]
A mediator of natural killer cells and an activator of neutrophils	[53]
A pH and Ca^{2+} dependent candidacidal activity	[23]

IgG: Immunoglobulin G; Flk-1/KDR: Fetal liver kinase 1/Kinase insert domain receptor; CRISP-3: Cysteine-rich secretory proteins.

MSMB is reportedly involved in a variety of functions including those modulating prostate cancer (Table 1). MSMB is believed to have growth inhibitory and apoptosis properties in prostate epithelium[16]. Treatment with MSMB appears to decrease tumor growth, hypercalcemia and skeletal metastases associated with induced malignancy in rat models. These *in vivo* studies have been performed with MatLyLu rat prostate cancer cells transfected with full-length cDNA encoding parathyroid hormone-related protein, which is known to be the major pathogenetic factor for malignancy-associated hypercalcemia/bone metastasis[17]. Using synthetic peptides corresponding to various regions of MSMB it has been shown that these decreases are attributed to amino acids 31-45 of the MSMB protein[18]. According to Lamy *et al*[19] the synthetic peptide PCK3145 corresponding to amino acids 31-45 of the MSMB protein is reported to decrease markers related to tumor vessel density in an *in vivo* model of rat prostate cancer. These studies have further shown anti-vascular endothelial growth factor (VEGF) effects in *in vitro* models[19]. This *in vitro* study has also shown a possibility for PCK3145 to inhibit the VEGF binding to tyrosine kinase receptor Flk-1/KDR expressed by endothelial cells. MSMB also inhibits the secretion of a matrix metalloproteinase that is implicated in tumor metasta-

sis[20]. Suppression of MSMB expression in immortalized (previously normal) prostate epithelial cells has shown a significant increase in anchorage-independent colony growth[21], a property associated with tumorigenicity[22]. This feature was unique to prostate epithelial cells as breast epithelial cells from the same study have shown no such changes in tumorigenicity by MSMB suppression. In addition MSMB is reported to have a pH and Ca^{2+} dependent candidacidal activity localised to amino acid region 66-76[23].

MSMB VARIANTS

According to records in Ensembl[24], human MSMB has six splice variants of which two are protein coding[25]. A full-length (long) and a short isoform have been reported for human MSMB mRNA with the full-length isoform and the short isoform translating to MSMB of 94 and 57 amino acids (also known as PSP57) respectively[26,27]. Using reverse transcriptase polymerase chain reaction and Southern blotting with primers corresponding to the human prostate *MSMB* gene, it has been shown that endometrium, myometrium, ovary, breast, placenta and the human endometrial cancer cell lines carry this long variant[26]. Human endometrial tissues additionally recorded the short isoform while human ovary, breast, placenta and endometrial cancer cell lines, show only the full-length isoform[26]. Xuan *et al*[27] record that exon 3 is deleted in the short isoform while showing a frame-shift at exon 4. The two isoforms possess identical exons 1 and 2 including identical secretion signal peptide[25,27]. According to Xuan *et al*[27] the short isoform is localised in the nuclear fraction of prostate cancer cell lines. Predominant expression of these two splice variants have been observed from normal prostate, urinary bladder, lung, and stomach tissues[25]. Ohnuma *et al*[25] have further shown that the prostate cancer cell line LNCaP-FGC, derived from the subclavicular lymph node, carry proportionately higher amounts of the long variant compared to the short variant. Both variants are found in lower proportions compared to normal prostate tissue[25]. However, in prostate cancer cell lines DU145 and PC3 derived from brain and bone metastasis respectively, both variants were barely detected[25]. A study by Pomerantz *et al*[21] also indicates that tumor cells have a relatively higher expression of the long variant compared to the short variant while both isoforms are expressed in significantly lower levels in prostate cancer tumors compared to normal prostate tissue. Harries *et al*[28] analysed the mRNA expression of these MSMB isoforms in patients with benign prostate hyperplasia (BPH) and prostate cancer. In BPH the short isoform was predominant (98%), whereas in prostate cancer the full-length isoform was predominant (96%)[28].

Fusion transcripts between MSMB and nuclear receptor coactivator 4 (NCOA4) have been reported including multiple androgen response elements in the MSMB/NCOA4 region. The presence of the fusion transcript indicates that these fusion genes could be subjected to androgen receptor regulation[29]. Genome-wide association studies on prostate cancer have identified MSMB promoter variant rs10993994:C >T as producing one of the largest effects on relative disease risk, with about 25% per allele increase in the relative risk of the disease[30,31]. This risk allele is also found to be linked to a significant lower level of MSMB in both normal and prostate tumor tissue[21,32]. It is also reported that this single nucleotide polymorphism (SNP) variation also influences the expression of five NCOA4 splice variants with the risk allele being associated with higher levels of NCOA4 variant expression[21].

Using a series of truncated *MSMB* promoter constructs it has been shown that deletion from -27 to -58 eliminates *MSMB* promoter activity, showing that the region around rs10993994, located at -57, is critical for MSMB promoter activation[29]. Protein encoded by the *MSMB* gene is found to be carrying a gene repressive histone mark[33] of trimethylated histone H3 on Lys27 in androgen-refractory, but not in androgen-sensitive prostate cancer cells[34]. Lou *et al*[35] cloned DNA fragments containing SNP rs10993994 into the pGL3 vector and subsequently measured the promoter activities in the transfectants in 293T (human primary embryonal kidney cell line 293 carrying a plasmid containing the temperature sensitive mutant of SV-40 large T-antigen), PC3, and MCF7 (human breast cancer cell line). The transcriptional activities of the *MSMB* promoter fragments with allele C at rs10993994 were higher than those of fragments with allele T at this position.

MSMB promoter activity increase has been attributed to the presence of a possible cyclic adenosine monophosphate response element binding protein (CREB) binding site[36] in the gene sequence carrying allele C[35]. Lou *et al*[35] have further confirmed that the addition of anti-CREB antibody shows consistent reduction of the intensity of the oligonucleotide-protein complex. Lonz *et al*[37] note that CREB is among a few genes that support expression of multiple splice variants of a multitude of genes. Lonz *et al*[37] also report CREB phosphorylation in neurons with hypoxia and oxidative stress. Nicotine exposure has also shown CREB phosphorylation in rat prefrontal cortex[38]. Tobacco specific carcinogens have also been shown to activate CREB protein in human adenocarcinomas of the lungs, pancreas, and breast[39]. It is possible that CREB binding in the area around rs10993994 allele C is partially responsible for producing variation in the amounts of splice variants transcribed from the *MSMB* gene. Over 55% of prostate cancer patients from our New Zealand cohort have been tobacco smokers compared to 38% of healthy men or 45% of patients with benign disease[40]. The rs10993994 T allele frequency in this prostate cancer cohort stands at 43% whereas the 36%-39% of benign and healthy cohorts carry the T allele frequency[40]. Considered together, our prostate cancer cohort may have a higher tendency of producing activated CREB but difficulties with delivering the correct MSMB splice variants due to a relatively higher representation of MSMB rs10993994 T variant.

MSMB BINDING PROTEINS

MSMB has binding motifs to a few known proteins. Binding of MSMB to certain serum proteins via disulphide bonds has been shown to be very stable[12]. Its binding to immunoglobulin G (IgG) earns the name immunoglobulin binding factor and is thought that this binding occurs through the fragment crystallisable portion (Fc factor) of IgG[41]. Jagtap *et al*[15] has shown that the reduction of disulphide bonds in the MSMB molecule does not affect its IgG binding ability[15]. The Cysteine-rich secretory protein (CRISP) family including CRISP-2 and -3 binds to MSMB[42,43]. The structure of CRISP-1 is also considered as having a possible binding capability to MSMB[42]. With multi-dimentional NMR it has been shown that only one strand of the dimeric MSMB molecule binds with CRISP-3[44]. Udby *et al*[42] also suggests that CRISP-3 binds to dimeric MSMB in seminal plasma where the concentration of MSMB is several fold higher compared to that of CRISP-3. The amino acids Y(3), F(4), P(56) and the C-terminal β-strand of MSMB are essential for interacting with CRISP-3 and this interaction is reported to have inhibitory effects on ion channel regulation[43]. MSMB homologues from various animal species carry conserved cysteine residues[45]. It is suggested that both MSMB and CRISPs are evolutionarily conserved across various animal species and their interaction may play an important physiological role[43]. A 50-300 fold increase in CRISP-3 mRNA expression has been shown with Gleason grade 6 prostate epithelial cells compared to normal cells[46]. The N-terminous region of CRISP-3 proteins share similarity to pathogenesis related proteins of group 1 expressed in plants in response to pathogenic insults[47,48]. CRISP-3 proteins are also present in neutrophils[49,50]. Therefore, considered together Breed suggests CRISP-3 as having a role in the innate immune system[43]. Therefore increased levels of CRISP-3 in parallel to decreased levels of MSMB could be related to boosting innate immunity in prostate cancer cells. An increase in PSP94 binding protein (considered as another CRISP protein) has shown a reduced risk of prostate cancer recurrence following radical prostatectomy[51].

MSMB has shown to interact with monoclonal natural killer-associated antibody anti-Leu 11b[52] and polyclonal anti-FcγRⅢ (anti-Fcγ receptor Ⅲ) antibodies, but not with other anti-FcγR antibodies[13]. Monoclonal anti-FcγRⅢ antibodies have been shown to activate neutrophils[53]. The aforementioned indicates that MSMB is a mediator of natural killer cells and an activator of neutrophils. According to Liang *et al*[13] the 16 kDa form of MSMB binds human IgG and murine anti-Leu-11b antibody; whereas the 27 kDa form is inactive. MSMB bound to prostatic acid phosphatase (PAP) secreted by the prostate gland has been recorded from human seminal plasma[54]. According to Kuciel *et al*[55] this PAP protein is also dimeric and is dissociated to its monomers at low pH. The activity of this PAP protein is also affected when it is in its monomeric form[56]. According to Mori *et al*[57], MSMB derived from human seminal plasma and cervical mucus gets converted to its monomeric form in the female reproductive tract and interacts with IgG in preventing immune activation against allogeneic sperms. Therefore, the modulation of antibodies could be an additional role of MSMB.

STABILITY AVAILABILITY AND ACTION OF IGGS

Due to MSMB binding ability to IgGs to prevent a downstream impact of IgG activated immunity, it is important to consider the general attributes of stability, availability and action of IgGs as well. Using hybrid bispecific antibodies it has been shown that the efficient binding of the Fc factor of IgGs activate human FcγRs, macrophages, dendritic cells, and natural killer cells[58-61]. Binding of the Fc factor of IgG to the neonatal Fc receptor (FcRn) has been shown to regulate the homeostatic half-life of IgG[62]. The binding of IgG to FcRn is also pH dependent where lower pH enhances binding and physiological pH causes no binding or release[63-65]. A comparison of the concentration of anti-tetanus toxoid specific IgG in serum and fluids secreted from the prostate gland and seminal vesicles of rats has shown that the latter two tissues have only 0.3% and 0.1% respectively of the titres in serum[66]. This study has also reported the mRNA levels of FcRn in different tissues of the male reproductive tract where ductuli efferentes has shown the highest levels, while prostate and testis have shown comparatively lower levels. This indicates that the prostate gland has limited levels of IgG and in addition, lower levels of FcRn that controls the half-life of IgG. It has been suggested that these lower levels could be related to the avoidance of autoimmunity against spermatozoa[67]. Therefore, in a normal prostate the IgG binding nature of MSMB could support the control of free IgG and its downstream effects. In the event of prostate cancer development this mechanism may need reversing to produce IgG activated immunity through the support of circulating IgG. However, immunoreactivity experiments have shown local synthesis of IgG distinguished from the depositions from circulating IgG in prostate cancer cells and significantly higher levels in prostate cancer compared to benign prostate hyperplasia[68]. In a study by Liu *et al*[68] it was shown that levels of γ chain specific IgG is positively correlated to Gleason score and histological grade.

MSMB EXPRESSION IN ADVANCE PROSTATE CANCER

High staining intensities of MSMB in prostate biopsy samples have been correlated with a Gleason score of < 7 at radical prostatectomy[69]. Whitaker *et al*[7] have shown that the decrease in MSMB level is associated with the histopathological grade of prostate cancer. Their studies have shown that the tissue areas with Gleason grade 4 show no expression while grade 3 tissues have shown reduced expression of MSMB. Consistent with this, ex-

pression of MSMB is lost or lowered in metastatic prostate cancer compared to primary disease[70,71]. A human polycomb repressive complex protein Enhancer of Zeste Homolog 2 (EZH2), shows increased expression in metastatic prostate cancer and is correlated with a loss of the tumor suppressor protein MSMB[70,71]. Beke *et al*[34] tested methylation and acetylation levels of the *MSMB* gene in prostate cancer cell lines (LNCaP and PC3) representing features of advanced prostate cancer. The *MSMB* gene was shown to be carrying gene repressive histone marks[33] with highly trimethylated histone H3 on Lys27 in PC3 and showed very little activity of the MSMB protein, whereas LNCaP prostate cancer cell line that expresses considerable levels of MSMB protein, is only mildly trimethylated at this histone position[34]. Further, Beke *et al*[34] showed the involvement of CpG methylation in the promoter region of the *MSMB* gene in both PC3 and LNCaP and deacetylation in Lys 9 of Histone H3 in PC3, thus repressing MSMB levels.

Patients with advanced prostate cancer are generally treated with androgen deprivation therapies (ADT). MSMB expression levels have shown a significant decrease in patients undergoing ADTs[72]. Dahlman *et al* [72] also showed that although prostate derived CRISP-3 was not affected by short-term ADTs, the level was high in castration-resistant prostate cancer (CRPC) and metastases[72].

MSMB AS A PROSTATE CANCER MARKER

MSMB protein is increasingly being studied as a prostate cancer diagnostic and prognostic marker due to high levels recorded from normal or benign prostate tissue, while lower or undetectable levels are recorded from prostate cancer tissue[7]. Haiman *et al*[73] has shown that with increasing serum MSMB levels there is a progressive reduction of odds ratio of prostate cancer risk when compared in men in the highest decile, with men in the lowest decile of MSMB, regardless of *MSMB* rs10993994 genotype or ethnicity. These odds ratios further increased when the MSMB level was adjusted by serum PSA levels. Reduced MSMB levels have also been reported in invasive neoplasms of the breast and lower levels were associated with reduced survival rates[74].

However, contradictory evidence on the value of MSMB level as a prognostic marker is also recorded in literature[8,9,75]. Girvan *et al*[9] reports that the presence of MSMB in the highest Gleason area as an indicator of risk for biochemical recurrence and disease progression. Dahlman *et al*[8] have recorded the intensity of MSMB in tumor sections in a scale between 0-255. They have reported that the sections with MSMB intensity above 155 are at a lower risk of biochemical recurrence[8]. Another approach of considering the ratio of bound to free MSMB has been considered by Bauman *et al*[75]. Here they report that those with a higher ratio may indicate worse outcome post-radiotherapy. Higher levels of MSMB have been associated with idiopathic male infertility[76,77]. Meanwhile infertile men have been shown to have an increased risk of subsequently developing high-grade prostate cancer[78].

The above contradictory data related to the action of MSMB in prostate cancer etiology, recurrence and metastasis needs explanation. One possible reason could be a reduction of MSMB to make way for IgG induced immunity against developing cancer cells. In the event of prostate cancer initiation, it is required to up-regulate IgG activated immunity. Hemstreet *et al*[79] have shown the induction of IgG responses in men with prostate cancer when immunotherapy is used with autoantigens for prostate cancer. A review by Helo *et al*[80] discusses the utility of the human monoclonal antibody of the IgG2 subtype as a useful therapy to prevent skeletal events related to androgen deprivation therapy on prostate cancer patients. Therefore, a decrease in MSMB could be a protective mechanism to support up-regulation of circulating IgG induced immune responses. Therefore, the prediction of biochemical recurrence associated with retained MSMB activity in the highest Gleason area[9] could be representing tumor areas that have suppressed IgG levels leading to impaired IgG induced immunity. However, as reported by Harries *et al*[28] it is also possible that the tumor environment attempts to overcome the action of IgG induced immunity by producing the longer isoform of MSMB in place of depleting shorter isoform[28]. This could be a possible reason for the presence of MSMB in high Gleason areas[9]. It is also possible that high Gleason areas are producing fusion transcripts between MSMB-NCOA4[29] with multiple androgen response elements, and thus being subjected to androgen receptor regulation. Local production of tumor derived local IgG is also reported by Liu *et al*[68] as discussed before. The above led us to believe that aggressive tumor environments could produce long MSMB isoforms to counteract the hosts immune function; similarly the tumor environment could produce its own immunity by way of tumor specific IgGs for its survival as discussed by Liu *et al*[68].

MSMB AND PCA3

A genome-wide association study involving 1371 men, has shown a significant association of the prostate cancer antigen 3 (PCA3) level with MSMB promoter rs10993994 variant allele T[81]. This non coding RNA marker PCA3 is over expressed in prostate cancer and considered as having great potential as a clinical biomarker for predicting prostate biopsy outcome in subjects with elevated PSA (> 2.5 ng/mL)[82-85].

Our own unpublished data shows trends between urinary MSMB and PCA3 mRNA level variability with various prostate cancer treatments. We measured the PCA3 mRNA expression and PCA3 score[86] and the urinary and serum MSMB levels of a limited number of prostate cancer patients. It has been shown that prostate

Table 2 Relative expression of *PCA3* and *PSA* genes in cells captured from urine of patients undergone various treatments

Treatment type	Relative *PCA3* gene expression	Relative *PSA* gene expression	PCA3 score[1]
RP and RT	0	0	0
RP and RT	3.91	6.45	0.61
RP and RT	0	0	0
RP and RT	0	0	0
RP and RT	5.03	4.67	1.08
RT	0	0	0
RT	0	0	0
RT	4.76	0	-
RT	4.24	4.23	1
RT	4.94	1.86	2.66
ADT and RT	0	0	0
ADT and RT	0	0	0
ADT and RT	0	3.72	-
ADT and RT	0	0	0
ADT and RT	0	0	0
ADT and RT	0	0	0
RP + RT and ADT	0	7.39	0
ADT	0	0	0
ADT	4.39	5.47	0.8
ADT	0	0	0
ADT	0	0	0
ADT	0	0	0
ADT	0	0	0
ADT	0	0	0
RP and NFT	2.23	0	-
RP and NFT	7.97	2.83	2.82
RP and NFT	3.04	3.22	0.94
RP and NFT	0.39	0.34	1.15
RP and NFT	6.56	3.53	1.86
RP and NFT	6.05	4.67	1.3
RP and NFT	0	0	0
RP and NFT	2.33	1.53	1.52
AS	6.02	7.2	0.84
AS	2.83	2.14	1.32
AS	5.42	Not available	-
AS	3.18	2.93	1.09
AS	6.53	8.13	0.8

[1]PCA3 score is the ratio between relative PCA3 and relative *PSA* gene expression. PSA: Prostate specific antigen; *PCA3*: Prostate cancer gene 3; RP: Radical prostatectomy; RT: Radiation therapy; NFT: No further treatment; AS: Active Surveillance; ADT: Androgen deprivation therapy.

cancer cells from urine of prostate cancer patients can be captured and used in the subsequent analysis of gene expression[87,88]. Therefore, with urine samples collected from prostate cancer patients, we were able to capture prostate cells and measure the PCA3 and PSA mRNA expression levels and to estimate the PCA3 score for these patients (Table 2). For these assays urine collections were carried out without prostatic massage. Urine samples were transferred to the laboratory on ice on the same day of collection. A total of 20-40 mL was centrifuged at 300 *g* for 5 min at 4 ℃ and the supernatant was removed. RNA was extracted from the cell pellet using an RNeasy mini kit from Qiagen and cDNA conversions were carried out using QuantiTect Reverse Transcription kit from Qiagen. Gene expression assays were carried out in triplicate using TaqMan gene expression analysis using ABI 7900 real-time polymerase chain reaction machine from Applied Biosystems; *PCA3* gene expression was assayed using primers for assay ID Hs01371939_g1; *PSA* gene expression was assayed using primers for assay ID Hs02576345_m1 and the house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was assayed using primers for assay ID Hs02758991_g1. The PCA3 and PSA mRNA expression levels presented in Table 2 are standardised to the house-keeping gene, *GAPDH*. The level of PCA3 scores recorded among our patient cohort was < 5 for all patient groups including those under active surveillance. PCA3 scores > 100 are generally considered as associated with positive biopsies when urine is collected from patients after a prostatic massage[86]. A smaller proportion of our patients who have undergone radiation therapy or prostatectomy followed by radiation therapy, showed a PCA3 score < 3. The majority of patients who have undergone prostatectomy only, also showed a PCA3 score < 3. ADT on its own or combined with other treatments has resulted in a majority of patients with a PCA3 score of zero. However, a few of the patients who have undergone ADT still expressed PSA. The majority of patients that have recorded PCA3 expression also recorded a PSA expression. However, one patient each that have undergone radiation therapy, and prostatectomy have recorded a PCA3 expression while recording no expression of PSA.

We also measured the urinary and serum MSMB levels of a limited number of prostate cancer patients who had undergone various treatments. MSMB levels were measured using Cusabio Human Beta-microseminoprotein (MSMB/PRSP) ELISA kits (CSB-E17126h)[89]. Urinary MSMB levels in those who had undergone ADT or ADT and radiation therapy showed significantly lower urinary levels compared to those undergoing active surveillance (Table 3), as expected. Serum MSMB expression was also significantly lower among those on ADTs and radiation therapy compared to those on active surveillance (Table 3). The above data indicates that treatment with ADT suppresses the urinary and serum MSMB levels as well as PCA3 mRNA expression in cells captured from urine. The reduced levels of MSMB with ADT treatment are similar to observations made by Dahlman *et al*[72] with prostate cancer tissue sections[72]. The ratio of urinary MSMB and serum MSMB was significantly higher among those who had radiation therapy compared to those on active surveillance (Table 3). It is too early to know the association between biochemical recurrence of prostate cancer of this cohort of patients with increased urinary MSMB levels or PCA3 mRNA levels or PCA3 scores. However, for urine and cells captured in urine from patients who had undergone radiation therapy and surgical treatments to present MSMB or PCA3 mRNA expression respectively, could indicate a possibility of the presence of prostate cells or prostate cancer cells that have escaped treatment. Hypoxic regions are known to resist radiation therapy treatment[90]. Hypoxic tumor regions in theory could have very limited immune response related to IgG as the acidic environment favours monomeric MSMB protein that keeps free

Table 3 Relative β-microseminoprotein levels measured from urine and serum of prostate cancer patients undergone various treatments

	Treatment type (number of patients)	Mean (range)	Estimate (95%CI)	*P*
[Urine MSMB]:[Serum MSMB]	ADT (8)	1.397 (0.163-119.5)	0.396 (0.085-1.852)	0.227
	ADT and RT (9)	4.100 (0.989-50.50)	1.162 (0.19-7.099)	0.866
	RT (5)	25.00 (10.32-45.88)	7.089 (1.16-43.322)	0.035
	AS (5)	3.529 (0.746-70.32)	1	
[Urine MSMB]	ADT (8)	1.908 (0.639-80.88)	0.172 (0.04-0.741)	0.020
	ADT and RT (9)	1.677 (0.579-35.73)	0.151 (0.027-0.839)	0.032
	RT (5)	43.12 (16.73-83.51)	3.879 (0.698-21.57)	0.116
	AS (5)	11.11 (2.186-160.3)	1	
[Serum MSMB]	ADT (8)	1.366 (0.560-25.92)	0.434 (0.145-1.301)	0.129
	ADT and RT (9)	0.409 (0.081-0.720)	0.13 (0.036-0.471)	0.003
	RT (5)	1.723 (0.730-8.093)	0.547 (0.151-1.986)	0.343
	AS (5)	3.149 (0.624-10.21)	1	

Log-transformed β-microseminoprotein measurements were used in the analysis and the results presented above were determined by using the exponential (antilog) function. Generalised linear model was carried out to investigate MSMB level variability among four different treatments. All analyses were carried out using statistical package R[99] and SAS (V9.1 SAS Institute, Cary, NC, United States). ADT: Androgen deprivation therapy; RT: Radiation therapy; AS: Active surveillance.

IgG under control. Besides, under hypoxic conditions CREB protein could be activated[37] and could bind to and cause the *MSMB* gene to produce a splice variant if the gene variant C of rs10993994 is present, while allele T will produce impaired variants. As such, a possibility exists for the utility of these markers in identifying prostate cancers that have resisted radiation therapy or possible metastasis before or after radiation therapy or surgical procedures. However, further investigations with sufficient patient numbers with prospective monitoring for biochemical recurrence is required to confirm the utility of these markers.

MSMB RELATED THERAPIES

Very little information is available in the literature on MSMB related therapies for the treatment of prostate cancer. An MSMB derived peptide, PCK3145, containing amino acids 31-45 of the MSMB molecule, has been used as a therapeutic agent on prostate cancer patients with metastatic hormone refractory prostate cancer[20]. Treatment with this peptide has shown reduction of matrix metaloprotinase-9 (MMP-9) in *in vitro* experiments[91]. This therapy has also shown reduced levels of MMP-9 in patients with higher baseline MMP-9 levels[92]. Daigneault *et al*[93] reveals the clinical utility of this peptide, the use of which can be monitored with various serum markers including levels of follicle stimulating hormone and MMP-9[93].

Moreover, MSMB or related proteins could have future directions in therapeutic use for prostate cancer. Among them is the control of the level of long splice variants in proportion to the short isoform. Tumor specific IgGs also could be considered as therapeutic targets for the control of biochemical recurrence of prostate cancer.

MSMB AND CHOLESTEROL

Several epidemiologic studies report that low levels of high-density lipoprotein levels are associated with the risk and prognostic factor of prostate cancer[94-97]. Recently, Pommier *et al*[98] have shown with mouse models that cholesterol homeostasis regulated by Liver X Receptors (LXR) are involved in maintaining MSMB levels. They have shown that in LXR knockout mice fed high cholesterol diets; there is an increased expression of the EZH2 that in turn down regulates MSMB expression levels. Further studies are needed for evaluating the link between cholesterol levels in prostate cancer patients and their association with MSMB levels.

CONCLUSION

The major properties of MSMB are related to anticancer functions and immune modulation. Anticancer properties have been mapped to amino acid region 31-45. However, in the event of prostate cancer, increased expression of a longer splice variant transcribed additionally by the 3rd exon is reported for this protein. The MSMB promoter SNP rs10993994 with the C allele polymorphism, functions as an activated CREB binding site. This could be responsible for the production of required splice variants under variable conditions ranging from normal to hypoxic and oxidative stress/hypercholesterol/tobacco smoke exposure scenarios. MSMB or PCA3 as urinary markers for detecting aggressive prostate cancers that have resisted radiation and surgical treatments, seems possible, but needs further investigation. MSMB, as a marker, will be useful if the levels are measured in surgical samples from the highest Gleason area of the tumor where the presence of MSMB is indicative of a higher tendency for biochemical recurrence. MSMB level decline below 155 units out of a maximum score of 255 units in the overall tumor is also considered as a factor predicting outcome after radical prostatectomy. Gene expression levels of MSMB in prostate cancer cells, if measured as a ratio of exon 3 region against the expression of exons 1, 2 or 4, could also be a possible approach in understanding prostate cancer severity with higher ratios indicating severe

disease. MSMB with amino acid region 31-45 has been trialled as a therapeutic against prostate cancer. Targeted therapies against long splice variants of MSMB carrying additional amino amino acids coded through exon 3 could also be useful in prostate cancer treatment.

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MINIREVIEWS

Systematic review of the non surgical management of Peyronie's disease

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Abstract

This systematic review shows the "Status quo" on medical treatment for Peyronie's disease (PD). PD is a connective tissue disorder, characterised by the formation of a fibrotic lesion or plaque in the tunica albuginea, which leads to penile deformity. The aetiology of PD is unknown. Nowadays the most widely accepted hypothesis proposed by Gonzalez-Cadavid *et al*, is repetitive microvascular injury or trauma to the tunica albuginea. Physicians have proposed several medical alternatives for treatment of this disease with few effective results. Nevertheless, as of today nonsurgical options are currently available, and some of them are able to stabilize or even reduce deformity while improving pain relief and sexual function. A systematic literature search throughout the Medline database was carried out. The controlled vocabulary of the medical subject headings database employs the specific term "penile induratio" for PD. A total of 50 articles on PD were found. Studies were selected based on clinical relevance. The recommended standard of care for PD involves an initial treatment in the acute phase. Several non-operative treatment options have been used. Unfortunately no further substantial, quality evidence on the use of medical therapy currently exits. There is, however, an increasingly enhanced interest in this disorder and basic scientific and clinical research will eventually lead to a more effective methodology to study the disease.

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Key words: Peyronie's disease; Non surgical; Dupuytren; Extracorporeal shockwave therapy

Core tip: Ince the first medical publication on Peyronie's Disease, physicians have proposed several medical alternatives for treatment of this disease. As of today nonsurgical options are currently available. A consistent number of non-surgical treatment options that offer some benefit with respect to disease stabilization, alleviation, as well as reduction of deformity and improved sexual function are available including oral treatment with potassium para-aminobenzoate, intralesional treatment with interferon, iontophoresis with verapamil 5 mg and dexamethasone 8 mg and extracorporeal shock-wave treatment.

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INTRODUCTION

Peyronie's disease (PD) is a connective tissue disorder, characterised by the formation of a fibrotic lesion or plaque in the tunica albuginea, which leads to penile deformity[1]. The true prevalence of PD is unknown. An evaluation of existing epidemiological data on PD revealed a prevalence rate of 3.2%. However the actual prevalence of PD may be even higher, considering many patients' reluctance to report this embarrassing condition to their physicians. The latest version of the European Association of Urology guidelines[1] reports a prevalence rate of 0.4%-9%.

The aetiology of PD is unknown. Nowadays the most widely accepted hypothesis proposed by Gonzalez-Cadavid *et al*[2], is repetitive microvascular injury or trauma to the tunica albuginea. PD starts with an acute inflammatory process. The progression of inflammation causes a proliferation of the tunical fibroblasts, some of which differentiate into myofibroblasts with a disproportionate production of collagen, the persistence of fibrin, and resulting elastin fragmentation. This prolonged inflammatory reaction generates the remodelling of connective tissue into a dense fibrotic plaque. As a result of Penile plaque the penis develops a curvature which, in the case of severe bending, can prevent vaginal intercourse. Comorbidities and risk factors associated with the disease are diabetes, hypertension, lipid abnormalities, ischaemic cardiopathy, erectile dysfunction, smoking, and excessive consumption of alcohol[3]. An accompaniment with Dupuytren contracture is reported in 9%-39% of PD patients. Men experiencing sexual dysfunction after Radical Prostatectomy have a higher PD incidence rate then the general population and therefore should be routinely evaluated for PD. Younger men and Caucasian men are at increased risk for PD[4]. The natural course of PD indicates that penile curvature stabilizes in 47%-67% of patients or worsens in 30%-50% of patients. As well, spontaneous improvement is reported in only 3%-13% of patients[5]. In later phases, once the plaque becomes calcified the possibilities that penile curvature can be corrected are not as likely as in the early stage of the disease. Thirty-five percent to forty-five percent of patients can experience referred pain at early stages of the disease. However in 90% of men the pain tends to abate with time, usually during the first 12 mo after onset.

Nelson *et al*[6] reported that, besides the physiologic and functional alteration of the penis, 48% of men with PD experience mild-to-moderate depression as revealed through validated mental health questionnaires.

Since the first medical publication on PD, physicians have proposed several medical alternatives for treatment of this disease with few effective results. The most recent findings on the molecular pathophysiology of PD, though not conclusive, nonetheless give a bigger picture of the mechanisms underlying the evolution of the plaque formation and may one day guide to medical treatment. Nevertheless, as of today nonsurgical options are currently available, and some of them are able to stabilize or even reduce deformity while improving pain relief and sexual function. Unfortunately the studies found in literature, though providing evidence of the efficacy of these options, are compromised by the small size of the sample or the absence of a placebo control. Moreover, PD assessment lacks a validated questionnaire that allows for the interpretation of the data outcomes, and the analysis of the data is complicated by the fact that the spontaneous improvement rates reported are 5%-12%[7-10].

In light of existing Nonsurgical options for PD pain treatment and curvature including oral, intralesional, topical, and combination therapies, the aim of the current paper is to investigate, through a systematic review, the currently available Medical approaches for treating PD.

METHODOLOGY

A systematic literature search throughout the Medline database was carried out. The controlled vocabulary of the medical subject headings (MeSH) database employs the specific term "penile induratio" for PD. In order to identify relevant articles, the search included the MeSH terms, penis abnormalities, male, penile curvature and the terms "Peyronie's Disease" and "Induratio Penis Plastica". A total of 50 articles on PD were found. Relevant English-language articles were abstracted and reviewed using the reference list to identify additional potential articles for review. Studies were selected based on clinical relevance. If more than one article was published in the same study population, the study with the larger sample size was selected.

NON SURGICAL TREATMENT

The recommended standard of care for PD involves an initial treatment in the acute phase (first year) of this condition when symptoms are present and the plaque is not yet densely fibrotic or calcified[9]. Several non-operative treatment options have been used. Up to the present the European Medical Association has not approved any medication for the treatment of PD with the except of potassium para-aminobenzoate (Potaba) which has been classified as "possibly effective" for PD by the United States Food and Drug Administration.

The studies collected for this review showed contradictory results on conservative treatment for PD making it difficult to provide recommendations in an everyday real-life setting.

ORAL TREATMENT

Vitamin E

Vitamin E (tocopherol) is a fat-soluble compound that acts as a natural antioxidant to reduce the number of oxygen-free radicals produced in the energy metabolism. It has also been shown to play a role in DNA repair and in immune modulation[11]. The widely accepted use of tocopherol in the treatment of PD attests to the hypothesis that it inhibits fibrosis by acting as a scavenger of oxygen free radicals. *In vitro* studies examining the effect of free

radicals on human cavernosal cells have shown a direct association with increased collagen production[12]. Based upon these findings it is logical to conclude that inhibition of free radicals (*i.e.*, with use of tocopherol) should decrease the rate and degree of fibrosis. However, *in vivo* data has failed to reveal any concrete benefits in PD patients[13,14]. To date, tocopherol is commonly prescribed by most urologists in once- or twice-daily doses of 400 IU because of its wide availability, low cost, and safety. In 1983, Pryor *et al*[10] conducted a double-blind, placebo-controlled crossover study evaluating vitamin E for the treatment of PD in 40 patients. No significant improvements were noted in plaque size or penile curvature. The authors therefore did not recommend vitamin E for the treatment of PD as there was no meaningful evidence of its benefit in placebo-controlled trials.

Potassium para-aminobenzoate

Potassium paraaminobenzoate (Potaba, Glenwood) is a vitamin B complex and is believed to exert an antifibrotic effect through an increase in oxygen uptake by the tissues, a rise in the secretion of glycosaminoglycans, and enhancement of the monoamine oxidases activity in tissues thereby decreasing local levels of serotonin and, in turn, possibly decreasing fibrogenesis. A prospective double-blinded controlled study by Shah *et al*[15] analyzed 41 patients with PD and showed that, while penile pain was significantly improved by potassium para-aminobenzoate (12 g/d for 12 mo) neither penile curvature nor penile plaque size were affected. Weidner *et al*[16], in a prospective randomised double-blind placebo-controlled trial evaluating 103 patients with PD, found that potassium para-aminobenzoate (3 g/d four times daily for 12 mo) decreased penile plaque size significantly but had no effect on penile curvature or penile pain. The Treatment-emergent adverse events reported were: nausea, anorexia, pruritus, anxiety, chills, cold sweats, confusion, and difficulty concentrating and therefore no serious adverse events have been reported.

Tamoxifen

Tamoxifen is a nonsteroidal oestrogen receptor antagonist. The action mechanism which should determines changes in PD is the modulation of the transforming growth factor (TGF)b1 secretion by fibroblasts. Teloken *et al*[17] published a placebo controlled randomised study performed in 25 patients in the late stages of PD with a mean disease duration of 20 mo. The results of this study showed that Tamoxifen 20 mg twice daily for 3 mo failed to demonstrate significant improvement in pain, curvature, or plaque size[17].

Colchicine

Colchicine is a medication that is commonly employed in the treatment of acute attacks of gout. Its anti-inflammatory effects suggested its possible use in treating PD. Kadioglu *et al*[18] reported on the results of a study on 24 men, half of whom were given colchicines (0.6-1.2 mg daily for 3-5 mo) and whose painful erections and penile curvature were shown to improve in 50% of them with penile plaque decreasing or disappearing completely. Akkus *et al*[19] presented the results of a study involving 60 men treated with colchicine 0.5-1 mg daily for 3-5 mo and subsequently increased to 2 mg twice daily. Following this treatment penile pain was resolved in 95% of the cases while penile curvature improved in 30%. The reported treatment-emergent adverse events from the use of colchicine were gastrointestinal effects (nausea, vomiting, diarrhea), which were improved by dose escalation.

Finally, Prieto Castro *et al*[20] investigated the combination of vitamin E and colchicine (600 mg/d and 1 mg every 12 h, respectively) in patients with early-stage PD for 6 mo. The results of this study showed a significant improvement in plaque size and curvature, but not in pain when compared with ibuprofen 400 mg/d for 6 mo.

Acetyl esters of carnitine

It has been suggested that carnitine can reduce intracellular calcium levels in endothelial cells which, in turn, may eventually suppress fibroblast proliferation and collagen production thereby reducing penile fibrosis. To date, only 1 randomised double-blind study evaluating the efficacy of acetyl-L-carnitine has been conducted by Biagiotti and Cavallini[21]. In 48 patients with early-stage PD, subjects were given either tamoxifen 20 mg twice daily or acetyl-L-carnitine 1 g twice daily for 3 mo. After 3 mo, acetyl-L-carnitine was significantly more effective than tamoxifen in reducing pain and curvature and in inhibiting disease progression, but not in reducing penile plaque size (both tamoxifen and carnitine significantly reduced plaque size.

Another study investigated the combination of propionyl-l-carnitine (2 g/d for 3 mo) with intralesional verapamil (10 mg weekly for 10 wk). The results showed that this combination therapy significantly reduced penile curvature, plaque size, and disease progression when compared with intralesional verapamil combined with tamoxifen (40 mg/d) for 3 mo[22].

Pentoxifylline

Pentoxifylline is a nonspecific phosphodiesterase inhibitor that downregulates TGFb1 and increases fibrinolytic activity. Valente *et al*[23] found that normal human and rat tunica albuginea as well as PD plaque tissue expresses phosphodiesterase (PDE)5A-3 and PDE4A, B and D. In their in vitro study, PD fibroblasts were cultured with pentoxifylline and found to have increased cyclic adenosine monophosphate levels and reduced collagen I levels as compared to controls. An increase of nitric oxide levels may be effective in preventing the progression of PD or reversing fibrosis. Brant *et al*[24] performed a study on 62 patients with PD, and showed that treatment with pentoxifylline for 6 mo appeared to stabilize or reduce calcium content in penile plaques and also tended to improve penile curvature.

Phosphodiesterase type 5 inhibitors

The rationale for the use of a phosphodiesterase type 5 (PDE5-I) in PD comes from animal studies which show

that PDE5- I can reduce the collagen/smooth muscle and collagen Ⅲ/ I ratios and increase the apoptotic index in PD like plaque[25]. In a recent retrospective controlled study Chung *et al*[26] investigated the role of daily tadalafil (2.5 mg for 6 mo) which resulted in a statistically significant ($P < 0.05$) resolution of the septal scar in 69% of patients compared with 10% in the control group (no treatment). However, this study included patients with isolated septal scars without evidence of penile deformity[26].

INTRALESIONAL TREATMENT

The injection of a medication directly into the penile plaque results in restricted delivery and higher drug concentrations inside the plaque.

Steroids

The powerful anti-inflammatory effect of steroids makes them obvious agents for intralesional therapy of PD. Steroids operate by counteracting the inflammation responsible for Peyronie's plaque progression *via* inhibition of phospholipase A2 and suppression of the immune response and by diminishing collagen synthesis. In 1954, Bodner *et al*[27] reported improvement in 17 patients treated with intralesional hydrocortisone and cortisone. In 1975, Winter *et al*[28] showed no difference between patients treated with dexamethasone injections and the natural history of the disease. Cipollone *et al*[29], in a single-blind placebo-controlled study with intralesional administration of betamethasone, reported that no statistically significant changes were seen in penile deformity, penile plaque size, nor penile pain during erection. Adverse effects included tissue atrophy, thinning of the skin, and immune suppression.

Verapamil

Verapamil is a calcium channel antagonist that is thought to selectively inhibit calcium ion flux in both cardiac muscle and cells responsible for intracardiac conduction, as well as in coronary and systemic arteries. The rationale for its use in the intralesional treatment of patients with PD is based on in-vitro data that demonstrates transport of extracellular matrix molecules that include collagen, fibronectin, and GAGs as a calcium-dependent process[30]. A concomitant increase in collagenase activity, modification of the inflammatory response in the early phase of the disorder, and inhibition of fibroblast proliferation in the plaques are other proposed mechanisms. Shirazi *et al*[31] reported in a randomized placebo-controlled study that no statistically significant differences in plaque size, penile curvature, penile pain during erection, and plaque "softening" were seen.

Clostridial collagenase

Clostridial collagenase is a chromatographically purified bacterial enzyme that selectively attacks collagen and is known to be the primary component of the PD plaque. Collagenase was first studied *in vitro* by Gelbard *et al*[32] in 1982. A subsequent clinical trial by that group demonstrated subjective improvement in 64% of patients within 4 wk of treatment[33]. A decade after their initial study, the group published their findings of a double blind trial in 49 men[34]. In this study which compared the effects on plaque size and penile deformity of intralesional purified clostridial collagenase (6000-14000 U) and saline placebo, the overall response was 36% with clostridial collagenase compared with 4% with placebo ($P < 0.007$). Follow-up was only 3 mo. Response rates were even higher in patients with smaller plaques and curvature < 60°. The efficacy of intralesional collagenase injections (three injections of clostridial collagenase, 10000 U/0.25 cm^3 per injection, administered over 7-10 d and subsequently administered over 7-10 d at 3 mo) was assessed over a non-placebo-controlled short-term follow up study in a small population of men with PD[35]. This study showed a significant reduction from baseline in the deviation angle, plaque thickness, and plaque extension, but it was biased by an incorrect scientific approach. The most commonly reported side effects were penile pain, contusions, and ecchymosis.

Interferon

Duncan *et al*[36] reported in 1991 that interferons decrease the rate of proliferation of fibroblasts in Peyronie's plaques in vitro, reduce the production of extracellular collagen, and improve the activity of collagenase. Hellstrom *et al*[37], demonstrated that intralesional injections (5×10^6 units of interferon a-2b in 10 mL saline two times per week for 12 wk) improved average curvature in the treatment group by 13° *vs* 4° in the placebo arm, and that 27% of patients in the treatment group had measurable improvement *vs* 9% of the saline group. Pain resolution was noted in 67% of the treatment patients *vs* 28% for the placebo[37]. Side effects include myalgias, arthralgia, sinusitis, fever, and flulike symptoms which can be effectively treated with nonsteroidal anti-inflammatory drugs before interferon injection.

TOPICAL TREATMENTS

Topical verapamil

Fitch *et al*[38] presented the results of a small randomised placebo controlled study on topical verapamil applied as gel 15% to the penile shaft twice daily. The penile curvature, plaque size, and penile pain were significantly improved. After 9 mo of treatment better improvement was reported compared with the results at 3 mo, demonstrating that a prolonged treatment period may be important. However, a lack of evidence exists supporting that topical verapamil applied to the penile shaft produces adequate levels of active compound within the tunica albuginea.

Iontophoresis

To overcome limitations of topical therapies, emphasis has more recently been placed on testing modalities such as iontophoresis, which enhances the local uptake of drugs. Iontophoresis involves the application of an exter-

nal electric force to induce further (electromotive) penetration of topical medication. Di Stasi *et al*[39] presented their results of a randomised double blind controlled study, demonstrating that iontophoresis with verapamil 5 mg and dexamethasone 8 mg resulted in a statistically significant improvement in penile curvature and plaque size. On the other hand, Greenfield *et al*[40] with a randomized double-blind placebo controlled study showed that penile curvature was not statistically improved after iontophoresis with verapamil 10 mg. No significant events with Iontophoresis were reported.

Extracorporeal shock wave lithotripsy

The mechanism of action involved in extracorporeal shock wave lithotripsy (ESWL) for PD is still unclear, but there are two hypotheses. In the first hypothesis, ESWL may work by directly damaging and remodeling the penile plaque. In the second hypothesis, ESWL may increase the vascularity of the area by generating heat, resulting in an inflammatory reaction with increased macrophage activity causing plaque lysis and eventually leading to plaque reduction. Hauck *et al*[41] in an exploratory meta-analysis detected that ESWT seems to have an effect on penile pain during erection and on the improvement of sexual function. Pain seems to resolve faster after ESWT than during the course of the natural history. The effect on plaque size and penile curvature is less impressive. This result were confirmed by Palmieri *et al*[42] that published the only existing prospective randomised double-blind placebo-controlled study where they investigated four weekly treatment sessions of ESWL, with each session consisting of 2000 focused shock waves which resulted in significant improvement for penile pain only.

Traction devices

The use of tissue expanders has long been a mainstay of treatment in the orthopedic, oral-maxillofacial and plastic surgical fields. A continuous traction in Dupuytren contracture increases the activity of degradative enzymes. This first leads to a loss of tensile strength and subsequently to solubilization. This is followed by an increase in newly synthesized collagen. Levine *et al*[43], in an uncontrolled study, applied this technique on 10 patients with PD whereby application of the the FastSize Penis Extender was the only treatment for 2-8 h/d for 6 mo. Penile curvature was reduced in all men from 10° to 45°, with an average reduction of 33% (range: 51°-34°). Stretched penile length increased to 0.5-2.0 cm, and erect girth increased to 0.5-1.0 cm, with a correction of the hinge effect in four of four men. No adverse events such as skin changes, ulcerations, hypoesthesia, or diminished rigidity were reported.

Vacuum devices

When it comes to vacuum devices the same principles as traction devices are followed. Raheem *et al*[44], evaluated the efficacy of vacuum devices in an uncontrolled study assessing 31 patients. The vacuum device was applied over a 12-wk period (Osbon ErecAid, MediPlus, High Wycombe, United Kingdom) for 10 min twice daily. Penile pain was reduced significantly ($P = 0.012$). Stretched penile length also increased significantly ($P = 0.029$) with a mean of 0.5 cm. Reduction of the curvature was reported in 67% of patients. 10% had worsening curvature and 23% showed no change. Half of the patients were satisfied with the outcome while the remainder had their curvature corrected surgically. Vacuum therapy can improve or stabilize PD curvature, is safe to use in all stages of the disease, and could reduce the number of patients requiring surgery.

COMBINATION THERAPIES

In 1999, Mirone *et al*[45] prospectively examined patients treated with ESWT or ESWT and perilesional verapamil injections. A 52% improvement in plaque size by ultrasound was noted in the ESWT-only group compared to 19% for the combination therapy. A follow-up study by the same investigators involving 481 patients demonstrated a 49% improvement in plaque size among those treated with combination therapy[46]. A recent study by Palmieri *et al*[47] investigated the effects of extracorporeal shock wave therapy (ESWT) plus tadalafil 5 mg once daily in the management of patients with PD and erectile dysfunction who had not not previously been treated. One hundred patients were randomly allocated to receive either ESWT alone for 4 wk ($n = 50$) or ESWT plus tadalafil 5 mg once daily for 4 wk ($n = 50$). They concluded that ESWT and tadalafil 5 mg once daily may represent a valid strategy for the conservative management of selected PD patients complaining of ED as it significantly improves erectile function and quality of life when compared to ESWT alone. However, ESWT plus tadalafil 5 mg once daily was not able to significantly improve plaque size and curvature degree in our subset of patients.

A placebo controlled study by Prieto Castro *et al*[20] randomized 45 patients to receive vitamin E and colchicine or ibuprofen. Statistically significant improvements in curvature and plaque size were noted in the group treated with vitamin E and colchicine as compared to the group receiving ibuprofen. Patients in the vitamin E and colchicine arm reported a greater decrease in pain, although this did not reach statistical significance.

In 2002, Cavallini *et al*[22] randomized 60 men to receive intralesional verapamil plus oral carnitine or intralesional verapamil plus oral tamoxifen. Statistically significant subjective improvements in curvature, plaque size and erectile function were found in the carnitine group. No difference in improvement of pain was noted between the two groups.

CONCLUSION

In our review we showed the "Status quo" on non-surgical treatment for PD (Table 1). Unfortunately no further substantial, quality evidence on the use of medical therapy currently exits. There is, however, an increasingly

Table 1 Efficacy of non surgical treatments on Peyronie's disease

Therapy	Therapeutic activity and indication
Oral treatment with potassium para-aminobenzoate	Reduction in penile plaque size and penile pain as well as penile curvature stabilization
Oral treatment with vitamin E and tamoxifen	Not associated with significant reduction in penile curvature, plaque size or penile pain
Other oral treatments (acetyl esters of carnitine, pentoxifylline)	Not recommended
Intralesional treatment with verapamil	No statistically significant differences in plaque size, penile curvature, penile pain
Intralesional treatment with steroids	Not associated with significant reduction in penile curvature, plaque size or penile pain
Intralesional treatment with interferon	Improve Penile curvature, plaque size and density, and pain
Intralesional treatment with clostridial collagenase	Significant decreases in the deviation angle, plaque width and plaque length
Iontophoresis with verapamil 5 mg and dexamethasone 8 mg	Improve penile curvature and plaque size
Extracorporeal shock-wave treatment	Beneficial for penile pain and stabilization of plaque
Topical verapamil gel 15%	Improve penile curvature and plaque size
Penile traction devices and vacuum devices	Reduce penile deformity and increase penile length
Combination therapies	Preliminary evidences to be clarified

enhanced interest in this disorder and basic scientific and clinical research will eventually lead to a more effective methodology to study the disease. In the meantime, a consistent number of non-surgical treatment options that offer some benefit with respect to disease stabilization, alleviation, as well as reduction of deformity and improved sexual function are available. In conclusion the most beneficial treatment identified are: (1) oral treatment with potassium para-aminobenzoate (may result in a significant reduction in penile plaque size and penile pain as well as penile curvature stabilization); (2) Intralesional treatment with interferon (may improve penile curvature, plaque size and density, and pain); (3) iontophoresis with verapamil 5 mg and dexamethasone 8 mg (may improve penile curvature and plaque size); and (4) Extracorporeal shock-wave treatment (may be beneficial for penile pain and stabilization of plaque).

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Chlamydial infections in urological disease: A challenging management

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Abstract

Chlamydia trachomatis (Ct) infections are the most prevalent sexually transmitted bacterial infections worldwide, causing considerable morbidity and socioeconomic problems. Moreover, Ct infections are asymptomatic in approximately 50% of infected men and 70% of infected women, with the risk for reproductive tract sequelae both in women and men. Recent studies have improved the comprehension of this infection and its natural history, also highlighting its role in decreasing male fertility. Severe complications can be avoided only by a proper early diagnosis and appropriate treatment. We reviewed the literature relating to the new findings in the treatment of Ct infection in sexually active young men. Articles from 1960-2012 were identified through a Medline search using the keywords "*Chlamydia trachomatis*" combined with "urethritis", "epididymitis", "prostatitis", "treatment" or "management". Currently, several studies have been published about the role of new antibiotic schedules and new associated compounds in order to improve the efficacy in terms of microbiological results and patient quality of life. In particular, several studies stress the fact that Chlamydia is only metabolically active in the host cell and therefore only targeted intracellularly by antibiotics. Even although the standard therapy includes intracellularly-accumulated antibiotics such as tetracyclines or macrolides, recent evidence highlights the role of quinolones. In particular, recent studies highlight the role of prulifloxacin in the treatment of chronic prostatitis for improving the patient's quality of life and decreasing the IL-8 level. However, future studies should focus on delineating the natural history of recurrent infections, paying particular attention to treatment failures.

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Key words: Chlamydia trachomatis; Prostate; Male; Fertility; Treatment; Review

Core tip: *Chlamydia trachomatis* (Ct) infection is the most prevalent sexually transmitted bacterial infection worldwide with a significant impact on young male fertility. Ct represents a challenge for the urologist, both for diagnosis and treatment. An accurate comprehension of the pathology, diagnosis and treatment of this entity is essential for the urologist in order to prevent persistent consequences and improve the patient's quality of life. We summarize the most current developments in the treatment of young men affected by chronic prostatitis due to Ct infection.

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INTRODUCTION

Chlamydia trachomatis (Ct) is the most common sexually transmitted bacterium worldwide, with over three million new infections per year[1,2]. The World Health Organization estimates that 92 million new cases of Chlamydia occur worldwide every year[3]. In particular, Chlamydia is the most frequently reported sexually transmitted infection in Europe and the number of cases is steadily increasing, with more than 255000 cases in people below 25 years of age[4]. The rate of transmission between sexual partners may be as high as 75%[5]. Therefore, partner notification and subsequent treatment are very important. However, approximately 75% of Ct infections in women and up to 50% of those in men are asymptomatic[6,7]. This aspect is extremely important due to the fact that although up to 13.3% of young men may have a genital chlamydial infection, only half of these will present with any symptoms and even fewer are likely to pursue treatment[8]. Moreover, the absence of symptoms increases the risk of infecting sexual partners and may also cause long-term complications in men, such as poor quality of semen and infertility[9-11]. Furthermore, several factors contribute to make it difficult to detect Ct by a conventional analysis[12]. To date, DNA recombination techniques are universally accepted as the gold standard to evaluate the presence of Ct in biological samples[13], even although immunological markers of Ct infection, such as immunoglobulin A (IgA) antibody and cytokines, have been detected in total ejaculate and seminal plasma samples to demonstrate their role in monitoring men with chronic prostatitis (CP)[14,15]. A recent review suggested that antimicrobial groups effective against Ct include the macrolides, tetracyclines, quinolones and penicillins[16,17]. The European Urological Association and the Centers for Disease Control and Prevention guidelines recommended the use of doxycycline and azithromycin in the treatment of chlamydial infections[4,18]. However, in the management and treatment of patients affected by Ct infections, the following factors should be taken into account: (1) chlamydia is only metabolically active in the host cell and therefore only targeted intracellularly by antibiotics; and (2) intracellularly-accumulated antibiotics are tetracyclines, macrolides and quinolones[4,18]. Although doxycycline and azithromycin are the most widely prescribed drugs in Ct infections treatment and recommended as the primary approach, other fluoroquinolones such as ofloxacin, levofloxacin or prulifloxacin are suggested as alternative drugs[4,19]. However, the approach to the management of Ct infection should include: (1) treatment of patients (to reduce complications and prevent transmission to sex partners); (2) treatment of sex partners (to prevent reinfection of the index patient and infection of other partners); (3) risk-reduction counseling; and (4) repeat chlamydial testing in women a few months after treatment (to identify recurrent/persistent infections)[20,21]. For these reasons, management of Ct is challenging for the urologist. We summarize the most current developments in the therapeutic approaches in Ct infections in sexually active young men.

EVIDENCE ACQUISITION

We conducted a search of the English language literature from 1960 through December 2010 with use of the Medline computerized database of the US National Library of Medicine (http://www.ncbi.nlm.nih.gov/pubmed). The Medline search was carried out using the following medical subject headings and free text terms. Ct and chlamydia infections (exploded) were combined with the terms treatment, therapy, antibiotic, drug, quinolones and tetracycline, then limited to humans, male and young adult, 19-24 years. Moreover, we searched reference lists of articles to identify potential additional references. All original papers and review studies of Ct treatment in young adults were considered for this review. We also considered guidelines from the National Institute for Health and Clinical Excellence, the European Center for Disease Prevention and Control, the US Center for Disease Control and Prevention, World Health Organization and European Association of Urology. From an initial literature search with 139 unique citations, a total of 19 articles were selected for the present review. A matched search between Ct and chlamydia infections (exploded) and quinolones found 3 articles.

RECOMMENDATIONS FROM THE INTERNATIONAL GUIDELINES

The Ct infection clinical features in men are: more than 50% are asymptomatic; burning with micturition; "penile tip irritation"; watery, viscous excretion ("morning milker"); urethral discharge; and proctitis[22,23]. Although several authors stated that many infections could spontaneously clear over time[24], the natural course of infection has not been studied in great detail and some infections may proceed to a chronic persistent state[25]. Recently, the European guidelines for managing chlamydial infection were issued[26]. The following indications for treatment were devised: (1) confirmed genital Ct infection; (2) infection with Ct in the partner; (3) in a patient with a confirmed *Neisseria gonorrhoeae* infection if laboratory tests for Ct are not available; and (4) in a patient with clinical signs of a chlamydial infection if laboratory tests for Ct are not available.

First line regimens include azithromycin 1 g orally as a single dose or doxycycline 100 mg orally twice a day for 7 d. Several alternative regimens have been proposed: erythromycin base 500 mg orally four times a day for 7 d; ofloxacin 200 mg orally twice a day for 7 d; roxithro-

mycin 150 mg orally twice a day for 7 d; clarithromycin 250 mg orally twice a day for 7 d; levofloxacin 500 mg once daily for 7 d; or ofloxacin 300 mg twice a day for 7 d [17,20,26].

Co-infections with other sexually transmitted infections

Men and women with a diagnosis of Ct infection should be offered a complete work-up for other sexually transmitted infections, such as gonorrhea, syphilis, mycoplasmata, human papillomavirus and HIV[11,26]. If co-infection with *Mycoplasma genitalium* is confirmed, patients should not be treated with a single dose of 1 g azithromycin, but with a short course of azithromycin: 500 mg on day 1 followed by 250 mg on days 2-5 (level Ⅲ, grade C) [26,27].

Rectal Ct infection

In cases of rectal non-LGV chlamydial infections, the first choice should be a course of doxycycline, 100 mg twice daily for seven days (level Ⅲ, grade B)[28].

Treatment failure

A repeated course or a longer course (10-14 d) with doxycycline or a macrolide has been suggested, but evidence is lacking (level Ⅳ)[26]. The most common reason for therapy failure is reinfection from an untreated partner (level Ⅱ)[26,29]. An interesting suggestion is the combined use of rifampicin and a macrolide[30]. Finally, the potential of Ct to develop antimicrobial resistance has not been well studied, although some case reports suggest resistance as a cause of treatment failure[31,32].

FIELDS TO BE DISCUSSED

Although there is a consensus about first line treatment regimens, future studies are needed in cases of treatment failure or infection persistence. Although little is known about Ct survival in the presence of fluoroquinolones, it is well known that after multiple cultivation passages resistant mutant for some fluoroquinolones were determined[33]. In a recent report, Smelov and co-workers suggested that ofloxacin could be recommended as the primary drug in the treatment of chlamydia-infected patients with CP due to its pharmacokinetic parameters[33]. Moreover, the same authors stated that the decision on the prescription of pefloxacin or lomefloxacin should be made individually, but ciprofloxacin treatment is not suggested[33]. The authors, however, concluded that the conditions of *in vitro* susceptibility studies are incompatible with the infection as it occurs *in vivo,* even although it may be useful to include investigations for antibiotic susceptibility in every patient prior to treatment[33].

NEW ACQUISITIONS FROM RCTS

A recent prospective, randomized and open-label study by Cai *et al*[19] of 221 patients affected by chronic prostatitis due to Ct infection who had received oral prulifloxacin 600 mg once daily for 14 d or doxycycline 100 mg orally twice daily for 21 d found that prulifloxacin was equivalent to the standard therapy. Moreover, in this study the authors showed that prulifloxacin was superior to standard therapy in microbiological efficacy rates in terms of mucosal IgA and interleukin-8 (IL)-8 levels decreasing[19]. This effect is probably due to an anti-inflammatory effect of quinolones.

Role of IL-8 evaluation in management of Ct infections

The role of pro-inflammatory cytokines, such as IL-6 or IL-8, in Ct infection is well established, discussed and used not only in diagnosis but also in management and therapy control[12,19,34]. Several reports, moreover, suggested that IL-8 evaluation should be used, not only as a Ct infection marker, but also as a marker of therapy efficacy[12,19]. The role of molecular markers in the management of Ct infections is, therefore, clinically useful and suggested. Mazzoli *et al*[12] recently demonstrated that patients with a higher mean value for IL-8 and massive presence of mucosal IgA had evidence of strong inflammation and a correlation with the higher level of pain and a worse quality of life, with a significant correlation between IL-8 and IgA values and NIH-CPSI subscale scores. Moreover, in clinical practice, Cai *et al*[19] found a good relationship between IL-8 and NIH-CPSI, demonstrating that an improvement in QoL (NIH-CPSI decreasing) is related to a decrease in IL-8 levels after therapy.

Role of IgA evaluation in management of Ct infections

Some authors demonstrated in an animal model that high production of IgA in genital tract secretions seems to be related to the presence, persistence and accumulation of Th2 MoPn cells in the genital tract during chronic infections, with the consequent inability to clear the infection[34]. Particularly, the presence of chronic infection in patients affected by Ct infection has been well correlated with the presence of high levels of anti-heat shock protein 60 (anti-HSP60) and major outer membrane prottein-P2 mucosal IgA antibodies. The anti-HSP60 immunization suggests chronic or repeated stimulation from an endemic source of the microorganism, proved by the presence of Ct DNA found in young sexually active patients affected by chronic prostatitis due to Ct infection[35].

THERAPY OF THE INFECTION SEQUELAE

It is well known that chronic prostatitis due to Ct infection not only decreases the quality of life[36] but also has a significant impact on a couple's reproductive health[9]. Indeed, Ct has a significant role in male infertility and eradication of the infection is critical to the recovery of the man's fertility[37]. However, eradication of the infection after antibiotic therapy does not always result in recovery of semen quality and other compounds are consequently needed. Recently, Cai *et al*[38], using a prospective, randomized and controlled study, demonstrated that L-arginine, L-carnitine, acetyl-L-carnitine and ginseng extracts, together with prulifloxacin, improved semen parameters in patients with Ct genital infection and

oligoasthenoteratozoospermia compared to treatment with prulifloxacin therapy alone. The enhanced quality of spermatozoa from an infertile status to a normal fertility index was determined through two mechanisms. The anti-inflammatory and antioxidative effects of ginseng improved the shape and concentration of spermatozoa[39] and L-arginine, L-carnitine and acetyl-L-carnitine enhanced sperm motility and function by stimulating the activity of endothelial nitric oxide synthase[40]. Two important aspects in the treatment of male infertility in patients affected by chronic prostatitis and oligoasthenoteratozoospermia due to Ct infection should be discussed. Firstly, treatment should be started concurrently with the antibiotic treatment. Then, the association of antibiotic therapy with L-arginine, L-carnitine, acetyl-L-carnitine and ginseng extracts together can produce good results in terms of semen quality recovery[38]. Recently, Miyashita *et al*[41] demonstrated the efficacy of a new-generation fluoroquinolone, sitafloxacin, against Ct infection. Its antimicrobial activity is unique compared to conventional fluoroquinolones, although few clinical studies have been reported[42,43]. In a recent study, the microbiological eradication rates of Ct, M. genitalium and U. urealyticum were 100% (33 of 33), 100% (11 of 11) and 80% (8 of 10), respectively[44]. Even although these results are promising, few clinical studies have been reported in the treatment of patients affected by non-gonococcal urethritis and no study in those with chronic prostatitis due to Ct infection.

FUTURE PROMISES BY VACCINES

The asymptomatic nature of Ct makes diagnosis and prevention of sequelae a challenge for the urologist. It is well known that host immunity induced by chlamydial infections is not long lasting and may take several months or years to develop[45,46]. Moreover, the pathogenesis of Ct has not been completely elucidated and the role of host immunology is unclear[46]. Several chlamydial vaccine trials have used the major outer membrane protein as a vaccine candidate[46]. However, studies using the major outer membrane protein have been inconclusive and immunity is generally short-lived[46].

CONCLUSION

Clinical trials continue to demonstrate equivalent efficacy and tolerability of azithromycin and doxycycline regimens, and both are still recommended as first line therapy, even although some experiences with other antibiotics have been published with promising results. Further evaluation of chlamydial etiology of prostatitis and infertility is required to make a definitive statement on the association between isolation of this organism and the diseases. Finally, clinical trials should be planned in order to evaluate the real frequency of Ct resistance to standard therapy.

PILLS TO TAKE HOME

Ct infection is one of the most prevalent sexually transmitted bacterial infections worldwide.

Chronic prostatitis due to Ct infection has a significant impact on young male fertility.

First line treatment includes azithromycin 1 g orally as a single dose or doxycycline 100 mg orally twice a day for 7 d.

IL-8 evaluation should be used as a marker of therapy efficacy.

A treatment schedule with prulifloxacin 600 mg once daily for 14 d is equivalent to the standard therapy in terms of microbiological eradication but is superior over standard therapy in terms of IL-8 levels decreasing.

Phytotherapeutic agents are able to improve semen parameters in patients with chronic prostatitis and oligoasthenoteratozoospermia due to Ct infection when administered together with antibiotic treatment.

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MINIREVIEWS

Role of magnetic resonance imaging in the diagnostic work up of clinical localized prostate cancer: A review

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Abstract

Imaging plays an increasingly important role in the work up of prostate cancer (PCa) and magnetic resonance imaging (MRI) is generally accepted as the most accurate and promising imaging modality in the local staging of PCa due to its high spatial resolution and excellent soft tissue contrast. The quality and performance of MRI of the prostate has improved dramatically during the last decade. Mainly, the combination of morphological information and functional information on cell density, tissue perfusion or metabolism as provided in multi-parametric prostate MRI (mpMRI) has led to a substantial increase in lesion detection and characterization. The correlation between functional parameters as provided by MRI and the aggressiveness of PCa as determined by the Gleason Score may help in differentiating clinically significant from indolent PCa non-invasively. Besides these pros, radiologists are confronted with an immense amount of information and standardized acquisition, interpretation and reporting of mpMRI is not yet a reality. Furthermore, prostate MRI availability is still limited to high volume centers in many countries; hence, it is not yet a routine tool in common daily practice. Hence, development of guidelines for standardized acquisition, interpretation and reporting of prostate MRI exams is urgently needed in order to provide useful information for treating clinicians. Preferably, multi-centric clinical studies comparing MRI findings to step-section histological specimens are mandatory during the coming years. Furthermore, simplification of the acquisition must be achieved in order to make this imaging modality applicable for daily use in common uro-radiological practice.

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Key words: Localized prostate cancer; Magnetic resonance imaging; Local imaging; Staging; Diagnostic work up

Core tip: This review gives an overview about the current strengths and pitfalls of magnetic resonance imaging in the work up of clinical localized prostate cancer as well as suggestions for steps in the future.

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INTRODUCTION

Imaging plays an increasingly important role in the work up of prostate cancer (PCa). An important mainstay of magnetic resonance imaging (MRI) is the local staging in patients with clinically localized PCa. In these patients, not only the information whether or not the disease is confined to the capsule is crucial, but also information on the aggressiveness of the PCa is desired. The latter information is especially important in PCa due to its heterogeneity in terms of histopathology and clinical outcome.

Exact characterization of PCa is a prerequisite in order to offer personalized treatment options and minimize the evident problem of over-diagnosis and over-treatment of indolent PCa[1]. Furthermore, because of prostate specific antigen (PSA) screening, even in opportunistic forms, most PCa are detected while still in a clinically localized stage[2]. In the end, a combination of tumor characteristics, such as stage and aggressiveness of PCa, and patient characteristics, such as the age, co-morbidities and preferences of the patient, will help decide on the best available (curative) treatment option. For men who suffer from aggressive localized PCa variants, meaning PCa with a Gleason score $\geqslant 7$, traditional whole gland approaches such as radical prostatectomy or irradiation of the prostate gland are still considered the gold standard treatment modalities. However, patients suffering from a less aggressive, so called "indolent", variants of PCa can nowadays be offered less aggressive treatment options which aim at preserving the prostate and thereby minimizing treatment related adverse events. Of these treatment options active surveillance (AS) should be mentioned first. In AS, patients with low risk PCa are closely observed and followed and curative intervention is only suggested in the case of disease progression during follow up. AS is considered a safe treatment strategy as long as inclusion criteria are strictly set and respected[3]. More recently, focal therapy emerged as another option. However, although short term safety of this approach has been described in the literature[4], long term outcome data are still lacking.

Deciding on the optimal treatment plan for a man suffering from PCa involves knowledge of differentiating limited from extensive tumor burden, organ confined from non-organ confined disease, and indolent from aggressive cancer variants. MRI may help in gathering this crucial information and is therefore an important test for urologists and radio-oncologists in order to offer personalized treatment to patients suffering from PCa.

THE ROLE OF MRI TODAY

Today, MRI is generally accepted as the most accurate and promising imaging modality in the local staging of PCa[5] due to its high spatial resolution and excellent soft tissue contrast. Furthermore, its ability to detect prostate cancer foci in anatomical locations within the prostate gland which are difficult to sample and therefore often missed by TRUS-Biopsy (*i.e.*, the anterior zone) has been shown by Sciarra *et al*[6]. The quality and performance of MRI of the prostate has improved dramatically during the last decade[5,7-9]. Mainly, the combination of morphological/anatomical information and functional information on cell density, tissue perfusion or metabolism as provided in multi-parametric prostate MRI (mpMRI) has led to a substantial increase in lesion detection and characterization[10,11]. The correlation between functional parameters as provided by MRI and the aggressiveness of PCa as determined by the Gleason score which has recently been discovered[11-15] may help in differentiating clinically significant from indolent PCa non-invasively.

THE ROLE OF MRI IN THE FUTURE

These promising developments have led to great enthusiasm and it is conceivable that the traditional ultrasound guided random prostate biopsy with its well known problem of under-sampling[16] might be completely replaced by MRI guided targeted biopsy procedures. Quite possibly overtreatment could be decreased due to the identification of only significant PCa[17] while indolent PCa remains undiscovered. However, there are several crucial and as yet unsolved problems with MRI in PCa today. As described by Gupta *et al*[7] and supported by others[18], radiologists are confronted with an immense amount of information in mpMRI and standardized acquisition, interpretation and reporting of mpMRI is not yet a reality. However, standardization is urgently needed in this field should MRI ever play a crucial role in PCa work up, as prospected by Choyke *et al*[17]. In this respect, there are currently promising developments[19,20] in progress. Furthermore, prostate MRI availability is still limited to high volume centers in many countries; hence, it is not yet a routine tool in common daily practice. As a consequence, reading prostate MRI correctly is not a routine task for most radiologists today but the experience of the radiologist in reading and interpreting prostate MR findings, probably more so than in other exams, is a crucial factor[21,22]. The role and responsibility of the radiologist will change as urologists and radio-oncologists will most likely base their treatment recommendations much more on MRI-findings than they used to. The quality and reproducibility of reading prostate MRI are important and mandatory prerequisites for a high quality care of patients with PCa.

NEXT STEPS

MpMRI has been shown to be an important and highly promising imaging modality in the work up of patients with PCa. As its technical potential seems to be far from fully exploited, mpMRI may truly revolutionize management of patients with PCa and subsequently personalize the treatment of PCa. However, proper interpretation of prostate MRI is still limited to some specialized radiologists and a certain number of prostate MRIs have to be interpreted on a regular basis in order to maintain a high quality of radiological reports. Development of guidelines for standardized acquisition, interpretation and reporting of prostate MRI exams is urgently needed in order to provide useful information for treating clinicians. In this regard, further and preferably multi-centric clinical studies comparing MRI findings to step-section histological specimens are mandatory during the coming years. Furthermore, simplification of the acquisition must be achieved in order to make this imaging modality applicable for daily use in common uroradiological practice.

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ORIGINAL ARTICLE

Development of a nomogram for predicting a positive repeat prostate biopsy

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Abstract

AIM: To find risk factors of cancer in patients who had a repeat biopsy and to develop the nomogram using our cohort.

METHODS: Among 3500 patients who had a prostate biopsy over 11 years between 2000 and 2010 at our hospital, we studied a total of 807 repeat biopsy sessions in 459 patients who had at least 1 initial negative biopsy. At each biopsy session, we recorded patient age, number of previous biopsy sessions, number of biopsy cores, number of previously negative biopsy cores, months from the initial biopsy, months from the previous biopsy, serum PSA, PSA slope, digital rectal examination findings, hypoechoic lesions suspicious for a cancer on transrectal ultrasonography, total prostate volume, transitional zone (TZ) volume, PSA density, PSA TZ density and history of high grade prostatic intraepithelial neoplasia (HGPIN) or atypical small acinar proliferation (ASAP). Clinical and pathological variables were correlated with the outcome of repeat biopsies. A nomogram was developed based on logistic regression analyses and calibration was performed.

RESULTS: Overall, 17% of repeat biopsies had a cancer. With receiver operating characteristics analyses, the highest area under the curve (AUC) was obtained based on all available 13 variables, which were age, PSA, digital rectal examination, PSA density, prostate volume, TZ volume, PSA TZ density, cumulative number of biopsy cores, HGPIN, ASAP, months from previous negative biopsy, initial negative biopsy and number of biopsy cores. Based on multivariable logistic regression analysis, a nomogram was constructed with an AUC of 0.74, which was greater than that of any single risk factor. The calibration plot seemed to be good.

CONCLUSION: Our nomogram for predicting a positive repeat biopsy can provide probabilities for cancer and may help clinical judgment on whether to do a repeat prostate biopsy.

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Key words: Prostate; Repeat biopsy; Prostate cancer; Nomogram; Prostate-specific antigen

Core tip: Although prostate cancer is found in about 30% of patients at the initial biopsy session, there is a need to identify those with a negative result but who are at high risk. Although individual risk factors have been found to be associated with cancer, patient counseling requires the integration of multiple risk factors to obtain a prediction for the individual. We developed a nomogram that predicts a positive biopsy after a previous negative biopsy session. It provides a wide range of probabilities for cancer and may help clinical judgment of whether to do a repeat prostate biopsy.

Iseki R, Ohori M, Piccorelli A, Yu C, Piccorelli A, Ohno Y, Tachibana M, Kattan MW. Development of a nomogram for predicting a positive repeat prostate biopsy. *World J Clin*

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INTRODUCTION

With the advent of serum prostatic specific antigen (PSA) and transrectal ultrasonography in the late 1980s, prostate needle biopsy has markedly increased for a diagnosis of prostate cancer[1,2]. About 70000 cases with prostate biopsies in a year in Japan[3] have been estimated, so roughly 50000 cases should have no cancer in biopsy specimens if the detection rate of cancer is about 25%. It is obvious that no cancer in the first biopsy session does not give the perfect answer so a repeat biopsy will be done over and over[4,5]. The procedure of prostate biopsy itself is not difficult but the complications, such as hematuria, dysuria and urinary tract infection, do exist and we should also not underestimate discomfort during the biopsy procedure.

On the other hand, the indication for rebiopsy has remained unclear. Clinicians tend to recommend a repeat biopsy because of high PSA levels, an increasing PSA, abnormalities on digital rectal examination and/or imaging studies, and the presence of high grade prostatic intraepithelial neoplasia (HGPIN) and/or atypical small acinar proliferation (ASAP) in a previous biopsy. Age, race and family history are also sometimes considered. Realistically, however, it is extremely difficult to get the right answers to make a clinical decision at the time of whether to do a biopsy with these many factors and backgrounds. In the past, many investigators studied to find risk factors to predict a cancer in a repeat biopsy and to construct models to provide the accurate prediction of a cancer. Among them, a nomogram, the mathematical model based on a logistic analysis with multiple factors, seems to be the best model to provide an accurate prediction for an individual patient. Therefore, we tried to find risk factors of cancer in patients with a repeat biopsy and develop the nomogram using our cohort.

MATERIALS AND METHODS

We studied 459 patients who had at least 1 initial negative biopsy and underwent a total of 807 repeat biopsy sessions at our hospital for 11 years between 2000 and 2010. The indication of repeat biopsy and number of biopsy cores varied among a total of 10 attending physicians. However, the number of biopsy cores has changed; 8 cores in 2000 to 2005, 10 cores in 2005 to 2007, and 12 cores in 2008 to the present. The majority of patients had a transrectal biopsy under local anesthesia of 10 cc of 1% lidocaine under the guidance of transrectal ultrasonography (TRUS). Also, the additional cores were taken if there were hypoechoic lesions suspicious for a cancer on TRUS. On TRUS, the volume of the prostate gland and transition zone were estimated with an ellipsoid formula, as previously described. PSA density[6] was calculated as PSA divided by prostate volume and PSA transition zone density (PSATZD)[7] was calculated as PSA divided by transition zone volume. PSA slope in ng/mL yearly was estimated by the difference between the most current serum PSA measured minus serum PSA at the previous biopsy, divided by elapsed time in years[8].

Pathology

We obtained the pathology outcome from the official pathology reports. The majority of pathological reports indicated HGPIN as an indicator of cancer precursor and we neglected the reports of low grade PIN. We also recorded ASAP if the pathological reports indicated that there were a few atypical cells which were not enough to diagnose a cancer.

Statistical analysis

In analyses, the association of positive repeat biopsy with each clinical and biopsy feature was evaluated using multivariable logistic regression analysis. All the predictor variables included in the multiple logistic regression were based on a systematic literature review and clinical relevance with model selection. Restricted cubic splines were used for numeric or ordinal variables to accommodate the non-linear relationship. Generalized estimating equations (GEEs) implemented to adjust the clustering effect resulted from the repeat biopsies from the same patients. To evaluate the predictive accuracy of each factor in combination, we used the area under the receiver operating characteristic (ROC) curve (AUC) as the discrimination metric. Also, in the calibration plots, we compared the predictive values with the actual outcomes. Bootstrapping was used to correct for over-fitting bias. Based on the logistic analysis, we developed a nomogram to predict the probability of a positive repeat biopsy. Statistical significance was determined with a P value less than 0.05. All analyses were carried out using the commercially available software, STATA (Stata Corporation, College Station, TX, United States) and S-plus (Insightful Corporation, Seattle, WA, United States) or the open-source statistical software R-2.12.2 (R Development Core Team 2011) with Design and Zelig packages added.

RESULTS

Overall, 17% of patients had a prostate cancer on repeat biopsy. Table 1 lists the characteristics of the patients. A mean of 1.7 repeat biopsy sessions (or 2.7 all biopsy sessions including the initial biopsy) per patient were performed. Table 2 shows the rates of a positive repeat biopsy according to each variable. The cancer detection rates at biopsy 2 to 4 were consistent at 16%-18%. The number of patients was too small but the detection rates were high at 38%-50% if palpable nodules were identified. As prostate volume measured with ultrasonography increased, detection rates of cancer significantly de-

Table 1 Clinical and pathological characteristics *n* (%)

Predictor variables		No cancer *n* = 675	Cancer *n* = 132	*P* value
Digital rectal examination	Negative	642 (95.1)	122 (92.4)	0.209
	Positive	33 (4.9)	10 (7.6)	
TRUS	Negative	603 (89.3)	108 (81.8)	0.015
	Positive	72 (10.7)	24 (18.2)	
HGPIN in previous biopsy	Negative	648 (96)	121 (91.7)	0.032
	Positive	27 (4)	11 (8.3)	
ASAP in previous biopsy	Negative	627 (92.9)	116 (87.9)	0.051
	Positive	48 (7.1)	16 (12.1)	
Age, yr	Mean	67	68.8	0.008
	SD	7.3	6.3	
No. of previous biopsy sessions	Mean	1.7	1.7	0.894
	SD	1	1.1	
No. of biopsy cores	Mean	11.3	11.9	0.006
	SD	2.4	2.5	
Cumulative No. of cores	Mean	16.1	15.8	0.791
	SD	12.3	11.4	
Months from the initial biopsy	Mean	26.8	25.7	0.576
	SD	21.0	22.7	
Months from the previous biopsy	Mean	16.9	15.7	0.353
	SD	13.7	13.6	
PSA slope (ng/mL per year)	Mean	1.2	1.3	0.824
	SD	5.7	13.2	
PSA (ng/mL)	Mean	9.8	11.6	0.011
	SD	6.7	9.8	
Prostate volume (cc)	Mean	53.0	43.8	< 0.001
	SD	25.8	27.2	
TZ prostate volume (cc)	Mean	30.2	21.2	< 0.001
	SD	20.4	17.9	
PSA density (ng/mL per cc)	Mean	0.2	0.3	< 0.001
	SD	0.2	0.4	
PSA TZ density (ng/mL per cc)	Mean	0.4	0.8	< 0.001
	SD	0.4	0.9	

TRUS: Transrectal ultrasonography; HGPIN: High grade prostatic intraepithelial neoplasia; ASAP: Atypical small acinar proliferation; TZ: Transitional zone.

Table 2 Association between cancer detection rates and clinical variables according to the number of biopsy sessions

Variable	Total No./No. Ca (%)			
	Biopsy 2	Biopsy 3	Biopsy 4	Biopsy 5
	459/75 (16.3)	208/34 (16.7)	82/15(18)	41/3(7)
Digital rectal examination				
Negative	437/73 (16.7)	195/29(14.9)	76/12 (15.8)	40/3 (7.5)
Positive	22/2 (9.1)	13/5(38.5)	6/3 (50)	1/0 (0)
Prostate volume on TRUS, (cc)				
Less than 20	15/7 (47.7)	6/4 (66.7)	0/0 (0)	0/0 (0)
20-40	181/39 (21.5)	69/12 (17.4)	24/6 (25.0)	14/1 (7.1)
40-60	145/19 (13.1)	75/12 (16.0)	35/5 (14.3)	16/2 (12.5)
60-80	71/7 (9.9)	32/6 (18.8)	13/2 (15.4)	5/0 (0)
80 or Greater	47/3 (6.3)	26/0 (0)	10/2 (20.0)	6/0 (0)
PSA density (ng/mL per cc)				
Less than 0.15	196/16 (8.2)	82/11 (13.4)	29/6 (20.7)	10/0 (0)
0.15-0.30	185/33 (17.8)	84/10 (11.9)	36/6 (16.7)	22/3 (13.6)
0.30-0.45	46/14 (30.4)	20/3 (15.0)	10/1 (10.0)	7/0 (0)
Greater than 0.45	32/12 (38.5)	22/10 (45.5)	7/2 (28.6)	2/0 (0)
PSA TZ density (ng/mL per cc)				
Less than 0.25	156/9 (5.8)	58/7 (12.1)	19/3 (18.8)	10/0 (0)
0.25-0.5	168/22 (13.1)	85/11 (12.9)	42/7 (16.7)	18/2 (22.2)
0.5-1.0	98/26 (26.5)	40/6 (15.0)	15/3 (20.0)	12/2 (18.3)
Greater than 1.0	37/18 (48.6)	25/10 (40.0)	6/2 (33.3)	1/0 (0)
PSA (ng/mL)				
Less than 4	18/1 (5.6)	1/1 (100)	0/0 (0)	0/0 (0)
4-10	313/48 (15.3)	136/19 (14.0)	49/8 (16.3)	20/2 (10.0)
10-20	108/21 (19.4)	54/11 (20.3)	25/5 (20.0)	17/1 (5.9)
Greater than 20	20/5 (25.0)	17/3 (17.6)	8/2 (25.0)	4/0 (0)
PSA slope (ng/mL per year)				
Less than 0.75	237/30 (12.7)	102/15 (14.7)	44/8 (18.2)	21/1 (4.8)
0.75 or greater	222/45 (20.3)	106/19 (17.9)	38/7 (18.4)	20/2 (10.0)
Previous HGPIN				
Negative	437/67 (15.3)	200/32 (16.0)	79/14 (17.7)	40/3 (7.5)
Positive	22/8 (36.4)	8/2 (25.0)	3/1 (33.3)	1/0 (0)
Previous ASAP				
Negative	427/68 (15.9)	190/30 (15.8)	75/13 (17.3)	38/2 (5.3)
Positive	32/7 (21.9)	18/4 (22.2)	7/2 (28.6)	3/1 (33.3)
Normal histology	406/61 (15.0)	184/29 (15.8)	72/12 (16.7)	37/2 (5.4)
Total	459/75 (16.3)	208/34 (16.4)	82/15 (18.3)	41/3 (7.3)

Results of cases with 6 or more biopsy sessions are not shown because of low numbers. HGPIN: High grade prostatic intraepithelial neoplasia; TZ: Transitional zone; ASAP: Atypical small acinar proliferation.

creased ($P < 0.001$). Of 38 repeat biopsies with HGPIN in previous biopsy specimens, 11 (32%) had cancer compared to 116 (15%) of 769 patients with no HGPIN ($P = 0.032$). Similarly, of 64 biopsies with ASAP, 16 (25%) had a cancer compared to 116 (16%) of 743 biopsies with no ASAP ($P = 0.05$).

Table 3 shows the results of the multivariable logistic regression analysis in predicting a positive biopsy after adjusting the clustering effect with GEEs. Of all variables, age, number of previous negative biopsy sessions, number of biopsy cores, cumulative number of biopsy cores, PSA slope, PSA density and history of ASAP were statistically significantly associated with repeat biopsy findings (all $P < 0.05$).

A nomogram was constructed incorporating all predictors (Figure 1). Although some variables were not statistically significant predictors of a positive repeat biopsy, they were retained in the nomogram because omitting them from the model tended to decrease accuracy. Also, other factors become falsely better if clinically important factors are omitted. The nomogram is used by first locating the patient position on each predictor variable scale. Each scale position has corresponding prognostic points (top axis). Point values for all the predictor variables are determined consecutively and summed to arrive at a total point value. This value is located on the total point axis and directly below is the prediction for finding cancer. The nomogram was internally validated with 1000 bootstrap resamples and achieved an AUC of 0.74.

Figure 2 shows the calibration by plotting the nomogram predicted probability for positive repeat biopsy against the patient's observed probability. In general, the performance of the nomogram appears to be reasonably accurate while the predicted probability seems to overestimate a positive repeat biopsy in the higher range of predicted probability.

DISCUSSION

In 2003, Lopez-Corona *et al*[19] first reported the nomo-

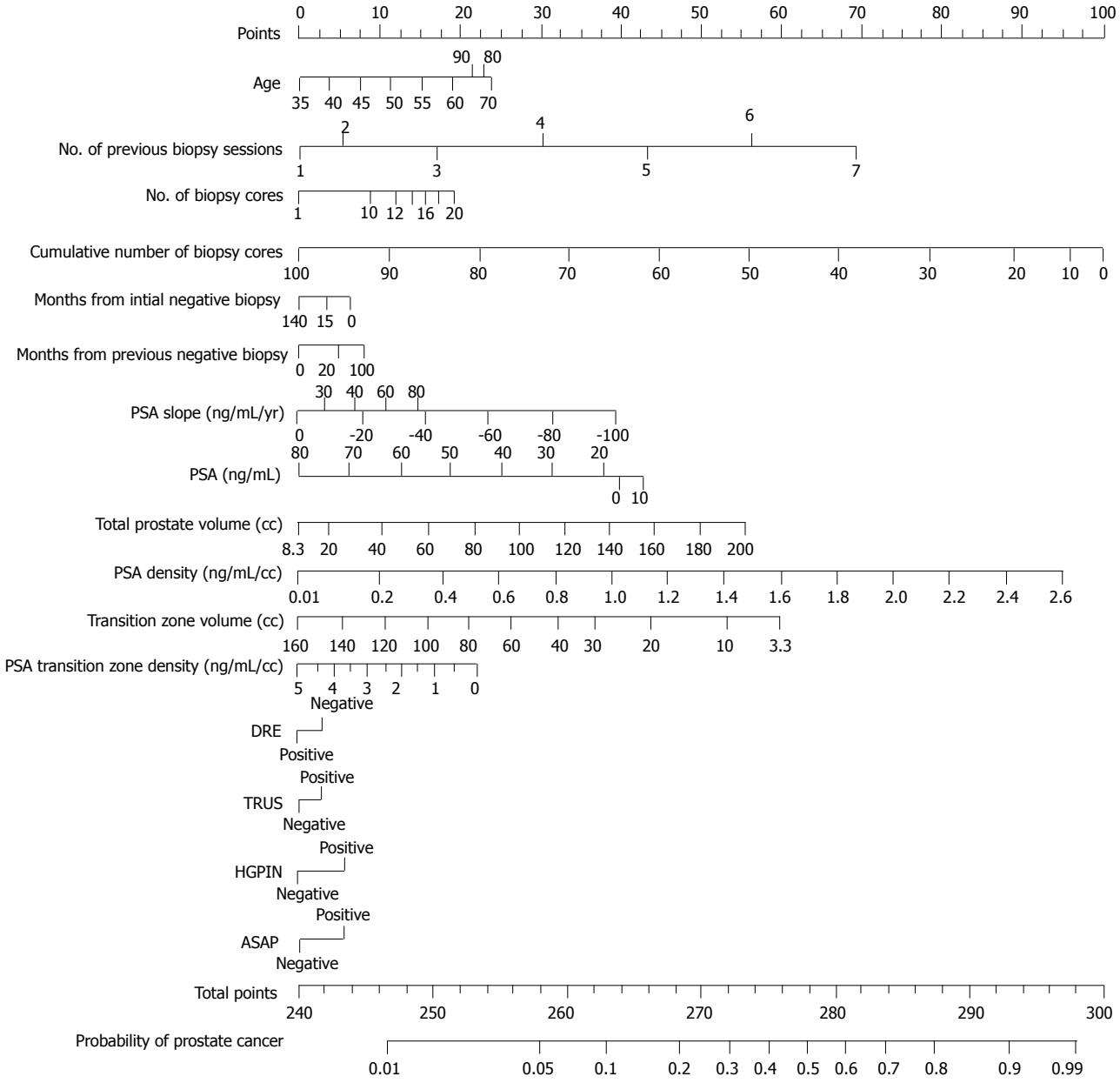


Figure 1 Nomogram (age, number of previous biopsy session, number of biopsy cores, number of previously negative biopsy cores, months from initial biopsy, months from previous biopsy, prostate specific antigen slope, prostate specific antigen, digital rectal exam, transrectal ultrasonography, prostate volume, transition zone volume, prostate specific antigen density, high grade prostatic intraepithelial neoplasia, atypical small acinar proliferation, prostate specific antigen transition zone density) for predicting the probability of a positive repeat biopsy. Nomogram is used by first locating the patient's position on each factor axis. Each factor has corresponding prognostic points (top axis). The points for each factor are added to achieve a total point that is subsequently plotted on the total point axis (the second scale from the bottom). The probability of prostate cancer is estimated by drawing a straight line downwards to the bottom line from the location of the total point .TRUS: Transrectal ultrasonography; HGPIN: High grade prostatic intraepithelial neoplasia; ASAP: Atypical small acinar proliferation; TZ: Transitional zone; PSA: Prostatic specific antigen; DRE: Digital rectal exam.

gram to predict a cancer in 661 patients with a previous negative biopsy using 8 factors, such as age, digital rectal examination, a total number of biopsy cores, HGPIN, ASAP, PSA, PSA slope and family history. Since then, to our knowledge there are a total of 8 published nomograms[9-18], including 2 with an extended biopsy strategy[10,18], to predict a positive biopsy in a setting of repeat biopsy. We have summarized these nomograms in Table 4. All nomograms, except 2, included age, PSA and DRE as standard predictive factors. Among variables in the previous nomograms, 4 nomograms included the results of %free PSA which seemed to be most valuable predictor since it had a large scale of points in the nomograms with a relatively higher AUC of 0.76 to 0.856[10,11,14,18]. Following %free PSA, prostate volume seemed to be important since 6 out of 9 nomograms included it as a significant predictor. Only Sakura *et al*[18] reported that age was the largest scale in the nomogram, while another 7 nomograms showed a short scale for age. There were many other factors and none of 9 nomograms used identical factors, which may represent the complexity of the situation of a repeat biopsy.

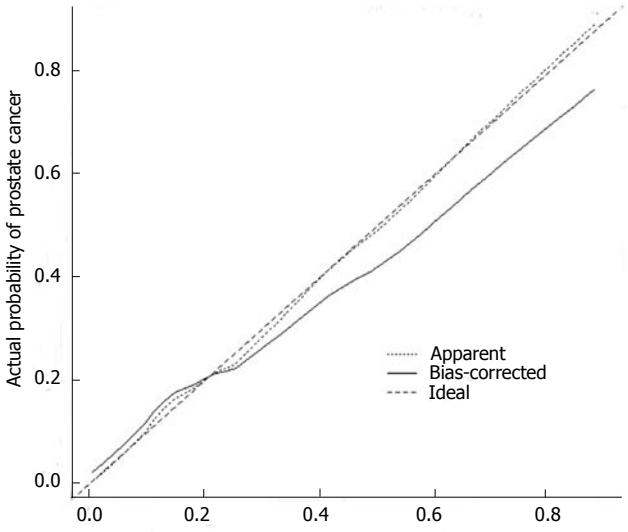


Figure 2 A calibration plot of the nomogram predicting the probability of a positive repeat biopsy.

Table 3 Multivariable logistic regression analysis in predicting positive repeat biopsy with generalized estimating equations adjustment for clustering repeat biopsies from the same patients

Predictor variables	Q1	Q3	Odds ratio	Lower 0.95	Upper 0.95	*P*
Age, yr	62	73	1.6	1.1	2.5	0.002
No. of previous biopsy sessions	1	2	2.3	0.8	6.2	0.002
No. of biopsy cores	10	12	1.6	1.3	2.1	0.001
Cumulative No. of cores	8	20	0.3	0.1	0.8	0.001
Months from the initial biopsy	10.3	36	0.6	0.2	2.0	0.727
Months from the previous biopsy	7.1	22.7	1.5	0.6	3.8	0.651
PSA slope (ng/mL per year)	-0.3	2.4	0.9	0.9	1.0	0.007
PSA (ng/mL)	6	11.4	1.0	0.3	4.0	0.098
Prostate volume (cc)	33.5	60.5	3.3	0.5	20.8	0.081
TZ prostate volume (cc)	15	36.1	0.1	0.0	1.0	0.116
PSA density (ng/mL per cc)	0.1	0.3	2.5	0.3	24.7	0.045
PSA TZ density (ng/mL per cc)	0.2	0.6	0.8	0.1	6.9	0.328
DRE-positive: negative	NA	NA	0.7	0.3	1.7	0.393
TRUS-positive: negative	NA	NA	1.4	0.8	2.6	0.218
HGPIN-positive: negative	NA	NA	2.2	0.9	5.2	0.080
ASAP-positive: negative	NA	NA	2.2	1.1	4.7	0.035

TRUS: Transrectal ultrasonography; HGPIN: High grade prostatic intraepithelial neoplasia; ASAP: Atypical small acinar proliferation; TZ: Transitional zone; PSA: Prostatic specific antigen; DRE: Digital rectal exam.

Early studies reported relatively high rates of cancer detection on repeat biopsy after an initial biopsy finding of HGPIN, but recent studies have reported substantially lower rates[19,20]. Park *et al*[21] analyzed 45 patients with ASAP and 43 with HGPIN on initial biopsy and found cancer in 51% of repeat biopsies in each group. Abouassaly *et al*[22] reported that the findings of ASAP were associated with a high likelihood of cancer on repeat biopsy regardless of the number of cores taken on initial biopsy. In our study, in 32 patients with ASAP and 22 patients with HGPIN in the previous biopsy session, cancer was found in 21.9% of repeat biopsies in the ASAP group and in 36.4% in the HGPIN group. Since the proportion of ASAP and/or HGPIN of the entire group is small, the impact on the detection rates of cancer may not be huge but we still think that they should be included in the nomogram since they are a different entity from the other markers and it would be important to include the findings by pathologists.

In studies by Walz *et al*[10] and ourselves, PSA density was the best factor to predict a cancer in a repeat biopsy. Benecchi *et al*[13] also included PSA density, which was the second predictor after %free PSA, while Walz *et al*[10] indicated that PSA density was a stronger predictor than %free PSA. Previously, Keetch *et al*[23] noted that PSA density and PSA slope were the best predictors of cancer on a repeat biopsy. In men with a PSA density of $\geq$ 0.15 ng/mL per milliliter and a PSA slope of 0.75 ng/mL per year, the rate of cancer detection was 46% compared to 13% in men with a PSA density of < 0.15 ng/mL per milliliter and a PSA slope of < 0.75 ng/mL per year ($P < 0.001$)[23]. In contrast, Fowler *et al*[24] found that percent free PSA was the best predictor of cancer compared with the other PSA derivatives. Eventually, the inclusion of these important factors enhances the detection rate of cancer in a repeat biopsy and a nomogram would be the best method to combine these factors to provide an accurate prediction for the individual patient.

There are several limitations in the present study. Firstly, we could not include the results of %free PSA since it was frequently not measured at our clinic. However, all variables in our nomogram are readily available so it is not necessary for patients to come to the clinic frequently to get the result of %free PSA. Also, in the present study, we showed a similar accuracy to predict a positive repeat biopsy without %free PSA. Thus, our nomogram can help many patients and their physicians after appropriate validations. Secondly, there are 16 variables in our nomogram to achieve the highest AUC. The accuracy, AUC 0.74, seems to be reasonable but some may feel that there are too many variables to calculate the probability at a busy clinic. We agreed that the calculation on paper may not be realistic so we have put our nomogram on the web. Thirdly, because the nature of a nomogram is based on multivariable analysis, the values of some variables were reversed. For example, the points on the nomogram increase with a decrease of the PSA transition zone density. This happened because we included similar variables, such as transition zone volume, PSA density and prostate volume. Therefore, it may slightly confuse clinicians when they put each value of variables into our nomogram. However, the inclusion of such variables is valuable to get accurate estimations. Fourthly, in the present study, we did not do model selection that would be accomplished on the modeling data alone. Instead, we built the model by including all predictors that were clinically relevant to the disease based on the literature whether they were statistically significant or not on the current data. With enough number of events in the modeling data, theory driven models normally have better generalizability than data driven models when applied to other patient populations and

Table 4 Published nomograms to predict positive prostate biopsy in repeat biopsy

Ref.	No. of patients	AUC	AUC of external validation	No of factors	Nomogram factors	Mean of no of biopsy cores	Ca detection rate, %
Lopez-Corona *et al*[9,12]	343	0.700	0.710	8	Standard factors + PSAV, HGPIN, ASAP, No. of total cores, biopsy history, family history	9.15	20
Walz *et al*[10]2	161	0.720	-	9	Age, PSA, %free PSA, PSA density, TZ density, prostate volume, TZ volume, no of previous biopsy, no of cores.	24	41
Chun *et al*[11]	1082	0.760	0.68-0.78	6	Standard factors, prostate volume, biopsy history, %free PSA, sampling density	11.1	30.2
Benecchi *et al*[13]	419	-	0.856	7	DRE + PSA slope, HGPIN, %free PSA, PSA density	-	31
Rochester *et al*[14]	110	0.818	0.696	7	Standard factors + PSA velocity, HGPIN, biopsy history, %free PSA	-	30-31
Chun *et al*[15,16]	809	0.700	0.73-0.75[1]	6	Standard factors + prostate volume, biopsy history, PCA3	15	39.1
Moussa *et al*[17]	408	0.720	0.620	12	Standard factors + prostate volume, PSA velocity, HGPIN, ASAP, No. of total cores, family history, time from initial/previous biopsy, BMI	19.1	31.6
Sakura *et al*[18]2	515	0.791	-	5	Age, %free PSA, prostate volume, previous extended biopsy, PSA doubling time	26	31.6
Present series	459	0.740	-	16	Age, No. of biopsy core, PSA, PSA density, prostate volume, TZ volume, PSA TZ density, TRUS, DRE, HGPIN, ASAP, months from initial biopsy and previous biopsy, No. of previous negative biopsy, PSA slope	11.4	17

[1]Standard factors include age, PSA and digital rectal examination; [2]Extended biopsy. AUC: Area under the curves; TZ: Transitional zone; TRUS: Transrectal ultrasonography; HGPIN: High grade prostatic intraepithelial neoplasia; ASAP: Atypical small acinar proliferation; PSA: Prostatic specific antigen,DRE: Digital rectal exam; BMI: Body-mass index.

preserve the predictive performance that was evaluated based on the modeling data[25,26].

In conclusion, we developed a nomogram to predict a cancer in a repeat biopsy using 16 readily available clinical factors with reasonable calibration. This helps both patients and clinicians to decide whether to do a repeat prostate biopsy.

COMMENTS

Background

Prostate cancer is found in about 30% of patients at the initial biopsy session but there is a need to identify those with a negative result who are at high risk. Although individual risk factors have been found to be associated with cancer, patient counseling requires the integration of multiple risk factors to obtain a prediction for the individual.

Innovations and breakthroughs

The authors developed a nomogram that predicts a positive biopsy after a previous negative biopsy session.

Applications

It provides a wide range of probabilities for cancer and may help clinical judgment of whether to do a repeat prostate biopsy.

Peer review

This paper is well-written and the topic is timely and appropriate.

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INSTRUCTIONS TO AUTHORS

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16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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REVIEW

Laparoendoscopic single site, laparoscopic or open surgery for adrenal tumors: Selecting the optimal approach

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Abstract

Numerous surgical modalities are available to treat adrenal lesions. Minimally-invasive approaches for adrenalectomy are indicated in most circumstances, and new evidence continues to be accumulated. In this context, current indications for open surgical adrenalectomy (OS-A), minimally-invasive adrenalectomy (MI-A), and laparoendoscopic single-site adrenalectomy (LESS-A) remain unclear. A comprehensive English-language literature review was performed using MEDLINE/PubMED to identify articles and guidelines pertinent to the surgical management of adrenal tumors. A comprehensive chart review was performed for three illustrative cases. Clinical recommendations were generated based on relevant literature and the expertise of the investigator group. MI-A offers advantages over OS-A in properly selected patients, who experience fewer complications, lower blood loss, and shorter hospital stays. Robot-assisted laparoscopic and retroperitoneoscopic adrenalectomy may offer advantages over transperitoneal surgery, and LESS-A may be an even less-invasive option that will require further evaluation. MI-A remains the surgical treatment of choice for most adrenal lesions. Tumor size and stage are the primary indications for selecting alternative treatment modalities. OS-A remains the gold standard for large tumors (> 10 cm) and suspected or known advanced stage malignancy. LESS-A appears to be an appropriate initial approach for small tumors (< 4-5 cm), including pheochromocytoma and isolated adrenal metastases.

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Key words: Adrenal masses; Surgical approach; Indications; Open adrenalectomy; Laparoscopic adrenalectomy; Laparoendoscopic single-site adrenalectomy

Core tip: Minimally-invasive adrenalectomy remains the surgical treatment of choice for most adrenal lesions. Tumor size and stage are the primary indications for selecting alternative treatment modalities. Open surgical adrenalectomy remains the gold standard for large tumors (> 10 cm) and suspected or known advanced stage malignancy. laparoendoscopic single-site adrenalectomy appears to be an appropriate initial approach for small tumors (< 4-5 cm), including pheochromocytoma and isolated adrenal metastases.

Riedinger CB, Tobert CM, Lane BR. Laparoendoscopic single site, laparoscopic or open surgery for adrenal tumors: Selecting the optimal approach. *World J Clin Urol* 2014; 3(2): 54-65 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i2/54.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i2.54

INTRODUCTION

Neoplasms of the adrenal gland consist of a broad spectrum of pathologies, ranging from benign non-functioning cortical adenomas (or "incidentalomas") to locally-invasive malignancies, such as adrenocortical carcinoma or metastasis from a distant site. Adrenalectomy

Table 1 Indications and relative contraindications for each surgical approach

Indications	Relative contraindications
OS-A	
Adrenocortical carcinoma with radiographic evidence of extra-adrenal extension (stage Ⅲ-Ⅳ)	Amenable to minimally-invasive approach
Extension of adrenal vein tumor thrombus into IVC	
Size larger than 10-12 cm	Size < 10 cm
Concomitant open procedure	
Paraganglioma	
Multiple prior abdominal surgeries	
MI-A	
Non-functioning and functioning adrenal tumors, including pheochromocytoma	Locally-advanced tumors (stage Ⅲ-Ⅳ)
Isolated adrenal metastases	Known, relatively large adrenocortical carcinoma (complete resection is essential for cure)
Size < 10 cm	Size larger than 10-12 cm
Adrenocortical carcinoma, consider only if stage Ⅰ-Ⅱ and < 10 cm	Multiple prior abdominal surgeries (or discussion of possibility of conversion)
BMI ⩾ 30 kg/m^2	
LESS-A	
For patients who consider cosmesis to be of great importance	Surgeon inexperience with LESS-A
Size < 4-5 cm	Size > 5 cm
Non-functioning and functioning adrenal tumors, including pheochromocytoma	Adrenocortical carcinoma
Isolated adrenal metastases	Multiple prior abdominal surgeries
BMI < 30 kg/m^2	

OS-A: Open surgical adrenalectomy; MI-A: Minimally-invasive adrenalectomy; LESS-A: Laparoendoscopic single-site adrenalectomy; BMI: Body mass index.

is the definitive therapy for these tumors and is indicated when either hormone secretion and/or increased risk for malignancy is present[1]. Risk factors for adrenal malignancy include evidence of a functional adrenal tumor, large tumor size, and concerning radiographic findings such as hyperdensity or heterogeneity, irregular tumor margins, local invasion, lymphadenopathy, or distant metastases[2-4].

Open surgical adrenalectomy (OS-A) using a dorsal lumbotomy, midline, subcostal, or thoracoabdominal incision is the historical gold standard, but has largely been replaced by minimally-invasive alternatives. Minimally-invasive adrenalectomy (MI-A) using a transperitoneal laparoscopic approach was first described in 1992[5] and has since replaced OS-A as the operation of choice for resecting most adrenal lesions[6]. Two notable exceptions to this include known adrenal malignancy and tumors that are excessively large or difficult to approach laparoscopically. MI-A is known to offer improved analgesia, hospital stay, blood loss, and complication rate compared to OS-A[7]. Several different techniques, including robot-assisted approaches, have been proposed to address various clinical scenarios where the traditional transperitoneal approach is technically difficult. Even more recently, laparoendoscopic single-site adrenalectomy (LESS-A) has emerged as an appropriate initial surgical approach for tumors smaller than 4-5 cm[8].

Some might suggest that the least invasive approach should be used in all circumstances, but our clinical experience would suggest that the optimal approach in a given scenario may depend on patient and tumor features, such as specific adrenal pathology, prior history of abdominal surgeries, tumor size, patient body habitus, and experience of the operating surgeon (Table 1)[9]. This report details a contemporary approach to surgical decision-making for adrenal tumors, with a review of the literature regarding OS-A, MI-A, and LESS-A.

LITERATURE REVIEW

A comprehensive English-language literature review was performed using MEDLINE/PubMed to identify articles and guidelines pertinent to the indications, techniques, perioperative results, and oncologic outcomes for various treatment modalities for adrenal tumors. Combinations of MeSH search terms adrenal tumor, pheochromocytoma, adrenal cortical carcinoma, metastasis, adrenalectomy, indications, laparoscopic, open, laparoendoscopic single site, transperitoneal, retroperitoneal, and robotic were used. A comprehensive chart review was conducted for the three patients in order to detail the factors determining the elected surgical approach. Approval was obtained from the institutional IRB. Clinical implications presented incorporate relevant literature and the expertise of the investigator group.

OS-A

Case 1: A 54-year-old female presented with worsening left lower abdominal pain that radiated to the left flank. Computed tomography with Ⅳ contrast revealed a large complex solid and cystic mass in the left retroperitoneal space measuring 13 cm and displacing the left kidney laterally and inferiorly. No retroperitoneal lymph nodes or visceral metastases were identified and subsequent chest X-ray was normal. She reported no history suspicious for a functional adrenal tumor, denying uncontrolled

Table 2 Outcomes from select studies comparing minimally-invasive adrenalectomy to open surgical adrenalectomy

Ref.	*N*	Mean tumor size (cm)	ACC or MET (%)[1]	OT (min)	EBL (mL)	LOS (d)	Conversion (%)	Complications (%)
Assalia *et al*[116]	581 MI-A	2.8	0.9	184	154	2.9	-	10.9
	753 OS-A	4.1	5.8	162	309	7.2	-	35.8
Lee *et al*[59]	358 MI-A	-	13.6	174	-	4.1	-	3.6
	311 OS-A	-	44.5	234	-	9.4	-	17.4
Eichhorn-Wharry *et al*[119]	1980 MI-A	-	4.1	146	-	2.8	-	1.8
	592 OS-A	-	18.4	186	-	6.7	-	7.6
Lombardi *et al*[120]	30 MI-A	7.7	100	135	-	5.3	0	3.4
	126 OS-A	9	100	129	-	9.3	-	5.6
Mir *et al*[121]	18 MI-A	7	100	298	1500	4	24	5.0
	26 OS-A	13	100	273	1100	6	-	20.0
Donatini *et al*[79]	13 MI-A	5.5	100	-	-	7	0	8.0
	21 OS-A	6.8	100	-	-	9	-	14.0
Bittner *et al*[122]	356 MI-A	3.2	5.9	159	-	2.5	6.2	11.0
	46 OS-A	8.5	28.2	197	-	9.1	-	50.0

[1]Preoperative indication of ACC or isolated adrenal metastasis. ACC: Adrenocortical carcinoma; MET: Metastasis to adrenal gland; OT: Operative time; EBL: Estimated blood loss; LOS: Length of hospital stay; OS-A: Open surgical adrenalectomy; MI-A: Minimally-invasive adrenalectomy.

blood pressure, new hair growth or other features of Cushing's syndrome. Functional work-up was pursued with serum potassium, urinary cortisol, and plasma and urinary metanephrines. After this negative functional evaluation was completed, the patient underwent OS-A *via* an anterior subcostal incision. The patient was discharged after an uneventful 5 d hospital stay and has remained without evidence of disease recurrence more than 3.5 year since surgery.

With expansion of the indications for MI-A, fewer OS-A are being performed worldwide. Nevertheless, there remain situations in which OS-A remains the best option, such as the case just described. Tumors larger than 10-12 cm should be resected *via* OS-A, given the increased likelihood of these lesions being malignant and the technical difficulties associated with laparoscopic removal of large tumors. OS-A remains the standard treatment for all patients with preoperative radiographic evidence of extra-adrenal tumor invasion. Tumors with associated adrenal and renal vein thrombus, which can extend into the inferior vena cava and right atrium, should also be resected *via* OS-A (Table 1). All these scenarios require maximal exposure to complete the procedure safely and extract the tumor intact. A summary of the evolution of indications for and outcomes of OS-A compared to MI-A are included in (Table 2).

MI-A

Case 2: The patient is a 54-year-old female who presents with a 7 cm × 5 cm × 4 cm right adrenal mass on follow up imaging obtained 12 mo after open right radical nephrectomy for pT2N0M0 clear cell renal cell carcinoma with negative lymph nodes. The patient has a relevant past medical history of renal cell carcinoma, rectal cancer s/p low anterior resection, and morbid obesity with a body mass index (BMI) of 61. Physical examination was unremarkable except for her large abdominal girth and prior incisions. Metastatic workup was negative for additional lesions. Biopsy of the adrenal mass was positive for metastatic renal cell carcinoma. After a discussion of risks and benefits of laparoscopic adrenalectomy and the possibility of open conversion, the patient underwent a transperitoneal laparoscopic adrenalectomy (TL-A). The procedure was successful, without any significant complications other than some delayed return of bowel function, and the patient was discharged home after a 5-d hospital stay.

Three aspects of this case indicate MI-A to be the appropriate surgical approach. MI-A is the procedure of choice for isolated adrenal metastases and for adrenal masses < 10 cm. MI-A is indicated for obese patients, as reduced morbidity and improved outcomes have been demonstrated when compared to OS-A[10]. An additional consideration in this case is the patient's past surgical history. This patient is likely to have adhesions, which may complicate laparoscopic surgery, but MI-A has been shown to be a feasible and safe initial approach to patients with previous abdominal surgery[11,12]. MI-A is an appropriate initial approach, with conversion to OS-A for failure-to-progress when adhesions make laparoscopic surgery excessively difficult.

Laparoscopic adrenalectomy: Transperitoneal vs retroperitoneal

TL-A is the most widely performed MI-A. TL-A offers a large working space, familiar anatomical exposure, excellent visibility, and the ability to perform simultaneous transperitoneal procedures[13]. This approach is dependent upon the ability to retract and mobilize the abdominal organs required for adequate exposure, and may require additional instruments to accomplish this prerequisite step. Adhesions from prior abdominal surgery can complicate port placement and lysis of adhesions may lengthen operative times and increase intra-operative risk of bowel injury, and should be considered carefully when deciding upon the surgical approach in any given scenario.

Retroperitoneal laparoscopic adrenalectomy (RL-A)

Table 3 Summary of the most recent meta-analyses comparing laparoscopic techniques for adrenalectomy

Ref.	RAL-A *vs* MI-A Brandao *et al*[30]	TL-A *vs* RL-A Nigri *et al*[27]	LESS-A *vs* MI-A Wang *et al*[41]
n	600	1205	443
Mean tumor size (cm)	3.86, 3.78	4.0, 3.3	2.7, 3.43
(Odds ratio, CI)	NA	0.48 (-0.21-1.18)	- 0.69 (-1.11--0.26)
P-value	NS	0.17	0.002
Mean operating time (min)	175, 148	132, 136	113.1, 92.7
(Estimate, CI)	5.88 (-6.02-17.79)	-11.07 (-41.38-19.24)	14.97 (4.69-25.24)
P-value	0.33	0.47	0.004
Mean EBL (mL)	44, 69	115, 85	74.2, 79.7
(Estimate, CI)	-18.21 (-29.11--7.32)	29.7 (-10.32-69.72)	-1.4 (-9.72-6.91)
P-value	< 0.0001	0.15	0.74
Mean LOS	3.78, 3.17	6.4, 5.5	3.82, 4.38
(Estimate, CI)	-0.43 (-0.56--0.30)	0.66 (-0.11-1.43)	-0.5(-1.02-0.02)
P-value	< 0.0001	0.09	0.06
Mean % conversion rate	4.4, 7.1	7.23, 7.74	7.8, 1.2
(Odds ratio, CI)	0.82 (0.39-1.75)	NA	4.66 (0.88-24.64)
P-value	0.61	NA	0.07
Mean % complication rate	3.6, 6.8	8, 6	14.2, 10.1
(Odds ratio, CI)	-0.04 (-0.07--0.00)	0.923 (0.58-1.46)	1.83 (0.88-3.81)
P-value	0.05	0.73	0.1

RAL-A: Robot-assisted laparoscopic adrenalectomy; MI-A: Minimally-invasive adrenalectomy; TL-A: Transpeitoneal laparoscopic adrenalectomy; RL-A: Retroperitoneal laparoscopic adrenalectomy; LESS-A: Laparoendoscopic single-site adrenalectomy; EBL: Estimated blood loss; LOS: Length of hospital stay.

allows for direct access to the adrenal gland without bowel mobilization or interference from intraperitoneal organs or adhesions. The disadvantages of RL-A, compared with TL-A, are the smaller operating space and relative absence of anatomic landmarks. This led some authors to conclude that RL-A should be reserved for surgeons with considerable experience with retroperitoneal surgery and tumors smaller than 5-7 cm[13,14]. Recent studies have demonstrated the safety and feasibility of treating tumors up to and exceeding 10 cm[15,16]. BMI > 35 kg/m^2 has also been cited as a contraindication to RL-A because of the difficulty in establishing and maintaining this potential space, resulting in limited exposure[14,15,17].

Numerous studies have been published comparing these two approaches[13,17-24]. These generally favor the retroperitoneal approach. Meta-analyses of these studies have demonstrated that operative time, blood loss, duration of hospitalization, time to oral intake, overall and major morbidity, and mortality are equivalent or superior for RL-A compared to TL-A[25-27]. A summary of the meta-analysis performed by Nigri *et al*[27] is included in (Table 3). RL-A may be preferred to TL-A for a few select scenarios, such as patients with suspected adhesions from previous transperitoneal abdominal surgery. However, it should be noted that the retroperitoneal space is typically obliterated after nephrectomy, preventing insufflation of this space independent of the peritoneal cavity. Some surgeons use the prone position for RL-A, allowing bilateral procedures to be performed without repositioning the patient. For bilateral RL-A (or TL-A) in full or modified lateral decubitus position, repositioning is a necessity in all but the thinnest of patients. With RL-A, some have reported success with two surgical teams operating simultaneously to reduce operative time and surgical stress[28,29].

Robot-assisted laparoscopic adrenalectomy

Robot-assisted laparoscopic adrenalectomy (RAL-A) offers improved blood loss and hospital stay, and similar operative time, conversion rate, and postoperative complications when compared to traditional MI-A[30]. A summary of Brandao *et al*[30] meta-analysis comparing RAL-A to MI-A is included in (Table 3). This procedure may offer advantages in morbidly obese patients (> 30-35 kg/m^3) and those with larger tumors, by improving retraction and exposure[31-34]. The main disadvantages cited by most authors are the learning curve of the entire surgical team, particularly for those not regularly performing other robotic surgeries, and the potential added cost of robot-assisted surgery. Overall, this approach shows promise to enable a wider range of tumors to be addressed with MI-A, and is likely to become more commonly utilized in the future.

LESS-A

Case 3: The patient is a 72-year-old male presenting with an incidentally-detected adrenal mass found during staging evaluation of a suspicious lung lesion. The patient was not found to have signs or symptoms of a functioning adrenal tumor upon investigation. Relevant past medical history includes hypertension, coronary artery disease and hyperlipidemia, with no prior surgical history. The patient has a 58 pack-year smoking history and physical examination was unremarkable. The patient was referred to a multi-disciplinary clinic for evaluation of a

Table 4 Laparoendoscopic single-site adrenalectomy *vs* minimally-invasive adrenalectomy: Overview of the outcomes from available comparative studies (adapted from Rane *et al*[8] and Wang *et al*[41])

Ref.	*N*	Mean tumor size (cm)	ACC or MET (%)[1]	OT (min)	EBL (mL)	LOS (d)	Conversion (%)	Complications (%)
Jeong *et al*[36]	9 TLESS-A	2.9	0	169	178	3.2	11 (1 to MI-A)	11
	17 MI-A	4.3	0	145	205	3.5	5.8 (1 to OS-A)	5.8
Walz *et al*[40]	47 RLESS-A	2.3	2.1	56	< 10	2.4	8.5 (to TLESS-A)	8.5
	47 TLESS-A	2.6	0	40	< 10	3.1	0	6.4
Ishida *et al*[39]	10 TLESS-A	2.8	0	125	12	5.2	0	0
	10 MI-A	4.5	0	120	15	6.9	0	0
Shi *et al*[38]	19 RLESS-A	2.1	0	55	30	6	0	11
	38 MI-A	2.4	0	42	18	6	0	7.9
Kwak *et al*[123]	10 TLESS-A	3.3	0	127	-	4.5	0	10
	12 MI-A	3	8	113	-	4.1	0	-
Vidal *et al*[124]	20 TLESS-A	3	0	95	Min	3.0	0	0
	20 TL-A	3	0	80	Min	2.5	0	0
Wang *et al*[125]	13 TLESS-A	2	7.7	149	79	5.2	0	31
	26 TL-A	2.4	0	113	93	6.3	0	12
Tunca *et al*[126]	22 TLESS-A	3.3	0	64	48	2.45	-	0
	74 TL-A	4.7	4.1	68	38	3	-	0
Lin *et al*[127]	21 RLESS-A	-	0	145	Min	2	0	0
	28 MI-A	-	0	95	50	4	0	3.6

[1]Preoperative indication of ACC or isolated adrenal metastasis. ACC: Adrenocortical carcinoma; MET: Metastasis to adrenal gland; OT: Operative time; EBL: Estimated blood loss; LOS: Length of hospital stay; TLESS-A: Transperitoneal LESS-A; RLESS-A: Retroperitoneal LESS-A.

2.5 cm, spiculated right upper lobe lung mass. A recommendation was made for biopsy of the lung lesion and this revealed moderately differentiated adenocarcinoma. A staging evaluation with PET/CT imaging revealed only a single lesion suspicious for metastatic disease: a 1.2 cm, solid left adrenal mass. Initial recommendation was for percutaneous biopsy by interventional radiology, but based on the small size and location of the lesion adjacent to the aorta, the patient was advised that surgical excision of the lesion was the recommended course. After discussion of risks and benefits with the patient, including the possibility of conversion to multi-port TL-A or OS-A, the patient was scheduled for transperitoneal LESS-A. The patient was discharged home after an uneventful 1 d hospital course. He subsequently underwent thoracotomy and wedge excision of the lung mass and remained without significant disease progression until brain metastasis was detected 2.5 year later.

The first case of LESS-A was reported in 2008[35]. Since then, several studies have compared this emerging technique with MI-A, demonstrating reduced postoperative pain, shorter hospital stay, improved cosmesis, comparable blood loss and complication rate, but with longer operative times[36-40]. A summary of all studies comparing LESS-A to MI-A is included in (Table 4). Rane *et al*[8] published a meta-analysis for LESS-A in 2012. Cumulatively, they found that LESS-A was performed for 59 functioning adenomas (Cushing's syndrome or Conn's Syndrome), 28 pheochromocytomas, and 15 non-functioning masses (adenoma, adrenal metastasis, others). They proposed early and advanced indications for LESS-A based on surgeon experience. Accepted indications for surgeons early in their operative experience include adrenal tumors up to 4 cm in size, functioning or non-functioning, that are localized, and suspected to be benign, in non-obese patients without previous abdominal surgery. Indications for surgeons with advanced LESS experience might include any adrenal neoplasm up to 10 cm in size, with consideration of moderately obese patients and those with limited prior abdominal surgery. Wang *et al*[41] published an updated meta-analysis comparing LESS-A to MI-A in 2013. A summary of this article's findings is included in (Table 4).

Unfortunately, the data on this procedure is limited at present and long-term oncologic outcomes are not yet available. Four of the nine studies included by Wang *et al*[41] had less than 15 patients. Though a learning curve for LESS-A has not been formally demonstrated, extrapolation from other LESS procedures suggests that this is likely within the surgeon's learning curve. Additionally, the patients in these studies have been carefully selected, as demonstrated by the smaller tumor size compared to MI-A. These limitations notwithstanding, LESS-A appears to be an appropriate initial approach when cosmesis is of the utmost importance in the setting of small (< 4-5 cm) adrenal tumors or metastases, that can readily be converted to MI-A with the placement of one or more accessory ports to aid with retraction and exposure. These indications are subject to change as new literature arises, better informing the optimal utilization of this emerging technology.

DISCUSSION

Pathology-benign

MI-A is the surgical approach of choice for almost all benign adrenal tumors. Functional adrenalomas, including aldosteronomas, pheochromocytomas, and cortisol-secreting adenomas, have been demonstrated to be amenable to MI-A[42-49]. For pheochromocytomas,

recurrence rates following MI-A are low (6%-8%) and not significantly different from OS-A in reports that are somewhat limited by short follow-up durations (21-102 mo)[50-54]. Most experts believe that size and tumor biology, rather than surgical approach, are more likely to determine the chance of cure in this disease[55,56]. Based on this literature, many authors and guideline-producing societies have concluded that MI-A is an appropriate initial approach to pheochromocytomas[50-57]. Recurrence of other benign adrenal tumors is extremely rare and is generally limited to tumors that are later discovered to be malignant based on metastasis after initially benign or indeterminate pathology. Advantages of this procedure compared to OS-A are well documented and include lower or equivalent blood loss, improved postoperative pain control, shorter hospitalization, improved perioperative convalescence, and excellent cosmesis[7,58-60]. Results concerning operative time for MI-A compared to OS-A have been mixed, with some series reporting longer times for MI-A[61,62], others reporting comparable durations[63,64], and still others shorter times[59,65]. Operative mortality is very low for adrenalectomy, and has not been shown to be significantly different between the two procedures[60]. At this time, selecting amongst different MI-A approaches should be determined by surgeon familiarity and experience, as there is inadequate evidence to demonstrate superiority of any one MI-A approach for a specific benign adrenal pathology.

Pathology-malignant

Adrenocortical carcinoma (ACC) is a rare disease with an annual incidence of approximately 1 per million population[66]. Overall 5 years survival is approximately 38% to 46%[66,67]. Unfortunately, only 30% of cases are detected prior to extension outside the adrenal gland or metastasis[67]. Cure of ACC is dependent upon complete surgical resection, including regional lymph nodes and involved adjacent organs[68-71]. OS-A has been the gold standard for ACC for decades[3,4]. This status has been challenged in recent years, by reports of favorable perioperative and oncologic outcomes with MI-A for ACC[72-79]. However, the use of MI-A for known ACC remains controversial, in large part based on the poor outcomes reported during initial experiences with MI-A, which included intraoperative tumor fragmentation, port-site and local recurrences, and peritoneal carcinomatosis[80-83]. Two recent studies reported that MI-A was associated with increased frequency of positive margins and intraoperative tumor spillage, shorter time to recurrence, and worse overall survival for stage Ⅱ ACC compared to OA[84,85]. These authors concluded that MI-A is inappropriate in known or suspected ACC. In contrast, numerous recent reports have described improved perioperative and equivalent or even superior oncologic outcomes for MI-A performed with contemporary techniques[74,77-79,86]. These advocates argue that so long as standard oncologic principles are strictly adhered to, comparable outcomes can be achieved for stage Ⅰ and Ⅱ ACC tumors by those with the requisite experience and expertise. Current guidelines remain equivocal, with some suggesting that MI-A is acceptable as an initial surgical approach for stage Ⅰ and Ⅱ ACC < 10 cm and others recommending OS-A for all suspected or known ACC. All guidelines currently recommend conversion to OS-A when extensive tumor adhesion, invasion, or lymphadenopathy is identified intraoperatively[50,87-89].

Pathology-metastasis

Metastasis to the adrenal gland is commonly seen in cancer of the breast, lung, colon, melanoma and lymphoma. Adrenal metastasectomy has been shown to improve survival in patients with limited metastatic burden, with average survival rates of between 20 and 30 mo after surgery compared with 6 to 8 mo without resection. Nevertheless, this heterogeneous population of patients has approximately 25% 5 year survival[3,90]. Despite poor durable oncologic outcomes post-adrenalectomy, there is also a role for this procedure as a palliative measure[91]. For the treatment of isolated adrenal metastatic disease, MI-A was initially controversial, but has since become the standard approach. Published series have demonstrated that this is a safe procedure with very low morbidity and similar long-term outcomes to OS-A[90,92-95]. In the largest and most compelling comparison of MI-A and OS-A for adrenal metastases, Strong *et al*[94] reviewed 94 adrenalectomies (31 MI-A *vs* 63 OS-A) and found no differences in margin status, local recurrence, disease-free survival, or overall survival. They also demonstrated that MI-A provided significantly shorter operative time, lower estimated blood loss, shorter length of hospital stay, and fewer total complications. These authors concluded that MI-A should be recommended as an appropriate initial approach to isolated adrenal metastases. Several additional studies comparing MI-A to OS-A for adrenal metastases reported similar results[91,93,96]. In accordance with these findings, guidelines have recommended that MI-A is appropriate for solitary metastases to the adrenal gland, given that local invasion is not present[50,87].

Prior abdominal surgery

Previous abdominal surgery is a known risk factor for laparoscopic procedures[97]. Some authors have considered this to be a relative contraindication to transperitoneal MI-A and recommend a retroperitoneoscopic approach[14,98-100]. Morris *et al*[11] analyzed 92 patients with and 154 patients without previous abdominal surgeries undergoing TL-A. Operating time, blood loss, and perioperative complications were not significantly different between the two groups. They concluded that TL-A was safe in patients with previous abdominal surgeries, conversion to OS-A occurs infrequently, and is rarely attributed to adhesions, and that surgeons should perform the surgical method they are most comfortable with. The laparoscopic approach can be tailored to accommodate patients with previous transabdominal surgery

with modification of port placement or addition of accessory ports, and should not be contraindicated in these patients. Maintaining a low threshold for conversion to OS-A for failure-to-progress can maintain an equivalent level of safety with MI-A to that obtained with an initial plan for OS-A.

Tumor size

Historically, tumor size > 5 cm was considered to be a relative contraindication to MI-A given the increased risk of treating incidentally-found ACC, along with greater complexity of procedure, longer operative time and increased blood loss[101-104]. In recent years, numerous studies have demonstrated that MI-A is safe and feasible for large (> 5 cm) adrenal masses, offering favorable outcomes, reduced convalescence, and decreased morbidity compared to OS-A[105-112]. Boylu *et al*[111] compared MI-A for adrenal tumors < 8 cm and ≥ 8 cm. They found that operative time and blood loss were significantly higher for tumors ≥ 8 cm, but noted that results were comparable between the two groups concerning transfusion rates, length of hospital stay, and conversion to open surgery. They concluded that MI-A achieved favorable morbidity and surgical outcomes for larger lesions, despite being technically difficult operations. Other studies have demonstrated mixed results regarding the impact larger tumor size has on operative time, blood loss, hospital stay, and conversion to OS-A[110,113-115]. Hemal *et al*[109] recommended TL-A over RL-A for larger masses, given the larger working space it provides, and concluded that size alone should not eliminate MI-A as an option. Assalia and Gagner[116] performed a meta-analysis of 20 case-control studies examining MI-A versus OS-A. They noted that a few studies reported MI-A for lesions up to 14-15 cm, but found that most authors cited 10-12 cm as the maximum acceptable for MI-A. Overall, we feel that MI-A is appropriate for adrenal tumors up to 10-12 cm, in the absence of pre-operative imaging suggesting peri-adrenal infiltration or venous invasion or biopsy-proven ACC.

Body habitus

The body habitus of the patient, including both BMI and abdominal girth, must be accounted for when determining the approach for adrenalectomy. Laparoscopic procedures may be preferable in obese patients. Fazeli-Martin *et al*[10] compared open and laparoscopic approaches for adrenal and renal procedures in obese patients, demonstrating that patients undergoing laparoscopic procedures had decreased blood loss, less narcotic use, shorter hospital stay, and fewer complications, compared to open procedures. It is however, important to note that obesity has been independently associated with increased operative time and postoperative complications compared to non-obese patients for MI-A[117,118]. Two studies have been published comparing different MI-A approaches in this patient subgroup. Epelboym *et al*[23] analyzed 81 RL-A and 130 TL-A procedures in obese patients. They found that operative time (90 min *vs* 130 min; $P < 0.001$) and estimated blood loss (0 mL *vs* 50 mL; $P < 0.001$) were significantly less for RL-A, but failed to demonstrate significant differences for length of stay, overall mortality, incidence and severity of postoperative complications, and rates of readmission. Aksoy *et al*[32] compared 42 retroperitoneal RAL-A and 57 RL-A procedures in obese patients. They found no difference in perioperative outcomes between these two approaches. Given the paucity of data comparing MI-A approaches in obese patients, we cannot provide specific recommendations at this time as to which approach is best. Patient body habitus should be included in patient selection and determination of operative approach, as MI-A may be beneficial in these patients despite the increased procedural difficulty and associated morbidity.

CONCLUSION

While the indications for adrenalectomy have remained reasonably stable over the last two decades, the surgical approaches have become less and less invasive. Despite these technological advances, the least-invasive procedure (LESS-A) is not always the most appropriate. Various approaches to MI-A remain the preferred surgery for many situations, with LESS-A appearing to be a viable alternative for small tumors in relatively uncomplicated scenarios, though further validation is needed for this emerging technique. There remains a clearly defined role for OS-A for large and locally-advanced malignancies. Most importantly, the surgeon should engage each patient in the medical-decision making process for each adrenal tumor encountered.

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REVIEW

Employing extracellular vesicles for non-invasive renal monitoring: A captivating prospect

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Abstract

Extracellular vesicles (EVs) are fascinating nano-sized subjects extensively studied over the recent years across several disparate disciplines. EVs are endlessly secreted into the extracellular microenvironment by most cell types under physiological and pathological conditions. EVs encompass a variety of molecular constituents from their cell of origin, such as lipids, cell specific proteins and RNAs, thus constituting an informative resource for studying molecular events at the cellular level. There are three main classes of EVs classified based on their size, content, biogenesis and biological functions: exosomes, shedding microvesicles and apoptotic bodies. Besides cell culture supernatants, biological fluids have also been shown to contain different types of EVs. Amongst the various body fluids, the study of urinary extracellular vesicles (uEVs) as a source of candidate biomarkers gained much attention, since: (1) urine can be non-invasively collected in large amounts; and (2) the isolated uEVs are stable for a relatively long period of time. Here, we review the important aspects of urinary extracellular vesicles which are fast gaining attention as a promising future tool for the non-invasive monitoring of urinary tract. Recent advancements in the purification and analysis of uEVs and collection of their constituents in rapidly developing public databases, allow their better exploitation in molecular diagnostics. As a result, a growing number of studies have shown that changes in expression profile at the RNA and/or protein levels of uEVs reveal the molecular architectures of underlying key pathophysiological events of different clinically important diseases with kidney involvement.

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Key words: Urine analysis; Extracellular vesicles; Biomarkers; Kidney disease; Quantitative proteomics; Exosomes

Core tip: Urinary extracellular vesicles research is a fast growing field of biomarker discovery providing new attracting prospective for monitoring tissue alteration in easily accessible clinical samples. Over the past ten years intense research has identified the various urinary vesicular cargo molecules (*i.e.*, RNAs, proteins and lipids) and detected their alterations upon a number of renal diseases. With the number of diseases relating to kidney increasing it is essential to effectively utilize this invaluable tool for the early diagnosis. Here we provide a comprehensive overview of uEVs nicely

setting the stage for their utility in future clinical diagnostics.

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EXTRACELLULAR VESICLES: WHY ARE THEY FASCINATING?

Extracellular vesicles (EVs) are membrane-bound secretory vesicles which exhibit an array of proteins, bioactive lipids, nucleic acids and metabolites[1-4]. EVs are considered to play an important role in intercellular communication[5-8], regulating immune response[9-12], antigen presentation[13-15], transport and propagation of infectious cargo such as prions and retroviruses[2,16-18]. Based on the size and origin, EVs are classified into three main types: (1) "exosomes" (40-100 nm) vesicles derived from the endosomal compartment and released *via* fusion of the multivesicular bodies with the plasma membrane; (2) "microvesicles" (50-1000 nm) vesicles that result from the direct budding from the plasma membrane; and (3) "apoptotic bodies" (800-5000 nm) vesicles released by cells undergoing programmed cell death[19,20]. Despite some distinct features, numerous similarities exist among the different classes of EVs with respect to their physical characteristics and biochemical composition, which make the separation of different subsets challenging[21].

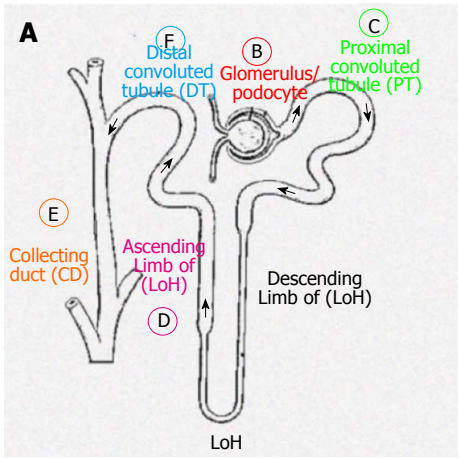
Nano- to micron-sized EVs have been isolated from many body fluids including urine[16,22-25], saliva[26,27], breast milk[9,27], cerebrospinal fluid[28], semen, pleural effusions and plasma[27,29]. Urinary extracellular vesicles (uEVs) are released from the renal epithelial cells, including glomerular podocytes, renal tubule cells, and the cells lining the drainage system[30]. They are promising starting material for biomarker discovery and also a great asset for non-invasive renal monitoring as they provide a full representation of the entire urinary system[3,16]. Recently developed quantitative workflows for uEVs transcriptomics, proteomics and lipidomics[31-34] including system biology approaches[35] enable researchers to study the expression profiles of the main bioactive constituents of uEVs in detail and with an increased rate and reproducibility than before. Research performed in the last 10 years highlighted that uEVs harbor 1%-3% of the total urinary proteins with a reduced dynamic range of protein concentration respect to the whole urine proteome. In this sense, the proteome of uEVs which today counts more than four thousand proteins can be considered as the urinary subproteome enclosed in double-layered vesicles. Despite the potential benefits of uEV analysis, there are barriers and limitations that must be dealt with. Foremost among these challenges is the reproducible isolation of pure vesicles suitable for downstream analysis[36]. In addition to proteins, uEVs encapsulate different RNA species amongst which messenger RNAs (mRNAs) and micro-RNAs (miRNAs) are the subjects of intense studies as possible biomarkers of renal diseases[3,37]. RNAs incorporated in uEVs are resistant to nuclease digestion and, similarly to proteins they originated from the different nephron regions[38]. For example, podocyte related mRNA expression of CD2-associated protein in uEVS was shown to correlate with renal function and level of proteinuria[39]. Moreover, miRNA-29c level in uEVs was shown to be a potential biomarker of renal fibrosis[40]. The relative abundance of different mRNAs do not necessarily correlate with cellular mRNA, suggesting a selective process of mRNA entry into vesicles[1]. As vesicle-enclosed mRNAs travel along the nephron, modification of nucleic acid cargo could permit targeted delivery of RNA to the kidney, a hypothesis which open a new area for the treatment of renal tubular disorders[41].

Here, we will focus on summarizing the current knowledge about uEVs, starting from the critical review of their isolation, biochemical characterization till their potential application in biomarker discovery and non-invasive renal monitoring.

URINARY SYSTEM ORIGIN OF uEVS

Exosomes are cell-derived secretory vesicles shed by proliferating cells through exocytosis[6,13,29]. During endosomal maturation, the formation of intraluminal vesicles (ILVs), ranging from 30-100 nm in diameter, inside the lumen of the endosome can be observed. The ILVs are generated by inward invagination of the endosomal membrane, and scission of vesicles from the limiting membrane into the endosomal lumen. The size of a fully matured late endosome, also called multivesicular body (MVB) is approximately 500 nm in diameter and, it contains several ILVs. The fate of MVBs may vary. They could fuse with lysosome and get degraded or fuse with the plasma membrane releasing the vesicles into the extracellular space. During the later process, the second inward budding of the endosome membrane results in a positive orientation of the ILVs lipid membrane. While the endosomal sorting complexes required for transport (ESCRT) machinery involved in sorting the cargo to lysosomes *via* MVBs within the endocytic system is well characterized (ESCRT-dependent endolysosomal pathway), protein sorting into exosomes until recently was less understood[6]. The involvement of ESCRT protein complexes and protein ubiquitination has been shown by different groups. Recently, Alvavez *et al*[3] revealed that syndecan-syntenin-*ALIX* might be a key regulator of the biogenesis of syntenin expressing exosomes. In particular, the interaction between syndecan and exosomes might thus support a novel role for proteoglycans in vesicular trafficking and cellular signaling. On the other hand, EVs displaying similar physicochemical characteristics like exosomes (density, size-distribution, presence of protein markers) have also been described to bud

A



B

UniProt Acc.Number	Protein name	Ref.
PODO_HUMAN	Podocin	48
PODXL_HUMAN	Podocalyxin	48
NPHN_HUMAN	Nephrin	48
CR1_HUMAN	Complement receptor type 1	48
KIRR2_HUMAN	Kin of IRRE-like protein 1	48
NEST_HUMAN	Nestin	98
VTDB_HUMAN	Vitamin D-binding protein	49
APT_HUMAN	Adenine phosphoribosyltransferase	62
LRRK2_HUMAN	Leucine-rich repeat kinase 2	50

C

UniProt Acc.Number	Protein name	Ref.
TR11B_HUMAN	Osteoprotegerin	49
LRP2_HUMAN	Megalin	44
CUBN_HUMAN	Cubilin	44
AMPN_HUMAN	Aminopeptidase N	44
AQP1_HUMAN	Aquaporin-1	44
CAH4_HUMAN	Type IV carbonic anhydrase	44
GGT1_HUMAN	γ-glutamyltransferase	44
SL9A3_HUMAN	Sodium/hydrogen exchanger 3	45
CD24_HUMAN	Signal transducer CD24	55
ANXA5_HUMAN	Annexin V	58
SCNNA_HUMAN	ENaC (epithelial sodium channel)	45
CHRDL1_HUMAN	Chordin-like protein 1	49
CYR61_HUMAN	Protein CYR61	49
ATF3_HUMAN	Activating transcription factor 3	22
LRG1_HUMAN	Leucine-rich alpha-2-glycoprotein-1	71
PKD1_HUMAN	Polycystin-1	45,46,54,62
MLL3_HUMAN	Isoform 1 of histone-lysine N-methyltransferase	16
GPC5B_HUMAN	G protein coupled receptor required for tubulogenesis	94
MMP9_HUMAN	Matrix metalloproteinase 9	77
CAH9_HUMAN	Carbonic Anhydrase 9	77
NGAL_HUMAN	Neutrophil gelatinase-associated lipocalin	91

D

UniProt Acc.Number	Protein name	Ref.
CD9_HUMAN	CD9 antigen	44
S12A1_HUMAN	Type 2 Na-K-2Cl cotransporter	44

E

UniProt Acc.Number	Protein name	Ref.
AQP2_HUMAN	Aquaporin-2	9
MUC1_HUMAN	Mucin-1	9
RHAG_HUMAN	Rh type C glycoprotein	9
TS101_HUMAN	Tumor susceptibility gene 101 protein	10-12
FLOT1_HUMAN	Flotillin	11,12

F

UniProt Acc.Number	Protein name	Ref.
S12A3_HUMAN	Thiazide-sensitive Na-Cl co-transporter	44

Figure 1 Schematics of Nephron showing the urinary extracellular vesicle proteins identified in the different segments. A: Core; B: Glomerulus/podocyte; C: Proximal convoluted tubule (PT); D: Ascending Limb of (LoH); E: Collecting duct (CD); F: Distal convoluted tubule (DT). UniProt Acc.Number: UniProt Database Accession Number. LoH: Loop of Henle.

from the plasma membrane. For example, podocyte-positive membrane vesicles in urine has been shown to originate from tip vesiculation of podocyte microvilli[42]. These vesicles however cannot be easily distinguished from endosome-derived exosomes and therefore their separate analysis remains a problem.

Exosomes are released from cells of different tissues or organs and while they share a common group of proteins related to their biogenesis they also harbor tissue specific proteins that reflect the origin and biological functions of their parental cells[43]. In fact, EVs isolated from biological fluids have organ and tissue specific protein and RNA profiles. The urinary system consists of the two kidneys, ureters, the bladder, and the urethra. Nephrons are the kidney functional and structural units with the main role to regulate water balance and inorganic ions by filtering the blood. More than a decade ago, the key finding of the first proteomic analyses performed on isolated uEVs was the presence of proteins known to be highly and differentially expressed in various kidney tissues from the glomerular podocyte to the epithelial cells lining the various nephron segments[44,45]. Later on proteins from the transitional epithelium of the urinary bladder were also identified, confirming uEVs may be shed from cells throughout the renal[46]. Targeted proteomics applied recently to perfused isolated rat kidney model to identified kidney originated proteins in human urine[47]. Out of the 990 kidney originated proteins with human analogues 74 were present only in plasma and 240 only in urine (including uEVs) but not in plasma. Screening the 240 kidney originated urinary proteins we have found that the major part (206) is listed in the human urinary dataset of EVpedia 2.0 database. This could be a core set of kidney originated vesicle proteins with high renal pathophysiology relevance for further studies.

Figure 1 shows a schematic diagram of a nephron

indicating sets of uEVs proteins highly expressed in the epithelial cells of glomerular podocyte[48] and to the different segments of renal tubule[44,45,49]. *Podocalyxin* (PODXL) specific for glomerular epithelial cells, aminopeptidase (AMPN) specific for proximal tubule cells, AQP2 specific for distal tubule, programmed cell death 6 interacting protein (PDCD6IP or ALIX) and *uroplakin-1* and *uroplakin-2* (UPK1 and UPK2) specific for bladder are frequently used as protein markers of human uEVs. Basal epithelial cells of collecting duct are hypothesized to be the major contributors toward the urinary pool of EVs including the expression of *leucine-rich repeat kinase-2* (LRRK2)[50]. More recently, Benito-Martin *et al*[49] showed that cultured human proximal tubular cell secrete exosomes containing osteoprotegerin (OPG) and this specific sub-group of exosomes can also be found in urine. Since, most of these have kidney relevant function, proteomic analysis of uEVs hold the promise to provide an insight into the physiological or pathophysiological processes in the various cell types facing the urinary space[44].

VESICLE ISOLATION: AN OBSTACLE TO OVERCOME

Isolation of EVs is still a major challenge in this rapidly growing field of research. The complex nature of body fluids and lack of standardized protocols makes isolation and characterization extremely difficult[34]. The research on EVs can be broadly divided into three categories depending on the end use of the vesicles: (1) discovery; (2) diagnostic; and (3) preparative, each of which demands a different level of purity, quality control and operating procedures. In most studies, ultracentrifugation is the commonly used technique for isolation of vesicles. Isolation of membrane vesicles by sequential differential centrifugations is complicated by the possibility of overlapping size distributions with microvesicles or macromolecular complexes. Recently, numerous alternate procedures were introduced, including immunoaffinity separation, filtration, microfluidic devices aided separation, and reagent based separation[20]. The choice of isolation procedure greatly depends on the source material and the goal of the EV research project.

Isolation of extracellular vesicles from urine has proven to be extremely difficult because of the presence of Tamm-Horsfall protein (THP), also known as uromodulin[36], and also due to very low vesicle load in this biofluid. Differential centrifugation is the most widely used technique for the isolation of uEVs. It includes several low-velocity centrifugation and ultracentrifugation steps with increasing centrifugal force; from 200 to 1500 g to remove cells and cellular debris, from 10000 to 20000 g to pellet microvesicles, and from 100000 to 200000 g to pellet nanometer-sized vesicles[50]. The efficiency to isolate EV depends not only on the size, shape and density of vesicles but on the volume, viscosity and temperature of the fluid in which the vesicles are present. Centrifugation time and the type of rotor used (fixed angle or swinging bucket) also influence the final yield and the purity of vesicles. Addition of dithiothreitol (DTT)[23,36,49,51] and 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS)[52] to the low-speed pellet has been shown to be efficient to disrupt the polymeric network formed by THP protein filaments, but do not solve the problem entirely[33,53]. Urinary EVs can be separated from non-membranous particles, such as protein aggregates by using their relatively low buoyant density, and differences in floatation velocity to separate differently sized classes of EV. Based on this, the crude uEVs containing pellet obtained by differential centrifugation can be further separated/purified using a sucrose density gradient[54,55] or sucrose cushion ultracentrifugation step[33,56,57]. It has been shown that both methods are efficient not only to separate exosomes from microvesicles but also to eliminate the interfering THP impurity[33,54-56]. The high sucrose concentration used in sucrose gradient centrifugation, however may negatively affect the biological function of EV. This can be avoided by layering the samples on top of the sucrose gradient or cushion in the tube subjected for centrifugation. Filtration through molecular filters (0.22-μm or 0.1-μm filters), which remove solvent and small molecule analytes while retaining and concentrating vesicles smaller than the pore size can also be used alone or in combination with centrifugation or ultrafiltration (MWCO 100 kDa) to isolate uEVs[25,58-60]. Among the primary advantages of the filtration method are the simplicity and easy scale-up. Though co-purification of abundant soluble urinary proteins and THP often compromises sample purity and the applied increasing forces with decreasing pore size has also been reported to result in artifacts[50]. The presence of characteristic surface proteins (CD9, CD63, CD81, Rab-5b, TSG101, Alix and A33) on certain EV classes is the basis for immunoaffinity isolation[57,61]. Immunoaffinity-based techniques employ antibodies thus have the potential for high specificity, an important consideration in the characterization of specific EV populations[57]. Because of the increasing interest in exosomes and other extracellular vesicles and their potential use in therapeutics or as biomarkers for disease, kits that allow for "easy isolation procedures" are being developed[16,62]. Commercial tests based on centrifugation (Total Exosome Isolation Reagent, Invitrogen™), filtration (Exomir ™ and Exo-Spin™), affinity capturing (Exotest™ and Exosome Dynabeads®) and proprietary exosome precipitation technologies (miRCURY™, ExoQuick-TC™ and DiagExo®) are already available in the market (Table 1). Such approaches for rapid purification are welcomed but they should be only considered as for bulk isolations because they often fail to pass quality test and fail to distinguish between differently sized EV and membrane-free macromolecular aggregates[50]. A widely accepted unique standardized protocol for the reproducible isolation and purification of uEVs suitable for the downstream analysis of the various RNA and protein constituents is still awaited.

Table 1 Methods used for the isolation of urinary extracellular vesicles

Technique	Type	Advantage(s)	Disadvantage (s)	Purity	Ref.
Centrifugation	Differential	Easy to perform	Co-purification of protein aggregates (THP) Inability to separate exosomes from microvesicles Lengthy Impractical for large-scale studies	++	[37,44,50,52,69,92]
	Sucrose gradient	No mechanical stress and hence allows the collection of morphologically intact particles Removes THP	High sucrose concentration may affect the biological functions of exosomes Inability to separate exosomes from particles with similar density and size Difficult and lengthy Impractical for large-scale studies	+++++	[23,50,54,55]
	Sucrose cushion	No mechanical stress and hence allows the collection of morphologically intact particles	Inability to separate exosomes from particles with similar density and size Difficult and lengthy Impractical for large-scale studies	+++++	[33,56,57,78,80]
Filtration	Nanofiltration	Easy to perform	Low purity grade Co-purification of protein aggregates (THP) Low exosome yield due to their lost on the surface of the nano-membrane	+++	[94]
	Microfiltration	Easy to perform Rapid Maintain vesicle structure	Co-purification of protein aggregates (THP) Inability to separate exosomes from microvesicles	+++	[59]
Immunoaffinity separation	Immunobeads	Allow rapid semi quantitative characterization of the surface phenotype can be tissue-specific	Not suited for large sample volumes Captured extracellular vesicles may not retain biological functionality even if successfully eluted from bead surface Co-purification of protein aggregates (THP) Low yield	++	[57,61,62]
Commercial kits	Total Exosome Isolation Reagent-Invitrogen™ Exomir™ Exo-Spin™ Exotest™ Exosome Dynabeads® miRCURY™ ExoQuick-TC™ and DiagExo®	Rapid and requires low sample volume Practical	Impractical for large-scale studies Reproducibility, yield and sample quality should be checked case by case Co-purification of protein aggregates (THP)	++	

THP: Tamm-Horsfall protein.

PHYSICOCHEMICAL AND ANALYTICAL CHARACTERIZATION OF URINARY EXTRACELLULAR VESICLES

Isolated uEVs are a heterogeneous vesicle population. Exosomes have a lipid bilayer membrane and a characteristic buoyant density ranging from 1.10 to 1.19 g/mL[55]. Determination of physicochemical properties, like integrity, morphology, size and concentration is generally the first step in the characterization of uEVs. Transmission electron microscopy (TEM)[16,23,33,52,58,59,63-65], dynamic light scattering (DLS)[66], nanoparticle tracking analysis (NTA)[67,68] and flow cytometry (FC)[69] are the methods most frequently used in this process. TEM imaging of numerous uEV samples has revealed intact vesicles with round morphology[16,23,33,52,58,59,63-65]. Depending on the isolation/purification method used, uEV samples typically show a heterogeneity in size ranging from approximately 30-100 nm in diameter (Figure 2A). Besides the determination of particle concentration and size distribution, the main advantage of using transmission electron microscopy (TEM) for uEV analysis is its ability to reveal the presence of known urinary contamination, like the long polymers of THP[36,52]. TEM[16,23,33,52,58,59,63-65] and cryo-EM[68,70] based morphological characterization, including observation of vesicle heterogeneity, therefore is a great support for the quality assessment of sample preparation[64]. Orthogonal techniques, like Dynamic Light Scattering (DLS) and nanoparticle Tracking Analysis (NTA) on the other hand, measures the Brownian motion of the particles in solution and calculate the hydrodynamic radii of the particles. Owning to its rapidity and simplicity, NTA is a quickly expanding in the field of exosome research. The mean diameter of human uEVs isolated by ultracentrifugation was measured by NTA

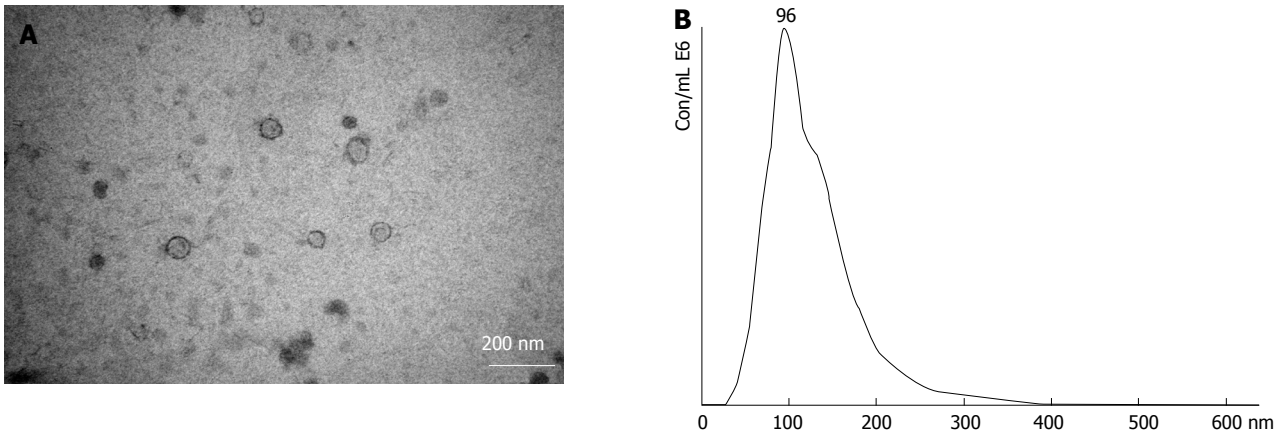


Figure 2 (A) Transmission electron microscopy and (B) nanoparticle tracking analysis images of urinary extracellular vesicles isolated by the sucrose cushion ultracentrifugation method.

around 150 nm ± 50 nm and concentration 10E9-10E11 particles/mL (Figure 2B)[68]. It should be noted that the hydrodynamic size of nanoparticles is expected to be higher to the core size measured with TEM, however the relationship between the two still need to be determined. Subpopulation of EVs can be studied by immunoelectron microscopy using antibodies to target membrane proteins, like aquaporin-2 (AQP2), aminopeptidase-N (AMPN), podocalixin (PODXL) and CD9[37,42,65,71]. Oosthuyzen *et al*[66] most recently demonstrated that NTA can also monitor specific subgroups of uEVs particles using fluorescent antibodies against specific surface proteins present on uEVs.

Analytical characterization of the components of urinary nano-vesicles (*i.e.,* proteins, mRNAs, mi-RNAs, lipids and small molecules) can be performed by transcriptomic[31,37,40,46,63,72,73], proteomic[16,19,23,25,31,33,35,44,47,48,50,52,59,60,62,63,65,66,71,74-79], metabolomic and lipidomic[2,31,46,68,75,80] tools[34]. One of the most exciting findings is that RNA transcripts exist in exosomes and maintain their function when transferred to other cells. Vesicle mediated route for cell-cell communication within the urinary tract opens new perspectives, yet its role in kidney development, function and pathogenesis needs to be elucidated[46]. Typically, commercially available extraction kits are used for total RNA isolation. Abundance of most RNAs is low in the in uEV which makes isolation and downstream RNA analysis challenging[37]. Most of the studies so far have used microarrays[72,81] or real time quantitative PCR (qPCR) assays[39,48,72] to examine exosomal RNAs, with a focus on miRNAs. Because of the inherent limitations of these methods, unknown miRNAs or other RNA species are often overlooked. To characterize RNA profiles systemically, Cheng *et al*[73] performed next-generation deep sequencing providing the base to identify miRNA biomarkers in urinary exosomes.

Since the first proteomic profiling reported by Pisitkun *et al*[44], protein content of urinary EVs has been extensively studied[16,19,23,25,31,33,35,41,45,47-50,52-54,59,60,62,63,65,66,70,71,74-80] both under healthy and numerous disease conditions. These studies let to comprehensive datasets of the identified proteins collected in the different exosome related public databases. It should be noted, that for the generation of many of the large datasets, uEV samples isolated by differential centrifugation (crude exosome preparation) was used[23,45]. As a consequence, public databases contain a relatively high percentage of urinary and other impurities related proteins. The protein content of urinary exosomes is about 1%-3% of that of total urine. The proteomic workflow generally starts with protein concentration assay (Bradford or BCA) followed by protein profile analysis which is usually performed by visualization of proteins separated by SDS-PAGE. The band at about 90 kDa corresponds to the highly glycosylated THP monomer[36] and its relative abundance is used to assess sample quality. Western blots are performed by using a set of urinary exosomal markers, such as Alix, CD9, TSG101, PODXL, AQP2, NES and Annexin V. Chemiluminescent western blotting gives valuable information for the quality of the preparation, but its use as a quantitative method for measuring the relative expression of the target proteins in the absence of appropriate normalization method is still debatable. To measure differences in protein expression between samples, quantitative proteomics is the method of choice. Both label-free quantitation[16,25,76] and stable isotope labeling by iTRAQ[33,78] and TMT reagents[50] in combination with SCX/C18 Multidimensional Protein Identification Technology (MudPIT) have been shown to be useful in the comparative analysis of uEVs. After appropriate enrichment steps, post translational modifications (PTMs), phosphorylation[45,50,79] and glycosylation[64,69] have also been studied by mass spectrometry-based proteomics in urinary exosomes. Since PTMs may affect important physiological process and its alterations may directly reflect early pathogenic events, further studies of PTMs alteration in uEV in various disease states are expected to come.

The bioactivity of exosomes is associated not only to their protein and RNA contents but also to their lipids. Compared to their cells, exosomes have been shown to be enriched in *Cholesterol* and *Sphingomyelin*. Total

exosomal lipids can be extracted by organic solvents according to Bligh and Dyer[82] or, alternatively using THF: H_2O (4:1)[2]. Different lipid classes (phospholipids and glycosphingolipids) can be purified and analyzed by thin layer chromatography (TLC), gas chromatography-mass spectrometry GC-MS and liquid chromatography-mass spectrometry (LC-MS). Recent progresses in electrospray ionization-tandem mass spectrometry-(ESI-MS/MS) based high-throughput lipidomics allowed the first comparative lipid analysis uEVs isolated from healthy individuals and renal cell carcinoma patient[2].

DATABASES FOR URINARY EXTRACELLULAR VESICLES RESEARCH

Recent studies have ignited significant interest on uEVs as possible players in kidney physiology and also as potential reservoirs of biomarkers. With such a huge interest, the amount of data accumulated has increased over time. High throughput proteomics study routinely identifies more than thousand proteins in human uEVs[23,45,50] and necessitates systematic classification of the data acquired[83]. Currently, there are two integrated manually curated web-based databases publicly available: Vesiclepedia[84] and EVpedia[85]. Both dedicate separate sections for the constituents of human urinary vesicles.

Vesiclepedia 2.1 (http://www.microvesicles.org) catalogs information from published non-mammalian eukaryotic and mammalian extracellular vesicles. Based on the 15 studies published on human uEVs, the current version contains 1162 unique proteins, 20 unique mRNAs and 690 unique miRNAs. To aid biomedical scientists in assessing the quality of the preparation Vesiclepedia also contains information on the methods used for the purification as well on the biophysical and molecular characterization of EVs.

EVpedia 2.0 (http://evpedia.info) provides a comprehensive lists of proteins, mRNAs, miRNAs, and lipids identified in EVs of both eukaryotic and prokaryotic (bacteria and archaea) origin, including non-mammalian eukaryotic extracellular vesicles[31,85]. Currently, out of the 263 studies included in this database 16 deal with urinary EVs. EVpedia 2.0 is cataloging a total of 12869 proteins isolated from human uEVs out of which 6275 are unique. 10 studies are related to the study of uEVs isolated from healthy donors and the number of unique proteins in this set is 4536. The most frequently identified 100 proteins are also listed in the database. Sets of proteins identified in different disease related samples (312 from early IgA nephropathy, 552 from autosomal recessive polycystic kidney disease, 621 from basement membrane nephropathy, 689 from bladder cancer, 480 from hernia, 184 from renal cell carcinoma) are particularly valuable components of this database. In the present 2.0 release of EVpedia there are no mRNAs, miRNAs and metabolites in the human urinary EVs subset reported. Regarding lipids, 28 different lipids were identified in human uEVs from healthy donors and 26 lipids were found in patients with renal cell carcinoma. Moreover, EVpedia offers a range of analytical tools: (1) search for and browse vesicular components; (2) Gene Ontology enrichment analysis of vesicular components; (3) network analysis of vesicular components; and (4) set analysis: a comparison of vesicular datasets by ortholog identification. Detailed methods for the isolation of extracellular vesicles and publications on extracellular vesicles are also listed in this database. An overall comparison of EVpedia with Vesiclepedia is beyond the scope of this paper and has recently been published[31,85]. Regarding urinary EVs, the current release of EVpedia contains more data on proteins and lipids while miRNAs and mRNAs are more represented in Vesiclepedia. There is a concern that a relatively high percentage of the entries in the single datasets included in the databases are not native constituents of uEVs but matrix impurities related to isolation/purification[83,86]. Complete workflow solution, starting from fast and efficient purification of EVs from urine without contamination by non-vesicular components (including abundant urinary proteins, protein aggregates), is a critical prerequisite for future high-throughput analyses. Important prerequisites for the efficient use of the databases are: (1) the definition of a reliable core set of uEVs' constituents; and (2) handling data redundancy of current databases, are still awaited.

EMPLOYING URINARY EXTRACELLULAR VESICLES IN BIOMARKER DISCOVERY

Biomarkers are defined as substances or characteristics that are objectively measured and evaluated as an indicator of normal or pathogenic processes, or pharmacologic responses to a therapeutic intervention. Amongst the different body fluid, urine is one of the most important sources of biomarkers for both urologic and non-urologic diseases. High throughput omics studies have resulted in a great number of potential urinary biomarkers which are publicly available[87]. While the characterization of the normal urinary proteome is steadily progressing, there are three major obstacles in the classical urine-based protein biomarker discovery: (1) sample instability; (2) high dynamic protein concentration range; and (3) the relatively high inter-individual and inter-gender variations of urinary proteome. Urinary EVs, on the other hand, possess some characteristics that make them particularly attractive for biomarker research, like increased stability, reduced complexity, lower dynamic range, and composition which is closely related to the cell of origin. Biomolecules enclosed in the extracellular vesicle are surrounded by a lipid bilayer which protects them against degradation by proteases and nucleases. Therefore, uEVs have been shown to be particularly stable over time[37,88]. In addition, extracellular vesicles exhibit a reduced complexity comparing that to the whole urine composition. The dynamic concentration range of proteins and RNAs was shown to be lower in uEVs when compared to whole urine[33,78]. Moreover, the content of EVs reflects

Table 2 Putative disease-associated protein and RNA biomarkers identified in urinary extracellular vesicles

Name of the putative marker	UniProt ID/RNA name	Molecular and biological function	Associated Kidney disease(s)	Disease/model	Ref.
Adenine phosphoribosyltransferase	APT_HUMAN	Enzyme involved in the purine nucleotide salvage pathway. Its deficiency can lead to urolithiasis and renal failure	APRT deficiency	Healthy	[62]
Low-density lipoprotein receptor-related protein 2 mRNA	LRP2_HUMAN (megalin), LRP2	Multi-ligand binding receptor; mediates endocytic uptake of complexes between the steroid 25(OH) vitamin D3 and vitamin D-binding protein in kidney proximal tubules	Donnai-Barrow syndrome; Renal aminoglycoside accumulation; Nephrotoxicity	Healthy	[38,78]
Polycystin-1, Polycystin-2	PKD1_HUMAN PKD2_HUMAN	Integral membrane glycoproteins, associated with structural and/or functional defects in the primary apical cilium of epithelia and polycystic kidney disease	ADPKD	Healthy	[45,46,54,62]
Solute carrier family 12 member 2 (Sodium potassium chloride cotransporter-2)	S12A2_HUMAN	Membrane transporter: aids in the active transport of sodium, potassium, and chloride into and out of cells; NKCC2 mutations lead to type I Bartter syndrome	Antenatal Bartter syndrome type 1	Healthy	[33,41,62]
Myosin-9	MYH9_HUMAN	MYH9 polymorphisms have been shown to associate with glomerulosclerosis and non-diabetic end stage renal disease	Fechtner syndrome and Epstein syndrome	Healthy	[62]
Aquaporin-2 mRNA	AQP2_HUMAN AQP2	Molecular water channel in the basolateral and apical plasma membranes of the collecting duct	Autosomal dominant and autosomal recessive nephrogenic diabetes insipidus	Healthy; Various kidney diseases	[24,33,38,39,44,54,58,62,95]
Neprilysin (CD10)	NEP_HUMAN	Surface metallo-endopeptidase highly expressed in the kidney brush-border membranes	Membranous glomerulonephritis	Healthy; Diabetic nephropathy, Renal cell carcinoma	[25,33,77,95]
Osteoprotegerin	TR11B_HUMAN	Decoy receptor of proximal tubular cells	chronic kidney disease-mineral and bone disorder	Healthy; ADPKD CKDD patients	[49]
Podocalyxin	PODXL_HUMAN	he major sialoprotein of kidney glomerulus; Involved in the regulation of cell adhesion, cell morphology and cancer progression	Autosomal recessive steroid–resistant nephrotic syndrome	Healthy; Glomerular disease; Renal cell carcinoma	[42,58,62,77,95]
Solute carrier family 12 member 3 (Thiazide-sensitive Na-Cl cotransporter; NCC)	S12A3_HUMAN	Membrane transporter highly expressed in the kidney: aids reabsorbing sodium and chloride ions from the tubular fluid into the cells of the distal convoluted tubule of the nephron	Gitelman syndrome; Aldosteronism	Healthy; High blood pressure; Aldosteronism	[41,62,79]
Protein AMBP (alpha-1-microglobulin; bikunin precursor)	AMBP_HUMAN	Membrane glycoprotein with serine protease inhibitor activity	Diabetic nephropathy	Healthy; DN patients in advanced disease stages	[16,45]
Leucine-rich alpha-2-glycoprotein-1	LRG1_HUMAN	Involved in protein-protein interaction, signal transduction, and cell adhesion and development; Expressed during granulocyte differentiation	NSCLC	Healthy; NSCLC	[71]
Matrix metalloproteinase 9	MMP9_HUMAN	Involved in extracellular matrix remodeling	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Basigin , Extracellular Matrix Metalloproteinase Inducer	BASI_HUMAN	Involved in extracellular matrix remodeling	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Ceruloplasmin	CERU_HUMAN	A ferroxidase enzyme in serum	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Dickkopf related protein 4	DKK4_HUMAN	Involved in extracellular matrix remodeling	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Carbonic Anhydrase 9	CAH9_HUMAN	A metalloenzyme that catalyzes the reversible hydration of carbon dioxide	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Dipeptidase 1	DPEP1_HUMAN	A kidney membrane enzyme that hydrolyzes a variety of dipeptides and is implicated in renal metabolism of glutathione and its conjugates	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]
Syntenin-1	SDCB1_HUMAN	Scaffold protein Pbp1 Syndecan-binding protein	Renal cell carcinoma	Healthy; Renal cell carcinoma	[77]

Leucine-rich repeatkinase2	LRRK2	A member of the leucine-rich repeat kinase family; Associated to risks to inflammation-linked diseases that include Crohn's disease and mycobacterium infection	Parkinson's disease	Healthy; Parkinson's disease	[50]
Activating transcription factor 3	ATF3_HUMAN miRNA494	Transcription factor with protective role in renal ischemia-reperfusion injury	AKI	AKI	[22]
Fetuin-A	FETUA_HUMAN	Plasma binding glycoprotein; inhibitor of calcification, regulator of cell-dependent process of osteogenesis	AKI	AKI	[46,96]
Wilm's tumor protein	WT1_HUMAN	Zinc finger protein with essential role in the normal development of the urogenital system	Early podocyte injury in diabetic nephropathy; steroid responsiveness or renal pathological conditions in patients with idiopathic nephrotic syndrome	FSGS patients; Diabetic Patients with or without Proteinuria; idiopathic nephrotic syndrome	[22,30,92]
Isoform 1 of histone-lysine N-methyltransferase	MLL3_HUMAN	Histonemethyltransferase which methylates "Lys-4" of histone H3, a specific tag for epigenetic transcriptional activation	Diabetic nephropathy	DN patients in advanced disease stages	[16]
Voltage-dependent anion-selective channel protein 1	VDAC1_HUMAN	Porin ion channel of the outer mitochondrial membrane	Diabetic nephropathy	DN patients in advanced disease stages	[16]
GPRC5B	GPC5B_HUMAN	G protein coupled receptor required for tubulogenesis	Acute Kidney Disease	AKD	[94]
Aquaporin-1 mRNA	AQP1_HUMAN AQP1	Molecular water channel in the basolateral and apical plasma membranes of the proximal tubules.	Renal ischaemia reperfusion injury	Renal transplantation; Renal cell carcinoma	[38,77,97]
Neutrophil gelatinase-associated lipocalin	NGAL_HUMAN	Iron-trafficking protein involved in multiple processes such as apoptosis, innate immunity and renal development. In the kidney it is produced by distal nephron	kidney transplantation	kidney allograft recipients	[91]
Immunoglobulin light chain	IGLL1_HUMAN IGKC_HUMAN	Immunoglobulin light chain is the small polypeptide subunit of an antibody	Light Chain Amyloidosis	Light chain amyloidosis; multiple myeloma; monoclonal gammopathy; non-paraproteinemia related kidney disease	[65]
Panel of 24 proteins _HUMAN	SERPINA1, A2M, APOA1, APOA2, CA1, CD5L, DPEP1, FGA, FGB, FGG, FN1, GSTT1, SLC2A1, HBA1, HBB, SERPIND1, HP, HPR, ITIH1, PON1, RAB27B, S100A8, S100A9, TACSTD2	-	Bladder cancer	Bladder cancer; Hernia	[77]
PSA	KLK3_HUMAN	Serine protease with diverse physiological functions; implicated in carcinogenesis	Prostate cancer	Prostate cancer	[56]
Glutamate carboxypeptidase 2 (Prostate-specific membrane antigen-PSMA)	FOLH1_HUMAN	Folate hydrolase and N-acetylated-alpha-linked-acidic dipeptidase activity; Involved in prostate tumor progression	Prostate cancer	Prostate cancer	[56]
microRNA	MicroRNA-29c	-	Renal fibrosis	Healthy; CKD	[40]
microRNA	miR-130A	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]

microRNA	miR-145	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]
microRNA	miR-155	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]
microRNA	miR-424	-	Type 1 diabetes	type 1 diabetic patients with and without incipient diabetic nephropathy	[81]
mRNA (CD2-associated protein)	CD2AP	-	Podocyte injury	Healthy; diabetic nephropathy; focal segmental glomerulosclerosis; IgA nephropathy;membranous nephropathy	[39]
mRNA (Prostate cancer antigen 3)	PCA-3	-	Prostate cancer	Healthy; Prostate cancer	[32]
mRNA (TMPRSS2/ERGa fusion transcript)	TMPRSS2:ERG	-	Prostate cancer	Healthy; Prostate cancer	[32]

CKD: Chronic kidney disease; AKI: Acute kidney injury; PSA: Prostate specific antigen; NSCLC: Non-small cell lung cancer; APRT: Adenine phosphoribosyltransferase deficiency; CD2AP: CD2-associated protein; PCA-3: Prostate cancer antigen 3; ADPKD: Autosomal dominant polycystic kidney disease.

the cell of urinary tract they shed from. Therefore, it was expected that biomolecules of uEVs could provide clinically more specific information for both early diagnosis of disease and also for monitoring drug responsiveness than that of urine[89]. Since the first publication of uEVs[44], the majority of the works in this field is focused on exploring the potential use of uEVs in preclinical and clinical studies[2,6,24,25,33,35,37,39-41,71,74-78,81,89-92]. Since plasma derived EVs are too large to pass through the glomerular filtration barrier, urinary tract originated vesicles (Table 2)[41,44] carry cell specific markers from the specific regions of the kidney with relevant physiological and pathophysiological information[41].

Proteomic analysis has revealed proteins and RNAs that have been isolated from healthy individuals but are associated with different human diseases (Table 2): Adenine phosphoribosyltransferase in APRT deficiency[62]); Low-density lipoprotein receptor-related protein 2 in Donnai-Barrow syndrome, renal aminoglycoside accumulation and Nephrotoxicity; Polycystin-1 and Polycystin-2 in ADPKD[54]; Neprilysin in Membranous glomerulonephritis[41]; Non-muscle myosin heavy chain IIA in Fechtner syndrome and Epstein syndrome[62]; Sodium potassium chloride cotransporter-2 and Thiazide-sensitive Na-Cl cotransporter in Antenatal Bartter syndrome type 1 and Gitelman syndrome, respectively[41,62]. The presence of theses disease associated analytes in uEVs is promising but their expression levels in well-defined disease cohort and conditions still need to be determined.

In disease cohorts including bladder[57,74,93], prostate[32,56,75] and renal cell carcinoma[2,77] altered expressions of vesicle derived proteins, RNAs and lipids have been demonstrated (Table 2) and panels of putative protein biomarkers have been set up for validation. Vesicle expressed aquaporin water channel proteins (AQP1 and AQP2) have been found to be differentially expressed in a number of different renal diseases too. Other putative biomarkers related to urinary system diseases, like activating transcription factor 3 and fetuin-A in acute kidney injury[22,46]; podocalyxin in autosomal recessive steroid-resistant nephrotic syndrome[62]; and Wilm's tumor-1 in early podocyte injury[22,92] have also been described. There are only a few studies though which report putative biomarkers related to treatment, like the effect of low sodium diet and infusion of aldosterone on the phosphorylation of Thiazide-sensitive Na-Cl cotransporter and prostatin in uEVS[79], or renal transplantation[91].

The reservoir for biomarker discovery could however extend beyond diseases of the urinary tract[41]. Conde-Li *et al*[70] investigated a rat model of liver injury induced by galactosamine and reported a change in urinary exosomes that coincided with liver injury. Gildea *et al*[71] reported that leucine-rich a-2-glycoprotein (LRG-1) was increased in both human urinary exosomes from patients with lung cancer and the lung cancer tissue. High-level of LRRK2 was found in uEVs of patients affected by Parkinson's disease[50]. These observations open a novel

scenario towards the future application of uEVs also in non-renal diseases.

CONCLUSION

Chronic kidney disease (CKD) is increasingly recognized as worldwide public health problem. CKD increases the risk for many adverse health outcomes, including cardiovascular disease, end-stage renal disease, and mortality. Because CKD usually progresses asymptomatically until its advanced stages, detection of early-stage CKD requires laboratory testing. The two key markers used for the definition, classification, and monitoring of kidney function are urine albumin and estimated glomerular filtration rate (eGFR). Kidney dysfunction is indicated by eGFR of less than 60 mL/min per 1.73 m^2, while kidney damage most frequently is manifested as increased urinary albumin excretion. When less invasive blood and urine tests are insufficient fine-needle aspiration (FNA) or renal mass biopsy (RMB) is performed especially to substantiate the diagnosis in renal masses. While these traditional markers are certainly of great utility they also present several limitations. Protein (albumin) concentration in urine is not very specific. Levels may rise with use of certain non-steroidal anti-inflammatory drugs, rheumatoid arthritis, lupus and cancers etc. Creatinine level used to estimate GFR, on the other hand, provides little information about the underlying cause of kidney injuries, and lacks specificity in case of low muscle mass and unusual diets.

Because of the above limitations of currently used kidney function markers intensive research is going on to find more accurate ones. Many genes have been shown to be differentially expressed in the kidney upon glomerular injury, endothelial dysfunction, inflammation, fibrosis, cardiovascular dysfunction, metabolic disorders and cancer with the corresponding protein products appearing in plasma and urine. In urine, a unique biomarker source with great potential, a number of new candidate protein biomarkers have already been proposed: cystatin, N-acetyl-β-o-glucosaminidase (NAG) and liver-type fatty acid-binding protein (L-FABP), neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1), just to mention a few, are potential biomarkers awaiting administration (FDA) approval. NGAL is suggested to be used as a urinary biomarker of delayed graft function (DGF), a frequent complication after kidney transplantation too. Recently Alvarez *et al*[90] have demonstrated that NGAL is mainly secreted into urinary vesicles and that the expression level of NGAL in uEVs is a sensitive measure of DGF, findings which might support the clinical management of patients undergoing kidney transplantation.

To become a clinically approved, a putative biomarker should be validated and implemented into clinical tests. Translating a novel discovery into clinical practice is however extremely challenging, consequently there are only a few urine-based protein biomarker assays which have been developed and approved so far (*i.e.*, BTA and NMP-22 for the diagnosis of bladder cancer and PCA3 for the diagnosis of prostate cancer). Rapidly expanding urinary extracellular vesicle research represents an interesting field relevant to the development of disease specific, non-invasive methods for clinical diagnostics as well as to the development of new therapeutic approaches. Especially, growing evidence suggest that EV-imprinted genetic and proteomic information may well reflect the state of their parental cells. In this sense, expression analysis of proteins and RNAs in circulating urinary vesicles could provide specific information about the change in the state of specific nephron segment(s) and/or of the epithelial cells of urogenital tract. Urinary EV-mediated cell-cell communication within the nephron is another interesting aspects which can have major impact in our current understanding in renal physiology. However, how to translate these captivating ideas to a non-invasive renal monitoring system, there is still a lot more to understand.

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REVIEW

Kidney stones over 2 cm in diameter-between guidelines and individual approach

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Abstract

The prevalence of urolithiasis has been observed to increase during last decades. Kidney stones over 2 cm in diameter are the common urologic problem. European and American Associations of Urology has published guidelines on Urolithiasis and presented the most effective tools to treat large stones. On the other hand many experienced endourologic centres choose other modalities from their armamentarium. All treatment methods are characterized by their efficacy and safety which are usually inversely proportional. It is crucial for patients and physicians to find a golden mean. Percutaneous lithotripsy is still considered treatment of choice with more than 95% efficacy. Less invasive retrograde intrarenal surgery is also less effective, but burdened with lower complication rate. Extracorporeal shockwave lithotripsy is feasible in paediatric patients with acceptable stone free rates. Open surgery (pylolithotomy and anatrophic nephrolithotomy) are almost obsolete techniques. All methods have their pros and cons. Physicians should share decisions regarding treatment modalities with patients.

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Key words: Kidney stones; Percutaneous lithotripsy; Shockwave lithotripsy; Retrograde intrarenal surgery; Pylolithotomy; Anatrophic lithotomy

Core tip: There are various modalities for treatment of kidney stones over 2 cm in diameter. Guidelines indicate the most appropriate methods. Percutaneous lithotripsy is considered first line treatment while retrograde intrarenal surgery or shockwave lithotripsy are optional approaches. Apart from guidelines physicians should share decisions regarding optimal treatment with patients.

Bryniarski P, Miernik A, Schoenthaler M, Zyczkowski M, Taborowski P, Paradysz A. Kidney stones over 2 cm in diameter-between guidelines and individual approach. *World J Clin Urol* 2014; 3(2): 81-86 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i2/81.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i2.81

INTRODUCTION

Nephrolithiasis is one of the most common diseases afflicting mankind. It has been reported in various medical writings since antiquity. In United States its prevalence has doubled since the sixties being now between 2% and 7%. Similarly in countries of Western Europe like Germany, Spain and Italy its prevalence has also been rising[1,2]. It has recently been shown that the real prevalence might even be higher reaching 8.4%. Men are afflicted more frequently than women (10.6% *vs* 7.1%). Racial differences are evident. The most commonly afflicted are white males. African-American females develop least likely urinary stones, while other races are in-

between[3].

Urolithiasis manifest itself clinically mostly between 30 and 50 years of patients' age. The risk of recurrent renal colic after the first stone episode is roughly 15% during the first 3 years and grows up to 50% for the next 7 years[4,5]. In patients with more than one stone diagnosed during their first renal colic this ratio might increase to 75%. After every urolithiasis treatment, the patients should be stratified and accordingly assign to low or high risk group of stone recurrence. Urolithiasis promoting factors as patients' age, recurrent stone formers, familial urolithiasis, calcium hydrogenphosphate (brushite), uric acid, cystine, and so called infection stones have to be analysed and appropriately considered for the further management. This group requires thorough metabolic evaluation and a close follow-up. However, only in 20% of the patients a systemic disease predisposing to stone formation can be identified[6].

Over the last centuries a significant shift in stone location has been observed from the lower to the upper urinary tract. A disease considered previously as male ailment is now gender blind. Metabolic diseases such as obesity and diabetes are strongly associated with urolithiasis. It seems that diet and lifestyle play an important role in disease development[7]. Nephrolithiasis might be an effect of other systemic diseases such as: inherited and acquired renal tubular acidosis, primary and secondary hyperparathyroidism, gout, various neoplasms, primary hyperoxaluria, gastrointestinal diseases, sarcoidosis, recurrent/persistent urinary tract infection, metabolic syndrome and cystinuria. Some anatomical abnormalities of the urinary tract are also associated with lithogenesis and comprise: horseshoe kidney, ureteropelvic junction obstruction, medullary sponge kidney, calyceal diverticulum and vesicoureteral reflux[8]. Urinary tract infections play an important role in stone formation and thus need a special clinical attention and management. As it can be seen in the example of staghorn calculi, most of them have an infectious origin and consist of magnesium ammonium phosphate-struvite or carbonate apatite- dahlite or ammonium urate[9,10].

The aim of our study was to present and compare several treatment methods that can be offered for patients with renal stones over 2 cm in diameter.

Medline was search for articles published between 1977 and 2013. The following keywords were entered: "kidney stone", "Percutaneous nephrolithotripsy", "extracorporeal shockwave lithotripsy" and "retrograde intrarenal surgery". Only English written papers were included. Only papers most relevant for the purpose of this review were included.

DO WE HAVE TO TREAT KIDNEY STONES?

Until now this crucial question remains at least partially unanswered. What is the appropriate clinical management for small asymptomatic calyceal stones, that do not grow? For all other renal stones active treatment is recommended. Referring to this, Guidelines on Urolithiasis of the European Association of Urology changed in 2011. Previously, active stone removal had only been recommended for calculi > 6 mm. In accordance to the literature, a rate of spontaneous passage was estimated to be 1% in comparison to smaller stones (*i.e.*, < 3 mm) when almost all stones can be expelled[11]. Currently guidelines state that all stones over 15 mm in diameter should be removed. This recommendation is based on a trial revealing, that there are no differences between active and conservative approach in asymptomatic calyceal kidney stones < 15 mm in diameter in terms of stone free rate, symptoms, quality of life and renal function[12]. Therefore patients who elect observation instead of active treatment of their kidney stones should be informed about the possible course of the disease. In that situation, in three years 77% of asymptomatic patients with kidney calculi will progress and 26% will require active treatment. Moreover, lower pole stones grow more frequently than middle and upper pole stones (61% *vs* 47%). The rate of growth is positively correlated with uric acid concentration in serum and urine[13]. Therefore individual approach for each patient is advised with abovementioned consideration and taking into account other clinical information obtained from patients history (*e.g.*, occupation, *etc.*). For that reason, even small asymptomatic calyceal stones should be actively treated in jet pilots, travelers, *etc.*

There is no such question in terms of larger stones. Staghorn calculi inevitably lead to unresolved/persistent urinary tract infections with loss of renal parenchyma, chronic pylonephritis and eventually loss of kidney function[9,14-17]. Untreated large stones may also cause life-threatening urosepsis which in some circumstances requires intensive care management or even nephrectomy.

METHODS OF TREATMENT

Renal stone treatment has gone through significant changes over last decades from mainly open surgeries to minimally invasive ones. The treatment modality depends mainly on stone size, hardness and position within the kidney. The last Guidelines on Urolithiasis of the European Association of Urology (EAU) recommend endourology as a treatment option for renal calculi over 20 mm in diameter stating simultaneously superiority of percutaneous lithotripsy (PNL)[8]. However, optional approaches in large stones are feasible and comprise retrograde intrarenal surgery (RIRS) with flexible(fRIRS) or semirigid ureterorenoscopes (rRIRS), endoscopic combined intrarenal surgery (ECIRS), shockwave lithotripsy (SWL) and exceptionally open surgery (pylolithotomy and anatrophic nephrolithotomy).

PNL and open surgery

Until the last year, PNL had been the gold standard in the treatment of renal stones over 2 cm in diameter. Still

this technique is being chosen among other methods as first line therapy for large renal calculi. It remains also an alternative for smaller stones formed by cystine, brushite and whewellite which are usually very hard and associated with lower stone free rates when treated with different modalities. It was shown that stone density over 970 Hounsfield units on non-contrast computed tomography are efficiently destructed by SWL in 38% in comparison with softer stones where such ratio reached 96%[18]. Usually 3-5 ineffective sessions with SWL also should prompt physician to offer more invasive methods to the patient.

There are many different PNL techniques. None appears to be more efficient than the others. The procedure can be conducted in prone and supine position. Originally PNL was described in prone position with specially invented metal dilators[19]. This kind of patient positioning offers an unlimited access to the kidney even in terms of a multi-track approach. Subsequently supine position was proposed by Valdivia Uría *et al*[20] to improve direct anesthesiological access to the patient's chest and to minimize the vena cava-syndrome. A further miniaturization of the equipment allowed to perform PNLs in children[21,22]. As standard PNL procedures are performed with 28-Fr or 30-Fr channel mini PNL offers smaller sheaths between 12-Fr and 20-Fr. Unfortunately ultrasonic disintegration is technically unfeasible in these systems. The next step of miniaturization called ultra-mini-PNL (UMP) has been shortly presented[23]. The procedure is carried out using a 3.5-F telescope and special inner and outer sheaths. After puncturing the kidney, tract dilatation up to 13-F is performed. Stones are disintegrated with a 365-μ holmium laser fibre and actively evacuated by creating an eddy current of saline in the instrument shaft. Further miniaturization has allowed to disintegrate stones with the so called "all-seeing needle" (4.8-Fr). Using this, micro PNL device, renal stones can be disintegrated but neither actively extracted nor washed out. Concerning the size of the instrument, it can be excellently used in paediatric urology[24,25].

To make PNL more convenient for patients tubeless (without nephrostomy) and totally tubeless (without nephrostomy and ureteral catheter) variations of procedure were proposed. Conventional PNL comprise insertion of nephrostomy tube after completion of surgery. This allows free drainage of clots and remnant stones as well acts as hemostat when closed for a short period after procedure. However, hospitalization and operation times are significantly longer in comparison with tubeless procedures[26-29].

Over the last three decades PNL has supplanted pylolithotomy and anatrophic nephrolithotomy in treatment of larger stones mostly due to its significantly decreased invasiveness with only marginally worse efficacy. The last comparison between open stone surgery and percutaneous nephrolithotomy was performed in the late 90's and showed that pyelolithotomy or nephrolitotomy was superior in terms of SFRs[30,31]. Despite inferior SFRs, PNL replaced open surgery in treatment of large kidney calculi. The reason for that might have been the acceptance of SFRs in favour of lower complication rate during PNL compared to the open approach. Due to continuous increase of expertise in PNL, both methods seem to have similar efficacy (see Table 1). Another endoscopic alternative in treatment of large renal stones might be a laparoscopic approach. As a minimally invasive procedure, PNL similar SFRs burdened with longer operative times were documented[32]. It is worth emphasizing that PNL is not free from complications. The most common are infections occurring in up to 35% of patients. Significant bleeding at 7.8% and mortality rates at up to 0.5% were estimated[33,34].

Shockwave lithotripsy for large renal stones

In 1984 first SWL machine was introduced for the treatment of kidney stones. Dornier Human Model 1 was a prototype while model number 3 was the first generation lithotripter that was widely used in the clinic[35,36]. First interventions were performed under general anaesthesia. The patient was positioned in a large basin filled with degassed fluid. Until now HM-3 Dornier has had the highest known efficacy throughout all shockwave lithotripters.

The mechanism of stone fragmentation is based on a rule that focused ultrasound waves can cause hard object disintegration through tear forces, spalliation, cavitation and squeezing[35,37-41].

With only 45%-60% stone free rate SWL efficacy in kidney stones over 2 cm in diameter may be consider as disappointing[42,43]. In older studies SFRs up to 70% with low complications rate were reported[44]. Fortunately, significantly higher SFRs reaching 85% can be achieved in paediatric patients[45-47]. In comparison PNL in children shows the same treatment efficacy as in adults. PNL stone free rates in this group ranges from 68% to 100%[48,49].

In conclusion SWL in patients with stones over 20 mm in diameter is inappropriate except paediatric patients, where an individual approach should always be aimed.

RIRS

Continuously mastered lithotripsy through natural body orifices (as ureteroscopy) has nowadays allowed to achieve satisfactory results with low complication rates. Although lower SFRs in comparison to PNL, natural orifice transluminal endoscopic surgery (NOTES) is characterized by low morbidity rates, pain and hospitalization times[50]. RIRS may be done using flexible (fRIRS) and semirigid instruments (rRIRS). Flexible ureterorenoscopy is characterized by a small shaft calibre, usually less than 10-F. In stones over 2 cm prolonged operation times can be observed (mean 82.5 max up to 215 min). Excellent SFRs above 90% are reported in centres with profound experience in urinary stone management and high case load. It was shown that 1.6 procedures per patient are needed to

Table 1 The summary of procedures feasible in treatment of renal stones over 2 cm in diameter

	Stone free rate[2]	Complications rate[3]	Ancillary procedures	Operating room time, min
Open surgery[30,31][1]	71%-84%	46%	-	130
PNL[26-29,32,34]	75%-98%	0%-33%	9%-33%	52
ESWL[42-47]	45%-60%–adults 85%–children	6%	18%	50-70
fRIRS[50,51-56]	90%	8%-10%	3%-13%	82-94
rRIRS[50,54]	Aug-81%	8%-15%	12%-5%	85-98

[1]Data from 1986; [2]Including insignificant small stone fragments; [3]Including minor and major complications. PNL: Percutaneous lithotripsy; ESWL: extracorporeal shockwave lithotripsy; RIRS: Retrograde intrarenal surgery.

achieve superior results in terms of RIRS treatment[51,52]. Complication rates were calculated to occur in 10% of patients while major complications contribute approximately to half of them[53]. Instrument costs for flexible ureterorenoscopy are high and appear to be a limiting factor. Due to a very fragile laser fibres which is frequently bent within the working channel of the instrument, its breakage and a consecutive damage of the scope might occur. On contrary, the latest comparison of costs between PNL and fRIRS revealed a vast economic advantage towards ureteroscopy ($ 19845 *vs* $ 6675) at least in the United States health care system[53].

The main disadvantages of NOTES-based techniques using semirigid ureterorenoscopes in comparison to fRIRS is their inability to disintegrate stones in lower and middle calyces, potentially high renal fluid pressure, limited intraoperative manoeuvrability and occasional inability to pass the scope through a tight ureter. The main advantage is the ability to pass stone extraction devices through wide working channels and high irrigation flow significantly improving visibility. In the last years we observe many efforts to increase the disintegration rate while lowering the morbidity[54,55]. However, even with this improvements reported SFRs are lower in comparison to fRIRS (90% *vs* 81.8%)[50,51,53,54,56]. On the other hand the number of ancillary procedures is inferior for rRIRS (see Table 1)[51]. The costs for rRIRS are lower than for fRIRS strongly depending on scope damages during procedure.

One of the most important questions regarding urolithiasis therapy has still to be answered. "Can we achieve high stone free rates with low morbidity only in experienced institutions specialized in urinary stone treatment or is it also feasible for all centres".

HOW TO ASSESS TREATMENT SUCESS?

The answer seems to be simple at first sight–lack of stones after the procedure. In most cases stone free status is estimated on the basis of ultrasound and X-ray, rarely on computed tomography (CT). It was shown that results documented by CT and ultrasound + X-ray may tremendously differ (62.3% *vs* 20.8%) in the same treatment group[57]. Noncontrast enhanced computer tomography (NCCT) has become a new diagnostic standard for evaluation of acute flank pain. Its sensitivity for identifying urinary stones was estimated by 96%[58]. Sensitivity of ultrasound for identifying renal stones over 5 mm is also 96%[59]. X-ray is used mainly due to its high specificity (80%-87%) in detection of urolithiasis[60]. Taking into consideration the abovementioned facts one may think that X-ray and ultrasound could be equal to CT in identifying significant residual stones (> 4 mm). Indeed this is not true. Park *et al*[57] in their study shown that almost 50% of stones over 4 mm in diameter are visible on NCCT and are not visualized on X-ray (mean size 7.4 mm). These facts strongly support the need for performing NCCT to assess residual stones after lithotripsy. It is also very difficult to compare the results of studies where other than NCCT diagnostic methods of efficacy were applied.

The definitions of stone SFRs are various. Some authors conservatively consider a stone free status as a renal pelvis free of any remaining fragments. Some are more liberal and treat insignificant stones as no stones at all. That concept of insignificant stone is based on statistics which states that almost all stones < 3 mm are freely expellable. On the other hand, some date suggest, that even small persistent calculi might accelerate stone formation and significantly shorten recurrence free intervals.

At last, appropriate scheduling for postoperative evaluation and imaging is crucial. It was shown that up to 25% of patients may become stone free when assessed 1 mo postoperatively in comparison to a group examined one day after a rRIRS intervention[50].

WHICH TECHNIQUE SHOULD BE CHOSEN FOR KIDNEY STONES OVER 2 CM?

The last EAU guidelines on urolithiasis recommend endourology for the treatment of > 2 cm renal calculi[8,9]. Nowadays, a wide spectrum of procedures and therapeutic modalities is available and allows the surgeon to offer an individualized treatment strategy to the patients taking into account all relevant clinical and patient-related parameters. The patient should also be well informed about advantages and disadvantages of each option and be involved in the decision making process. While many

patients choose PNL as widely established standard for treatment of a > 2 cm kidney stone, others may benefit from less invasive procedures accepting lower efficacy and necessity for ancillary procedures. The summary of abovementioned procedures is given in Table 1.

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REVIEW

Mesenchymal stem cells for kidney transplantation

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Abstract

The long term consequence of immunosuppressive therapy in kidney transplantation has prompted investigation of alternative means to modify the immune response to the allograft. Cell based therapies are potentially attractive as they may provide a long lasting immunomodulatory effect, may repair tissues and reduce the necessity to take immunosuppressive drug therapy. Of the current cell therapies, mesenchymal stem cells have now been trialled in small numbers of human kidney transplantation with apparent safety and potential efficacy. Many issues however need to be resolved before these cells will become mainstays of transplant immunosuppression including *ex vivo* modification to enhance immunomodulatory properties, cell number, route and frequency of administration as well as cellular source of origin.

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Key words: Mesenchymal stem cells; Kidney transplantation; Immunosuppression; Solid organ transplantation; Cellular therapies

Core tip: This review summaries several of the most prominent cellular therapies currently being examined for use in immunosuppression. From the current evidence the reviewers make the argument that mesenchymal stem cells offer the best chance of a useful and functional cellular therapy for solid organ transplantation.

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INTRODUCTION

Kidney transplantation remains the optimal treatment for end stage renal disease (ESRD) providing excellent short term outcome with greater quality of life than that provided by dialysis[1]. Whilst short term graft survival is improving and acute rejection rates are dropping long term graft survival rates remain a major focus for clinical improvement. There are many factors that can impact the prognosis of a kidney transplant, from graft or donor considerations[2,3], factors involving the immunosuppressant regime[4,5], and issues concerning the recipient[6,7].

Tissue typing and stringent exclusion criteria are implemented pre-transplant to reduce the risk of donor related problems[3]. Issues with the recipient such as non-compliance and co-morbidity are much more difficult to manage and are often beyond a clinician's power to control[6,7].

When a suitable kidney donor is found, it is then important to make sure that the graft does not reject by suppressing the recipient's immune system. Current immunosuppressive drugs may be classified into five groups based on their mechanism of action: (1) regulators of gene expression; (2) alkylating agents; (3) inhibitors of de novo purine synthesis; (4) inhibitors of de novo pyrimidine synthesis; and (5) inhibitors of kinases and phosphatases[5]. Targeting each of these mecha-

nisms has its benefits and disadvantages and tailoring a drug schedule has the potential to impact long term graft function and the quality of life of the recipient. However all current drugs are associated with a range of adverse effects including renal toxicity, opportunistic infections, development of malignancy and metabolic complications[5]. A common trait among all these drug classes is the targeting of T cell function[5,8-10]. T cells play an important role in rejection via alloantigen recognition and the direction of an effector response that results in graft damage and dysfunction[11].

Of these issues it is the modification of immunosuppression that is an obvious place to try and improve patient outcomes, as more options will allow for customised treatment programs unique to each patients needs. Towards this end, there has been a recent increase in the development of alternative means of immunosuppression for organ transplantation. Utilizing cell-based therapies for immunosuppression is an alternative approach to traditional pharmacological methods and represents a change in paradigm for transplantation therapies.

CELL THERAPIES FOR ORGAN TRANSPLANTATION

The basic concept of cell therapy is to implant cells with desired properties into a patient in an attempt to treat or cure. Although this idea has been around since the 19th century, it was not until 1968 that it became a viable treatment with the first bone marrow transplant[12]. Since then, there has been a steady expansion in the type of cells transplanted and the conditions that can be treated. The purpose of this review is to examine the state of several cell types that are being evaluated for preclinical or early clinical trials in solid organ transplantation (SOT), including; T regulatory cells (Tregs), dendritic cells (DCs), and with a particular emphasis on mesenchymal stem cells (MSCs) which have shown the greatest progress and potential as a cellular therapy.

RGULATORY T CELLS

Tregs are naturally occurring T cells which express the cell surface markers $CD4^+CD25^+$ $FoxP3^+$ and a variety of differing cell surface markers (CD127, Helios)[13,14]. Tregs are concerned with the maintenance of immunological self-tolerance by suppressing self-reactive lymphocytes that escape clonal deletion[14]. Naturally occurring Tregs are formed from naive T cells in the thymus. However these naive T cells can be converted to Tregs *in vitro* using TGF-β induction of FoxP3[15], providing a second source of Tregs for cell therapy.

Tregs are able to suppress the immune system on many levels, combining inhibitory cytokine secretion(*e.g.*, *via* TGF-β, IL-10)[16,17], cytotoxicity and inhibition of NK cells[18,19], and direct modulation of antigen presenting cells[20-22]. This multifaceted approach to immunosuppression makes Tregs a promising therapy to facilitate long term graft survival. Recently there have been advances in the methods for Treg isolation and expansion, with large scale expansion from peripheral blood (PB), umbilical cord blood (UBC), and induced Tregs from naive peripheral blood precursors[23]. There have also been positive results from experimental animal models[24]. Of greatest interest are the clinical trials that have used Tregs as a cellular therapy in graft-*vs*-host disease (GVHD), a major and potentially lethal transplant complication that is particularly prevalent in patients who have undergone a hematopoietic stem cell transplant (HSTC)[25,26]. With generally positive outcomes from the GVHD trials[26], it is likely that we will see Tregs initially deployed as an adjunctive therapy in SOT before being used in patients who have a high risk of rejection or who have already experienced adverse effects from standard immunosuppression. This would allow for the efficacy of Tregs to be determined in a way that would be ethical and pose a minimal risk of complications.

In addition to their safety, there are several other important issues that need to be addressed in the pursuit of an effective Treg based therapy. As mentioned above, there have been advances in the isolation and expansion of Tregs. These advances go some way to addressing the large number of cells that would be required for an effective therapy, with some estimates placing the required number at 11×10^8 cells/kg[27]. Another concern is the source of the Tregs. Currently, the most appropriate source for therapy is unknown, with uncertainty focused on whether alloantigen or antibody mediated expansion is the safest and most effective method[23]. The stability of Tregs *in vivo* has also been found to be problematic with studies finding that Tregs can lose FoxP3 expression and develop an effector cell phenotype, becoming pathogenic[28]. Of relevance to the previous point about the source of Tregs is evidence suggesting that induced Tregs lose FoxP3 expression at a much higher rate than natural Tregs[29,30]. These are just a few of the issues surrounding the use of Tregs for SOT that the ONE study (www.theonestudy.org) hopes to address. Currently the ONE study is examining the use of polyclonally expanded Tregs and alloantigen driven Tregs in kidney transplantation at doses of 1, 3, 6 and 10 $\times 10^6$ Tregs/kg. As of writing this no results have been published[23].

DENDRITIC CELLS

Dendritic cells (DCs) are able to function as antigen presenting cells that drive graft rejection (immunogenic DC) or have a role in promoting graft acceptance (tolerogenic DC; TolDC) depending on their state[31]. Immunogenic DCs cause T cell activation and proliferation with the use of three signals: (1) they present antigens on MHC molecules; (2) They provide co-stimulatory molecules; and (3) they secrete pro-inflammatory molecules. Only when all three signals are present can DCs activate T cells[31]. TolDCs are also able to interact with regulatory

T cells to promote immune tolerance. The role that DCs play in immune tolerance is twofold. Firstly, they play a role in the deletion of self-reactive thymocytes in the thymus[32]. Secondly, and of relevance to transplantation, they aid in peripheral tolerance. They do this by the presentation of antigens while lacking the co-stimulatory molecules required for T cell activation[32,33]. This causes T cell unresponsiveness as well as Treg induction[33].

Two strategies for the use of TolDCs in transplantation are likely to be applied in the setting of allotransplantation. The first involves negative immunization by administering either autologous DCs that have been exposed to alloantigens or donor derived DCs, pre-transplant[34]. The second method involves the use of recipient derived DCs delivered on the day of transplantation[35]. Intravenous injection of immature DCs of either donor or recipient origin at the time of transplantation have prolonged allograft survival in SOT models[36]. There is a large amount of literature on the use of DCs in pre-clinical experimental models[36,37]. Clinical trials looking at DCs have been carried out in both type-1 diabetes[38] and rheumatoid arthritis[39]. This has shown that the use of DCs for immunomodulation is safe and effective.

Many of the issues that face Tregs are also pertinent in the consideration of DCs as a cellular therapy. Cell dose and the best method for the isolation and expansion of the cells is uncertain. The use of either recipient derived DCs or donor DCs is yet to be resolved and adding additional complexity to this issue is the question of negative immunization vs. recipient derived DCs delivered peri-transplant. Again, the ONE study aims to answer these questions and early trials of DCs in SOT are ongoing as of writing this.

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs) are a multipotent cell lineage that has great potential for use in cellular therapies and is already being widely tested in clinical trials. www.clinicaltrials.gov currently lists 396 studies using MSCs in conditions such as spinal cord injury, diabetes, Alzheimer's disease, and kidney injury.

The International Society for Cellular Therapy (ISCT) has set the minimal criteria for defining MSCs as being plastic adherent, capable of differentiation into osteoblasts, adipocytes, and chondroblasts, and expressing CD105, CD73, and CD90 while lacking expression of CD45, CD34, CD14 or CD11b, CD19, and HLA-DR surface molecules[40].

MSCs are capable of being isolated from many tissues including bone, fat, and placenta. When cultured they adhere to plastic and have a fibroblast-like appearance, possessing a long, thin body and a small number of protrusions[40]. MSCs have a role in the formation and homeostasis of connective and structural tissues *via* the production of extracellular matrix, stabilization and regulation of the tissue vascularisation, and the creation of new connective tissue cells[41,42]. In addition to this, they also play a role in the immune system by inducing tolerogenic[42] properties that can be enhanced by *in vitro* treatment[43]. These roles are able to be exploited to aid in regenerative medicine and in immunosuppression. Combined with the many tissues from which they can be isolated and their ability to remain stable while being expanded *in vitro*[44] it becomes clear why so much work is now being carried out using MSCs for a large number of clinical applications.

The immunosuppressive abilities of MSCs are mediated by either nitric oxide synthase (iNOS) in mice[45,46], or indolamine 2,3-dioxygenase (IDO) in humans[46]. iNOS results in the production of nitric oxide (NO) which is an immunosuppressive agent in high concentrations[47]. Alternatively, IDO degrades the essential amino acid tryptophan thereby resulting in immunosuppression. The accumulation of the tryptophan metabolite kynurenine is also known to mediate the immunoregulatory effects of MSCs[48].

The exact mechanisms of how two pathways cause immunosuppression are not fully understood. In addition to these key factors, there are several immunosuppressive molecules secreted by MSCs. These include; PGE-2, IL-10, HO-1, PD-L1, and IL-6[49].

In reaction to stimulus from interferon-gamma (IFN-γ and proinflammatory cytokines, MSCs also secrete chemokines and adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)[45]. This results in a close proximity of immune cells allowing the local immunosuppressive environment to have a more pronounced effect[49].

A substantial amount of work has been focused on the potential for MSCs to treat GVHD. Ringdén *et al*[50] treated 8 patients, who had developed steroid-refractory GVHD, with bone marrow derived MSCs. In 6 of these patients acute GVHD ameliorated. The same group later went on to perform a phase Ⅱ trial consisting of 55 patients with acute GVHD. In this trial, 30 patients completely recovered from GVHD and a further 9 showed improvement. None of the patients developed adverse reactions due to the administration of MSCs[51]. Another phase one trial administering MSCs for GVHD was carried out by Introna *et al*[52] This multicentre study looked at 40 patients (15 children and 25 adults) with steroid resistant GVHD and gave them a median of 3 third-party derived MSCs infusions. Here it was found that the MSCs had a 67.5% T cell mediated response rate with a 27.5% complete response, 86 adverse effects were reported however most of these were of an infectious nature (72.1%) and not due to the administration of MSCs[52]. They concluded that MSCs could safely be administered in addition to conventional immunosuppression (*e.g.*, cyclosporin, steroid). Despite these positive results, there is some concern over a phase III clinical trial that failed to meet its primary clinical end point (NCT00366145)[53]. In this trial, patients received 8 infusions of 2×10^6 cells/kg over 4 wk and 4 more infusions administered weekly after 28 d. The trial did not meet its primary end point of a significant increase of

complete response of steroid resistant GVHD. Galipeau *et al*[54] provides a comprehensive failure analysis of the trial. The main conclusion of this analysis is that there are significant differences between the Martin study and studies from Europe that could account for the failure, in particular the passage number of the cells used[54]. As such, this study is not damning of MSCs but rather provides more areas that require examination before they can be used more widely.

Unlike the other cell types, there are now completed early clinical trials that have deployed MSCs as a therapy for SOT. The largest comes from Tan *et al*[55] In their trial they had 159 kidney transplant patients split into 3 groups, with 2 groups receiving autologous MSCs with either standard dose calcineurin inhibitors (CNIs) or low dose CNIs and the control group receiving standard dose CNIs and anti-IL-2 receptor antibody. The major conclusions from this study were that the MSC groups had a lower incidence of glucocorticoid-resistant rejection, a faster recovery in renal function, and significantly decreased risk of opportunistic infections than the control group[55]. This study also addresses safety concerns over the use of MSCs as there were no adverse reactions reported in either of the test groups. However this trial was not without its problems. It was noted by the authors that the number of rejection episodes in the control group was higher than what would be expected. This made it appear that the MSC groups performed better than standard immunosuppression when this may not be the case[55]. Additionally, the major differences in graft function were only noticed in the first 2 wk. It is conceivable that this was due the regenerative abilities of MSCs repairing the reperfusion injury associated with all kidney transplants. And lastly, the major difference in opportunistic infections was noted in the MSC and low dose CNI group. As there was no control low dose CNI group, we cannot be certain that the observed reduction in infection is due to MSCs or simply due to the reduced use of immunosuppressive drugs.

In addition to the work from Tan there have been several case reports looking at the use of MSCs in a small number of SOT patients. Perico *et al*[56,57] have performed two pilot studies looking at the use of MSCs in kidney transplantation in 4 patients. In their first study they administered intravenous autologous MSCs 7 d after transplantation and followed the patients for 360 d. From days 7 to 14 post transplant, serum creatinine increased in 1 of their patients, however acute graft rejection was excluded *via* biopsy. They also noted an increase in patient Tregs and a decrease in T cell expansion post-transplant. Long term, both patients showed stable graft and the authors concluded that MSC infusion in kidney transplant recipients is feasible, allows increase of Treg in the peripheral blood, and controls memory $CD8^+$ T cell function[57]. In their second trial, they dosed two living-related kidney transplant recipients with autologous MSCs one day before transplantation. The change in dosing time was an attempt to avoid the acute graft deterioration observed to be caused by intragraft localization of MSCs when dosing 7 d post-transplant. Although both patients had no side effects to the MSC infusion and both had stable graft function at 12 mo, one of their patients did have an acute rejection episode 14 d post-transplant that was resolved with corticosteroid therapy[57]. The authors attribute the rejection episode to a higher number of HLA mismatches. They concluded that pre-transplant administration of MSCs avoided the cell induced graft dysfunction associated with post-transplant MSC administration and that this method is favourable for future trials. Peng *et al*[58] examined the effect of autologous MSCs on renal transplants by giving 6 patients MSCs combined with half doses of tacrolimus and comparing acute rejection, graft function, and graft survival at 12 mo to a control group of 6 patients receiving standard dose tacrolimus. The results of this showed no toxic adverse effects associated with MSC infusion and all patients survived with stable graft function to 12 mo with only 1 acute rejection episode in the control group. The one difference they did notice was elevated B-cell counts in the MSC group at 3 mo compared to the control[58]. They concluded that MSCs may provide benefits in renal transplantation by reducing the required dose of conventional immunosuppressive drug that is required for long term graft survival. The results of these case reports are consistent with those of the Tan study, with no adverse reactions, stable graft function, reduced rejection, and the ability to lower maintenance immunosuppression (Table 1).

From these early clinical trials, summarised in Table 1, it is evident that MSCs have an acceptable safety profile and have beneficial effects for transplantation. There still remain several very important questions to be answered before MSCs can obtain mainstream clinical use. The issue of whether autologous or allogeneic MSCs are better is significant, with arguments for both being put forward. Tan *et al*[55] employed autologous MSCs because of the issues surrounding MSC isolation from deceased donors. Furthermore, the use of autologous MSCs would avoid any potential for rejection of the cells and a subsequent loss of their function. However, there is some evidence that MSCs are immuno-evasive allowing them to escape recognition by the hosts immune system[59]. If this is the case then allogeneic MSCs are promising as obtaining them will not impact the eventual recipient who may have serious health issues that could be exacerbated by the collection of MSCs or could impact the quality of the MSCs. The immuno-evasive status of MSCs also opens up the potential for third party derived MSCs. This would invalidate concerns about obtaining MSCs in the cases of deceased donors. Nevertheless, issues pertaining to the immunogenicity of allogeneic or third-party derived MSCs has not been substantially addressed *in vivo* and have not been addressed in large animal models. There are preclinical studies demonstrating that allogeneic MSC monotherapy alone failed to prevent allograft rejection[60-69]. Studies reporting on the benefits of allogeneic MSCs have also shown short term prolongation of graft

Table 1 Summary of clinical trials using mesenchymal stem cells in kidney transplantation

Ref.	Patient number	Cell number	Cell source	Adverse reactions	Graft survival
Tan *et al*[55], 2012	106	1-2 × 10^6 cells/kg	Autologous, bone marrow	None	100% at 1 yr
Perico *et al*[56], 2011	2	2 × 10^6 cells/kg	Autologous, bone marrow	Acute graft dysfunction	100% at 360 d
Perico *et al*[57], 2013	2	2 × 10^6 cells/kg	Autologous, bone marrow	HLA induced rejection	100% at 1 yr
Peng *et al*[58], 2013	6	5 × 10^6 1st dose 2 × 10^6 cells/kg 2nd dose	Donor derived, bone marrow	None	100% at 1 yr

HLA: Human leukocyte antigen.

survival[64]. More importantly, in some studies, pre-transplant allogeneic MSC monotherapy accelerated allograft rejection thereby questioning the immunoprivileged status of MSC. There is evidence that allogeneic MSCs can trigger an anti-donor immune response resulting in accelerated allograft rejection[65-67]. The co-administration of allogeneic MSC with immunosuppressive drugs however showed better outcome of the allograft compared to MSC monotherapy[63,64,70-72]. Therefore, the synergistic effects of allogeneic MSC with immunosuppressive drugs need to be taken into consideration in MSC therapy. We have previously reviewed in detail the mechanisms associated with allogeneic or third-party derived MSC immunogenicity and the synergistic effects of MSC with immunosuppressive drugs, in Sivanathan *et al*[43]. Questions around the dose rate, the timing, the route of administration, what happens to the cells and what exactly the MSCs are doing and their mechanism of action still remain unanswered. Given the state of the field it is not possible to accurately speculate on the answers to these questions. Additionally there is the potential for the modification of MSCs that further expands the possible methods of application

MODIFYING MSC FOR ENHANCED IMMUNOSUPPRESSION

The *ex vivo* manipulation of MSCs with proinflammatory cytokines, particularly IFN-γ modification of MSC enhances the immunomodulatory, reparative and homing potential of MSCs[43]. The enhancement of these MSC properties would be beneficial in a transplant setting and may hasten the translation of MSC therapy into SOT patients.

Of key benefit, the priming of MSCs with IFN-γ is critical to active MSCs immunosuppressive function[73-75]. IFN-γ primed MSC have an enhanced ability to suppress T cell responses compared to untreated MSC[76-80]. Increase suppression of T cell responses is mediated by the induction of immunosuppressive factors such as iNOS andIDO[75,81]. IDO is also well known for its roles in preventing rejection and induction tolerance at the fetal-maternal interface[82]. In addition, MSC-expressed IDO have been shown to induce tolerogenic DCs and Tregs[83], which are two other cell based therapies that have gained significant interest in SOT, as we have discussed above. The upregulation of other MSC immunomodulatory factors, the enhancement of negative T cell signalling, the inhibition of proinflammatory T cell response and the increase in Tregs further support the benefits of administering IFN-γ primed MSC therapy for SOT.

Regardless of the potential therapeutic benefits of IFN-γ primed MSC therapy, it should be noted that IFN-γ upregulate MHC class I and induces MHC class II expression on MSCs[84-86]. This may render these cells more immunogenic in MHC-mismatched recipients[43], thereby decreasing their effectiveness at suppressing inflammation as reported in some studies[87,88]. Only two studies have directly addressed IFN-γ primed MSC immunogenicity *in vivo*[88,89] and this warrants further investigation. Thus, when considering IFN-γ primed MSC therapy, then administration of autologous MSC may be more beneficial. If allogeneic or third-party IFN-γ primed MSC were to be considered, the co-administration of these cells with immunosuppressive drugs would be necessary as an attempt to control anti-donor immune response towards MSC to enable MSCs to exert their beneficiary effects *in vivo*.

CONCLUSION

In summary, there are numerous cell based therapies that have shown potential for use in the immunomodulation of SOT in pre-clinical, small, and large animal models. Tregs and DCs have shown promise *in vitro* and in animal models as well as displaying safety and efficacy in clinical trials involving GVHD, diabetes, and rheumatoid arthritis. However, only MSCs have completed large clinical trials to date. MSC have shown the most promise having been tested in GVHD and in early clinical trials for kidney transplantation. Based on the GVHD experience and the early transplant work, it appears that MSC have an acceptable safety profile and potential therapeutic effect. However, much needs to be resolved, including the issue of autologous *vs* allogeneic (third party cells), frequency of administration and mechanism of action. The optimal immunosuppressive therapy to be co-administered should also be studied. The results from these early trials are positive but have presented numerous issues that need to be addressed before MSCs gain widespread clinical use.

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REVIEW

Comprehensive urodynamics: Being devoted to clinical urologic practice

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Abstract

As a combined electrophysiological system for evaluating the lower urinary tract (LUT), comprehensive urodynamics (UDS) aims at duplicating patient's micturition process, either normal or abnormal, and further seeking for possible causative origin, either neurogenic or non-neurogenic, in order to guide treatment. Through thorough analysis, some so-called cut-off values, for example, bladder outlet obstruction (BOO) degree or dyssynergic degree between the detrusor and sphincter, could be gained; however, in most cases, their qualitative description, such as stress urinary incontinence, idiopathic detrusor underactivity (DUA), detrusor overactivity (IDO), low compliance, and idiopathic sphincter overactivity (ISO), is more preferable and important. In aged neurologically intact male patients with symptoms of the LUT (LUTS) including benign prostatic hyperplasia, a combined UDS system, which coupled BOO with compliance, was constructed. The patients may be categorized into one of the seven subgroups, including equivocal or mild BOO with sphincter synergia with or without IDO (pattern A), equivocal or mild BOO with ISO (B), classic BOO with sphincter synergia (C) or ISO (D), BOO with only low compliance (E), BOO with both DUA and low compliance (F), and potential BOO with DUA (G). This new system can be used to optimize diagnosis and treatment according to a derived guideline diagram.

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Key words: Detrusor overactivity; Electromyography; Sphincter overactivity; Stress urinary incontinence; Urodynamics; Urology

Core tip: Scant progress during the last 2 decades and poor prognostic value of urodynamics (UDS) for benign prostatic hyperplasia interventional therapy may come from some technological problems, here we mean the underestimation of the role of electromyogram and some shortcomings of the UDS technology. Based on individualized UDS evaluation of more than 9000 cases, some so-called cut-off values, for example, degrees of bladder outlet obstruction and dyssynergia between the detrusor and sphincter, could be gained; however, in most cases, their qualitative description, such as stress urinary incontinence, idiopathic detrusor underactivity, detrusor overactivity, low compliance, and idiopathic sphincter overactivity, is more preferable and important.

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INTRODUCTION

Comprehensive urodynamics (UDS) performed for stress urinary incontinence (SUI) female patients has been challenged by two recent published papers[1,2]. Does it take no good for SUI or patients with symptoms of the lower urinary tract (LUTS)? This was just the same

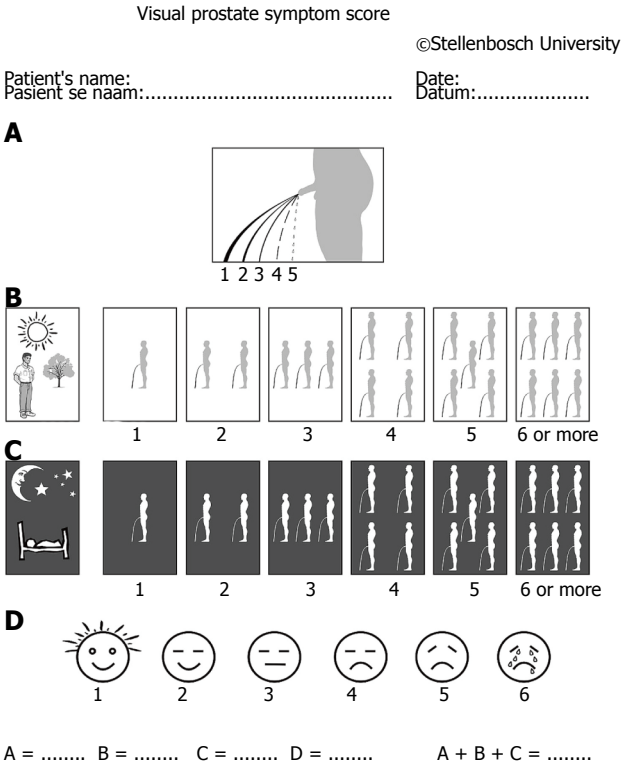


Figure 1 Visual prostate symptom score consisting of pictograms to evaluate (A) force of the urinary stream, (B) daytime frequency, (C) nocturia, and (D) quality of life[9].

as UDS for benign prostatic hyperplasia (BPH) patients in the late last century[3,4]. However, discerning experts indicated that the studies may have some common conceptual flaws, and there is a need to do more[5]. The authors therefore sighed, "scant progress has been made in the UDS recently[5]."

The NICE guidelines for the management of SUI[6] state that "the use of multichannel cystometry is not routinely recommended before surgery in women with a clearly defined clinical diagnosis of pure SUI". However, in a study of 6276 women with urinary incontinence, Agur *et al*[7] found that only 324 (5.2%) women had pure SUI. Although the symptomatic assessment had a specificity of 98%, its sensitivity was too low (11.4%).

As a combined electrophysiological system for evaluating the lower urinary tract (LUT), comprehensive UDS aims at duplicating patient's micturition process, either normal or abnormal, and further seeking for possible causative origin, either neurogenic or non-neurogenic, in order to guide treatment. Through thorough analysis, some so-called cut-off values, for example, bladder outlet obstruction (BOO) degree or dyssynergic degree between the detrusor and sphincter (TL value), could be gained; however, in most cases, their qualitative description, such as SUI, idiopathic detrusor underactivity (DUA), detrusor overactivity (IDO), low compliance, and idiopathic sphincter overactivity (ISO), is more preferable and important[8].

Whether a measuring system is inferior or non-inferior (*i.e.*, superior) is confirmed or based on large-sample population studies on one hand; however, decision on given patients should be made individually on the other hand. The important recording of electromyogram (EMG) was often absent in large sample trials and whether the patients had such finding as ISO or DUA was scant too[1,2,7]. Symptomatic analysis is usually preferable as compared with invasive measures, however, there are many uncertainties and variable factors. The most often used symptom score system IPSS (International Prostatic Score System) has been challenged by newly launched system VPSS (Visual Prostatic Score System) (Figure 1). A combination of VPSS > 8 and Qmax < 15 mL/s was used to select invasive evaluation during follow-up in men with urethral strictures[9]. Patients with bladder pain syndrome/interstitial cystitis (BPS/IC) and OAB patients had significant differences in their 3-d voiding diary records. Patients with BPS/IC had higher voiding frequencies and smaller maximal voided volume compared with OAB patients[10].

There are some differences between symptomatic and laboratory findings in clinical practice. Most clinical studies have relied on questionnaires as to the prevalence, symptoms, and treatment usage; however, the surveys must be interpreted with caution. So we should not let this prevent us from obtaining information *via* the use of laboratory tests including UDS combined with sphincter EMG. Furthermore, the symptom location is usually very factitious and clinical tests are necessary. For example, the pain caused by BPS/IC was often obscure. The study on BPS/IC prevalence could not find a single symptom-based definition of BPS/IC with ideal sensitivity and specificity to distinguish patients with BPS/IC from those with OAB, vulvodynia, or endometriosis[11]. The symptoms of irritable bowel syndrome, fibromyalgia, do overlap with those of BPS/IC[11]. The patients and doctors make every effort to reveal a sufficient description of the symptoms to prompt a rational diagnosis. The similarity of symptoms might have nosologic implications. If we want to locate the origin of symptoms and to validate the nature of the disease, necessary examinations, including UDS and EMG, had to be carried out[11].

The targets of invasive UDS test are: pursuing after completeness instead of simplicity[12] and selecting complete or appropriate UDS if feasible; clearly defining UDS entities and seeking cutoff values in order to subcategorize possible pathological process, although descriptive recording is often more preferable; and developing evidence-based or knowledge-based strategies based on UDS findings[13].

New studies should be performed using high quality UDS with possible cutoff parameters, and show treatment methods linked to UDS findings. This paper broadly reviews the fundamental concepts behind the technique, application, and interpretation of UDS testing and how they are applicable to general urologists in office settings. Some of the discussion is based on

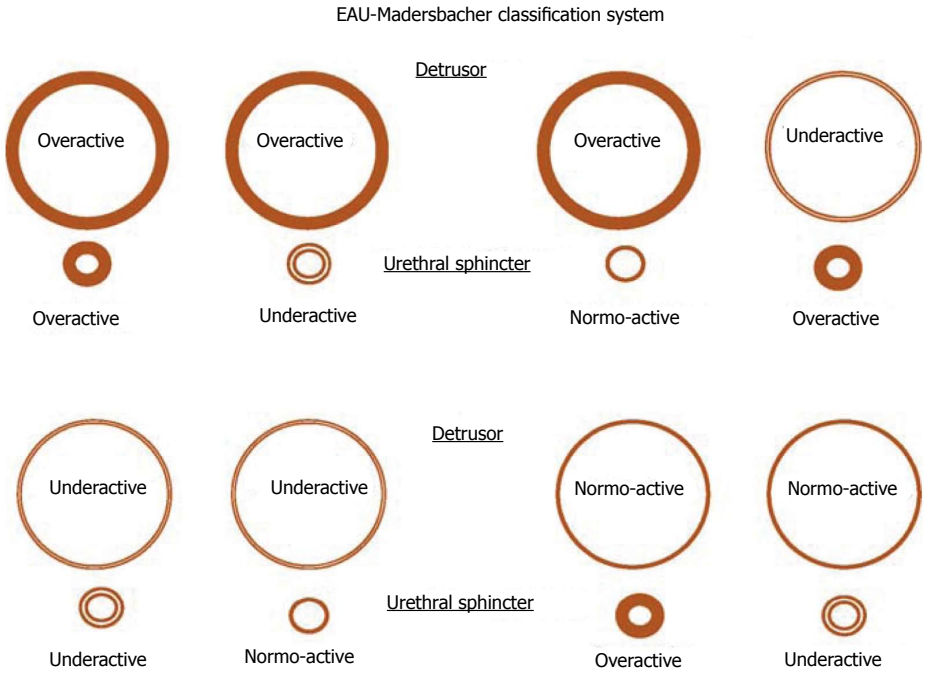


Figure 2 The European Association of Urology-Madersbacher classification system[15]. EAU: European Association of Urology.

the authors' experience and the existing literature in the field.

BASIC CONSIDERATION

Theoretical

The field of UDS has evolved significantly since its conceptual inception in the early twentieth century. UDS is a general term for a collection of techniques performed in an attempt to qualify and quantify the LUT activity during filling or storage, and emptying phases. Conceptually, normal, efficient bladder filling and storage require five components: (1) bladder compliance (distensibility); (2) bladder stability; (3) competence of ureterovesical junctions (*i.e.*, non-refluxing ureters); (4) closed vesical outlet at rest and during times of increased intra-abdominal pressure; and (5) appropriate bladder sensations. Bladder emptying requires: (1) constant detrusor contraction; (2) simultaneous relaxing of the smooth and striated sphincter; and (3) non-obstructed bladder outlet. Any abnormality of filling and storage or of emptying, regardless of causative pathophysiology, must result from a problem related to one of these factors. UDS studies can assist in categorizing and quantifying these problems[13]. Comprehensive UDS studies are a combination of noninvasive measures, such as initial uroflowmetry, and invasive measures, such as cystometrogram (CMG), pressure-flow study (PFS) with EMG, and urethral pressure profilometry (UPP). With the evolution of the personal computer, much development has been achieved in the field of UDS. The advent of smaller, less cumbersome, and less expensive machines has expanded the availability of complex UDS, including videourodynamics (VUDS) and ambulatory UDS (AUDS), to more practicing urologists[13,14].

PFS has been viewed as the urologic equivalent of cardiac catheterization[3]. These figurative words are right in some extent. There are still some differences between the two techniques: first of all, cardiac catheterization only needs patient's quiet recumbency, and not complex cooperation. UDS needs the patient's full cooperation to fulfill the process of storage and emptying. We would rather like to analog a patient as an actor or actress, and an urodynamicist as a director whose duty is to guide the patient to show his/her micturition process as actually as possible. Through their performance (complete storage and voiding behaviour, not only storage) by not only the leading role (detrusor), but also the co-star (sphincter), we could know whether the detrusor relaxes and the sphincter contracts during storage phase, and the detrusor contracts and the sphincter relaxes during empty phase or not, or vice versa. The guideline of normal detrusor and sphincter is "stretch out whenever necessary, and do not stretch out whenever unnecessary". Breach of the principle leads to dysfunction of the LUT: overactivity means "stretching out whenever unnecessary", and underactivity means "unable to stretch out whenever necessary". These demands and disability of the patients with potential lesions involving neurogenic system were exhibited in neurogenic LUTD guidelines (Madersbacher classification system)[15], in which the interrelationship or mutuality of the detrusor and sphincter was evaluated (Figure 2). In male patients, sphincter underactivity may exist clinically, however, its standard is not practical and data about its prevalence is scant. Furthermore in non-neurogenic LUTD, dysfunction of the detrusor or sphincter was presented separately, not integratedly as in neurogenic LUTD (NLUTD)[16] (Table 1).

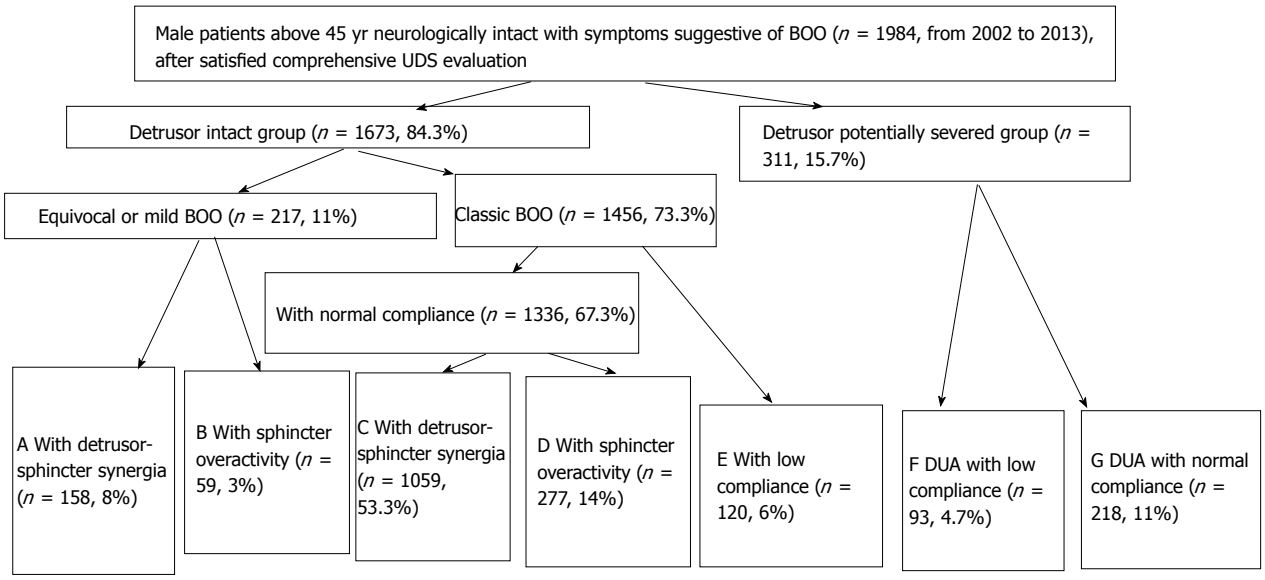


Figure 3 Study design, flow diagram and final outcome of male patients above 45 years neurologically intact with symptoms suggestive of male symptoms of lower urinary tract and bladder outlet obstruction[17]. UDS: Urodynamics; DUA: Detrusor underactivity; BOO: Bladder outlet obstruction.

Table 1 Expanded functional classification[16]

Failure to restore
Because of bladder
Detrusor hyperactivity
Involuntary contractions
Neurogenic diseases, injury, or degeneration
Bladder outlet obstruction
Inflammation
Idiopathic
Decreased compliance
Neurogenic disease
Fibrosis
Idiopathic
Detrusor hypersensitivity
Inflammatory
Infectious
Neurogenic
Psycologic
Idiopathic
Because of outlet
Stress incontinence (hypermobility related)
Nonfunctional bladder neck-proximal urethra (intrinsic sphincter dysfunction)
Failure to empty
Because of bladder
Neurogenic
Myogenic
Psycogenic
Idiopathic
Because of outlet
Anatomic
Prostatic obstruction
Bladder neck contracture
Urethral stricture
Urethral compression
Functional
Smooth sphincter dyssynergia
Striated sphincter dyssynergia

We think in urology practice, dysfunction of the detrusor, sphincter, detrusor compliance, incontinence state, and BOO had better been parceled on the basis of individualization. We have attempted this work and published a paper[17], which aimed at developing a UDS pattern system for aged male patients who complained of non-neurogenic LUTS to create a reference guideline for their diagnosis and treatment by a retrospective analysis. A retrospective analysis of UDS data was carried out in 1984 male patients neurologically intact with symptoms suggestive of BOO aged older than 45 years (2002-2013). On the basis of their UDS characteristic findings, the patients were classified into 1 of 7 subgroups: equivocal or mild BOO with sphincter synergia with or without IDO (pattern A); equivocal or mild BOO with ISO (B); classic BOO with sphincter synergia (C) or ISO (D); BOO with only detrusor low compliance (E); BOO with both DUA and low compliance (F); and equivocal BOO with DUA (G). The feasibility and rationality of this system were confirmed. The distribution of 7 patterns (pattern, case number, %) was A 158, 8%; B 59, 3%; C 1059, 53.3%; D 277, 14%; E 120, 6%; F 93, 4.7%; and G 218, 11% (Figures 3 and 4). The Abram-Griffiths (A-G) numbers (PdetQmax-2Qmax) in patterns C, D and E were 103.1-141.4, higher than those in other patterns ($P < 0.001$), and functional pressure lengths (FPL) in patterns C and D were 7.0-7.2 cm, longer than those in other patterns ($P < 0.001$). At last, a practical UDS pattern system for aged male patients with LUTS suggestive of BOO was constructed, which helps us to optimize the diagnosis and treatment[17]. For those with patterns A and B, medicinal therapy with 5a reductase inhibitor, antimuscarinics, or baclofen was administered first, whereas surgical intervention was reserved as an alternative option if medicinal therapy failed or their symptoms of BOO aggravated later. Those with patterns C, D and especially with E received transurethral resection of the prostate, and those with patterns F and G received 4-6

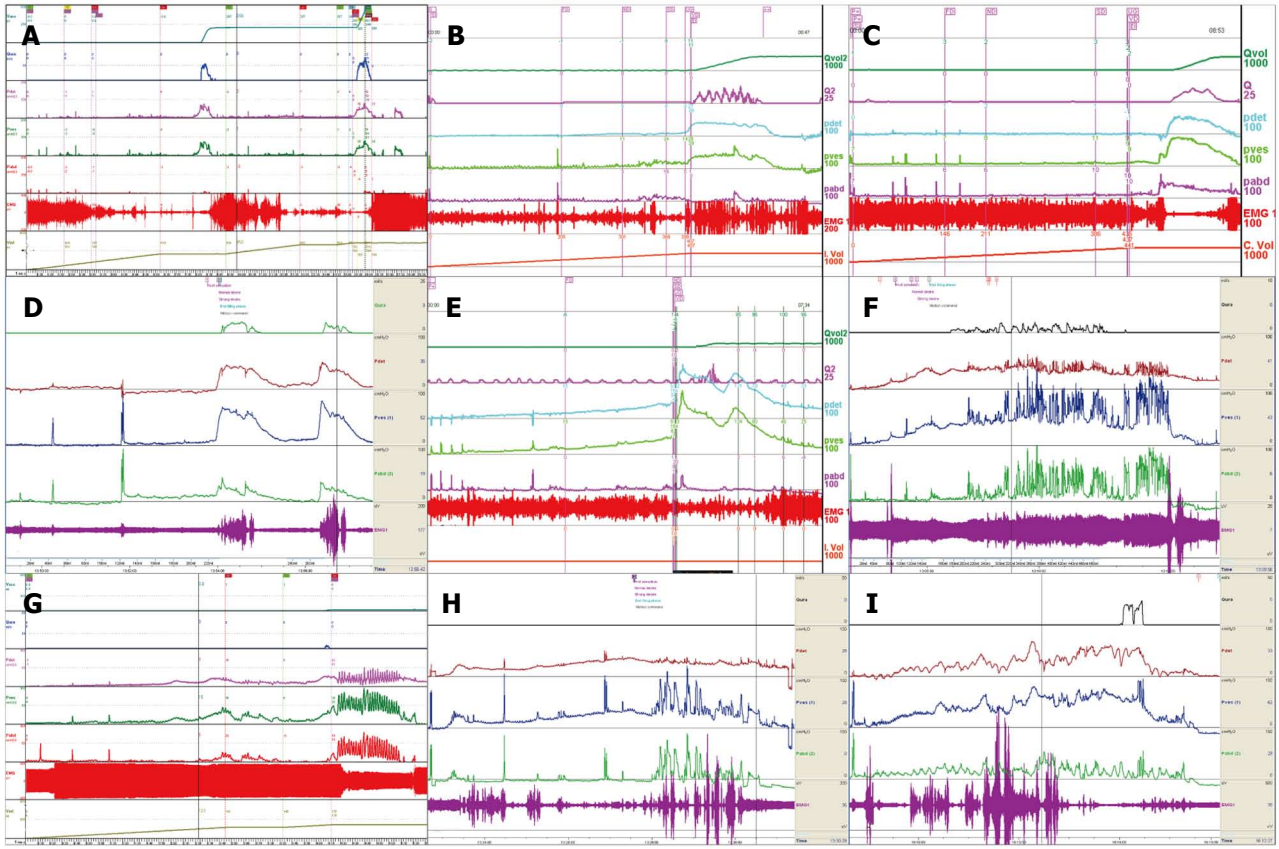


Figure 4 Urodynamic patterns of male patients older than 45 years neurologically intact with symptoms of lower urinary tract/bladder outlet obstruction[17]. A: Pattern A [equivocal or mild bladder outlet obstruction (BOO) with sphincter synergia with or without idiopathic detrusor overactivity]; B: Pattern B (equivocal or mild BOO with idiopathic sphincter overactivity); C: Pattern C (classic BOO with sphincter synergia); D: Pattern D (classic BOO with idiopathic sphincter overactivity); E: Pattern E (BOO with only detrusor low compliance); F: Pattern F (BOO with both detrusor underactivity and low compliance); G: Pattern G (equivocal BOO with detrusor underactivity); H: Urodynamic study of a patient with pattern F; I: The same patient with pattern F as in H after 4 wk urinary draining, whose detrusor contraction recovered and urodynamic study pattern was transformed into pattern E.

wk indwelled catheterization and administration of pyridostigmine bromide, baclofen, and decoction of Chinese medicinal herbs in an attempt to promote recovery of the detrusor contraction (Figure 5). These affordable principles may enrich the therapy of male LUTS using medical and/or conservative methods[18]. An integrated UDS pattern trial of 2195 female LUTS patients had been conducted, which revealed different results from those obtained from male patients[19]. At first they were divided into SUI and non-SUI groups and low compliance was seldom seen in neurologically intact women and was omitted in the calculation. At last, the distribution of UDS patterns were NA (normal detrusor-sphincter function) 50.2% (1101 cases), IDO 18.3% (401 cases), ISO 13% (286 cases), IDO + ISO 7.6% (167 cases), and DUA 10.9% (240 cases). We think the applicable principles from male population suit to female population too[19].

Technological

EMG technology: Scant progress during the last 2 decades and poor prognostic value of UDS for BPH interventional therapy may come from some technological problems, here we mean the underestimation of the role of EMG and some shortcomings of the UDS technology. Surface or patch electrode was routinely used as the standard option during UDS and the abnormal EMG findings were usually blamed as artifacts[20]. There are surface electrode, concentric needle electrode (CNE), and needle-guided wire electrode (simple as wire electrode[8]) available in practice. CNE was superior over surface electrode[21]. From our experience, needle guided wire electrodes were superior to CNE[22]. From our experience of about 9000 patients from 2002 to 2014, nearly 70%-80% so-called "artifacts" or bad recordings of EMG came from technical errors and we can record detrusor sphincter synergia or dyssynergia well using Solar from MMS (Netherlands), or Andromeda from Germany, as Janus from Life-tech (United States). We used the anal sphincter instead of the urethral sphincter to obtain EMG information[23]. Although some authors stated that anal and urethral sphincter EMG had the same significance[24], we believe that finding abnormal EMG signs in neurogenic diseases is more important than interpreting potential differences between the two routes. Anal external sphincter has a bigger mass than urethral external sphincter, less potential of pain and bleeding when needle is inserted into them, and less ar-

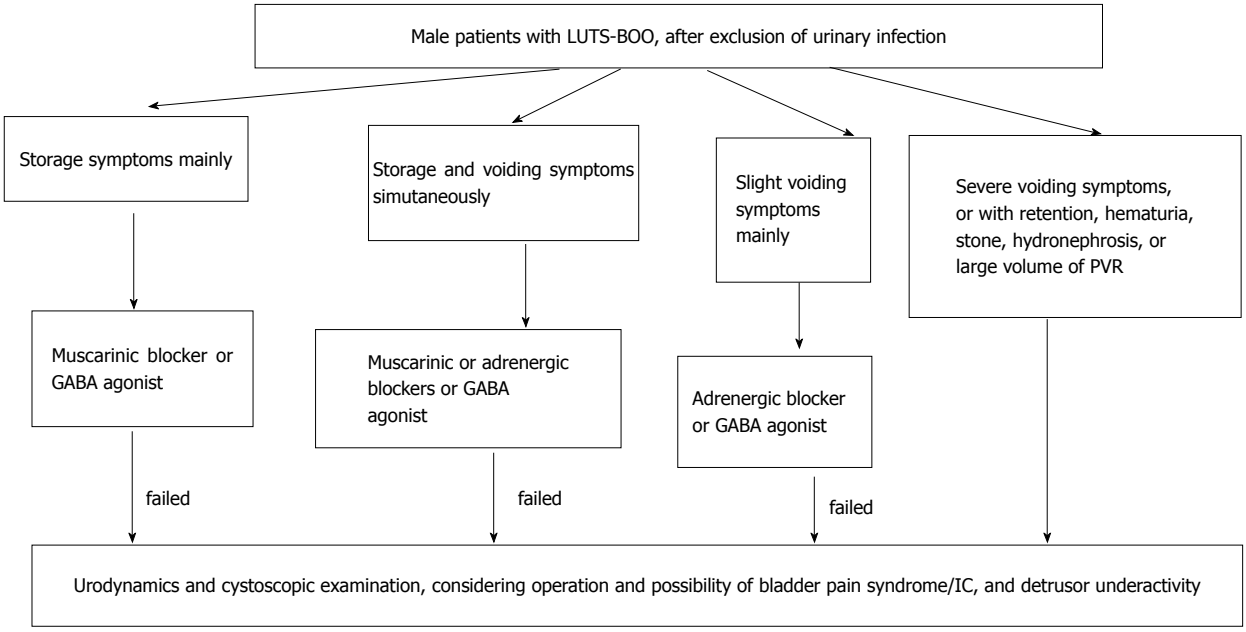


Figure 5 A proposed supplement to the guideline of male symptoms of lower urinary tract/bladder outlet obstruction[17]. BOO: Bladder outlet obstruction ; LUTS: Symptoms of the lower urinary tract.

tifact possibility. In our laboratory CNE, anal plug electrodes and urethral catheter containing concentric electrodes are abandoned because of lower accuracy. CNE has also two poles indeed: one in the core of the needle, the other in the outer coating of the needle.

Patient position during UDS procedures: The position should be as natural and physiologic as possible, and we prefer sitting for female patients, sitting or standing for male patients. For patients whose sitting or standing position is unfeasible, they may take the supine position on the UDS bed with the barrow together. If they could pass urine during PFS stage, the simultaneous imitation of urinary flow into the commode is conducted. For patients whose original position does not fit PFS, their position should be changed, *i.e.*, from sitting to squatting or standing. After position change, the same magnitude of increase of abdominal and bladder pressure produces, and their detrusor pressure is stable and no adjustment is required. The abdominal catheter may be ejected out by excessive abdominal strain during emptying phase in patients with DUA. If this happens, the abdominal pressure (*Pabd*) no longer works as a reference value for detrusor pressure, which means the difference between the vesical pressure *(Pves)* and *Pabd* (*Pdet* = *Pves-Pabd*). Measures should be taken to adjust *Pabd*, usually zeroing it.

UDS catheter and infusion medium selection: Catheters of UDS include urethral catheter and anal catheter. The former, either one, double or three-lumen, is usually afforded by appropriate companies and is disposable. Now an 8F double-lumen transurethral catheter (Xubu Medical Appliance Company, Dantu District, Jiangsu Province, China) is used for *Pves* recording and infusion of normal saline in our institution. The *Pabd* is recorded using a 12F transrectal balloon catheter (Cook Urological Incorporated, IN)[17]. The balloon covered with an envelope is lubricated and inserted into the anus and then semi-filled with saline, which assures satisfactory recording of the pressure. As far as the infusion medium is concerned, saline and air are used at the early stage, and normal saline is more suitable than air because of its physiologic property and ability to suit PFS[25].

UDS manufacturers: There are many UDS manufacturers or companies around the world, including MMS (Netherland), Life-Tech (United States), Andromeda (Germany), Leborie (Canada), Wearnes (Chengdu, China), *etc*. The design and performance are nearly the same. Our institution has the experience with products from all these companies.

CLINICAL CONSIDERATION

Early diagnosis and treatment are essential for patients with LUTS[15]. UDS findings, either as cut-off values or descriptive conclusions, may indicate possible lesions that later emerge, give preoperational forecasting of surgery for BOO, evaluate possible organic lesions other than functional origin, and main dysfunction sites, either the bladder or urethra or both.

LUTS followed by demonstrable abnormal UDS findings

Uroflowmetry curve: Uroflowmetry usually indicates whether the bladder outlet is obstructed or the detrusor is unable to contract. Here we report a special case with sustained urinary incontinence after birth and her low and smooth "uroflowmetry" curve represented infused fluid leakage. This was a 24-year-old unmarried Chinese

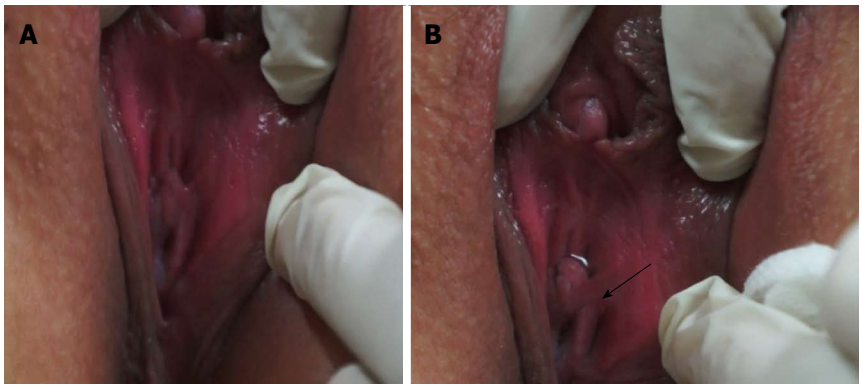


Figure 6 Perineal view of a female patient with urethral external sphincter agenesis before urodynamic evaluation. A: Urethral orifice and hymen as the urethral orifice was dried with gauze; B: Urine expelled out as solid arrow indicated.

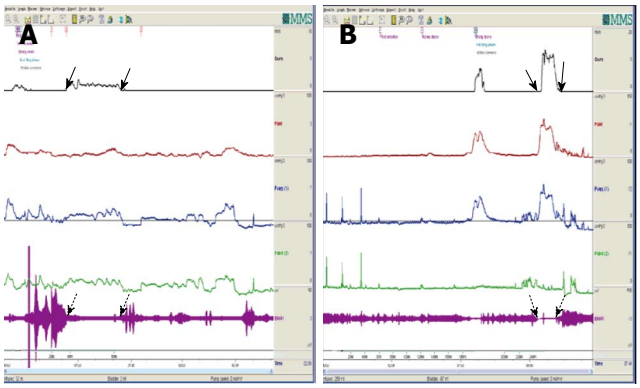


Figure 7 Comprehensive urodynamic evaluation of a patient with urethral sphincter agenesis. A: As saline was infused into the bladder, saline and her urine were expelled steadily (solid arrows) in the initial examination, however, during this phase, anal sphincter electromyogram showed synergia (as dotted arrows indicated); B: The storage and voiding function was recovered to normal and incontinence cured 4 mo after sphincteroplasty.

female referred for evaluation of congenital urinary incontinence. A transperitoneal anastomosis of both ureters to the bladder was carried out based on an assumption of ectopic ureteral orifices in the bladder neck and failed to cure her incontinence five years ago. She was neurologically normal and denied the presence of any other symptoms. She had good pelvic support and normal external genitals including the hymen. No abnormal neurologic signs were found. Ultrasonic test revealed that her kidneys, uterus, ovary and bladder were all normal. However, transrectal ultrasonography revealed that circular low echogenic zone of the peripheral region of the mid urethra, which represents the urethral striated sphincter[26], was absent. An intravenous urogram showed normal kidneys, ureters and bladder, too. There was no visible endoscopic evidence of vesicourethral lesions except for urinary leakage. When an inspection of her perineum in lithotomy position was performed, a rhythmic leakage of urine was observed, which was much like the way that urine jets ejected from ureteral orifices into the bladder being observed during cystoscopy both in rhythm and quantity (Figure 6). Each time she could only pass less than 30 mL with a maximum flow rate of 5 mL/s. UDS revealed that her bladder had no storage function at all, and we observed that more than 400 mL liquid was expelled after 360 mL normal saline had been infused at a rate of 50 mL/min. The flow or leakage rate was 1 mL/s, *i.e.*, 60 mL/min. During storage phase with virtually constant leakage, the detrusor had no contraction (Figure 7A), and as infusion ended, the anal sphincter EMG recovered to normal storage state (Figure 7A), which meant normal anal sphincter function, but not urethral sphincter function. Congenital urethral sphincter agenesis (much severe than intrinsic sphincter deficiency, ISD) was a supposed diagnosis. In order to reconstruct a functional urethral sphincter, urethral external sphincteroplasty using an autologous fascial sling which was obtained from her left fascia lata, was performed successfully. Double rolls of the sling around the urethra were formed, one fixed to the public union and the other to the sheath of the rectus. The patient achieved full continence thereafter and could pass urine ideally in an interval of 30 min to 2 h two weeks after the operation. At the follow-up 4 months after the procedure, she could pass urine with more than 250 mL in an interval of 2-3 h, and UDS showed that her detrusor could contract normally with a Qmax of 23 mL/s and her anal sphincter worked as preoperational status (Figure 7B).

Low-smooth uroflowmetry curve is also associated with constrictive BOO, for example, urethral stricture (Figure 8A). This type of BOO is different from compressive BOO, such as that due to BPH[27]. Constrictive BOO produces a decreased slope of the passive urethral resistance relation (PURR) curve and a normal minimal opening pressure (Pmuo) (Figure 8B), whereas compressive BOO produces a steep slope of the PURR curve and a higher Pmuo[27] (Figure 8C). In typical cases, low-smooth uroflowmetry curve and characteristic finding of constrictive BOO aid the diagnosis of urethral stricture, especially in female patients with a UTI history.

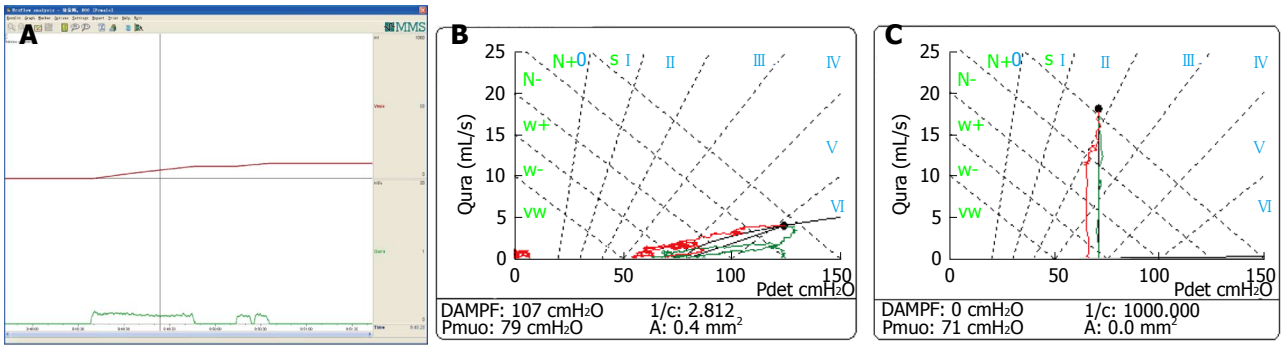


Figure 8 Low-smooth uroflowmetry curve in a female patient confirmed with urethral stricture, constrictive bladder outlet obstruction and compressive bladder outlet obstruction. A: Low-smooth uroflowmetry curve in a female patient confirmed with urethral stricture; B: Constrictive bladder outlet obstruction (BOO) produces a decreased slope of the passive urethral resistance relation (PURR) curve and a normal minimal opening pressure (Pmuo); C: Compressive BOO produces a steep slope of PURR curve and a higher Pmuo[25].

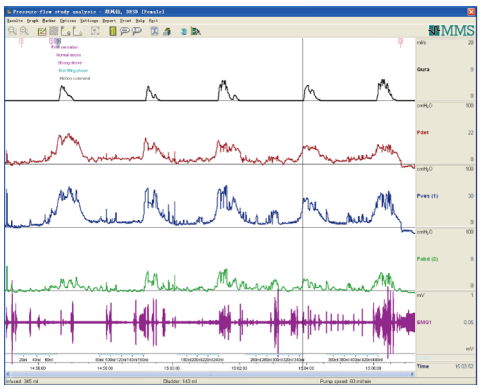


Figure 9 Repeated urodynamic curve of a female patient aged 69 years with urinary urgency and frequency for more than 20 years, proved as idiopathic sphincter overactivity with a functional bladder capacity of 80 mL and cured by baclofen 10 mg, *tid*. The characteristic findings of idiopathic sphincter overactivity were equivocal in the first and fourth urodynamic evaluations and obvious in the remaining ones.

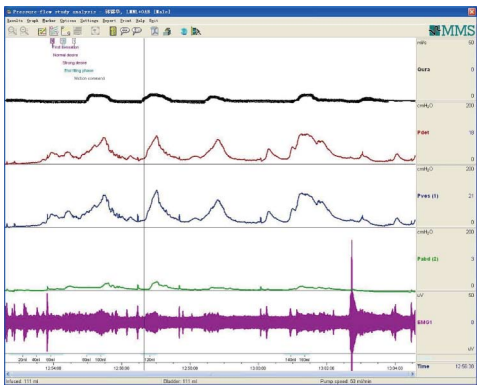


Figure 11 Repeated urodynamic curve of a male patient aged 37 years with urinary retention after lumbar tumor resection for one month, proved as neurogenic detrusor overactivity (*i.e.*, neurogenic detrusor hyperreflexia) and non-sympathetic overactivity (*i.e.*, detrusor-external sphincter dyssynergia).

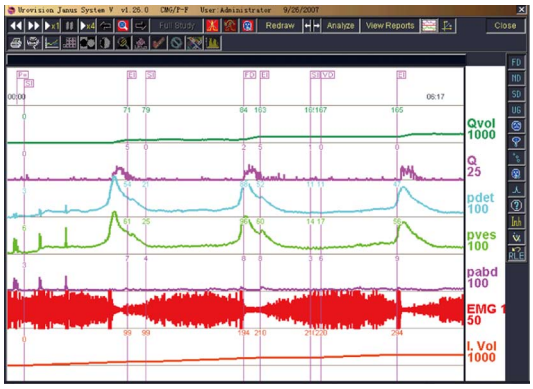


Figure 10 Repeated urodynamic curve of a male patient aged 80 years with urinary urgency and incontinence associated with acute myelitis for more than 11 years, proved as NDO, which had full response to administration of muscarinic blocker.

CMG-PFS-EMG curve: Normal CMG-PFS-EMG curve tells us how the bladder and urethra are performing storage function (bladder distention and urethra contraction) and how they are performing emptying function (at first the bladder contracts and the urethra actively relaxes[28], and then urinary flow produces). Active opening out of the urethra has major effects during emptying stage by stretching backwards the urethra and ceasing extension of urethral elasticity. So the external sphincter would actively relax during micturition: opening of the urethral tube, even to double the original diameter of the urethra[28]. After careful analysis and discussion of the UDS data, ideal options, such as IDO, ISO, DUA, SUI in LUTD, NDO, NSO (neurogenic sphincter overactivity, or detrusor-external sphincter dyssynergia, DESD), DUA in NLUTD, are produced. The process should be repeated more than one time if the curve is doubtful in order to gain a reproducible, stable and typical curve (Figures 9-11).

The main difference between NDO and IDO, or between NSO and ISO, is whether they are related to neurogenic origin or not. Grossly looking the patients may be non-neurogenic, just in the majority of patients with OAB (those with IDO), however, if more meticulous image examination, for example, brain functional magnetic resonance imaging (fMRI), is undertaken, some positive findings could be found in patients with OAB or LUTD responsive to Interstim (sacral neuromodula-

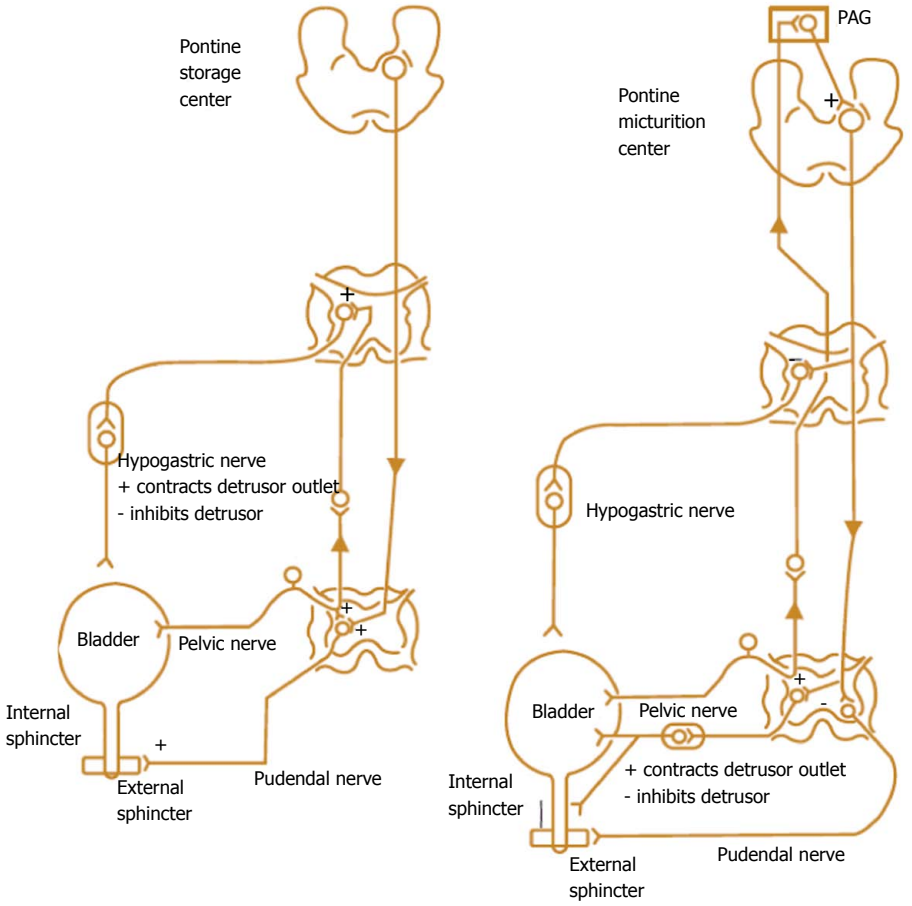


Figure 12 Nerve supply to the bladder and urethra and nerve signal transduction during storage and emptying phase[36]. PAG: Periaqueductal gray.

tion) treatment[29-31]. So many patients may be situated in the equivocal region between neurogenic and non-neurogenic LUTD.

There was some abnormal findings in fMRI in patients with Fowler's syndrome who were responsive to the Interstim procedure[29]. The primary abnormality of the syndrome may be an overactive urethra[29]. This central reflex and sacral guarding reflex have the same nature.

As far as small-vessel diseases of the brain affecting the deep white matter were concerned, they may be associated with some bladder abnormalities. Generally speaking, when we cared for elderly OAB patients, both the brain and the bladder should be looked at[30,31].

IDO and NDO may be treated by monotherapy or combination therapy[32-35]. And now, baclofen, a GABA agonist, may also go into the regimen[17,23]. Combination therapies with α1-blocker plus antimuscarinic, α1-blocker plus 5α-reductase inhibitor, α1-blocker plus PDE inhibitor, and α1-blocker plus 5-ARI have been attempted[32]. The pathophysiologic mechanisms and targets for pharmacotherapy for male LUTS, and nerve supply to the bladder and urethra are displayed below (Figures 12 and 13).

ISO and NSO are displayed by excellent EMG curves. Synonyms of ISO are dysfunctional voiding (DV), non-neurogenic neurogenic bladder, Fowler's syndrome, and Hinman syndrome[38-40]. Chronic idiopathic intestinal pseudo-obstruction (CIPO)[41] may co-exist with ISO.

In patients who complained of symptoms of frequency or urge may actually suffer from ISO, to which baclofen (a GABA-ergic receptor agonist) may be administered as a rational option and obtain good response shown by way of TL value[17,23].

Both storage and emptying symptoms may be caused by ISO. This double link could be explained by guarding reflex[8]. The storage symptom of the patient whose curve was shown in Figure 9 was resolved completely by administration of baclofen. Another female patient complained of urge incontinence for 10 yrs was also cured with baclofen after being confirmed as ISO (Figure 14). A woman aged 26 years complaining of poor-weak flow, voiding difficulty, intermittent or continuous catheterization for 18 years, and even receiving transurethral resection of the bladder neck twice, was also confirmed as ISO (Figure 15). She was eventually cured with baclofen too.

DUA and low compliance curve: DUA, either associated with NLUTS or LUTS, is known as detrusor underactivity, bladder underactivity, underactive bladder, or bladder underactivity/underactive bladder syndrome, either in analogy with the ICS definition of overactive bladder syndrome or not[42,43]. There are many different options as to its terminology, definition, and diagnostic methods. The approach is needed now to gain a consen-

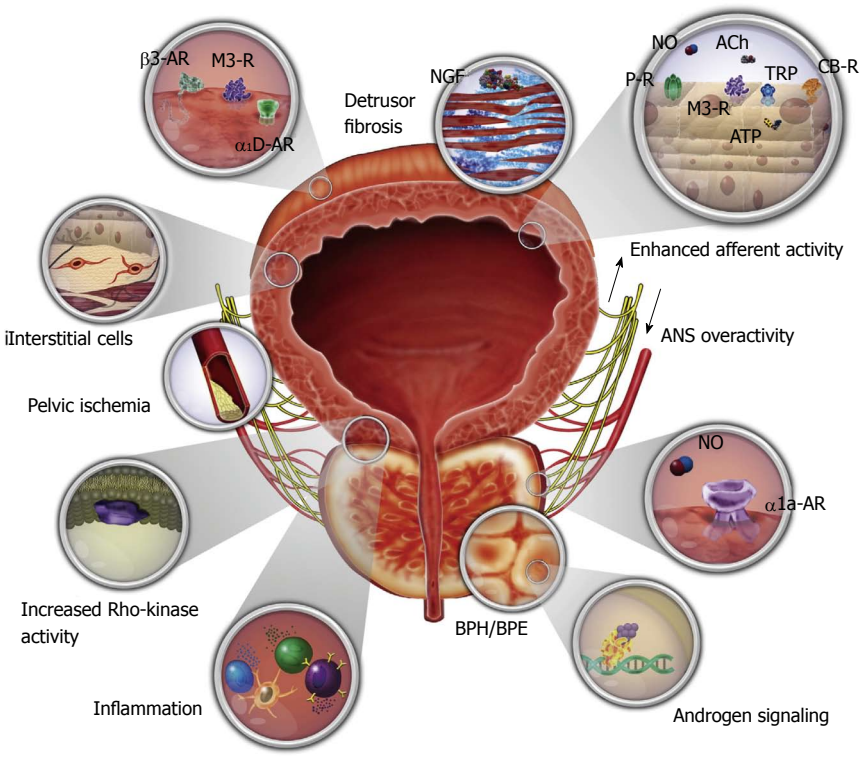


Figure 13 Various autonomic receptors of the bladder and urethra associated with pharmacotherapy for male symptoms of the lower urinary tract[37].

sus on these elements to allow standardisation of the literature and the development of optimal management[43]. No matter what the cause is, the main task of UDS is to show how the detrusor works: either unable to contract or relying on abdominal strain (Figure 16). DUA is associated with dysfunction of the sensory (afferent) nerve, the central nervous system (CNS), the efferent nerve and the target organ, the vesical detrusor itself. Furthermore, impaired voiding function has an age-associated prevalence[42] (Figure 17). As to the treatment of patients with DUA of any cause, the combination therapy (Chinese medicinal herbs, baclofen, and pyridostigmine) and continuous bladder drainage are proposed as feasible options[17]. After UDS, the patients with UDS patterns F and G could not pass urine at all and still needed catheterization, urinary diversion or even artificial urinary sphincter[44], but 56% and 12% of those with patterns F and G had their patterns changed into E or C, respectively, after such conservative treatment. The main function of Chinese medicinal herbs (common clubmoss herb, toothed achyranthes root, semen vaccariae, and so forth) were relaxing the urethral sphincter, decreasing outlet resistance, and promoting diuresis. Baclofen was used to relax the overactive sphincter, and pyridostigmine was used to strengthen the detrusor. And perhaps, the most important thing was absolute resting of the bladder without tube clamping for more than 4-6 wk[17]. Low frequency electrotherapy is also a rational option for selected female DUA patients suffering from neuromuscular deficiency[45].

Normal compliance is more than 10 mL/cmH_2O[46]. Low compliance could co-exist with or without DUA in patients with LUTS or NLUTS. The low bladder compliance patterns in patients with NLUTD had three groups[46]: gradual increase, Group A; terminal increase, Group B; abrupt increase and plateau, Group C. Careful analysis seeking for dominant disorder of the detrusor or sphincter is vital for the patients. Little or no detrusor contraction is needed for complete voiding in some women in whom normal sphincter relaxation is enough to finish the micturition process. These patients are considered to be "normal"[47]. This occult modality of DUA should be considered as asymptomatic DUA (Figure 18 A and B). Furthermore, in male patients with DUA and low compliance potentially related to neurogenic lesions, this pattern of voiding could occur too (Figure 18C).

Low compliance may lead to bilateral hydronephrosis in patients suffering from diabetes insipidus. We found that children with polyuria, nocturnal enuresis and MRI-confirmed pituitary abnormality (hypointensities on T1-weighted MRI) and diabetes insipidus usually had hydroureteronephrosis, enlarged bladder capacity and low bladder compliance at second-half storage phase. Their detrusor and sphincter function had to be evaluated carefully as the first procedure. If the detrusor could contract and sphincter could relax during the voiding phase, the prognosis is good (Figure 19), and vice versa.

Different opinions existed about the newly constructed somatic-autonomic reflex for patients complaining of dysuria and incontinence after spinal cord injury or with tethered spinal cord[48,49]. Whether the operation succeeds or not depends upon the exhibition of detrusor contraction and sphincter dyssynergia or synergia. We have shown the detrusor contraction with or without sphincter overactivity in some patients suffering from SCI who received a successful artificial somatic-autonomic reflex for bladder

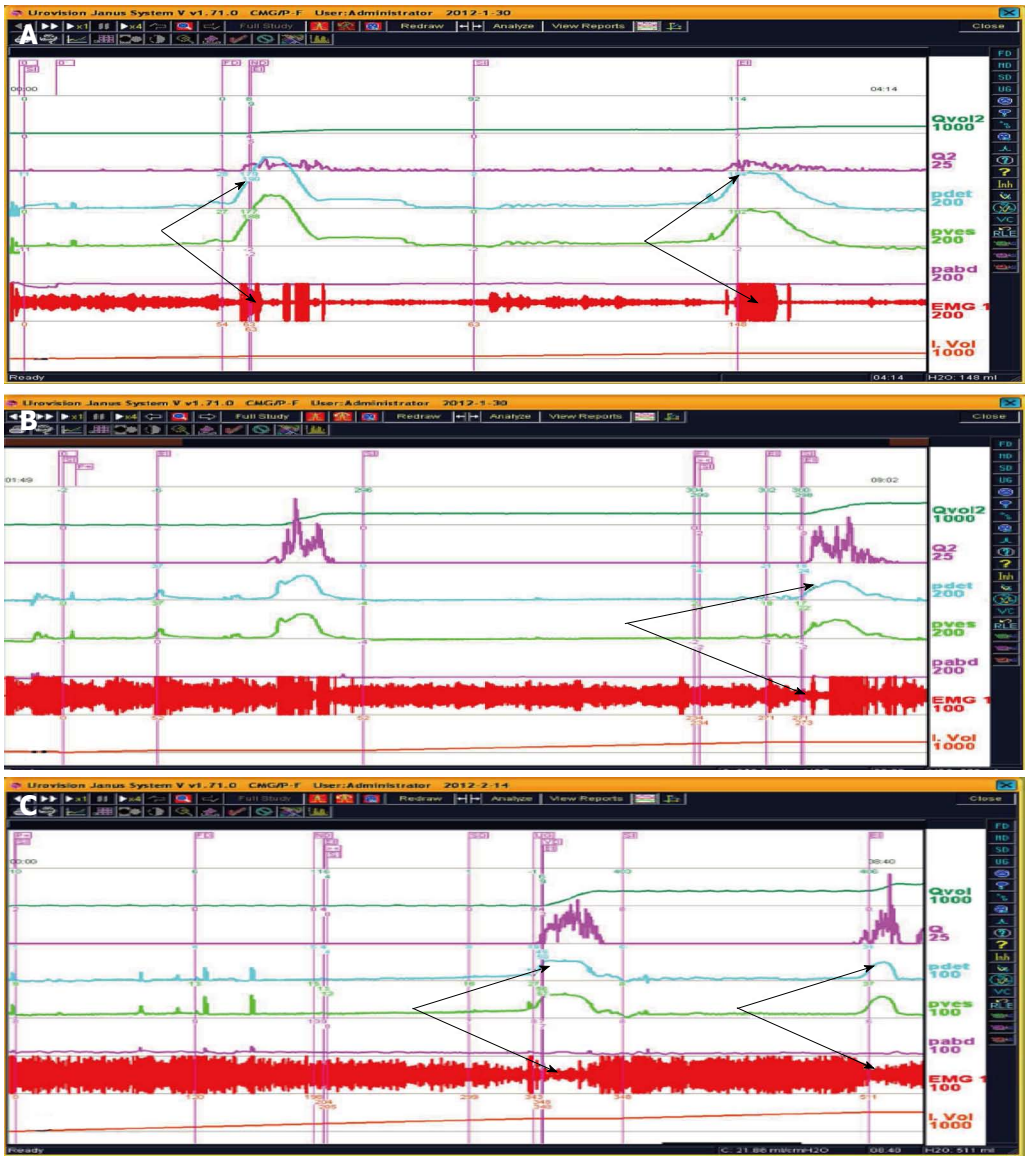


Figure 14 Severe urge incontinence caused by idiopathic sphincter overactivity was cured with baclofen. A: A 39-year-old woman presented with urge incontinence for 10 year. Urodynamics (UDS) showed that she had idiopathic sphincter overactivity (ISO); B: The ISO improved obviously after administration of baclofen for 2 wk; C: Six weeks after the first consultation, her ISO appearance disappeared absolutely.

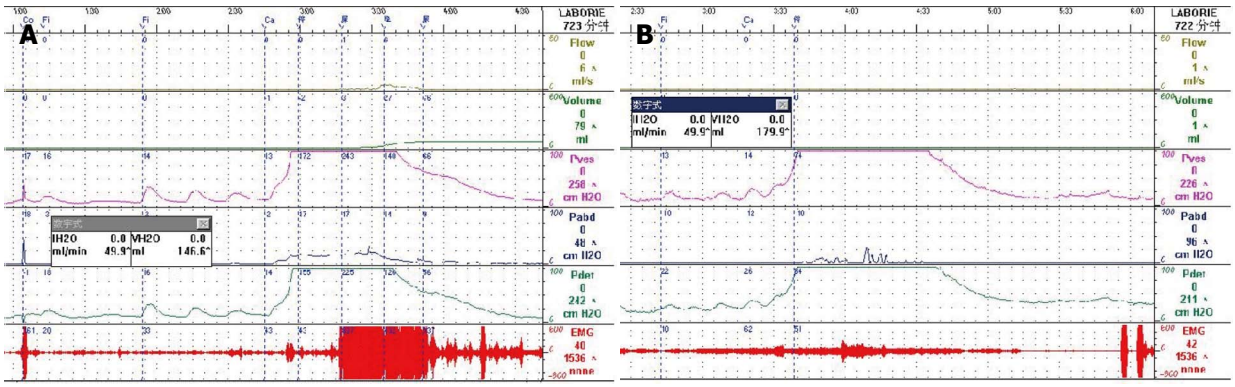


Figure 15 Refractory idiopathic sphincter overactivity-induced bladder outlet obstruction for 18 years cured with baclofen within three weeks. A: Urodynamic (UDS) curve of the woman aged 26 years whose complaints were poor-weak flow and voiding difficulty, undertaken before consultation; B: UDS curve undertaken four years ago.



Figure 16 Various detrusor underactivity manifestations coupled with idiopathic sphincter overactivity and abdominal straining. A: A woman complaining of urinary incontinence was confirmed with detrusor underactivity (DUA) and idiopathic sphincter overactivity (ISO); B: A male patient aged 86 years complaining of poor-weak flow after benign prostatic hyperplasia (BPH) operation was confirmed with DUA and abdominal straining with detrusor-sphincter synergia; C: A female patient suffering from bladder overdistention was confirmed with DUA and ISO; D: A female aged 55 years suffering from incontinence was proved with DUA, ISO and nearly normal Qmax.

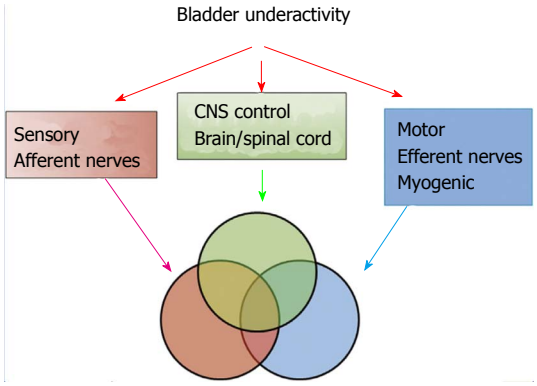


Figure 17 Mechanisms involved in bladder underactivity[42]. CNS: Central nervous system.

control in this institution (Figure 20).

UPP curve: UPP examination is one of the UDS menus and cannot be omitted casually. The UPP curve gives us functional parameter of the urethra and its morphological evaluation, which is very useful for surgical selection of male BPH patients (Figure 21).

LUTS and UDS findings inadequate to explain the disease

UDS findings other than abnormality, in short, subnormal or equivocal UDS findings, may lead to further seeking for potential pathologic factors. At this stage, cystourethroscopic examination, mucus membrane biopsies or imaging study of the LUT, and consult of associated physicians are necessary. Acidophilic cystitis[50] (Figure 22), slight bladder neck contracture, and glandular cystitis may be the possible responsive factors. In female and young male patients, BOO may be associated with inflammation of the bladder neck. In these patients, bladder neck obstruction or contracture, primary or secondary to longstanding ISO, squamous metaplasia or glandular cystitis-like appearance of the bladder neck are always observed. The lining of the bladder neck demonstrates a nontransitional epithelial appearance with epidermoid (squamous metaplasia) or glandular (adenomatous metaplasia) development and later formation of von Brunn's nests in the lamina propria. Squamous metaplasia or glandular cystitis-like lesions in the bladder neck may be responsible for primary bladder neck obstruction in female or young male patients. PFS data gained during emptying phase could reveal the nature of obstruction more precisely (Figure 23).

Extra or intra-LUT symptoms and UDS findings precede pathological findings

LUTS may display ahead of schedule in some disease related or not related with LUT. Multiple system atrophy

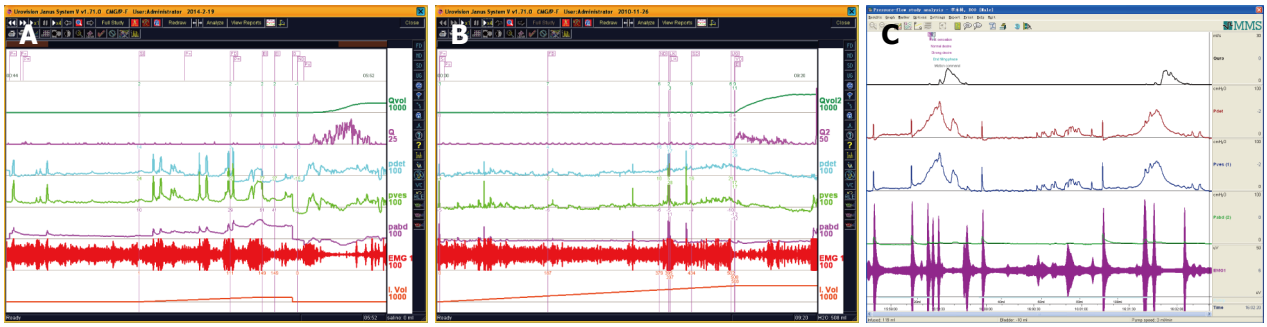


Figure 18 By means of efficient relaxation of the sphincter, satisfactory micturition fulfilled even without detrusor contraction in patients with symptoms of the lower urinary tract or neurogenic symptoms of the lower urinary tract. A: A female patient suffering from stress urinary incontinence had a satisfactory pressure-flow study; B: A woman passed urine fluently without action of the detrusor; C: A male patient with detrusor underactivity and low compliance voided by efficient sphincter relaxation.

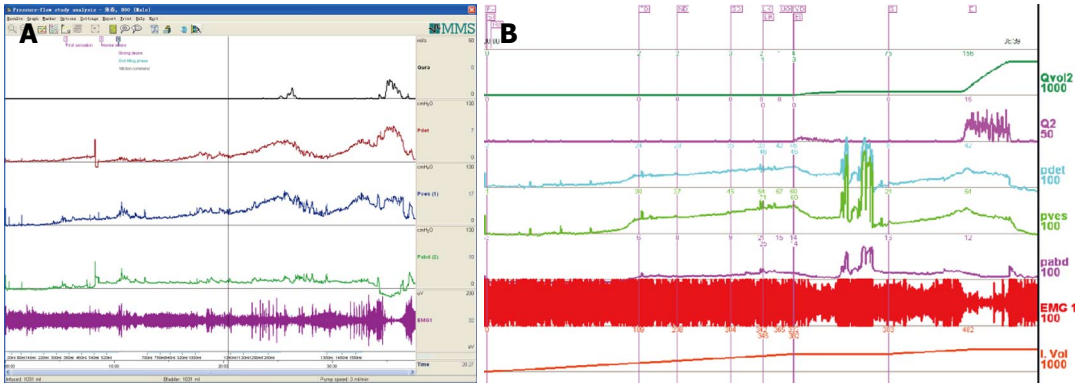


Figure 19 Bilateral hydroureteronephrosis due to bladder low compliance associated with diabetes insipidus and pituitary disorder. A: A male patient aged 42 years complaining of polyuria had low compliance and intact detrusor and sphincter. The residual urine reached 1550 mL after 700 mL saline voided out; B: A male patient aged 23 years had low compliance and no detrusor underactivity or detrusor-external sphincter dyssynergia. His detrusor could contract violently based on raised detrusor pressure and void 900 mL urine.

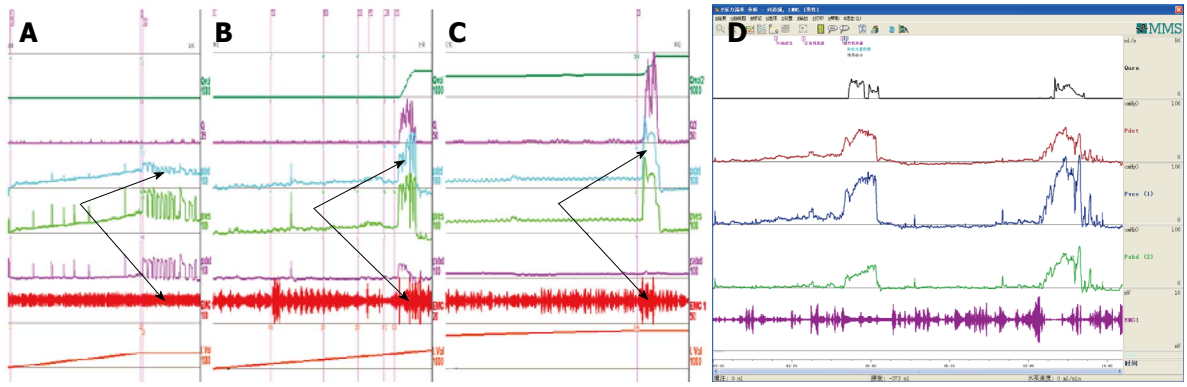


Figure 20 Effective neuroanastomosis resulting in recovery of micturition reflex and the patients getting rid of catheter-dependent state. A female patient aged 46 years had her new reflex succeeded 5 and 9 years after the anastomosis (B and C) as compared with preoperation (A); D: A male patient aged 39 years gained satisfying lower urinary tract function 2 years after the anastomosis, especially with detrusor-sphincter synergia now.

(MSA), multiple sclerosis (MS), spinal cord tumors, idiopathic normal pressure hydrocephalus (iNPH)[51,52] and other occult neurogenic lesions may be responsible for slight degree of DUA or incontinence in some patients (Figure 24). Two patients presented in Figure 23 showed extra or intra-LUT symptoms: incontinence (A) and hydronephrosis (B). The former was proved as MSA two years later after our first consultation, and the potential neurogenic lesion of the latter has not been found so far. Perhaps functional MRI of the nervous system or some forthcoming techniques may aid diagnosis. Whether or not the patients complained of incontinence or hydronephrosis depends on the safe volume of the bladder (bladder volume before *Pdet* reaches 40 cmH_2O) irrespective of the existence of vesico-ureteral reflux. If the functional bladder capacity exceeds it, both condi-

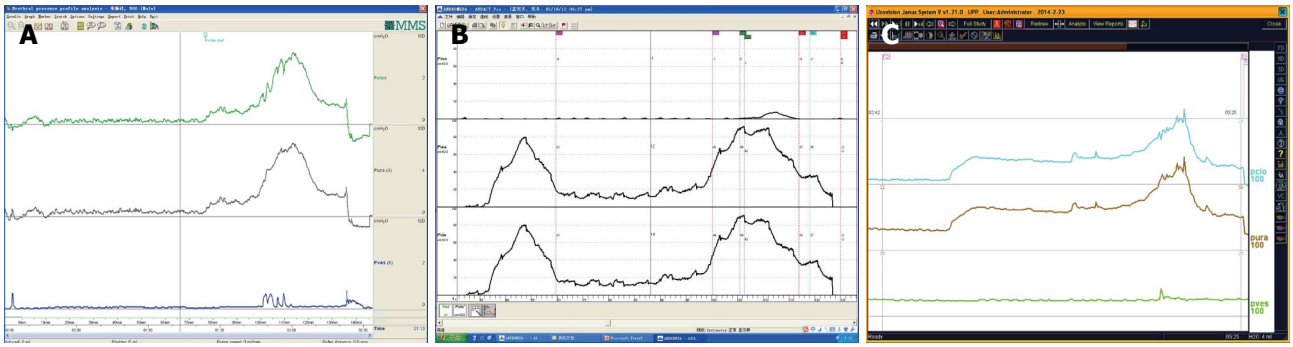


Figure 21 Typical urethral pressure profilometry curves in male patients suffering from benign prostatic hyperplasia. A: Classic urethral pressure profilometry (UPP) curve of male patients with benign prostatic hyperplasia induced bladder outlet obstruction; B: Bimodal UPP curve of patients with enlarged middle lobe; C: Severely big prostate possesses a long functional profile length.

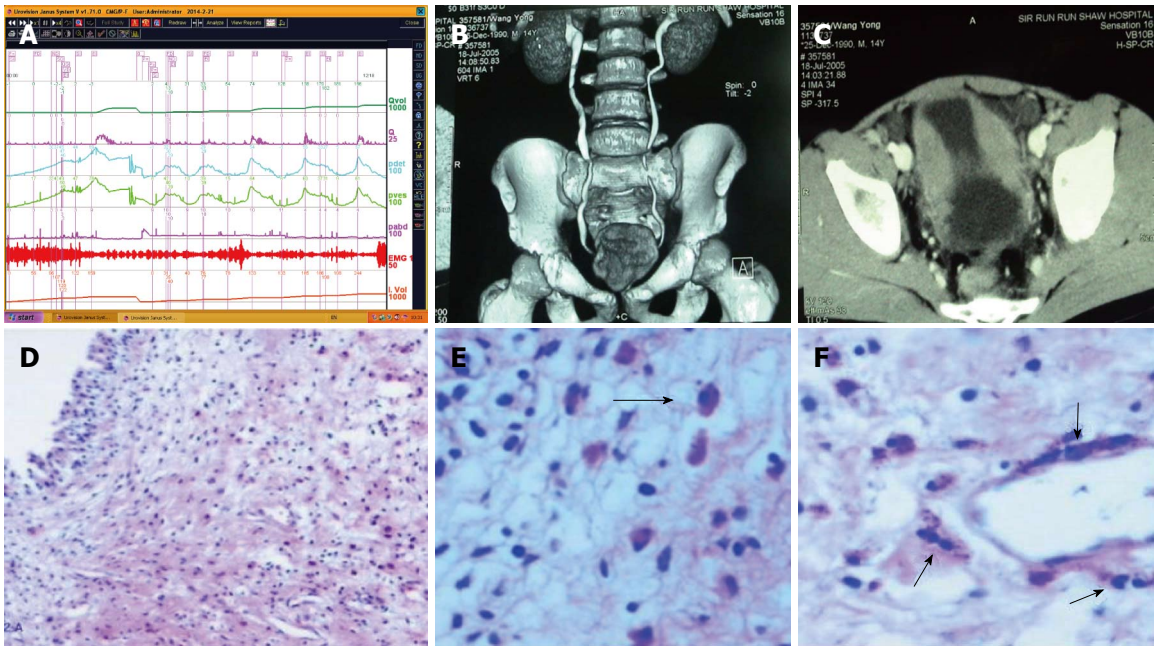


Figure 22 Acidophilic cystitis resulting in poor-weak flow and vomiting in a 15-year-boy was proved as normal detrusor and sphincter function, low compliance, and cured with steroid[50]. A: Although there was neither bladder outlet obstruction (BOO) nor idiopathic sphincter overactivity (ISO), his compliance was low; B: Bladder computed tomography (CT); C: Bladder CT with 3D imaging; D-F: Bladder biopsy specimen: eosinophilic cell suffusion infiltration and proliferation of spindle cells (HE staining).

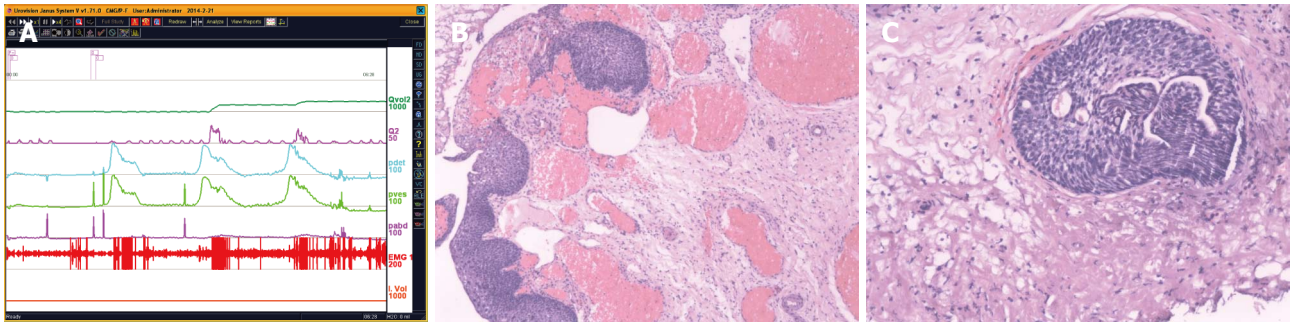


Figure 23 Squamous metaplasia or glandular cystitis of the bladder neck may be caused by longstanding idiopathic sphincter overactivity and produces bladder outlet obstruction-like symptoms. A: A 58-year-old woman presented with urinary obstruction and idiopathic sphincter overactivity and transurethral resection of bladder neck was performed with satisfactory results; B: The bladder transitional epithelium had entered the process of metaplasia, and Brunn nest was forming; C: A Brunn's nest was evident and cavity was in the nest core.

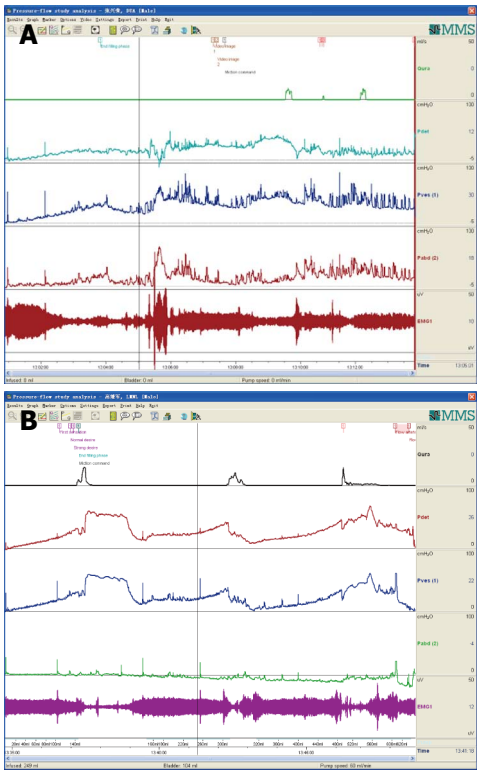


Figure 24 Extra or intra-lower urinary tract symptoms and urodynamic findings in some cases precede pathological abnormality. This phenomenon makes us be convinced of the present medical state. A: A 60-year-old male patient complaining of poor-weak flow and nocturia was proved as having detrusor underactivity (DUA) and low compliance, not responding to TURP, and was proved as having multiple system atrophy 2 years after the first consultation; B: A 52-year-old male patient having received bilateral nephrostomy because of hydronephrosis, was proved as having DUA and low compliance. He could void by efficient sphincter relaxation. However, no abnormal image sign was found at time of consultation.

tions will occur. If the functional capacity is less than the safe volume, distention of the upper urinary tract will not emerge. The latter patient had no imaging confirmed vesico-ureteral reflux indeed. However, bilateral nephrostomies were undertaken because of severe hydronephrosis at our first consultation.

CONCLUSION

Clear definition of UDS entities by means of cutoff values is ideal; however, descriptive recording is often more preferable in practice. TL value is a clear cutoff value for diagnosis of ISO or NSO. By overall view of the whole performance process of micturition (*i.e.*, both the detrusor and sphincter during storage and emptying phases), normal or abnormal UDS findings give us concrete and demonstrable contour of LUTS entities, such as NA, IDO, ISO, DUA, NDO, NSO, BOO and SUI, guide the further option if LUTS and UDS findings are inadequate to explain the disease, and make us be convinced of the present medical state since in some cases extra or intra-LUT symptoms and UDS findings precede pathological abnormality.

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MINIREVIEWS

SCAD syndrome: A vicious cycle of kidney stones, CKD, and AciDosis

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Abstract

Cumulative evidence has shown that kidney stone formers are at high risk for developing end-stage renal disease (ESRD) and cardiovascular disease. The aim of this mini-review is to summarize the present knowledge about the close relationships among kidney stone formation, chronic kidney disease (CKD), and plasma and urine acidosis (SCAD). Part of the cause of the positive relationships between higher risk of developing ESRD and cardiovascular diseases in stone formers may be explained by inflammation and cell death due to the components of kidney stones. In CKD patients, acidic urine and loss of anti-crystallization factors may cause stone formation. Acidosis can promote tissue inflammation and may affect vascular tone. Correction of plasma and urine acidosis may improve renal and cardiovascular outcome of stone formers and CKD patients. More intensive and long-term interventions, which include correction of plasma and urine pH in patients with reduced renal function and correction of urine pH in patients with normal renal function, may be considered in treating patients with SCAD syndrome.

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Key words: Endoplasmic reticulum stress response; End-stage renal disease; pH; Endothelial; Epithelial

Core tip: This minireview is written for urology and internal medicine physicians who see kidney stone formers in their daily practice. It is our responsibility to make more serious consideration on the long term outcome of developing end-stage renal disease and cardiovascular diseases in kidney stone formers. The significance of appropriate intervention on acidic condition for these subjects are often neglected. By naming "SCAD syndrome", we can promote more attention on this significant, but sometime forgotten pathological condition.

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INTRODUCTION

Patients with kidney stones usually visit only urologists. However, cumulative evidence indicates that stone formers are at high risk for developing end-stage renal disease (ESRD) and cardiovascular disease. This review will summarize present knowledge about the relationships among stone formation, chronic kidney disease (CKD), and plasma and urine acidosis. Incorporation of these three pathological conditions is needed for the awareness

of urologists and general physicians. To promote such awareness, we would like to give the name SCAD (stones, CKD, and AciDosis) syndrome to this pathological condition.

THE RELATIONSHIPS BETWEEN STONE FORMATION AND RENAL INJURY

Epidemiology

In patients with rare genetic diseases such as hyperoxaluria, cystinuria, Dent disease, and adenine phosphoribosyltransferase deficiency, it is well established that kidney stone formation causes renal damage[1,2]. In contrast, little attention has been paid to the pathological role of commonly observed kidney stones in the development of renal damage. As was reviewed by Rule *et al*[1] and Gambaro *et al*[2], cumulative evidence has shown a significant association between kidney stone formation and the risk of developing ESRD. Alexander *et al*[3] clearly showed in their prospective cohort study that even a single episode of kidney stones can cause a 2.16-fold higher risk for developing end-stage renal failure in both males and females. Hippisley-Cox and Coupland demonstrated a significant association between kidney stones and the development of end-stage kidney failure only in females in their prospective cohort study[4]. Chen *et al*[5] found an association between sonographically-determined nephrolithiasis and the estimated glomerular filtration rate (eGFR) in their cross-sectional study. Saucier *et al*[6] determined in their case-controlled retrospective cohort study that struvite stone formers and uric acid stone formers are more likely to develop CKD. These associations may not be simply explained by renal damage due to occlusion of the tubules or the urinary tract by stones. Alexander *et al*[7] discovered that a single kidney stone episode can cause a 1.40-fold higher risk of acute myocardial infarction and a 1.26-fold higher risk of stroke.

Mechanisms

Part of the cause of the positive relationship between higher risk of developing ESRD and cardiovascular disease in stone formers may be explained by inflammation and cell death due to the components of the stones. Oxalate has been shown to activate inflammatory cytokine signaling pathways, including the interleukin (IL)-2 and IL-6 signaling pathways, in renal tubular cells[8,9]. Oxalate has also been shown to induce cellular death in vascular endothelial cells, which is enhanced by hypoxia[10,11]. Crystalized uric acid activates toll-like receptors 2 and 4 and promotes inflammation[12]. An increase in intracellular uric acid causes oxidative stress[13]. In addition, during the process of uric acid generation, xanthine oxidase causes oxidative stress[14-16]. Struvite stones are generated at the place of inflammation due to bacterial infection[17]. Hamamoto *et al*[18] demonstrated the similarity in the mechanisms of pathogenesis for stone and atherosclerosis. They have identified the involvement of osteopontin in both pathological condition.

THE RELATIONSHIPS BETWEEN SERUM AND URINE ACIDOSIS AND STONE FORMATION

Epidemiology

Due to the higher prevalence in stone formers of developing CKD, it is difficult to clearly demonstrate the higher incidence of stone formation in CKD subjects. However, several mechanisms have been identified to help speculate that CKD subjects are at high risk for crystal formation.

Mechanisms

According to Coe *et al*[19], there are two major pathways for kidney stone formation. One pathway is based on plaque formation in the basement membrane of the thin limbs of loops of Henle. Stone is formed by the overgrowth of plaque and detaches to the tubular space. Plaque formation correlates with urine volume, pH, and calcium. Another pathway is crystallization in the tubular space. Supersaturated solute, including uric acid or calcium oxalate in the urine, forms crystals. The solubility of these solutes is urine pH dependent. These substances are less soluble in low pH conditions. Overall, urine pH has already been established as the major cause of kidney stone formation. In CKD subjects, reduced eGFR is often associated with decreased excretion of calcium and decreased urine concentration capability[20]. Indeed, Marangella *et al*[21] have demonstrated that subjects with lower GFR may have a lower recurrence rate of calcium stones. Along the same line, metabolic acidosis is often associated with CKD due to the limited capability of acid excretion into urine. But, in contrast to calcium, this does not mean that urine pH is high in CKD subjects, for the following reason. To excrete sufficient acid with limited reduction in urine pH, the kidney uses titration acids, including NH_4^+ and $H_2PO_4^-$. These titration acids can also be reduced in the urine of CKD subjects. Therefore, the pH level easily becomes low in the urine of CKD subjects. Related to this idea, Stettner *et al*[22] recently showed that sulfatide-deficient mice developed metabolic acidosis with lower urine pH in response to acid overload. Low pH causes the crystallization of uric acid and calcium oxalate by limiting their solubility. Systemic acidosis may also promote stone formation by increasing the solute overload into urine. Acidosis promotes calcium release from bone. Starke *et al*[23] showed that, in renal transplant patients, normalization of metabolic acidosis by the administration of potassium citrate has the potential to preserve bone quality, as assessed by bone biopsy. Regarding the molecular mechanism, Geng *et al*[24] showed that serum bicarbonate inhibits osteoclast formation though the activation of soluble adenylyl cyclase. Krieger *et al*[25] showed that metabolic acidosis directly increases fibroblast growth factor 23 (FGF23)

mRNA and protein in mouse bone. CKD and metabolic acidosis may also affect the expression of the Tamm-Horsfall glycoprotein and other factors that inhibit the growth of crystal[26].

THE RELATIONSHIPS BETWEEN SERUM AND URINE ACIDOSIS AND KIDNEY INJURY

Epidemiology

In CKD subjects, high and low serum bicarbonate levels are associated with a risk of mortality and the development of ESRD. Kovesdy *et al*[27] showed in their retrospective cohort study that the group of patients with serum bicarbonate level of 26-29 mmol/L had the lowest mortality rate. The retrospective cohort study of Navaneethan *et al*[28] showed that the group of patients whose serum bicarbonate level was 23-32 mmol/L had the lowest mortality rate. Kanda *et al*[29] showed in their retrospective cohort study that subjects with high serum bicarbonate level (28.8 mmol/L) are less likely to develop ESRD than patients with low serum bicarbonate level (23.4 mmol/L). Recently, several studies have shown the beneficial effect of correcting metabolic acidosis on the decline of GFR in CKD subjects. Susantitaphong *et al*[30] systematically reviewed the effects of sodium bicarbonate in the long term (> 2 mo), and showed an improvement in the eGFR and a lower incidence of initiating dialysis therapy. The beneficial effect of alkali therapy on eGFR in CKD subjects has been shown by the administration of potassium citrate[31].

In non-CKD subjects, acidic urine has been shown to be associated with diabetes and metabolic syndrome (Mets)[32,33]. Maalouf *et al*[32] showed a positive association between acidic urine and a number of the components of Mets in non-CKD subjects. These authors speculated that part of this association may be explained by impaired urine buffering capability due to insulin resistance.

Mechanisms

As was reviewed by Souto *et al*[34], metabolic acidosis can lead to the development of several risk factors for cardiovascular disease, including inflammation, hypertension and disturbed glucose tolerance, due to decreased insulin sensitivity.

Effects on inflamation: Bellocq *et al*[35] and Kellum *et al*[36] showed that acidic condition promotes tumor necrosis factor alpha (TNF-α)-dependent nuclear factor kappa-light-chain-enhancer of activated of B cells (NF-κB) activation in macrophages. More recently, Rajamäki *et al*[37] and Edye *et al*[38] showed pH-dependent secretion of IL-1β and activation of caspase-1 in macrophages. These investigators also have demonstrated the significant role of damage-associated molecular patterns (DAMPs) in this process. Nikoletopoulou *et al*[39] showed that acidosis switches TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis to regulated necrosis in cancer cells. Further investigations are expected to test whether cell death under an acidic condition also shows switching from apoptosis to necrosis. An increase in the proportion of necrosis may promote more inflammation to the injured area. Chen *et al*[40] showed that acidic condition induces the production of cell adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1), E-selectin, and vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells. Acidic condition has been shown to activate complement system. Emeis *et al*[41] and Curley *et al*[42] has shown that acidosis activates C3 and C5. Morita *et al*[43] showed that the administration of sodium bicarbonate in subjects with proteinuria decreases the renal excretion of complement activation products (CAP).

Effects on vessel function: The role of acidosis on vascular tone is controversial. As reviewed by Smith *et al*[44], Smith *et al*[45] and Wray *et al*[46], extracellular and intracellular decreases in pH have been shown to promote vasoconstriction. However, several studies have shown that acidosis enhances nitric oxide (NO) production and promotes vasodilatation[47]. Part of this inconsistency may be explained by oxidative stress. The intracellular acidic condition causes an increase in the fraction of free iron to protein-bound iron in cells, which causes oxidative stress by a Fenton-type biochemical reaction[48]. Oxidative stress itself has been shown to promote vasoconstriction. It reacts with NO and generates the highly toxic peroxynitrite anion ($ONOO^-$). Enhanced NO production also causes high endothelial permeability[49]. Dong *et al*[50] showed that endothelial cells detect the extracellular acidic condition by the proton-sensing G-protein coupled receptor 4 (GPR4), which activates inflammation and the endoplasmic reticulum (ER) stress response.

Effects on tubules: In epithelial cells, adaptation mechanisms to acidic conditions have been well investigated. This is because epithelial cells, including in the intestine and the kidneys, are in the location to be exposed to acidic condition even under physiological conditions. Therefore, these cells are resistant to extracellular acidification. Sodium-hydrogen exchangers (Na^+/H^+ exchangers, NHEs) have an established role in the maintenance of internal pH. Muthusamy *et al*[51] has shown that, in intestinal epithelial cells, acid induces the NHE2 Na^+/H^+ exchanger to regulate internal pH through the induction of early growth response protein 1 (EGR-1). Preisig *et al*[52] and Kwon *et al*[53] showed an increase in NHE3 and the Na/HCO3 cotransporter (NBC1) in the renal proximal tubules. Odunewu and Fliegel showed that acute sustained intracellular acidosis activated NHE1 in human embryonic kidney 293 (HEK293) cells and in Madin-Darby canine kidney (MDCK) cells[54]. Renal epithelial cells have also been shown to activate glutamine transporters, including SN1[55] and mitochondrial

glutamine transporter[56], to increase the production of ammonia, which acts as a titration acid. Ibrahim *et al*[57] proposed that, in proximal tubules, a change in intracellular pH may promote the ER stress response followed by the stabilization of corresponding mRNAs for ammoniagenesis. As for the protective mechanism of renal tubular cells against extracellular acidification, Namba *et al*[58] have demonstrated the significant role of autophagy in the proximal tubules.

CONCLUSION

Kidney stone formation, chronic kidney disease or cardiovascular disease, and metabolic acidosis influence each other and form a vicious cycle. Even a single episode of stone formation in a stone former or an asymptomatic stone former may place those persons at higher risk for the development of CKD and cardiovascular disease. More intensive and long-term interventions, which would include correction of plasma and urine pH in subjects with reduced renal function and correction of urine pH in subjects with normal renal function, may be considered in the strategy for treating subjects with SCAD syndrome.

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Laparoscopic single site surgery: Experience in pediatric urology

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Abstract

Laparoendoscopic single-site surgery (LESS) has been developed to benefit patients by enabling surgeons to perform scarless surgery. In this review we aimed to summarize and critically analyze the available evidence on the current status and future prospects for LESS in pediatric urology, with special emphasis on our experience with LESS in children. The clinical data available clearly demonstrate that LESS can safely and effectively be performed in a variety of pediatric urology settings. As clinical experience increases, expanding indications are expected to be documented and the efficacy of the procedure to improve. So far, the quality of evidence of all available studies remains low; mostly being small case series or case-control studies from selected centers. Thus, the only objective benefit of LESS remains improved cosmetic outcome. Prospective randomized studies are awaited to determine which LESS procedures will be established and which are unlikely to stand the test of time. Technological advances hold promise to minimize the challenging technical nature of scarless surgery. In this respect, robotics may be a driving force in the development of LESS.

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Key words: Laparoendoscopic single-site surgery; Pediatric urology; Laparoscopy; Review

Core tip: Laparoendoscopic single-site surgery (LESS) has been developed to benefit patients by enabling surgeons to perform scarless surgery. Clinical data demonstrate that LESS is safe and effective in many pediatric urology settings. As clinical experience increases, expanding indications are expected, along with improved efficacy. Prospective randomized studies are awaited to determine whether LESS procedures will be established as routine and will be able to stand the test of time. Technological advances hold promise to minimize the challenging technical nature of scarless surgery. In this respect, robotics is likely to drive a major paradigm shift in the development of LESS.

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INTRODUCTION

Laparoscopic surgery is beginning to gain acceptance as a standard of care in many intra-abdominal procedures in adult and pediatric urology[1]. Today, laparoscopic procedures are commonly performed and have become widely accepted as alternatives to open surgery, if not the gold standard in some procedures, such as radical or partial nephrectomy[2]. Even the more technically demanding procedures, such as laparoscopic pyeloplasty, laparoscopic-assisted bladder reconstruction, and laparoscopic ureteral reimplantation, have achieved widespread acceptance and are now routinely performed at many centers worldwide. With increasing experience



Figure 1 Laparoscopic single site surgery.

in the laparoscopic environment, efforts are now directed at further minimizing morbidity and improving cosmetic outcomes. This has led to the development of techniques, multichannel single-access ports, and novel bent/articulating instruments that allow the laparoscopic procedure to be performed through a single skin incision, often hidden within the umbilicus or utilizing the nature orifices of the human body in order to seal surgical incisions completely. Following this concept natural orifice transluminal endoscopic surgery (NOTES) and laparoendoscopic single-site surgery (LESS) have been developed in an attempt to reduce further the morbidity and scaring associated with surgical intervention[3-5]. Conceptually, these techniques share a common underlying hypothesis that has driven their development-namely, that a reduction in the number of transcutaneous points of access may benefit patients in terms of port-related complications, recovery time, pain, and cosmesis by potentially performing scarless surgery. The first documented one-port single-incision laparoscopy was cholecystectomy in 1997. Ten years later, the first single-port nephrectomy was done. Since then urologists have successfully performed various procedures with LESS, including partial nephrectomy, pyeloplasty, orchiectomy, orchiopexy, varicocelectomy, ureterolithotomy, sacrocolpopexy, renal biopsy, renal cryotherapy, and adrenalectomy[6,7].

In this review, we describe the rationale of the technique, the current clinical applications, the advantages and disadvantages compared to standard laparoscopy, and the results of LESS in pediatric urological surgery, with some attention in robotic surgery.

DEFINITION

LESS

LESS is a minimally invasive surgical procedure in which the surgeon operates almost exclusively through a single entry point, typically the patient's navel. Unlike a traditional multiport laparoscopic approach, LESS leaves only a single small umbilical scar, usually not larger than 2 cm. This particular access can be achieved through a single fascial incision site with a single trocar with multiple ports, or through a single skin incision site with multiple fascial incisions with individual trocars. The most popular technique is the first mentioned above: a single port with multiple channels. Like conventional laparoscopy, there are two principal approaches for renal, adrenal and ureteral surgery: transperitoneal and retroperitoneal[8]. Although the first mentioned above is the best known and usually performed, today sufficient clinical studies have shown the effectiveness and safety of LESS through a retroperitoneal approach, especially in nephrectomy for nonfunctioning kidney and in other extirpative retroperitoneal procedures[9].

With the time and development of the technique, the concept of LESS was diversified and the surgeons proposed different acronyms for LESS and associated procedures. They include: single port access, single incision laparoscopic surgery (SILS), natural orifice transumbilical surgery, transumbilical endoscopic surgery, single-access site laparoscopic surgery, single-site access, one-port umbilical surgery, transumbilical laparoendoscopic single-site surgery, transumbilical laparoscopic assisted surgery, and embryonic natural orifice transluminal endoscopic surgery[10-14]. The common factor is a single small skin incision, usually at the umbilicus (Figure 1).

Robotic LESS

Robotic LESS (R-LESS) uses the Da Vinci Surgery System *via* a one single-port approach to improve ergonomics that limit conventional single-port laparoscopy[15].

HISTORY

Minimally invasive surgery is a changing and evolving field. Since the first documented laparoscopic procedure in humans performed by Hans Christian Jacobaeus in 1910, there has been great progress that has expanded throughout the surgical specialties. In 1918, Goetze developed the first automatic pneumoperitoneum needle. In 1929, Kalk introduced the forward oblique (135 degrees) view lens systems, and in 1938, Veress developed a specially designed spring-loaded needle. Veress did not promote the use of his needle for laparoscopic purposes. He used the Veress Needle for the induction of pneumothorax. To date, the Veress Needle is the most important instrument to create pneumoperitoneum. The real credit for videoscopic surgery goes to Hopkins, who discovered in 1953 the rigid rod lens system that revolutionized the field of laparoscopic surgery. As a result of this development, in 1970, gynecologists started to embrace laparoscopy and thoroughly incorporated the technique into their practice. General surgeons, despite their exposure to laparoscopy remained confined to traditional open surgery until 1977, when the first laparoscopic assisted appendectomy was performed by Dekok. In that setup, the appendix was exteriorized and ligated outside. In the same year, Semm first demonstrated the endoloop suturing technique in laparoscopic surgery. The first documented laparoscopic cholecystectomy was performed



Figure 2 QuadPort + (Olympus) port system.

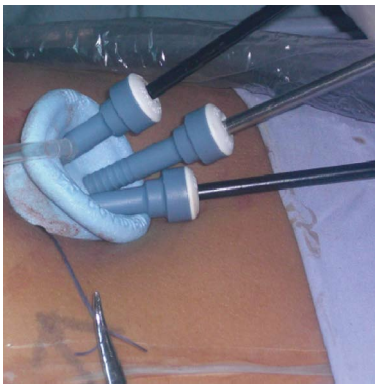


Figure 3 Single incision laparoscopic surgery (Covidien) port system.

by Mühe in Germany in 1985. In 1994, the first robotic arm was designed to hold the telescope, and in 1996, a live telecast of laparoscopic surgery was performed remotely *via* the internet (robotic telesurgery)[16]. As part of the natural development of minimally invasive surgery, in the late 1990s, LESS emerged[3]. The reason for the development of this type of procedure rests on the premise *"LESS pain, LESS scar"*, but the practical advantages in the field are many more than those. As already mentioned, the first urological use of LESS was reported in 2007 with the completion of single-port nephrectomy for a small nonfunctioning kidney, as well as transperitoneal ureterolithotomy[3,6]. To date, there is extensive experience with LESS in adult and pediatric urology in extirpative and nonextirpative procedures such as pyeloplasty, varicocelectomy and orchidopexy[3]. In other specialties such as gynecology, LESS has been used for several years, making single incision laparoscopic tubal ligation one of the most popular procedures in that field[17-19].

EQUIPMENT

Access devices

Multichannel ports can be used during LESS as one access approach. These devices allow for the insertion of instruments and a camera and involve a single fascial incision. TriPort (Advanced Surgical Concepts, Bray, Ireland) is the best-known FDA-approved access system. The size of the TriPort is fully adjustable, and allows a series of instruments to be introduced into any sized abdominal incision, from a 5 mm incision up to a hand-assisted laparoscopic surgery incision. Each device consists of a retractor component and a valve component, where the instruments are inserted. The valve component of TriPort is made of a unique elastomeric material that allows the passage of standard laparoscopic instruments and scopes simultaneously. The TriPort has three inlet valves: one for a 12-mm instrument and two for 5-mm instruments. QuadPort (Olympus, Advanced Surgical Concepts) is also available, and has four ports: two inlets for 12-mm instruments and two for 5-mm instruments. A separate insufflation port is provided through the valve housing in both devices. The high elasticity of the gel valve allows the removal of small specimens, whereas larger specimens are withdrawn into the distal end of the port and removed simultaneously with the device at the end of the procedure (Figure 2).

The Uni-X Single Port Access Laparoscopic System (Pnavel Systems, Cleveland, OH, United States) is a single port with three working channels, which all accommodate specialized 5-mm laparoscopic instruments. The device is placed through an open access technique and requires a 2-cm fascial incision. Once passed into the abdomen, the port is anchored in place using fascial sutures that are placed before attaching the device to the patient. As with TriPort, Uni-X has a separate valve port for insufflation. Once the procedure is complete, the port is untied and the specimen is removed through the initial incision[6]. The GelPOINT system from Applied Medical Technology (Brecksville, OH, United States) accommodates varying abdominal walls and incision sizes, provides continuous access, and ensures improved articulation of 5-12-mm instruments. The Alexis wound protector/retractor offers atraumatic retraction and protection, maintains moisture at the incision site, while providing convenient extracorporeal resection and specimen retrieval. The SILS Port designed by Covidien Tyco Health Care (Mansfield, MA, United States) consists of a blue flexible soft-foam port, with access channels for three cannulae. The 5-mm cannula may be interchanged at any time during the procedure with a 5-12-mm cannula. The SILS Port adapts its configuration to the size of the cannulae while maintaining pneumoperitoneum (Figure 3). We have utilized in all our patients the SILS Port (Covidien, Tyco Health Care). It is our preferential access devise for all LESS procedures. It has a foam port that expands after insertion to prevent air leakage. It is significantly cheaper compared with the others at least on the Israeli market. The access port is easy inserted *via* a 2-cm incision that is performed within the umbilicus. During the procedure the 5-mm trocars can be easily replaced by 10-12-mm trocars during surgery when needed.

Hand instruments

A basic tenet of laparoscopic surgery involves triangu-



Figure 4 Sixty centimeter, 5-mm telescope with right-angle light cord adapter (Karl Storz).

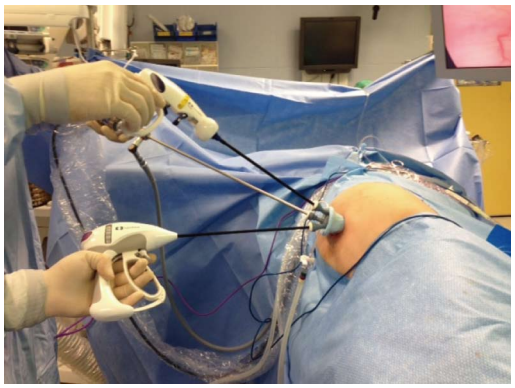


Figure 5 Operating field.

lation of instruments so as to produce adequate intracorporeal working space for anatomical dissection and manipulation of tissues. The parallel and close proximity of the right-hand and left-hand instrument shafts of standard laparoscopic instruments through a solitary port results in crowding of the laparoscope and the instruments, preventing appropriate triangulation. Articulated instruments were designed to overcome these challenges. Some of articulated instruments include the SILS Multiple Instrument Access Port manufactured by Covidien and the Laparo-Angle Articulating Instruments made by Cambridge Endoscopic Devices (Cambridge, MA, United States), articulating laparoscopic graspers (*e.g.*, Real Hand; Novare Surgical Systems, Cupertino, CA, United States and Autonomy Laparo-angle; Cambridge Endo, Framingham, MA, United States), endoshears (Cambridge Endo), and laparoscopic needle drivers (Cambridge Endo). A combination of conventional and flexible (articulating) instruments provides improved intraoperative ergonomics and further facilitates dissection during surgery.

Telescope

There are two types of telescope for LESS: 30° and 0°. Pelvic procedures require the use of a 30° lens directed upwards, whereas upper tract procedures need either a 30° lens facing downward or a 0° lens[20] (Figure 4).

We have utilized a 60-cm, 5-mm, 0° telescope (Karl Storz, Germany) for all LESS intra-abdominal or renal surgery, and a 30° telescope with a right-angle light cord adapter in order to move the camera further from the operating surgeon, minimizing incidental collision of instruments during pelvic surgery. We think that it is crucial to use a long 60-cm telescope with an adaptor that allows receiving a fair laparoscopic picture without interfering with the surgeons within the limited operative field (Figure 5).

The key problem with conventional laparoscopes is that they have a large extracorporeal profile, with a light cable exiting at 90°. This configuration leads to clashing of the instruments and camera during LESS. Thus, the ideal telescope for LESS should remove the light cord and camera head from the operative field. Low-profile camera systems have been introduced for this purpose.

Accessories

Park and colleagues have developed a transabdominal magnetic anchoring and guidance system (MAGS), which can be used to control an intra-abdominal laparoscope and multiple working instruments introduced through a single 1.5-cm port[21]. Once passed into the abdomen, instruments are affixed to the abdominal wall using external magnetic anchors. Currently, the MAGS incorporates an internal camera system, two types of passive tissue retractors, and a robotic arm cauterizer. By fixing internal instruments to external magnetic anchors, this platform allows for unrestricted intra-abdominal movement of surgical instruments, creating the potential benefits of LESS while maintaining an operative perspective similar to that of standard laparoscopy. This system has the added benefit of allowing the surgeon to reposition instruments intraoperatively without additional incisions.

TECHNIQUES

LESS is performed through a single abdominal incision, usually at the umbilicus. We and others have modified routine laparoscopic procedures in order to overcome the limitations of LESS. In general, a single port with multiple channels is used through which the laparoscope and the operative instruments are passed. The procedure usually involves two surgeons. As we have mentioned, pelvic procedures require the use of a 30° lens directed upwards, whereas upper tract procedures need either a 30° lens facing downward or a 0° lens[20].

We have performed LESS nephrectomy by a transperitoneal approach while the patient is in a flank position, utilizing the usual technique. In this setting, the retroperitoneal space is entered through the Told line utilizing Ligasure 5 mm-37 cm (Covidien). An articulating grasper (Covidien) and an articulating dissector (Cambridge Endo) are used in order to develop an operative space. Using both articulating instruments at this stage avoids extracorporeal hand cross and intracorporeal instrument collision. However, we have found particularly useful the use of both articulating instruments only at the initial stage of the surgery. After the initial dissection and the formation of an operative space, the articulating dissector can be easily replaced by a straight instrument such as Ligasure, allowing not only the dissection, but

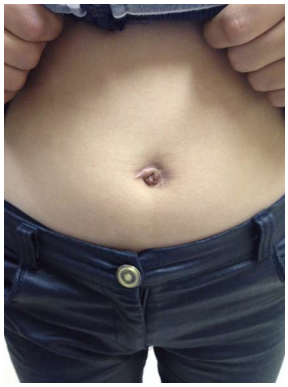


Figure 6 Final incision.

also rapid hemostasis, which shortens the time of the operation. After dissection of the colon away from the kidney, the ureter is identified and transected. Ligation of the hilum vessels is performed utilizing large Auto Suture hemostatic clips (Covidien). The specimen is retrieved into a laparoscopic bag and removed through one of the ports or together with the LESS port. No drain is needed for these cases. In the cases of large hydronephrosis and right-sided kidney, trans-flank holding stitches through the renal pelvis, in the same manner as used in laparoscopic pyeloplasty, can be used in order to facilitate renal dissection.

For single site laparoscopic gonadectomy, the LESS port is inserted in the same manner as for nephrectomy. Vascular control is achieved solely by utilizing the Ligasure system. In the case of varicocelectomy, laparoscopic dissection of the spermatic vein is performed sparing the spermatic artery and dissecting away the lymphatic vessels. Ligation of the spermatic vein is performed utilizing Auto Suture hemostatic clips (Endo Clip; Covidien Tyco Health Care) or using sealing devices such as the Ligasure system only without hemostatic clips.

R-LESS is performed through the same umbilical incision as for conventional LESS. When the SILS port (Covidien Tyco Health Care) is used, a finger is placed to guide introduction of two robotic trocars adjacent to the fascial incision through two separate fascial stab incisions. If using a GelPort (Applied Medical Technology), the access device is placed through the fascial incision and the robot is subsequently docked. The robotic cannulae utilized vary from 8 to 5 mm to accommodate the Endowrist (Intuitive Surgical, Sunnyvale, CA, United States) monopolar shears and the Endowrist Schertel Grasper, depending on the procedure to be performed.

ADVANTAGES AND INDICATIONS

Beyond the obvious better cosmetic results, advantages of LESS include reduced postoperative pain, reduced operative complications related to trocar insertion (*e.g.* wound infections, epigastric vessel injury and organ herniation), and easier specimen removal through a larger incision (specimens may be fragmented in the laparoscopic bag)[22,23]. Those benefits are especially relevant in pediatric and young populations in whom the esthetic outcome is crucial (Figure 6).

LIMITATIONS OF THE TECHNIQUE

Not all patients will be candidates for single-site surgery[24]. As with any other minimally invasive surgical technique (laparoscopy or robotics), patient selection is a composite of clinical judgment, risk, benefits, alternatives and a well-informed patient. Other limitations of this technique are the added cost and the technical challenges of the procedure. The major limitation is the lack of working space. The surgeon and the assistant must maneuver in a small space, resulting in hand and instrument collisions. The laparoscopic surgery concept of triangulation is challenged with the single-port procedure, and the ability to move the scope is significantly limited by other instruments[25].

Specialized equipment for single-port procedures can be used to help overcome these technical challenges, including the use of articulating instruments, a flexible laparoscope or a 30° laparoscope, and instruments of varying lengths. Articulating instruments can help with triangulation because the operator is able to work with two instruments in a similar location inside the abdomen while his or her hands are separated on the outside of the abdomen. Other disadvantages of LESS are related to operative time and learning curve.

CLINICAL STUDIES IN PEDIATRIC UROLOGY

Almost all body cavities can be entered through a small skin incision, therefore, the theoretical applications of LESS seem to be unlimited. However in a practical way, this statement is not entirely correct. Although LESS has successfully been proved for almost all diagnostic, extirpative and reconstructive surgery, there are limitations inherent to patient selection, surgical skills of the team, operative time, setup of the operating room, and availability of devices. In urology, LESS has been principally described for renal, ureteral, and prostatic surgery. In the most specialized centers it is now used for adrenal, bladder and testicular minimally invasive surgery as well. Despite the slower introduction of LESS in the pediatric population, today various LESS procedures have been described in pediatric urology with apparently good results.

Nephrectomy for nonfunctioning kidney is a good example. In 2010 Koh *et al*[26] reported outcome in 11 LESS nephrectomies in pediatric patients (age range: 0.1-16.2 years, with a mean age of 5.7 years) using an umbilical incision. None of the patients required conversion to conventional laparoscopy or open surgery. However, an accessory port was used in five of 11 cases. Of the 11 patients, two were infants, aged 39 d and 3.5 mo. The mean operative time was 139 min (range:

85-205 min), and the mean hospital stay was 1.5 d (range: 1.0-2.1 d)[26]. Ham *et al*[27] reported their results in four LESS nephrectomies and two nephroureterectomies through a homemade transumbilical port in children, without intraoperative or postoperative complications. The median operation time was 112 min (range: 90-148 min), and the median blood loss was 30 mL (range: 0-50 mL). All patients were discharged on postoperative day 2. As the surgeon had gained experience, the length of the umbilical incision was decreased from 2.0 to 1.0 cm[27]. In another recent study, Ganpule *et al*[28] reported on 10 patients who underwent different LESS procedures through the umbilicus. Seven patients underwent nephrectomy and three pyeloplasty. Mean age of the nephrectomized patients was 3.14 ± 1.7 years; the mean operating room time was 97.5 ± 12.54 min. All procedures were technically successful[28].

Another usual application of LESS in pediatric urology is varicocelectomy. Kaouk *et al*[29] reported three consecutive adolescent patients who underwent transumbilical varicocelectomy without placement of any additional ports or conversion to open surgery. The mean operative duration was < 1 h and all patients were discharged on the same day as their surgery and none required rehospitalization. There was no varicocele recurrence, or intraoperative or postoperative complications including wound infection, hydrocele, or incision site herniation[29].

LESS pyeloplasty is another popular but technically demanding procedure. Desai *et al*[30] performed 17 pyeloplasties; two with robotic assistance. The mean operative time and blood loss were 236 min and 79 mL, respectively. There were no complications, but all cases required an additional 2-mm port to aid suturing. One case was converted to conventional laparoscopy. All patients were symptom-free post-procedure and postoperative imaging showed unobstructed drainage in 15 of the 16 patients for whom data were available[30]. White *et al*[7] performed eight pyeloplasties; one with the aid of the Da Vinci robotic platform. The mean operative time and blood loss were 233 min and 62.5 mL, respectively. Renographic follow-up was documented as within normal limits and there were no complications apart from a wound site hernia[7]. One of the most recent studies was done at the Bayi Children's Hospital and included 24 pediatric patients with ureteropelvic junction obstruction who underwent transumbilical LESS pyeloplasty. All operations were successful. None was converted to open surgery and no additional sheath tube or incision besides umbilicus was needed. No intraoperative complications occurred. The mean operative time was 145 min, and the average blood loss -10 mL. Two patients had postoperative urinary fistula, which naturally disappeared at 4 and 7 d postoperatively, respectively. In follow-up, 23 of 24 patients demonstrated a significant decrease in renal pelvis diameter[31].

Orchidopexy has been performed with LESS as well. Noh *et al*[32] published the results of LESS orchidopexy in 17 patients with a median age of 11 mo (range: 3-43 mo). The study included two bilateral procedures and five primary Fowler-Stephens (FS) procedures. One patient underwent a staged FS orchidopexy, with the LESS technique utilized during the second stage. Median laparoscopic dissection time for each testis was 35 min (range: 22-40 min). There was no blood loss or intraoperative complications. In follow-up, all testes were noted to be in the scrotum without testicular atrophy[32].

Other LESS procedures have also been performed in pediatric urology, such as ureteral reimplantation and bladder augmentation. The data for this type of surgery is limited to case reports and small series[33,34] (Table 1).

OUR EXPERIENCE

Since 2011 a total of 18 patients underwent 23 procedures at our department: eight patients underwent nephrectomy due to nonfunctioning kidneys; four had removal of bilateral intra-abdominal gonads; four had high ligation of spermatic vein (HLSV); one underwent hysterectomy; and the remaining one had bilateral HLSV. A 1-year-old child who required hysterectomy was diagnosed with 46 XY ovotestis disorder of sexual differentiation and was raised as a boy. He required the removal of ovary and hypoplastic uterus. In all the patients a multichannel single laparoscopic port (Covidien) inserted through a 2-cm skin incision was used in order to obtain access into the abdominal cavity. All the patients underwent LESS without complications within a reasonable operating time. No one required conversion to open or conventional laparoscopic surgery. In two patients with large hydronephrosis we utilized a transcutaneous holding stitch, which was introduced through the renal pelvis and allowed additional manipulation of the severely hydronephrotic kidney, facilitating dissection and avoiding a need for additional trocar insertion. All but one patient were discharged on the day of surgery or on the day after[35] (Table 1).

LIMITATIONS OF CLINICAL STUDIES

Thus far, LESS is no longer an experimental technique; however, there are only a few retrospective studies with a significant number of cases that can prove the efficacy and safety of this technique for different indications. The advantages of LESS still exist at a theoretical level, because no clear benefit on postoperative course and patient convalescence has been definitively proven. The only potential benefit of LESS remains the claimed cosmetic outcome. Another obvious limitation is the lack of comparative studies between LESS and standard laparoscopy in terms of clinical outcome. Only a few retrospective case-control studies have compared LESS with standard laparoscopic techniques. In one such study, LESS nephrectomy (11 procedures) demonstrated no difference in median operating room time (122 min *vs* 125 min), change in hemoglobin levels, analgesic use, length of hospital stay, or complication rate compared to

Table 1 Clinical studies in pediatric urology

Ref.	Type of study	Procedure	Nopts	Operating time	Need for conversion	Complications/events	Blood loss
Koh *et al*[26]	Retrospective	Nephrectomy	11	139 min	No	Acc. port in 5	20 mL
Kocherov *et al*[35]	Case Control	Nephrectomy	8	65 min	No	None	None
Ganpule *et al*[28]	Retrospective	Nephrectomy	7	97.5 min	No	None	None
Ham *et al*[27]	Prospective	Nephrectomy	4	112 min	No	None	0-50 mL
Ham *et al*[27]	Prospective	Nephro-UBil	2	112 min	No	None	0-50 mL
Kocherov *et al*[35]	Case Control	Gonadectomy	4	37.5 min	No	None	None
Kocherov *et al*[35]	Case Control	Varicocelectomy	6	26 min	No	None	None
Kaouk *et al*[29]	Retrospective	Varicocelectomy	3	< 1 h	No	None	None
Noh *et al*[32]	Retrospective	Orchidopexy	17	35 min	No	None	None
Desai *et al*[30]	Retrospective	Pyeloplasty	17	236 min	No	Acc. port in all	79 mL
White *et al*[7]	Prospective	Pyeloplasty	8	233 min	No	None	136 mL
Zhou *et al*[31]	Prospective	Pyeloplasty	24	145 min	No	Two urinary fistulas	10 mL

standard laparoscopic nephrectomy (22 procedures)[22]. A limitation of this study, in addition to it being retrospective, was that patients had their nephrectomy specimens removed through an extension of the umbilical incision up to 4-6 cm, thus obscuring the possible benefits of LESS, such as shorter convalescence and reduced postoperative pain, compared with standard laparoscopy. These results might not indicate any advantages of LESS over standard laparoscopy[36]. We have also identified a similar historic group of patients from our database who underwent conventional laparoscopy and have compared their outcome to those patients undergoing the LESS technique[35]. This group included two patients with androgen insensitivity that underwent gonadectomy; four with nonfunctioning kidneys who underwent nephrectomy; and four who underwent HLSV. All patients in this group had similar parameters in terms of age and indications for surgery as the LESS group. Outcome data regarding operative time, narcotic requirements, length of hospitalization, and complication rate were obtained following chart review. In spite of the fact that in those patients who underwent LESS the operating time seemed to be longer, there was no difference in the length of surgery and intraoperative narcotic requirements between conventional laparoscopy and LESS. None of the patients in the LESS group required narcotic administration compared with three patients from the conventional laparoscopy group (one gonadectomy and two nephrectomies) who required postoperative narcotic treatment. LESS patients had shorter hospitalization compared with the conventional laparoscopy group, but only in the nephrectomy group.

CONCLUSION

LESS has proved to be immediately applicable in the clinical field of pediatric urology, being safe and feasible in the hands of experienced laparoscopic surgeons in well-selected patients. We believe that one of the future challenges for LESS in the pediatric population may be the treatment of nephrolithiasis. Despite promising early outcomes, the benefits of LESS are not obvious at present, with the only claimed advantage being cosmetic. Prospective randomized studies are required to define the benefits of this technique for patients as well as to elucidate the cost-effectiveness of the approach.

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RETROSPECTIVE STUDY

Cardiopulmonary bypass with brain perfusion for renal cell carcinoma with caval thrombosis

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Abstract

AIM: To compare a modified technique preserving brain circulation during cardiopulmonary by-pass (CPB) for radical nephrectomy and caval thrombectomy, to the standard technique.

METHODS: Retrospective evaluation of an institutional database that collects the data of patients submitted to nephrectomy and removal of caval thrombosis with CPB since 1998. In period between 1998 and 2007, CPB followed a standard technique (group sCPB); then, since 2008, a variation in the perfusional technique was introduced, allowing the anterograde perfusion of brain circulation during circulatory arrest (group CPB + BP) with the aim to reduce the risk of ischemic damage to the brain and also the need of deeper hypothermia. Patients (age, gender, comorbidity) and tumor characteristics (side, histology, staging, level of thrombosis), as well as parameters of CPB (times of CPB, aortic clamping and circulatory arrest, minimum temperature reached during hypothermia), intra- and perioperative morbidity (complications in general, bleeding, renal and hepatic failure) and mortality were analyzed and compared between 2 groups (sCPB *vs* CPB + BP)

RESULTS: The data of 24 patients, respectively 9 in sCPB group and 15 in CPB + BP group, have been reviewed. No differences in the characteristics of patients and tumors were observed. Only 1 (11.1%) and 4 (26.0%) of sCPB and CPB + BP patients, respectively, didn't experience any event of complication. In sCPB group were observed 15 events of complication (5 of which Clavien ≥ 3, 33% of the events), for a mean of 1.66 events/patient; 29 events (10 Clavien ≥ 3, 30.3%), in the CPB + BP group, for a mean of 2.1 events/patient. 1 (11.1%) and 2 (14.2%) deaths occurred, respectively. For patients submitted to CPB + BP, the minimum temperature reached was significantly higher (29.9 ℃ *vs* 26.4 ℃, $P = 0.001$), the time of circulatory arrest was longer (17.4 min *vs* 13.7 min, NS), but the overall time of CPB shorter (76.1 min *vs* 92.5 min, NS), albeit these latter differences were not statistically significant. No differences in terms of bleeding, impairment of renal function (post-operative Cr > 2.0 mg/dL respectively in 44.4% *vs* 35.7% of cases, in the two groups, NS) or hepatic insufficiency (post-operative GOT or GPT > 50 U/L respectively in 44.4% and 66.7% of patients, NS) were noted. Average follow-up was 51 mo in patients undergoing a sCPB and 12 mo in the CPB + BP group of patients; at the last follow-up, 7 patients had died of progression of the condition (4 in the first group and 3 in the second group, respectively), 7 were alive in progression and 10 had no evidence of the disease.

CONCLUSION: The perfusional technique that maintains brain perfusion during circulatory arrest limits hypothermia and lowers time of CPB, without rising the risk of renal and hepatic injury.

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Key words: Renal carcinoma; Vena cava thrombosis; Cardiopulmonary bypass; Cerebral perfusion; Circulatory arrest

Core tip: Surgery for renal cell carcinoma with caval thrombosis extended to the diaphragm or right atrium is burdened by a high risk of complications. The adoption of a modified technique of cardiopulmonary bypass that maintans the perfusion of brain circulation, doesn't add morbidity to the procedure and can be in principle of benefit, since it shortens the duration of surgery and requires a less deep hypothermia.

Antonelli A, Bisleri G, Mittino I, Moggi A, Muneretto C, Cosciani Cunico S, Simeone C. Cardiopulmonary bypass with brain perfusion for renal cell carcinoma with caval thrombosis. *World J Clin Urol* 2014; 3(2): 127-133 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i2/127.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i2.127

INTRODUCTION

Renal carcinoma (RCC) is extremely angiotropic and may extend into the venous circulation, from the renal vein to the right sections of the heart[1]. Lacking an effective medical treatment, surgery is still the only available treatment, leading to 5-year cancer-specific survival rates of up to 70%[2,3].

When the thrombosis reaches the diaphragm, the surgical treatment requires a combination of abdominal and thoracic approaches in order to achieve an extensive removal of the thrombus within the inferior vena cava and the right atrium by means of cardiopulmonary bypass (CPB) and circulatory arrest. Even if other strategies have been also suggested to avoid circulatory arrest or even CPB (*i.e.*, CPB under mild hypothermia without cardiac arrest, veno-venous by-pass by means of cavo-atrial shunt and "milking" manoeuvre below the major hepatic veins without CPB[4]), CBP with circulatory arrest is probably the most widely accepted surgical strategy for these patients.

This procedure itself is burdened by a high risk of bleeding, which is in part due to the need of blood heparinization during the exanguination of the patient, but also to the coagulation disorders caused by deep hypothermia, used in order to minimise the potential parenchymal damage (in particular to the brain) caused by the circulatory arrest.

In an attempt to further improve patients' clinical outcomes, at our institution since 2007 a novel CPB technique has been adopted which allows for antegrade cerebral perfusion during circulatory arrest in mild hypothermia. The purpose of this study was therefore to review the results achieved by means of this novel technique (cardiopulmonary bypass with cerebral perfusion-CPB + BP, Figure 1) and compare it with the conventional technique (standard cardiopulmonary bypass without cerebral perfusion-sCPB), with respect to intra- and post-operative outcomes.

MATERIALS AND METHODS

This study included all patients undergoing a procedure including nephrectomy and removal of thrombosis with CPB at our Institution since 1998, because since that year a specific database to store all the surgical data of these patients was generated.

The indication for the removal of thrombosis with the aid of CPB was done for all those cases in which the preoperative assessments revealed that the upper level of thrombosis reached the right atrium or above. All the patients were studied by electronic computer X-ray tomography technique (CT), magnetic resnane iamge (MRI), trans-esophageal echocardiography and coronarography, in order to evaluate the extent and features of the thrombosis as well to rule out any potential concomitant heart disease.

The surgical approach implies a bilateral subcostal incision and a median sternotomy, separated by a short skin bridge, with the aim to improve the healing of the sternal incision by keeping it separate from the abdominal one. Once the abdominal vena cava and aorta have been isolated and the renal artery ligated, the kidney is mobilised while preserving only the renal vein; once the CPB has been instituted, before the cavotomy, the inferior vena cava caudal to the thrombosis and the contralateral renal vein are clamped with tourniquets. Then, once mild hypothermia is achieved, cardioplegia solution is delivered and the general circulation is arrested; finally, the right atrium and the lower abdominal vena cava are opened in order to remove the thrombosis and to complete the nephrectomy.

From 1998 to 2007, CPB has been carried out using a standard central cannulation technique, achieving moderate hypothermia (25 ℃) during circulatory arrest (sCPB group).

Instead, since 2007 to date, a variation of such technique has been adopted (CPB + BP group), to maintain cerebral perfusion so that mild hypothermia was sufficient during the circulatory arrest. As we previously described[5], once the pericardium is opened, the aortic arch is prepared as to allow exposure of the supra-aortic vessels; the ascending aorta is cannulated, as well as the right atrium and the superior vena cava. Once moderate-mild hypothermia has been reached (30 ℃), the aorta is clamped and a cardioplegic solution is administered; then, an additional clamp is positioned on the aortic arch between the left common carotid artery and the left subclavian artery. Following opening of the right atrium, venous drainage is obtained exclusively *via* the cannula previously inserted into the superior vena cava: therefore, cerebral perfusion is maintained through the aortic arch while the patient is on systemic circulatory arrest, allowing for a bloodless field both on the cardiac and abdominal sides.

In the present study, the level reached by the thrombosis was classified according to the Mayo Clinic system[6]; co-

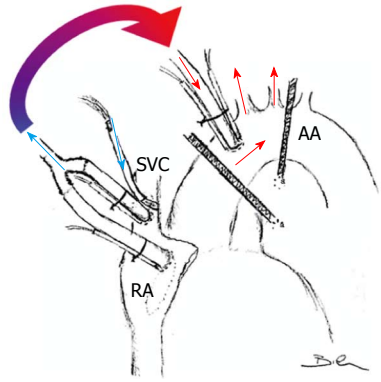


Figure 1 Sketch of the technique of incannulation adopted for CPB+BP: when right atrium and superior vena cava are cannulated, venous flow (blue arrows) after oxygenation is pumped into the aortic arch clamped (aclamps) between the brachio-cefalic trunk and the left carotid artery. RA: Right atrium; SVC: Superior vena cava; AA: Aortic arch.

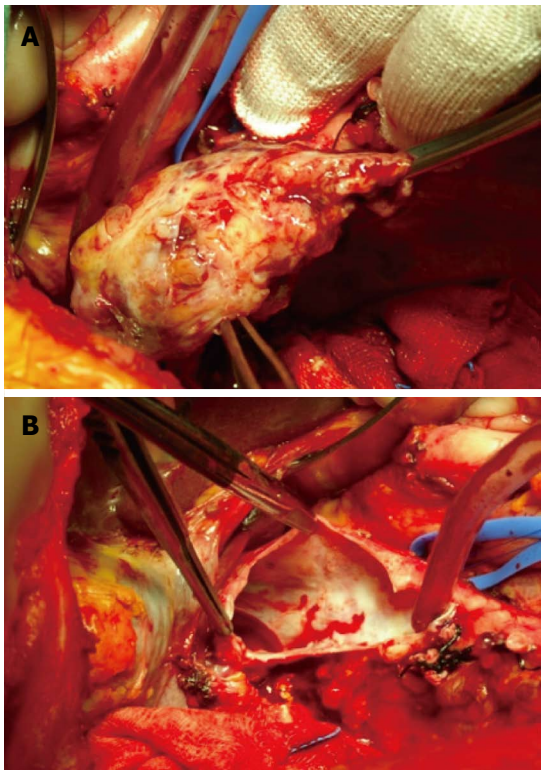


Figure 2 Extraction of the thrombus through a cavotomy (A) and examination of the caval lumen in a completely bloodless field (B).

morbidities were scored according to the Charlson-Romano score[7]; post-operative complications were recorded up to 30 d after surgery according to the Clavien-Dindo system[8].

Statistical analysis

Discrete variables were analysed by Fisher exact test or χ^2 test, while Mann-Whitney test was utilised for continuous variables (SPSS v.13, SPSS Inc, Chicago, IL, United States).

RESULTS

During the period of the study (1998-2013), 1477 patients underwent surgery for renal cancer at our Institution. Overall, 23 patients with venous thrombosis underwent surgery with the aid of CPB, 9 with sCPB, 15 with a CPB + BP technique.

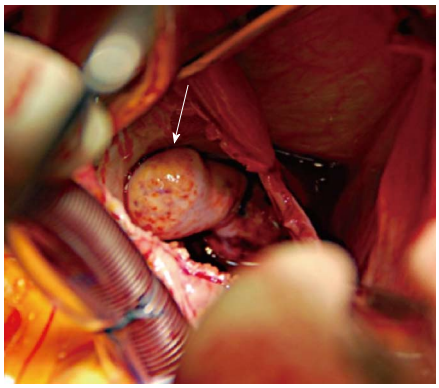


Figure 3 The head of the thrombus (arrow) seen from an upper point of view through the atriotomy.

The two groups had comparable characteristics, as listed in Table 1. Even in all the patients at the preoperative evaluation the head of the thrombosis was deemed as inside of the right atrium, in 12 patients at surgical exploration, it was close but outside the atrium, so that they have been down-classified as level 3, again in a similar rate in the two groups.

Only 1 (11.1%) and 4 (26.0%) patients in the sCPB and in the CPB+BP group, respectively, did not experienced any postoperative complications; 15 complications (5 of which were grade $\geqslant$ 3, equal to 33% of the events) happened in the sCPB group, which means on average 1.66 events per patient; 29 complications (10 of which were grade $\geqslant$ 3, equal to 30.3% of the events) in the CPB + BP group, which means on average 2.1 events per patient (Table 2).

Table 3 summarizes the comparison of CPB parameters and indicators of morbidity. For the patients of the CPB+BP group was noted a significantly less deep hypothermia and, even without statistical significance, a prolonged time of circulatory arrest (CPB + BP 17.4 min *vs* sCPB 13.7 min), but a shorter overall CPB time (CPB + BP 76.1 min *vs* sCPB 92.5 min) due to the faster cooling and rewarming of the patient. Finally, no differences in terms of indicators of hepatic, renal, brain and coagulative impairment were noted, with a shorter overall length of stay for the CPB + BP group.

Average follow-up was 51 mo in patients undergoing a sCPB and 12 mo in the CPB + BP group of patients; at the last follow-up, 7 patients had died of progression of the condition (4 in the first group and 3 in the second group, respectively), 7 were alive in progression and 10 had no evidence of the disease.

DISCUSSION

Renal cancer is extremely angiotropic, which may result in a macroscopic invasion of the large venous vessels in up to 10% of cases[9]. While a regression with targeted

Table 1 Details of the two groups

Patients and tumors characteristics	sCPB	CPB + BP	*P*
Age (yr)	64.0 ± 9.2	66.2 ± 10.2	NS
Gender			
Males	7/9 (77.8%)	9/15 (60%)	NS
Females	2/9 (22.2%)	6/15 (40%)	
Pre-operative ejection fraction	59.7% ± 0.8%	57.6% ± 4.6%	NS
Comorbidity			
Charlson score 0	2/9 (22.2%)	6/15 (40%)	NS
Charlson score ⩾ 1	7/9 (77.8%)	9/15 (60%)	
Tumor side			
Right	7/9 (77.8%)	10/15 (66.7%)	NS
Left	2/9 (22.2%)	5/15 (33.3%)	
Tumor diameter (cm)	9.6 ± 2.5	9.6 ± 2.9	NS
Level of thrombosis			
3	5/9 (56.6%)	8/15 (53.3%)	NS
4	4/9 (44.4%)	7/15 (46.6%)	
Histology			
Clear Cell renal carcinoma	7/9 (77.8%)	13/15 (86.6%)	NS
Other	2/9 (22.2%)	2/15 (13.3%)	
Infiltration of perirenal tissues	7 (77.8%)	10 (66.7%)	NS
Infiltration of venous wall	3 (33.3%)	4 (26.6%)	NS
N+	1 (11.1%)	2 (13.3%)	NS
M+	2 (22.2%)	5 (33.3%)	NS

NS: Not significant; CPB: Cardiopulmonary by-pass.

therapies has been occasionally reported[10,11], only radical surgery can effectively treat patients albeit with variable 5-year survival rates, ranging between 34% and 72%[2,3]: such outcomes may be influenced mostly by unfavourable prognostic factors which are frequently associated with the occurrence of neoplastic thrombosis rather than the cranial extension of the thrombosis itself[12].

Surgery of caval thrombosis is technically complex and has high morbidity and mortality rates, up to 22% in a recent series[11].

In patients in which the thrombosis has extended over the diaphragm, the use of a CPB with circulatory arrest has been widely utilised in order to allow for a better control of the cranial end of the thrombosis through an atriotomy as well as to achieve a bloodless surgical field once the inferior vena cava has been opened (Figures 2, 3). To reduce the metabolic requirements of the tissues and thus increase the resistance to ischemic damage during the circulatory arrest, deep hypothermia (< 20 ℃) has been routinely used[13]. In particular, the brain yields an higher risk of ischemic damage and its metabolism during circulatory arrest must be therefore significantly reduced by means of hypothermia, with an ischemic tolerance lasting up to 5 min at 37 ℃, 15 min at 25 ℃, 24 min at 20 ℃, and 45 min at 10 ℃[14-17].

There is a high risk of bleeding during CPB, which is related to the heparin infused when blood is diverted into the reservoir and the patient is exanguinated, and then antagonised by protamin when the physiologic circulation is restored, but also to hypothermia itself that could impair the platelet function and the production of coagulation factors.

The evidence that hypothermia may exert a negative impact is debated and recent publications, on the contrary, advocate its adoption on the basis of a better clinical outcome[18,19]. At the same time a diffuse concern exists, since several authors suggested alternative techniques to avoid the circulatory arrest[20,21], rather accepting a higher risk of a bloody surgical field, embolisms and tears to the caval wall due to the lack of a direct control over the cranial end of the thrombosis[22].

CPB with antegrade cerebral perfusion has been widely utilised for the surgery of the aortic arch[23], and it has also been reported in the treatment of thrombosis from renal carcinoma by our and other groups[5,24]. By the antegrade perfusion of the brain, hypothermia can be limited, thus reducing the theoretical risk of hypothermia-induced coagulation failure as well as the sure waste of time required to cool down and warm up the patient, but still providing the same bloodless operative field offered by a conventional deep hypothermic circulatory arrest.

The present study reviews the experience of a single academic institution with 23 consecutive patients treated in the last 15 years, using a CPB for caval thrombosis over the level of diaphragm. The number of patients, limited in absolute terms, is comparable to the main reports that have been published so far; in addition, for the present study the data collection has been planned for the specific purpose of estimating the morbidity of the procedure.

It has been confirmed that extensive caval thrombosis is associated with other unfavourable pathologic prognostic factors (invasion of peri-renal tissues, lymphnodes and distant metastasis), which may account for the poor prognosis[1,5,11]: in this study, the 5-year cancer-specific survival rate was 35%, but reached 62% in patients without lymphnodes or distant metastasis, thus confirming that in such cases surgery may promote a prolonged survival.

In the 9 cases treated in the first period of this study (sCPB), patients were cooled down to an average temperature of approximately 26 ℃ during the circulatory arrest, while in the 15 cases treated in the second period (CBP + BP), changing the technique of perfusion resulted in a remarkably lesser hypothermia, with an average minimum temperature of approximately 30 ℃. Such level of hypothermia was maintained for a more prolonged time of circulatory arrest (CPB + BP 17.4 min *vs* sCPB 13.7 min), but within a shorter overall CPB time (CPB + BP 76.1 min *vs* sCPB 92.5 min) due to the less time needed to cool and rewarm the patient.

No differences were found between the two groups in terms of pre-operative co-morbidity or characteristics of tumors. Post-operative complications, systematically recorded, were very frequent, with at least one event in 83% of cases and a 30-d perioperative mortality rate of 12.5%, as shown in Table 3. The number of events of complications and the rate of major complications were similar between the 2 groups, as the mortality rate. However the length of stay was slightly shorter in CPB + BP group, suggesting a less detrimental effect in these patients from the events of complication. More interest-

Table 2 Complications observed in the 2 groups, according to the Clavien-Dindo's scale

Clavien grade	sCPB	CPB + BP
1	4 acute renal failures	5 acute renal failures
2	3 transfusions	7 transfusions
	3 atrial fibrillations	7 atrial fibrillations
3a	1 sternal wound dehiscence	
3b	1 bleeding requiring re-laparotomy	1 bleeding requiring re-laparotomy
		1 ligature of the contralateral ureter, requiring ureteral stenting
4a	2 =respiratory failures	1 brain stroke
		1 pulmonary embolism
		3 respiratory failures
4b	-	1 multi-organ failure
5	1 (1st post-operative day)	2 (3rd and 25th post-operative day)

CPB: Cardiopulmonary by-pass. NS: not significant.

Table 3 Comparison in terms of intra- and peri-operative characteristics between the 2 groups

Intra- and post-operative variables	sCPB	CPB + BP	*P*
CPB time (min)	92.5 ± 35.7	76.1 ± 18.5	NS
Aortic Clamp time (min)	30.8 ± 15.8	26.5 ± 19.6	NS
Circulatory arrest time (min)	13.7 ± 8.9	17.4 ± 5.3	NS
Minimum temperature (℃)	26.4 ± 2.5	29.9 ± 1.9	0.001
Intraoperative blood units transfused (no.)	12 (4-12)	13.2 (6-20)	NS
ICU stay (in days)	2 (2-2)	2.7 (1-3)	NS
Hospital stay (in days)	27 (22-57)	21 (10-25)	NS
Post-operative Cr > 2 mg/dL	4/9 (44.4%)	5/15 (33.3%)	NS
Post-operative GOT or GPT > 50 U/L	4/9 (44.4%)	8/15 (53.3%)	NS
Post-operative bleeding	5/9 (55.6%)	8/15 (53.3%)	NS
Peri-operative mortality	1/11 (11.1%)	2/15 (13.3%)	NS

CPB: Cardiopulmonary by-pass. NS: not significant; GOT: Glutamic-oxalacetic transaminase; GPT: Glutamic-pyruvic transaminase.

ingly, the number of renal and liver failure events turned out to be comparable, confirming that these parenchyma may tolerate temperatures above 20 ℃-25 ℃, at least for the time of circulatory arrest that such kind of surgery requires. At the same time, from our results nor advantages neither disadvantages in terms of bleeding or neurologic sequelae cannot be proved, as the only neurologic event happened just in the CPB + BP group.

So, the present study shows that the CPB + BP technique is not inferior to a standard CPB in terms of radicality of surgery and overall complications, while gives an advangtage in terms of duration of surgery. Surely, these conclusions should be taken with caution, because the study suffers from some limitations. First, the retrospective design, in spite of the perspective manner in which data has been collected: even if it is reasonable that the maintenance of cerebral circulation could add an advantage on neurological functions, the study fails to offer sure evidences of this, lacking of an established indicator that could measure cerebral metabolism prior, during and after the intervention. Second, the limited number of patients, enrolled in a long timeframe, that lowered the validity of statistical comparisons between groups; however, RCC with caval thrombosis treated with CPB is a rare condition, so that a perspective study probably will never be designed, even with a multicentric data collection, since different institutions usually adopt a personal approach.

The variation of the technique of perfusion proposed limits the need for hypothermia to moderate levels (30 ℃), with the theoretical benefits on coagulation function and a sure advantages in terms of duration of surgery, without affecting the radicality of surgery or provoking a higher number of sequelae than a CPB with deeper hypothermia.

COMMENTS

Background

Surgery is the only option to cure some of the patients affected by a renal cancer that involves the inferior vena cava, However, this surgery suffers from high risks of intra and postoperative morbidity, which mainly depend on the cranial extension of the thrombus. Indeed, morbidity is higher for thrombosis extended up to the retroepatic vena cava or the right atrium, since in such conditions cardiopulmonary by-pass (CPB).

Research frontiers

The exsanguination by CPB is required to extract of caval thrombosis extended up to the diaphragm or above in a bloodless field after the vena cava and/or the atrium are opened. To permit CPB, deep hypothermia is required, so that the organs - and in particular the brain - will suffer in a less extent from ischemia. Hypothermia can provoke an impairment of coagulation and prolongs the duration of the operation, worsening the morbidity of the procedure. Many authors investigated means alternative to CPB to reduce the morbidity of surgery in renal cancer with extended thrombosis. These procedures avoid at all the CPB,

adopting an exclusive abdominal approach, by the complete mobilization of the liver and the exposition of the retrohepatic vena cava, or by a veno-venous bypass that diverts the venous flow from the vena cava but maintaining the cardiac activity and the arterial perfusion. There are, however, risks of incomplete radicality, if the thrombosis infiltrates a portion of the caval wall out which is not visible from the abdominal access, of embolism due to the detachment of part of the thrombus without the upper control through atriotomy, and of bleeding, related to the backflow from the lumbar veins.

Innovations and breakthroughs

The CPB with brain perfusion (CPB + BP) is a technique developed by cardiac surgeons with the intent to preserve cerebral perfusion during aortic arch replacement, showing a lower morbidity in respect to a standard CPB, in principle related to the less deep hypothermia required and to the maintenance of cerebral perfusion. At authors' institution this technique has been applied to the surgery of renal cancer with extended venous thrombosis. At now only a very few cases have been reported, while this study reported a larger number of patients, comparing the results with an homogeneous historical group in which a standard CPB was adopted

Applications

The tecnhique can be suggested to the institutions actually treating these patients by a standard CPB, since the authors showed a non-inferiority of this modified technique in terms of renal and hepatic impairment, as of morbidity in general, with a less deep hypothermia and shorter operative times. The study can be the basis for future studies to investigate the impact on cerebral metabolism of this technique.

Terminology

Cardiopulmonary by-pass: a procedure to convey blood from normal circulation to a machine that oxygenates blood.

Peer review

The article is a well-written manuscript comparing two techniques of cardiopulmonary bypass for excision of renal tumours with vena caval extension.

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RETROSPECTIVE STUDY

Penile prosthesis: Patient satisfaction, use and preference for malleable *vs* inflatable

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Abstract

AIM: To evaluate and compare long-term patient satisfaction and use after either malleable penile prosthesis (MPP) or inflatable penile prosthesis (IPP) implantation.

METHODS: we present a retrospective unicenter study of 108 patients implanted with either 2 or 3-piece American Medical System™ (AMS™) or Coloplast™ inflatable penile prosthesis (AMS 700CX™, AMS 700CXR ™, AMS Ambicor™ or Coloplast TITAN™) or malleable (AMS Spectra™ or Coloplast Genesis™) in our Centre between 1993 and 2011. We collected data from the medical record including follow-up, age and type of prosthesis. We used a four-question telephone survey designed ad hoc by urologist in our Department, with three multiple choice questions and a grading answer. After verbal consent was obtained, proposed questions concerned global satisfaction regarding to the procedure, quality of sexual intercourses graded from 0 to 10, frequency of sexual intercourse and about undergoing the same procedure again. SPSS™ version 20.0 was used for the descriptive analysis of the data.

RESULTS: Sixty seven (64%) patients underwent a MPP and 41 (36%) an IPP. The mean age was 52.6 ± 3.6 years in the MPP group and 57.2 ± 2.8 years in the IPP group (P = 0.02). Total respond rate was 55.5% (60/108). Twenty six out of 33 MPP patients (78.9%) and 19 of the 27 IPP subjects (70.3%) were satisfied or very satisfied with the procedure. The quality of sexual intercourse was rated 7.13 ± 0.39 points in the MPP group and 6.16 ± 0.47 points in the IPP group. Frequency of sexual intercourse was 1 or more times per week in 15 (46.9%) patients with MPP and in 12 (46.1%) of the IPP patients. Twenty-eight (84.9%) patients who received a MPP would undergo the procedure for the same device again as well as 24 (88.9%) of the IPP group. There were no statistical differences between groups regarding the four items in the survey.

CONCLUSION: Patients show high satisfaction rate and no statistical differences exist regarding to global satisfaction, use of the device and quality of sexual intercourse depending on the type of penile prosthesis.

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Key words: Penile prosthesis; Patient satisfaction; Erectile dysfunction; Medical survey

Core tip: retrospective study of 108 patients implanted with either malleable penile prosthesis or inflatable penile prosthesis designed to investigate results in

terms of use of the device and satisfaction. Patient satisfaction after prosthetic surgery is multifactorial and it should be considered when exposing the pros and cons of prosthesis to patient before surgery. We collected data with a four-question telephone survey. After analyzing our results, we concluded that patients show high satisfaction rate and no statistical differences exist regarding to global satisfaction, use of the device and quality of sexual intercourse depending on the type of penile prosthesis.

Rogel Bertó R, López-Acón JD, Luján Marco S, Ordaz Jurado DG, Delgado Oliva F, Conca Baenas MA, Boronat Tormo F. Penile prosthesis: Patient satisfaction, use and preference for malleable *vs* inflatable. *World J Clin Urol* 2014; 3(2): 134-138 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i2/134.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i2.134

INTRODUCTION

Surgical treatment for erectile dysfunction (ED) by implantation of penile prosthesis is considered a safe and efficient option to treat those cases non responding to pharmacological agents[1]. Both, prosthesis materials and prosthesis design, have evolved in time, in the same way that surgical techniques have, in order to achieve a better durability and quality of the device.

Patient satisfaction after the penile prosthesis surgery is considered multifactorial and depends on issues like presurgery expectations and the success of the implant as ED treatment option[2]. If compared with the other options to treat ED like phosphodiesterase five (PDE-5) inhibitors, intraurethral alprostadil, intracavernous injection of alprostadil or mechanical devices, penile prosthesis is the one showing better satisfaction rates[3].

The objective of our study was to evaluate and compare long-term patient satisfaction and use after either malleable or inflatable penile prosthesis implantation. Patients were offered both types of prosthesis if no contraindication. Most of the papers published so far concerning patient satisfaction with the device have used non-validated questionnaires designed by each Hospital or Investigation Group[1]. Two validated questionnaries exist concerning sexual intercourse satisfaction: the satisfaction domain of the Internation Index of Erectile Function (IIEF) and the Erectile Dysfunction Inventory of Treatment Satisfaction (EDITS)[4-7].

MATERIALS AND METHODS

We present a retrospective study on 108 patients implanted with either 2 or 3-piece American Medical System™ (AMS™) or Coloplast™ inflatable penile prosthesis (AMS 700CX™, AMS 700CXR™, AMS Ambicor™ or Coloplast TITAN™) or malleable (AMS Spectra™ or Coloplast Genesis™) in our Hospital between 1993 and 2011. We collected data from medical record like follow-up, age and type of prosthesis. We made contact with each patient for a telephone survey. Prior to the survey each patient was informed of the content and objective of the survey and consent was obtained. Survey was designed by the authors based in the penile prosthesis satisfaction papers published to date. We configured a first version which was examined by several other urologists in the Department to evaluate clarity and precision of the questions. After this first version, we configured the final version. We obtained a four-question telephone survey with three multiple choice questions and a grading answer question. First of them made reference to global satisfaction concerning the procedure with four possible answers being: (1) Not satisfied; (2) Partially satisfied; (3) Satisfied; and (4) Very satisfied. Second question asked about the quality of sexual intercourses graded from 0 to 10, being zero "very bad quality" and 10 "very good quality". We asked in question number three about the frequency of sexual intercourse being answer: (1) More than once per week; (2) Once per week; (3) Once per for night; (4) Once per month; and (5) Less than once per month; and last, we requested about the fact of undergoing the same procedure again, and the two possible answer were (1) Yes or (2) No.

Statistical analysis

Data were analysed using the SPSS™ 20.0 (IBM corp™). Statistical analysis was performed using Fisher test to detect differences between differents groups. A value of $P < 0.05$ was considered to be statistically significant.

Table 1 Etiology, infection rate and reoperation *n* (%)

		MPP	IPP	*P*
Main etiology	Diabetes mellitus	21 (31.3)	17 (41.5)	0.505
	Vasculogenic	14 (20.9)	8 (19.5)	
	LaPeyronie disease	9 (13.4)	5 (12.2)	
	Radical Prostatectomy	2 (3)	3 (7.3)	
	Neurogenic	8 (11.9)	4 (9.7)	
	Unknown	13 (19.4)	4 (9.7)	
Implant Infection		7 (10.5)	5 (12.2)	0.202
Reoperation		10 (14.9)	7 (17.5)	0.787

MPP: Malleable penile prosthesis; IPP: Inflatable penile prosthesis.

RESULTS

A total of 67 (64%) patients underwent a MPP and 41 (36%) an IPP. The mean age was 52.6 ± 3.6 years in the MPP group and 57.2 ± 2.8 years in the IPP group ($P = 0.02$). Total respond rate was 55.5% (60/108); 27 (25%) had deceased and the remaining 21 (19.4%) did not respond. Among those who attended to the survey, 33 patients (55%) had MPP and 27 (45%) had IPP. There were no statistical differences between the groups regarding to etiology of erectile dysfunction (ED) ($P = 0.505$), incidence of implant infection ($P = 0.202$) or reoperation rate ($P = 0.787$) (Table 1).

The median time from surgery to the survey was 161 (6-199) mo for the MPP group and 37 (3-161) for the IPP group. As shown in Table 2, 26 of the 33 MPP

Table 2 Results of the survey *n* (%)

Question	MPP		IPP		*P*
Satisfaction	No satisfied	2 (6.1)	No satisfied	6 (22.2)	0.157
	Partially satisf.	5 (15.2)	Partially satisf.	2 (7.4)	
	Satisfied	15 (45.5)	Satisfied	13 (48.1)	
	Very satisfied	11 (33.3)	Very satisfied	6 (22.2)	
Quality	7.13 ± 0.39		6.16 ± 0.47		0.314
Frequency	> 1/wk	8 (25)	> 1/wk	7 (26.9)	0.413
	1/wk	7 (21.9)	1/wk	5 (19.2)	
	1/15 d	5 (15.6)	1/15 d	5 (19.2)	
	1/mo	3 (9.4)	1/mo	3 (11.2)	
	> 1/mo	9 (13.4)	> 1/mo	6 (23.1)	
Undergo again	YES	28 (84.9)	YES	24 (88.9)	0.774
	NO	5 (15.1)	NO	3 (11.1)	

MPP: Malleable penile prosthesis; IPP: Inflatable penile prosthesis.

patients (78.9%) and 19 of the 27 IPP subjects (70.3%) were satisfied or very satisfied with the procedure. The quality of sexual intercourse was rated 7.13 ± 0.39 points in the MPP group and 6.16 ± 0.47 points in the IPP group. Frequency of sexual intercourse was 1 or more times per week in 15 (46.9%) patients with MPP and in 12 (46.1%) of the IPP patients. Twenty eight (84.9%) patients who received a MPP would undergo the same device procedure again as well as 24 (88.9%) of the IPP group. There were no statistical differences between groups regarding the four items investigated in the survey.

DISCUSSION

Penile prosthesis as an ED treatment option is considered to have a high satisfaction rate among the patients implanted[3]. On the other hand, there is a small number of unsatisfied patients with surgery, esthetic and/or functional results. When perceived, it is about rigidity, length, infection of the device, spontaneous deflation or mechanical failure the main reasons for those cases of patient dissatisfaction. Patient has to know before surgey which are the real expectations, the way prosthesis will modify or not penile length and girth, penile sensitivity, glans status, if circumcision is going to be performed as well as infection, mechanical failure and prosthesis removal rates[1,8].

In our unicenter retrospective study we have obtained a high rate global satisfaction in both groups showing no statistically significant differences between them. Another result to pay attention is that most of patients are located in highest or lowest frequency groups of use, at the expense of the middle positions. More than 1 per week is 25% in MPP group and 26.9% in IPP; and the opposite position, less than 1 per month is 13.4% in MPP group and 23.1% in IPP. Concerning to satisfaction, most of the patients in both groups would undergo the procedure again.

Focusing in global satisfaction with the inflatable prosthesis, our percentages of 70.3% of satisfied or very satisfied, are less evident than the ones in other references. In a study that involved 145 patients implanted with IPP AMS 700 Ultrex™, after the satisfaction questionnaire, 85% were satisfied against 76% of partner satisfaction[9]. Another study of 207 patients implanted with IPP AMS 700CX™, performing a telephone survey, showed 79% use the device at least twice monthly and 88.2% would recommend an implant to a relative or friend[10]. Two other studies, one conducted with 200 consecutive patient who underwent IPP AMS Ultrex™ and CX™ showed an overall satisfaction of 92%[11]. The other one, 80 cases implanted with IPP AMS 700CX™ responded to a nine-point telephone survey and 97% of patients use the device frequently; 69% affirmed they never had problems with its use; and 97% reported they would suggest this treatment to other people[12].

In relation to IPP Mentor alpha-1™, one study showed that 89% of men had fulfilled expectations with the prosthesis. Regarding intercourse ability, confidence and device rigidity and function satisfaction rate was 80% or greater[13].

One study was conducted to rate patient satisfaction with 3 types of penile prosthesis. A random sample of 330 patients (of 1298 patients implanted) with either AMS700™, Mentor Alpha 1™ or Mentor Alpha NB™ responded to a computer assisted telephone survey. The overall satisfaction rate was 69%, and there was no significant difference by implant type[14].

Another multicentre study comprising several types of prosthesis (IPP AMS700 CX™, AMS Ambicor™ and AMS 600-650™) in terms of satisfaction, use the EDITS validated questionnaire. Patient satisfaction rates were 97%, 81% and 75% respectively[15].

Two different papers included two-piece IPP patient satisfaction. The first one evaluated 146 patients implanted with IPP AMS Ambicor™ and found that 91% said that it was easy to use. Overall patient satisfaction was 85%, and 86% would recommend the prosthesis to friends or undergo the procedure again if necessary [4]. The second paper regarding IPP AMS Ambicor™ satisfaction involved 131 patients and they collected date from their own mailed questionnaire and from a modified EDITS mailed questionnaire. Overall patient satisfaction was 96.4% and 92.9% would recommend it to others. Of the 85 men who completed the modified EDITS survey,

90.6% were satisfied and 82.6% were very satisfied with the prosthesis[16].

A review article published recently 2012 concluded that nine studies, which met their criteria for review over the past 20 years, showed high satisfaction rates with the 3-piece IPP[1].

The limitations of this study are our low number of patients collected, which downs the statistical potency. In the same way, results might have been affected by the fact of being unicenter and retrospective. We consider that prostheses conditions and characteristics through time and the different surgeons performing the implantation could have modified the patients satisfaction as well, and it has not been taken into account to perform the analysis. Another important issue is that we have not used a standarized and validated questionnaire which makes our results difficult to correlate or compare with the ones published by other authors using them.

On the other hand, we present a long term follow up study with low representation in the literature by the fact that we compare malleable and inflatable two or three component in terms of satisfaction. This results offer a new extra tool for the urologist. Because of the absence of differences between malleable or inflatable penile prosthesis in terms of satisfaction, frequency or quality of sexual intercourse, these results could be used as an extra parameter to consider and should be added to the ones used normally to assist ourselves and the patient to choose the more suitable type of prothesis.

Using a non validated questionnaire in our retrospective, unicenter study, our results show high satisfaction rate in patients implanted with either IPP or MPP, similar to literature, and indicate that there are no statistical differences with regard to patient global satisfaction, frequency use of the device and quality of sexual intercourse depending on the type of penile prosthesis. More prospective studies using validated questionnaires are needed in order to obtain more powerful results and conclusions regarding satisfaction in patients implanted with penile prosthesis. In terms of sexual satisfaction, those studies should consider partner satisfaction as well.

COMMENTS

Background

Surgical treatment for erectile dysfunction (ED) by implantation of penile prosthesis is considered a safe option. High satisfaction rates have been reported in the literature.

Applications

Satisfaction rates should be used as an extra item to consider and should be added to the ones used normally (infection and mechanical failure rates, possibility of prosthesis removal, patient preference, anatomical conditions and comorbidities) to assist ourselves and the patient to choose the more suitable type of prothesis.

Peer review

This is a interesting article using a retrospective design, but less innovation. The results of the data are acceptable. Overall the manuscript is well written especially for the authors with English as non-native language.

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OBSERVATIONAL STUDY

Bone markers predict survival in castration-resistant prostate cancer patients treated with docetaxel

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Abstract

AIM: To investigate the relationship between clinicopathological features and bone turnover markers in castration-resistant prostate cancer (CRPC) patients treated with docetaxel.

METHODS: Thirty-three patients were enrolled in this study. Serum levels of carboxyterminal cross-linked telopeptide of type 1 collagen generated by metalloproteinases (1CTP) and alkaline phosphatase (ALP) were measured at the start of docetaxel chemotherapy. We examined the relationship between clinicopathological features and serum levels of 1CTP and ALP levels in CRPC patients treated with docetaxel.

RESULTS: For the total patient group, the mean ± standard deviation (SD) values for docetaxel chemotherapy dose, dose intensity, dosage interval, and number of cycles were 59.3 ± 10.6 mg/m^2, 13.9 ± 5.2 mg/m^2 per week, 4.7 ± 1.2 wk, and 11.2 ± 7.4, respectively. Fourteen patients died from prostate cancer. Patients were divided into two groups according to mean + SD of serum 1CTP (8.2 ng/mL) and ALP (538.2 IU/L) levels at the start of docetaxel chemotherapy. Patients with lower levels of serum 1CTP and ALP had significantly better survivals than those with higher serum levels ($P < 0.05$).

CONCLUSION: Serum levels of 1CTP and ALP are predictors of survival in patients with CRPC who are treated with docetaxel.

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Key words: Prostate cancer; Docetaxel chemotherapy; Carboxy-terminal pyridinoline cross-linked telopeptide parts of type-1 collagen; Alkaline phosphatase; Prognostic factor

Core tip: This study examined the relationship between clinicopathological features and serum levels of carboxy-terminal pyridinoline cross-linked telopeptide parts of type-1 collagen (1CTP) and alkaline phosphatase (ALP) in castration-resistant prostate cancer patients treated with docetaxel. Patients were divided into two groups according to mean + SD of serum 1CTP (8.2 ng/mL) and ALP (538.2 IU/L) levels at the start of docetaxel chemotherapy. Patients with lower levels of serum 1CTP and ALP had significantly better survivals than those with higher serum levels. Serum levels of 1CTP and ALP are predictors of survival in patients with CRPC who are treated with docetaxel.

Endo T, Kamiya N, Suzuki H, Oka R, Lee FC, Utsumi T, Yano M, Kamijima S, Kawamura K, Imamoto T, Ichikawa T. Bone markers predict survival in castration-resistant prostate cancer patients treated with docetaxel. *World J Clin*

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INTRODUCTION

Prostate cancer (PCa) is the most common cancer among men in Western countries[1] and is the second leading cause of death in men[2]. In Japan, the incidence of PCa is rapidly increasing[3]. The clinical course of this cancer varies markedly due to its biological heterogeneity[4]. PCa is androgen-dependent, so androgen deprivation therapy (ADT) is generally used for patients with locally advanced and metastatic PCa[5,6]. ADT is effective for several years, but the disease may ultimately evolve into castration-resistant prostate cancer (CRPC)[7,8].

Docetaxel chemotherapy is the first chemotherapy regimen to demonstrate a survival benefit in CRPC patients[9,10], but not all patients gain benefit from this therapy. Thus, identification of factors that can predict favorable responses to docetaxel would be of benefit for rational selection of therapy for patients with CRPC.

PCa commonly metastasizes to bone, and these types of metastases are associated with various complications and significant morbidity, including severe bone pain, prolonged hospital stay, reduced mobility, hypercalcemia, and pathologic fractures. Furthermore, skeletal-related events correlate with reduced overall and median survival and quality of life of patients with PCa. Biochemical markers of bone metabolism may be useful, non-invasive, and sensitive surrogates of skeletal health. Carboxyterminal cross-linked telopeptide of type 1 collagen generated by metalloproteinases (1CTP) is a marker of bone resorption and is a metabolic product of mature type 1 collagen resorption[11]. Several studies have reported that serum 1CTP levels were significantly higher in PCa patients with bone metastasis than in PCa patients without bone metastasis[12]. Alkaline phosphatase (ALP) is a marker of bone formation and is widely used for assessing bone metastases[13]. We previously reported that serum 1CTP and ALP levels were reliable for the detection of bone metastatic spread and for the prediction of survival in PCa patients with bone metastasis[14].

The goal of the present study was to focus on bone resorption and formation markers by examining the relationship between clinicopathological features and serum levels of 1CTP and ALP in patients with CRPC treated with docetaxel.

MATERIALS AND METHODS

A total of 33 patients treated at our medical institutions between 2005 and 2009 were investigated in this study. All patients had histologically confirmed PCa. They all eventually became resistant to ADT, progressed to metastatic CRPC, and received docetaxel chemotherapy. The dose of docetaxel was 88.1 ± 24.7 (range, 40-130) mg/body, and the median number of docetaxel chemotherapy cycles was 11 ± 8.5 (range, 1-33). Other patient characteristics are described in Table 1.

Blood samples were taken with informed consent. Serum samples, such as prostate specific antigen (PSA), C-reactive protein (CRP), 1CTP, and ALP, were measured at the start of docetaxel chemotherapy. All serum samples were immediately frozen and stored at -20 ℃ until analysis. Serum PSA (ARCHITECT, Abbott Laboratories, Abbott Park, IL, United States) and serum CRP (Alpha Diagnostic Intl. Inc., San Antonio, TX, United States) were measured. Serum 1CTP levels were measured by radioimmunoassay (Immundiagnostik, Bensheim, Germany) to avoid the instability of radioiodinated reagents. Serum ALP levels were measured with IATROLQ ALP (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The rate of decrease in PSA was stratified according to > decline in 30%PSA and < decline in PSA. AEs were classified using common terminology criteria for adverse events (CTCAE) v4.0.

Statistical analysis

Statistical significance was examined using the Mann-Whitney *U*-test and Student's *t*-test, and cause-specific survival curves were created using the Kaplan-Meier method with the log-rank test. For all analyses, differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

For the total patient group, the mean ± SD values for docetaxel chemotherapy dose, dose intensity, dosage interval, and number of cycles were 59.3 ± 10.6 mg/m^2 (range 20-75 mg/m^2; median 60 mg/m^2), 13.9 ± 5.2 mg/m^2 per week (range 3.3-25 mg/m^2 per week; median 13.8 mg/m^2 per week), 4.7 ± 1.2 wk (range 3-8 wk; median 4 wk), and 11.2 ± 7.4 (range 4-37; median 9), respectively. Mean follow-up time after the start of docetaxel chemotherapy was 16.2 ± 12.0 mo (range, 0.2-38.6 mo). During the follow-up period, 14 patients (42%) died from PCa. The median survival period of all patients was 16.2 ± 12.0 mo (range, 0.2-38.6 mo).

Fourteen patients had a PSA decrease > 30%, and 19 patients had a PSA decrease < 30%; thus, the PSA response rate was calculated to be 42.4%. The age at the start of chemotherapy, PSA level at the start of docetaxel chemotherapy, with or without PSA flare , Gleason sum and Extent of disease (EOD) score were not statistically related to survival (data was not shown). However, the number of chemotherapy cycles was significantly related to survival (data was not shown).

A number of adverse events (AEs) were reported in this study. Nausea was reported in seven (21%) cases, including two (6%) cases with grade 2 nausea, and three (9%) cases with grade 3 nausea. Grade 2 constipation was reported in three (9%) cases. Grade 3 lymphedema

Table 1 Clinicopathologic characteristics of castration-resistant prostate cancer patients *n* (%)

Variable	Value
Patients, *n*	33
Median (range)	
Age at start of chemotherapy, years	71.5 ± 7.4 (55-83)
PSA levels at start of chemotherapy, ng/mL	93.3 ± 131.3 (6.3-744.5)
PSA levels (initial diagnosis), ng/mL	877.8 ± 2259.1 (21.0-12490)
Dose of docetaxel, mg/body	88.1 ± 24.7 (40-130)
Dose of docetaxel, mg/m^2	53.3 ± 13.1 (30-70)
Number of cycles, *n*	11 ± 8.5 (1-33)
Survival period after chemotherapy, mo	16.2 ± 12.0 (0.2-38.6)
Clinical T stage	
3	28 (84.8)
4	5 (15.2)
Lymph node status	
0	15 (45.5)
1	18 (54.5)
Gleason sum (initial diagnosis)	
< 6	3 (10.0)
7	7 (23.3)
8	5 (16.7)
9	11 (36.7)
10	4 (13.3)
EOD score (initial diagnosis)	
0	12 (36.4)
1	3 (9.1)
2	10 (30.3)
3	8 (24.2)

CRPC: Castration-resistant prostate cancer; PSA: Prostate specific antigen; EOD: Extent of disease.

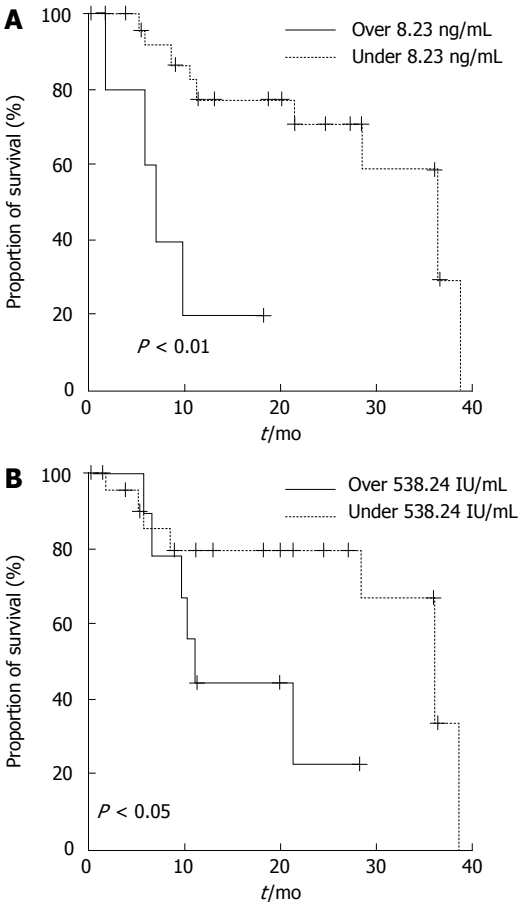


Figure 1 Cause-specific survival curves of castration-resistant prostate cance patients according to serum carboxy-terminal pyridinoline cross-linked telopeptide parts of type-1 collagen levels (A) and alkaline phosphatase levels (B).

of the limb was seen in two (6%) cases; this complication interfered with activities of daily living and resulted in a decreased quality of life. Grade 3 leukopenia was found in five (15%) cases.

During docetaxel chemotherapy up to five cycles, PSA flare was found in five (15%) cases. However, statistical analysis using the Kaplan-Meier method showed that PSA flare was not related to survival.

CRPC patients were stratified according to higher versus lower serum 1CTP levels using the mean + SD (8.23 ng/mL). Six patients had higher levels, and 27 patients had lower levels. Using the Kaplan-Meier method to construct cause-specific survival curves, we established that patients with higher serum 1CTP levels showed statistically significantly worse survival when compared with those with lower levels of 1CTP ($P < 0.01$) (Figure 1A).

CRPC patients were also stratified according to higher versus lower serum ALP levels using the mean + SD (538.24 IU/L). Nine patients had higher ALP levels, and 24 patients had lower ALP levels. Patients with higher serum ALP levels showed statistically significantly worse survival than those with lower ALP levels ($P < 0.05$) (Figure 1B).

Finally, CRPC patients were stratified according to higher (≥ 1 mg/dL) versus lower (< 1 mg/dL) serum CRP levels. Twenty-four patients had higher levels of CRP, and four patients had lower levels of CRP. Statistical analysis using the Kaplan-Meier method to construct cause-specific survival curves showed that patients with higher serum CRP levels had significantly worse survival than those with low CRP levels ($P < 0.01$) (data not shown).

There was no significant difference in the dose of docetaxel when comparing patients with higher and lower levels of 1CTP, ALP, or CRP, respectively.

DISCUSSION

Two large randomized phase Ⅲ studies (SWOG 99-16 and TAX 327) showed that docetaxel, when combined with either estramustine phosphate (EMP) or prednisone, significantly prolongs overall survival in men with metastatic CRPC when compared with the former standard treatment[9,10,15]. The SWOG 99-16 study using EMP in combination with docetaxel confirmed the survival advantage of docetaxel-based chemotherapy[15]. The TAX-327 study randomized 1006 patients to receive either docetaxel or mitoxantrone, each given with low-dose prednisone, and showed an extension of overall survival, improvement in quality of life, pain control, PSA decline, and objective tumor response[9]. Data from these two landmark trials and from other studies demonstrating promising activity for docetaxel against CRPC have resulted in the use

of docetaxel-based chemotherapy as a standard treatment for metastatic CRPC[16,17]. Indeed, docetaxel-based chemotherapy against metastatic CRPC is recommended by the National Comprehensive Cancer Network (NCCN) and the European Association of Urology (EAU) guidelines and is widely used in Japan.

Since the data described above were published, further data have become available from TAX-327, and prognostic factors and nomograms for docetaxel therapy have been proposed[18-21]. In 2010, Armstrong *et al*[20] reported that four independent risk factors predicted a ⩾ 30% increase in PSA within 3 mo of starting chemotherapy, pain, as well visceral metastases, anemia, and bone scan progression following treatment with docetaxel. Furthermore, a nomogram integrating several pretreatment factors (*e.g.*, pain, performance status, ALP, number of sites of metastatic disease, liver metastases, hemoglobin, PSA, and time since diagnosis) was validated for the prediction of post-progression survival. Armstrong *et al*[21] described evidence for the benefit of continuation of chemotherapy beyond progression only for men who had isolated worsening of pain. We similarly demonstrated that serum ALP level was a prognostic factor and that serum 1CTP level was also a useful prognostic factor for cause-specific survival in patients with CRPC who are treated with docetaxel chemotherapy.

Bone metastases are present in almost all CRPC patients receiving docetaxel therapy. In our previous study, we reported that serum ALP levels were useful for the detection of bone metastatic spread and for predicting survival probability in PCa patients with bone metastasis[14]. Sonpavde *et al*[22] investigated patients with bone metastasis and high baseline ALP who were treated with docetaxel and reported that normalization of ALP by day 90 was predictive of better survival independent of whether or not a 30% decline in PSA was achieved. An increase in ALP by day 90 was also predictive of poor survival independent of whether or not a 50% increase in PSA occurred.

Serum ALP level is a useful biomarker in patients with prostate cancer that is characterized by osteosclerotic bone metastasis. Based on data from the present study, serum 1CTP (a bone formation marker) may also be a reliable biomarker in CRPC patients. Indeed, serum 1CTP level was an independent predictor of bone metastasis according to univariate and multivariate analysis in our previous paper. Furthermore, as the EOD score increased, serum levels of 1CTP also significantly increased. Serum 1CTP level was also a significant independent predictor of cause-specific survival according to univariate and multivariate analysis[13]. Patients with higher levels of 1CTP showed worse prognosis, and our analysis suggests that therapies other than docetaxel should be considered for patients with higher levels of 1CTP.

A previous study reported that CRP is an independent prognostic factor for overall survival in patients with CRPC treated with docetaxel[23]. Similarly, we found that patients with lower CRP levels (< 1 mg/dL) had better outcomes than those with higher CRP levels (⩾ 1 mg/dL) ($P < 0.01$). These data suggest that CRP level can predict outcomes in patients with CRPC treated with docetaxel. Narita *et al*[24] used multivariate analysis to demonstrate that serum lactate dehydrogenase (LDH) was an independent prognostic factor for overall survival. Another analysis suggested that levels of serum markers of angiogenesis [*e.g.*, endothelin-1 (ET-1) and tissue factor (TF)] and/or markers of vascular damage [*e.g.*, circulating endothelial cells] could predict overall survival in CRPC patients treated with docetaxel[25].

Measurement of serum bone turnover markers, such as 1CTP or ALP, is useful when PCa patients are diagnosed with CRPC. If high serum bone turnover marker levels are observed, the patient will need to be treated by immediate and adequate intervention (*i.e.*, docetaxel chemotherapy or bone targeted therapy). This study has several limitations, further investigation is necessary to confirm our results.

In conclusion, the present study demonstrates that serum levels of 1CTP and ALP are predictors of survival in patients with CRPC who are treated with docetaxel. The novel agents (*i.e.*, abiraterone acetate, enzalutamide, radium-223, and cabozantinib) offer new options for the treatment of patients with CRPC, including those with disease that is resistant to docetaxel chemotherapy in Western countries.

COMMENTS

Background

Docetaxel chemotherapy has been widely administered to men with metastatic castration resistant prostate cancer (CRPC). To investigate the relationship between clinicopathological features and serum levels of bone turnover markaers in CRPC patients treated with docetaxel.

Innovations and breakthroughs

Serum bone turnover markers can be determined frequently and easily, with negligible disturbance to the patient. This present study suggested that both bone formation markers [alkaline phosphatase (ALP)] and bone resorption markers [type 1 collagen generated by metalloproteinases (1CTP)] might be useful predictors of survival in patients with CRPC who are treated with docetaxel.

Applications

The authors might help the treatment strategy for CRPC in using a novel agents (*i.e.*, abiraterone acetate, enzalutamide, radium-223, and cabozantinib), and avoid the need for frequent electronic computer X-ray tomography technique or bone scintigraphy by using bone formation markers (ALP) and bone resorption markers (1CTP).

Peer review

The article supplements the known literature on risk factors for progression on chemotherapy in patients with hormone refractory prostate cancer.

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PROSPECTIVE STUDY

Alternative mechanisms for prostate-specific antigen elevation: A prospective analysis of 222 transurethral resections of prostate patients

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Abstract

AIM: To investigate the relationship between prostate-specific antigen (PSA) levels and (1) bladder outlet obstruction (BOO) and (2) the severity of prostate inflammation.

METHODS: Two hundred and twenty-two consecutive patients undergoing transurethral resection of the prostate (TURP) were prospectively included. Patients with proven urinary tract infection and/or known prostate cancer were excluded. PSA levels, International Prostate Symptoms Score (IPSS), prostate weight, post residual volume and pressure flow parameters were determined. A histopathological assessment of the presence and severity of inflammation was also performed.

RESULTS: Patients had a mean age of 69.1 ± 8.6 years (45-90 years), with mean preoperative PSA levels of 4.7 ± 5.4 ng/mL (0.2-32.5 ng/mL) and IPSS of 15.7 ± 6.9 (0-32). Mean $PdetQ_{max}$ was 96.3 ± 34.4 cmH_2O (10-220 cmH_2O). The mean resected prostate weight was 39.4 ± 27.3 g (3-189 g). Correlations were observed between PSA (logarithmic) and resected prostate weight (r = 0.54; P < 0.001), PSA (logarithmic) and $PdetQ_{max}$ (r = 0.17; P = 0.032), and resected prostate weight and $PdetQ_{max}$ (r = 0.39; P < 0.001). Furthermore, low correlations were observed between PSA (logarithmic) and active (r = 0.21; P < 0.0001) and chronic (r = 0.19; P = 0.005) inflammation.

CONCLUSION: In this study we showed a correlation between BOO ($PdetQ_{max}$) and PSA (logarithmic). Furthermore, we demonstrated a weak correlation between PSA (logarithmic) and active as well as chronic prostatic inflammation.

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Key words: Transurethral resection of the prostate; Prostate-specific antigen; Bladder outlet obstruction; Lower urinary tract symptoms; Prostate inflammation

Core tip: The goal was to investigate the relationship between prostate-specific antigen (PSA) levels and (1)

bladder outlet obstruction (BOO) and (2) the severity of prostate inflammation. We performed a prospective study on 222 consecutive patients undergoing transurethral resection of the prostate. Patients with proven urinary tract infection and/or known prostate cancer were excluded. PSA levels, International Prostate Symptoms Score, prostate weight, post residual volume and pressure flow parameters were determined. A histopathological assessment of the presence and severity of inflammation was also performed. In this study we showed a correlation between BOO ($PdetQ_{max}$) and PSA (logarithmic). Furthermore, we demonstrated a weak correlation between PSA (logarithmic) and active as well as chronic prostatic inflammation.

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INTRODUCTION

Since its introduction, prostate-specific antigen (PSA) has played a key role in prostatic evaluation[1]. Elevated or rising PSA levels might indicate prostate cancer, a highly prevalent cancer in men with an incidence rate surpassing 20%[2]. The downside of PSA testing is that it is not cancer specific but merely organ specific.

Consequently, elevated PSA levels are a challenging problem for urologists in assessing or excluding potential life-threatening prostate cancer. However, to confirm the diagnosis of prostate cancer, an additional histological evaluation from prostate biopsies is still required. These biopsies can be falsely negative and cannot be repeated infinitely[3]. Furthermore, taking a prostate biopsy is a moderately invasive examination that has, although infrequently, potential life threatening complications[4].

Therefore, a better understanding of other possible mechanisms causing PSA elevation is of utmost importance as this may help to avoid unnecessary biopsies and prevent patient anxiety. Furthermore, patients do not have to be bothered with other, sometimes expensive diagnostic tests, including magnetic resonance imaging[5], advanced transrectal ultrasound imaging[6] or molecular diagnostics[7].

Benign prostate hyperplasia (BPH) is a very common condition in ageing males. As prostatic enlargement can be asymptomatic, the exact incidence of BPH is unknown, ranging between 28% and 60% of the population[8,9]. Additionally, BPH seems to be the second most common reason for surgery in men over 60[10]. Currently, the exact pathogenesis of BPH is not fully understood. Amongst other factors, including hormonal influence, prostatic inflammation could stimulate prostatic growth. Moreover, data on the association between inflammation, prostatic volume, PSA levels and acute urinary retention risk have been published[11].

Therefore, the aim of this study was to investigate the relationship between PSA and the degree of prostate inflammation and to investigate whether PSA levels can be used as a biomarker for bladder outlet obstruction (BOO).

MATERIALS AND METHODS

The study was approved by the hospital's Ethics Committee (07.58/uro07.02) and was conducted according to the established GCP criteria. In this prospective study, 222 consecutive patients undergoing transurethral resection of the prostate (TURP) between May 2008 and June 2010 were included. A single high volume surgeon in a non-academic referral center operated on all patients. As indicated in the EAU guidelines[12], surgery was performed only on patients with a clear indication for TURP, including patients who did not improve after medical therapy, patients with (recurrent) acute urinary retention, high post void residual volume, obstruction characterised by pressure flow analysis or post renal kidney insufficiency. However, patients who were treated with 5ARIs were excluded because of the possible impact on PSA values. Patients with a proven urinary tract infection were excluded, except for patients with catheters, which are colonised by definition. Additionally, patients with known prostate cancer were excluded in order to prevent influence on PSA by cancer cells.

Before surgery, PSA levels were determined for all patients by GPs. The International Prostate Symptom Score (IPSS) was determined. Full urodynamic studies were performed in 154 patients using Laborie Medical Technologies INC/UDS-64-IIs and were evaluated by PIs. Urodynamic studies were not performed in cases of acute urinary retention or high post-residual volume. All patients were treated with low-dose quinolone prophylaxis for 48 hours, starting the day before urodynamic testing. Filling was done standing with a filling speed of 35 mL/minute, using a 6F-filling catheter (double lumen). When indicated, pressure flow analysis was performed according to the International Continence Society criteria[13]. Endoscopic procedures were performed under loco-regional anesthesia using an Olympus resectoscope 26 (6%) or 28 Charrière (94%), depending on the estimated prostate volume. The resected prostate specimens were weighed and the fragments were embedded until four cassettes, each containing 2 g of tissue, were filled. Each additional 10 g of prostate tissue was used to fill an extra cassette[14]. The tissue was fixed in formalin and embedded in paraffin. One slide was made from every paraffin block and examined after staining by hematoxylin and eosin. The inflammatory infiltrate was first divided into an active (mixed infiltrate of lymphocytes, plasma cells and polynuclear cells) and chronic (mononuclear infiltrate of lymphocytes and plasma cells) component, after which the density of both components

Reference tissue (A0 and C0)

A1	A2	A3
C1	C2	C3

Figure 1 Scores for active and chronic inflammation. A: Active inflammation; C: Chronic inflammation. Semi-quantitative scoring scale from 0 (no infiltrate) to 3 (severe infiltrate).

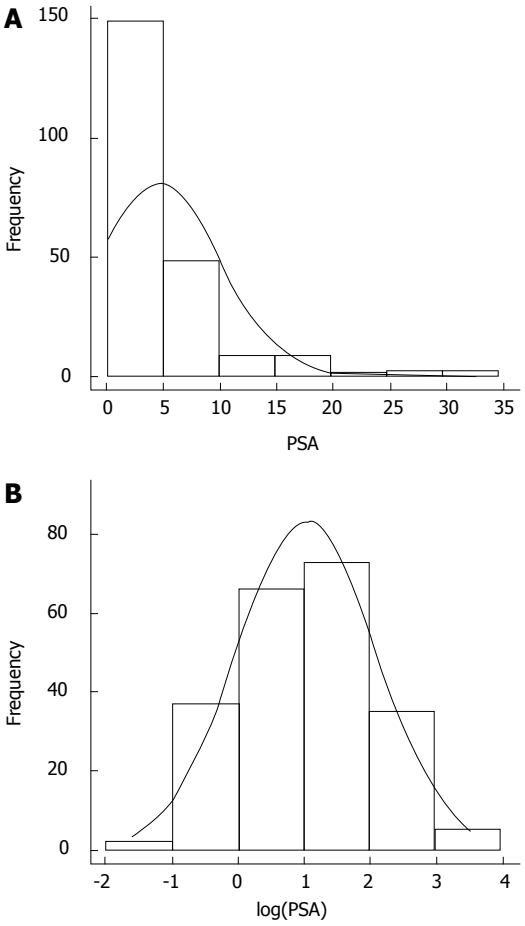


Figure 2 Histogram for prostate-specific antigen (A) and log prostate-specific antigen (B) with a normal density curve superimposed. PSA: Prostate-specific antigen.

was scored 0-3 according to a semi-quantitative scoring system: 0 being no infiltrate and 3 severe infiltrate (Figure 1). Two senior pathologists analyzed all tissue sections of each patient independently and were blinded for clinical data. They scored the mean value of the infiltrate, considering all tissue sections.

Statistical analysis

In an initial step, data were analyzed with descriptive statistics and Pearson correlation was used to investigate simple correlations between different variables. Appropriate linearity tests and lack of fit tests were performed to ensure that these relationships were indeed linear and adequate. Secondly, in a more in depth analysis the relationship between PSA levels and the different potential covariates was analyzed with a multiple regression model. Since the histogram of PSA is severely skewed due to the prevalence of lower PSA values in a normal population (Figure 2), PSA values were transformed with a natural log transformation, resulting in a more realistic normality assumption of the log PSA than the original PSA scale. Selection of the variables included in the model for log PSA was based on the AIC criterion and the adjusted r-square. With respect to the variable active inflammation, it might not seem realistic from a clinical point of view to assume that the difference between any consecutive levels of the active inflammation was the same or the increase was linear in nature. For this reason, the choice was made to treat inflammation as a categorical rather than a continuous variable. Furthermore this choice resulted in a better fit of the model.

Table 1 Characteristics of the 222 consecutive patients undergoing transurethral resection of the prostate between May 2008 and June 2010

	n	Mean	SD	Min	Max
PSA (ng/mL)	218	4.7	5.4	0.2	32.5
Age (yr)	222	69.1	8.6	45	90.0
UF (mL/s)	204	10.7	6.5	1	47.2
IPSS	222	15.7	6.9	0	32.0
PRV (mL)	208	83.8	130.3	0	890
P/F (cm H_2O)	154	96.3	34.4	10	220
Operation time (min)	222	31.1	11.9	10	110
TURP weight (g)	222	39.4	27.3	3	189

SD: Standard deviation; PSA: Prostate specific antigen; UF: Uroflow; IPSS: International prostate symptoms score; PRV: Post residual volume; P/F: Pressure flowmetry; TURP: Transurethral resection of the prostate; Min: Minimum; Max: Maximum.

RESULTS

Patient characteristics

The patients were between 45 years and 90 years old (mean age: 69.1 ± 8.6 years) (Table 1) and had an indication for TURP. Mean preoperative PSA value was 4.7 ± 5.4 ng/mL (0.2 to 32.5 ng/mL). Mean IPSS was 15.7 ± 6.9 (0 to 32). Mean peak flow was 10.7 ± 6.5 mL/s (1 to 47 mL/s). Post residual volume ranged from 0 to 890 mL with a mean value of 83.8 ± 130.3 mL. When indicated (n = 154), pressure flow analysis was executed and showed a mean value of $PdetQ_{max}$ of 96.3 ± 34.4 cmH_2O (10 to 220 cmH_2O). The mean resected prostate weight was 39.4 ± 27.3 g (3 to 189 g). Mean operating time was 31.1 ± 11.9 min (10 to 110 min).

Correlations with PSA

We investigated the relationship between log PSA and several parameters, including IPSS, maximum uroflow, post voided residual volume, pressure flowmetry parameter $PdetQ_{max}$ and the weight of the resected prostate tissue (Table 2). A significant correlation between log PSA and the resected prostate weight was found (r = 0.54; $P < 0.001$). Furthermore, log PSA was correlated with $PdetQ_{max}$ (r = 0.17; P = 0.032). A negative correlation was encountered between log PSA and IPSS (r =

Table 2 Correlation between log (prostate specific antigen) and the corresponding predictor variables

Variable	Linearity (*P* value)	Significance (*P* value)	Correlation (*r*)
IPSS	0.488	0.04	-0.14
Age (decades)	0.563	< 0.0001	0.29
UF (mL/s)	0.929	0.986	0.00
PRV (mL)	0.766	0.276	0.08
P/F (cm H_2O)	0.443	0.032	0.17
TURP weight (g)	0.504	< 0.0001	0.54

IPSS: International prostate symptoms score; UF: Uroflow; PRV: Post residual volume; P/F: Pressure flowmetry; TURP: Transurethral resection of the prostate.

Table 3 Multiple regression model results

Effect	Estimate(st.err)	*P* value
Age (decades)	0.18 (0.069)	0.010
IPSS	-0.02 (0.008)	0.008
Active_0	-1.58 (0.47)	0.001
Active_1	-0.96 (0.53)	0.073
Active_2	-0.78 (0.54)	0.146
Active_3	-0.80 (0.55)	0.150
TURP weight	0.034 (0.006)	< 0.0001
R^2 adj	0.377	

IPSS: International prostate symptoms score; Active 0: No active inflammation; Active 3: Most severe inflammation; TURP: Transurethral resection of the prostate.

-0.14; $P = 0.04$). No correlation was observed between log PSA and peak flow on uroflowmetry ($r = 0.00$; $P = 0.99$). The correlation between log PSA and the post residual volume ($r = 0.08$; $P = 0.28$) was not significant. Additionally, we did not find any correlation between IPSS and $PdetQ_{max}$ ($r = 0.051$; $P = 0.53$). Last but not least, a significant correlation between the weight of the resected tissue and $PdetQ_{max}$ ($r = 0.39$; $P < 0.001$) was observed. These relationships were confirmed in a multiple regression model (Table 3).

Correlations with inflammation (active and chronic)

We also investigated the relationship between inflammation (active and chronic) and log PSA, IPSS, age and the resected prostate tissue weight (Tables 4 and 5). A significant correlation between log PSA and active ($r = 0.21$; $P < 0.0001$) and chronic ($r = 0.19$; $P = 0.005$) inflammation was observed. Age was related to active (r = 0.24; $P < 0.0001$) but not to chronic ($r = 0.09$; $P = 0.08$) inflammation. Similarly, the weight of the resected prostate tissue was related to active inflammation ($r = 0.13$; $P = 0.011$) but not to chronic inflammation ($r = 0.1$; $P = 0.34$). However, IPSS was not correlated with active inflammation ($r = 0.03$; $P = 0.6$) or chronic inflammation ($r = - 0.03$; $P = 0.91$). Additionally, categorization of IPSS into 3 categories did not result in any association with active and chronic inflammations (Table 6).

We also evaluated the influence of pre-operatively placed suprapubic catheters, trans-urethral catheters or previous prostate puncture biopsies and TURP on the relationship with active and chronic inflammation (Table 7). Only a weak negative correlation was found ($r = -0.16$; $P = 0.003$) between active inflammation and the presence of a suprapubic catheter. No significant correlation was observed with the other variables (Table 7). However, for some categories of degree of inflammation no data were obtained, complicating comparisons.

DISCUSSION

PSA measurement is one of the cornerstones in prostate evaluation. Besides BPH and acute prostatitis, prostate cancer can be one of the reasons for PSA elevation, sensitizing and alarming many patients and care givers. Dealing with a patient with elevated/rising PSA levels is always a challenge, especially in ruling out potential life-threatening prostate cancer.

Relationship between PSA and BPH and/or BOO

Multiple papers have been published indicating the relationship between PSA levels and BOO. In patients with clinical BPH, PSA levels are shown to be positively correlated with the five year cumulative risk of invasive BPH treatment[15]. Additionally, in men with BPH, prostate volume and PSA have been evaluated as predictors of acute urinary retention[16,17]. Consequently, elevated PSA levels are predictive for the need of BPH-related surgery. PSA has also been shown to be a strong predictor of future prostate growth[18], which is related to a higher risk of acute urinary retention and, subsequently, to the need for BPH-related surgery. A broader approach for the use of PSA seems justified. In a multicenter study in men suffering from LUTS, a correlation between PSA and the category of BOO was observed[19], Above a PSA-level of 4 ng/mL, mild or definite BOO was observed (in 89% of the cases) and below 2 ng/mL, the chances of a patient not suffering from BOO were one in three[19]. In previous papers, we have shown that BOO can be expected in a very particular group of patients with elevated and/or rising PSA, (multiple) negative multisided prostate biopsies and minor LUTS (mild + moderate IPSS)[20,21]. In a retrospective analysis of 82 consecutive patients, 95.9% were clearly obstructed ($PdetQ_{max} \geqslant 40$ cm H_2O)[18]. Similar results were obtained in a prospective analysis ($n = 33$), with a mean $PdetQ_{max}$ of 80.3 cm H_2O[21]. A positive correlation between PSA velocity and $PdetQ_{max}$ was found ($r = 0.5014$; $P = 0.006$)[22]. As already mentioned, rising PSA levels can also be observed in patients with prostate cancer. Notwithstanding the presence of a tumor in these patients, the elevated PSA levels can also be influenced by BOO, implying that PSA should be included in the pre-treatment workup and the post-therapy evaluation of these patients. Therefore, PSA should be considered an additional indicator in the BOO decision tree.

We found some interesting correlations in this pro-

Table 4 Correlation between active inflammation and prostate related continuous variables

	Active inflammation				*P* value	Correlation, *r*
	0	1	2	3		
Log PSA, *n*	25	91	88	14		
Mean (SD)	1.4 (1.6)	4.4 (4.3)	5.9 (6.4)	4.6 (6.6)	<0.0001	0.21
IPSS, *n*	25	94	88	15		
Mean (SD)	14.4 (6.3)	16.2 (7.0)	15.3 (7.0)	16.6 (7.1)	0.595	0.03
Age, *n*	25	94	88	15		
Mean (SD)	59.7 (5.4)	70.1 (8.1)	70.8 (8.2)	68.4 (9.5)	<0.0001	0.24
PRV, *n*	25	90	81	15		
Mean (SD)	75.0 (103.9)	83.8 (110.6)	68.9 (110.0)	202.8 (303.2)	0.001	0.09
TURP weight, *n*	25	94	88	15		
Mean (SD)	24.7 (10.7)	39.5 (27.4)	44.2 (30.4)	32.6 (14.0)	0.011	0.13

SD: Standard deviation; PSA: Prostate specific antigen; IPSS: International prostate symptoms score; PRV: Post residual volume; TURP: Transurethral resection of the prostate.

Table 5 Correlation between chronic inflammation and prostate related continuous variables

	Chronic inflammation				*P* value	Correlation, *r*
	0	1	2	3		
Log PSA, *n*	4	109	91	14		
Mean (SD)	1.0 (0.7)	4.0 (5.0)	5.7 (5.9)	4.2 (4.4)	0.005	0.19
IPSS, *n*	4	110	93	15		
Mean (SD)	14.8 (7.9)	16.0 (6.5)	15.3 (7.2)	15.7 (8.3)	0.91	-0.03
Age, *n*	4	110	93	15		
Mean (SD)	61.5 (5.2)	68.4 (8.75)	70.5 (8.31)	67.6 (9.3)	0.08	0.09
PRV, *n*	4	105	86	13		
Mean (SD)	68.5 (137.0)	80.3 (129.5)	83.8 (121.8)	116.9 (191.4)	0.88	0.05
TURP weight, *n*	4	110	93	15		
Mean (SD)	25.0 (5.2)	36.8 (25.4)	42.5 (28.9)	40.1 (31.6)	0.34	0.1

SD: Standard deviation; PSA: Prostate specific antigen; IPSS: International prostate symptoms score; PRV: Post residual volume; TURP: Transurethral resection of the prostate.

Table 6 Relationship between International Prostate Symptoms Score (categorized) and inflammation

		IPSS Categorized			*P* value
		Mild	Moderate	Severe	
Active inflammation	0	4	16	5	0.68
	1	11	47	36	
	2	15	43	30	
	3	2	7	6	
Chronic inflammation	0	1	1	2	0.13
	1	10	65	35	
	2	18	41	34	
	3	3	6	6	

IOSS: International Prostate Symptoms Score. 0: No inflammation; 3: Most severe inflammation.

spective analysis of 222 consecutive patients undergoing TURP for bothersome LUTS. A significant correlation was shown between log PSA and $PdetQ_{max}$. In line with the literature[22], log PSA was also correlated with prostate volume (the resected prostate weight) (r = 0.54; P < 0.001). Interestingly, a negative correlation between IPSS and log PSA was observed (r = -0.14; P = 0.04), confirming the findings of our previous studies. This negative correlation could partly be explained by the correlation between high PSA levels and high detrusor pressures due to an increased compensation level of the detrusor for the urethral resistance, resulting in high flow rates and less symptoms.

On the other hand, no statistically significant correlation was observed between log PSA and post voiding residual volume, and between IPSS and the weight of the resected tissue. This might mean that the response of the detrusor to obstruction is more related to the symptoms than the degree of obstruction as such. Last but not least, a highly significant correlation between the weight of the resected tissue and $PdetQ_{max}$ (r = 0.39; P < 0.001) was observed, implying that bigger prostates are more frequently associated with obstruction and therefore are more prone to BPH-related surgery.

Relationship between PSA and prostate inflammation

The role of chronic prostate inflammation in PSA elevation in asymptomatic men is still unclear. Many papers have covered this subject with various and sometimes contradictory outcomes and conclusions. In a prospective analysis of a small group of asymptomatic patients (n = 51) who underwent prostate puncture biopsies, a statistically significant correlation was found between the inflammatory process and the PSA values (P = 0.02)[23].

Table 7 Correlation between inflammation (active and chronic) and risk factors for inflammation

		Active inflammation				*P* value	Correlation, r_c	Chronic inflammation				*P* value
		0	1	2	3			0	1	2	3	
SPC	Yes	1	6	6	5	0.003	-0.16	0	8	7	3	0.34
	No	124	88	82	10			4	102	86	12	
TUC	Yes	0	1	3	0	0.710	[1]	0	2	2	0	1
	No	25	93	85	15			4	108	91	15	
PPB	Yes	2	29	27	4	0.130	[1]	1	25	34	2	0.08
	No	23	65	61	11			3	85	59	13	
Re-TURP	Yes	2	13	11	2	0.930	[1]	0	14	12	2	1
	No	23	81	77	13			4	96	81	13	

[1]No significant correlation. SPC: Presence of a suprapubic catheter; TUC: Presence of a transurethral catheter; PPB: Prostate biopsy punctures; Re-TURP: Previous transurethral resection of the prostate.

Similarly, in a retrospective analysis of 238 men, a correlation between PSA and inflammation was observed[24]. In an analysis of the prostate puncture biopsy of 80 asymptomatic patients, the extent of inflammation was positively correlated with total PSA levels ($P < 0.001$)[25]. In all these studies, histological examination was performed on prostate puncture biopsies in asymptomatic men, limiting the results obtained.

In contrast to the previously mentioned reports, multiple papers claimed the opposite and concluded that there is no correlation between PSA and inflammation. In a retrospective analysis of cancer negative prostate biopsies ($n = 233$), it was concluded that the degree of chronic inflammation did not correlate with PSA levels[26].

Chronic prostatitis was encountered in 68.3% of 284 patients with negative prostate puncture biopsies, while active prostatitis was observed in 8.4% of patients[27]. However, inflammation did not correlate with total PSA levels. In a prostate puncture biopsy driven evaluation ($n = 49$), the presence of inflammation did not correlate statistically with the PSA levels[28]. Finally, Nickel *et al*[29] studied a cohort of 80 patients without a history of prostatitis who underwent TURP. After histological examination on the resected tissue, prostatic inflammation was found to be extremely common. On the other hand, no correlation was observed between inflammation and PSA levels.

We performed a prospective analysis in 222 patients undergoing TURP according to the EAU guidelines[30] but without proven infection, known prostate cancer and a history of chronic prostatitis. We made a histological distinction between active and chronic inflammation, each with 4 subcategories. Additionally, since we used TURP tissue and not prostate puncture biopsy derived tissue, we were able to evaluate a substantial prostatic tissue volume. Our results showed that most patients have some degree of inflammation. Especially in the active inflammation group, a correlation between log PSA and inflammatory parameters was observed ($r = 0.21$; $P < 0.0001$). However, we did not encounter a statistically relevant correlation between symptoms (IPSS) and inflammation. The correlation between age and active inflammation ($r = 0.24$; $P < 0.0001$) is interesting to note, which was not the case for the chronic inflammation group ($r = 0.09$; $P = 0.08$). A possible explanation for this finding could be the accumulation of debris in the prostate during the lifetime, resulting in more frequent active inflammations. This finding may also be the explanation for the correlation between prostate weight (TURP) and active ($r = 0.13$; $P = 0.011$) and chronic ($r = 0.1$; $P = 0.34$) inflammation.

In conclusion, elevated and/or rising PSA levels with regard to underlying prostate cancer and prostatic inflammations are well known. In this paper, we have shown that PSA could also be indicative of BOO, not only with respect to observational data but also with regard to statistically relevant correlations between PSA and $PdetQ_{max}$. Therefore, PSA levels should be taken into account and could even be used as a biomarker when a treatment strategy is determined or executed for patients suffering from clinical BPH. Additionally, the results obtained in this study indicate that inflammation can be correlated with PSA levels. This implies that elevated PSA levels should also be considered as predictive for prostate inflammation as well as for prostate cancer.

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COMMENTS

Background

Since its introduction, prostate-specific antigen (PSA) has played a key role in prostatic evaluation. However, it is not a cancer specific parameter but merely organ specific. Consequently, elevated PSA levels are a challenging problem for urologists in assessing or excluding potential life-threatening prostate cancer. A better understanding of other possible mechanisms causing PSA elevation is required. Therefore, the aim of this study was to investigate the relationship between PSA and the degree of prostate inflammation and to investigate whether PSA levels can be used as a biomarker for bladder outlet obstruction (BOO).

Research frontiers

The cause of elevated PSA serum levels is not always prostate cancer. In the area of interpreting PSA elevation, the research hotspot is to have an insight in to other mechanisms that can be responsible for a PSA elevation, apart from prostate cancer.

Innovations and breakthroughs

In this study the authors showed a correlation between BOO ($PdetQ_{max}$) and PSA (logarithmic). Furthermore, the authors demonstrated a weak correlation between PSA (logarithmic) and active as well as chronic prostatic inflammation.

Applications

PSA could also be indicative of BOO, not only with respect to observational data but also with regard to statistically relevant correlations between PSA and $PdetQ_{max}$. Therefore, PSA levels should also be taken into account and could even be used as a biomarker when a treatment strategy is determined or executed for patients suffering from clinical benign prostate hyperplasia (BPH). Additionally, the results obtained in this study indicate that inflammation can be correlated with PSA levels. This implies that elevated PSA levels should also be considered as predictive for prostate inflammation as well as for prostate cancer.

Terminology

BOO: a blockage at the base of the bladder that reduces or prevents the flow of urine into the urethra, the tube that carries urine out of the body. This condition is most common in aging men. It is often caused by BPH. As a man ages, his chance of developing these diseases increases dramatically; Prostate inflammation: inflammation of the prostate gland. There are four types of prostatitis: acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis without infection, asymptomatic inflammatory prostatitis; PSA (prostate-specific antigen): a protein manufactured exclusively by the prostate gland. PSA is produced for the ejaculate where it liquefies the semen and allows sperm cells to swim freely. Elevated levels of PSA in blood serum are associated with benign prostatic hyperplasia and prostate cancer.

Peer review

This prospective analysis investigates the association of high PSA levels and bladder outflow obstruction and prostatic inflammation. Its results, as described in the discussion, add to the literature and the controversy that exists in these issues among other studies.

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REVIEW

Non-pharmacologic options for the management of voiding dysfunction in multiple sclerosis

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Abstract

Multiple sclerosis is a neuroinflammatory condition that can cause significant bladder dysfunction manifesting either as overactive bladder or impaired bladder emptying. Patients will often complain of urgency, frequency, nocturia, urgency incontinence, hesitancy, straining to void, and incomplete bladder emptying. While these symptoms can be treated with pharmacologic agents, often patients will require more significant treatments. Patients should first be evaluated with urodynamics in order to adequately diagnose the pathologic condition causing their symptoms. These interventions include catheter use, injection of botulinum toxin, neuromodulation, urethral stenting, sphincterotomy, suprapubic catheter with bladder neck closure, bladder augmentation and urinary diversion. The purpose of this review is to examine the evidence supporting each of these treatment options so urologic providers can better provide for this unique and complex patient population.

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Key words: Multiple sclerosis; Neurogenic detrusor overactivity; Detrusor sphincter dyssynergia; Botulinum toxin; Sacral neuromodulation

Core tip: Patients with multiple sclerosis can present with a variety of different urologic symptoms. While they can be treated with multiple pharmacologic agents, at times they will require manipulation or surgical intervention. This article reviews the scientific evidence behind each treatment modality so providers may be more informed as they council patients.

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INTRODUCTION

Multiple sclerosis (MS) is the most common neuroinflammatory disease, affecting approximately 85 people in every 100000[1]. The disease causes demyelination plaques in central nervous system white matter. One of the most common manifestations of MS is bladder dysfunction. Bladder dysfunction results from interruption of neural pathways between the pontine micturition center and the sacral spinal cord by demyelinating plaques[2]. The clinical manifestations of neurogenic bladder dysfunction in MS include overactive bladder syndrome and impaired bladder emptying. Overactive bladder is defined by the International Continence Society as urinary urgency, with or without urgency incontinence, but usually with frequency and nocturia and can be neurogenic or idiopathic in origin[3]. Urodynamic findings of detrusor overactivity provide objective evidence of involuntary contractions of the bladder muscle, the presumed etiology of overactive bladder symptoms. Impaired bladder emptying in MS may range from symptoms of hesitancy and slow urinary stream to complete retention of urine requiring catheterization. Poor relaxation of the external urethral sphincter

is the culprit and can be demonstrated on urodynamic testing. During voiding, synergy of the bladder and urethra should occur allowing the bladder muscle to contract and the urethral muscle to relax, resulting in complete bladder emptying. In patients with MS, dyssynergy occurs such that the urethra does not relax during voiding resulting in high bladder pressure with poor or low flow through the urethra, this is termed detrusor sphincter dyssynergia (DSD).

Bladder dysfunction can be treated with pharmacologic agents and generally these are instituted prior to consideration of more invasive treatment options. Theoretically, pharmacologic agents for overactive bladder symptoms may worsen symptoms of emptying dysfunction in patients with MS and agents aimed at improving bladder emptying may worsen incontinence. In practice there are mixed results with these medications. Additional treatment options may be considered as the result of patient bother from bladder symptoms or poor bladder compliance with concern over upper urinary tract deterioration. Urodynamics can be useful in documenting the components of bladder dysfunction and monitoring bladder changes over time. For neurogenic detrusor overactivity non-pharmacologic treatment options range from simple measures such as condom catheters in men or incontinence pads in women to more invasive options such as Botulinum toxin injection or neuromodulation. For DSD with poor bladder emptying, treatment options range from urethral catheterization to sphincterotomy or intrasphincteric Botulinum toxin injection. Some patients may even require bladder augmentation or urinary diversion.

Patients with refractory bladder dysfunction as a result of MS have several therapeutic options that can be used as an adjunct to pharmacologic therapy or can be used as primary treatment when pharmacologic agents have failed. These therapies are described below.

URODYNAMICS

Many patients with MS will present with specific urinary complaints; however, it is important to evaluate these patients objectively prior to treatment, as some neurogenic bladder dysfunction may be present and may progress to renal decompensation in the absence of symptoms. Typical complaints are include urgency, frequency, nocturia, urgency incontinence, hesitancy, straining to void, and incomplete bladder emptying[4]. The best way to assess the pathologic condition causing these symptoms is to perform urodynamics. Urodynamics is a broad term that includes studies that assess bladder function including uroflometry, post void residual measures, voiding cystometry, urethral pressure profilometry, and electromyography. Having a patient maintain a bladder diary prior to urodynamics will provide further information when the urodynamic study is being interpreted. These studies may include concomitant fluoroscopic imaging to assess anatomy during voiding; this is called video-urodynamics. Common urodynamic findings in MS patients include bladder hypersensitivity, detrusor overactivity, low urinary flow rate, elevated post void residual urine volume, and DSD. Patients may also have evidence of impaired compliance and high pressure voiding suggesting obstruction. In a study by Gallien *et al*[4] in 1998, 149 patients with MS who underwent standardized urodynamic studies DSD was found in nearly 60% of the population. Approximately half of these patients also had detrusor overactivity. They did not, however, find any significant correlation between the urodynamic findings and symptom presentation. A higher post void residual was the main risk factor for developing upper tract urinary infections in this population.

Having urodynamic data can be helpful in the treatment of neurogenic bladder dysfunction as treatment options vary widely and depend primarily on the underlying dysfunction found and patient bother. While it is not a common occurrence, prevention of upper urinary tract deterioration as a result of impaired bladder compliance is critical and must be address in affected patients.

TREATMENT OF NEUROGENIC DETRUSOR OVERACTIVITY

Neurogenic detrusor overactivity (NDO) secondary to the demyelination of the dorsal columns is the most prevalent urodynamic finding in patients with MS, with anywhere from 34%-99% of patients displaying this abnormality[2]. In a prospective study comparing the urodynamic tracings of women with MS to those with idiopathic detrusor overactivity, Lemack *et al*[5] found that the initial detrusor contractions in the MS subset were of higher amplitude and suggested that neurogenic detrusor overactivity may be harder to control[5]. In fact, it has been noted that patients with NDO require higher doses of anticholinergics for effective treatment[6]. Given the not insignificant side effect profile for these drugs, in addition to the potential lack of efficacy, it is logical that patients would seek out alternative forms of treatment such as condom catheters, sacral nerve stimulation, dorsal penile or clitoral nerve stimulation, percutaneous tibial nerve stimulation and injection of Botulinum A neurotoxin.

Catheters

In patients with neurogenic injuries to the bladder causing detrusor overactivity one of the least invasive non-pharmacologic treatments is either an indwelling or condom catheter. The biggest concerns for these patients are the increased risk of urinary tract infections and local irritation and infection of the genital tissues. Additionally, with indwelling catheters there is a risk of urethral erosion that may result in urinary leakage around the catheter, urethral tissue loss, and splitting of the glans and shaft of the penis in men. With condom catheters, approximately 40% of patients will develop urinary tract

infections (UTIs) with long-term use. An additional 15% of users are at risk of ulceration, necrosis and gangrene[7]. Suprapubic catheters are also an option to avoid genital ulceration, but the patient is still at increased risk for urinary tract infection and may continue to leak per urethra from involuntary bladder contractions. In general, catheters are used as a treatment of last resort.

Neuromodulation

Stimulation of the bladder nerves has been shown to inhibit detrusor overactivity in patients with NDO. Neuromodulation is felt to work *via* somatic inhibition of hyperstimulation and overactivity of the sympathetic and sacral motor neurons however, the exact mechanism of action remains unknown[8]. Bladder neuromodulation can be accomplished by stimulating the S3 sacral roots, the tibial nerve, the pudendal nerve, or one of the pudendal nerve branches which include the dorsal nerve of the penis or clitoris. It has also been shown that the posterior tibial nerve, which contains fibers originating from L4 to S3 can have similar effects when stimulated by depolarizing somatic lumbar and sacral nerves to the bladder[2].

In 2005, sacral neuromodulation was recommended by the International Consultation on Incontinence as a second-line therapy for NDO. In the United States, the Food and Drug Administration (FDA) has approved the InterStim Device® (Medtronic, Inc., Minneapolis, MN), a subcutaneous implantable pulse generator attached to a lead that stimulates the S3 nerve root, for the treatment of idiopathic frequency, urgency, urgency incontinence and non-obstructive urinary retention. It has been studied in patients with neurologic disease and found that 66% of patient with NDO can experience significant improvements in their lower urinary tract symptoms after placement and 75% of these patients will have continued success at mean follow-up of four years[9]. In one study of 25 patients with MS, 15 patients showed improvements in their symptoms that were sustained six months after implantation of the InterStim device. Of the six who had detrusor overactivity, there was a significant reduction in frequency episodes from a mean of 18 to 9 (episodes per day), post-void residuals from a mean of 127 to 33 mL, and incontinence episodes from a mean of 13 to 3 per day. Additionally, voided volumes increased from a mean of 83 to 160 mL[10]. Patients appear to have more success if their neurologic conditions are localized[11] and, while patients may have good clinical outcomes, they still may display overactivity on urodynamic testing[12].

In spite of the potential for significant benefit, sacral neuromodulation has not been adopted as a standard treatment for two reasons. First, in the small case studies assessing efficacy it has been noted that when some patients undergo a relapse or experience MS disease progression stimulation parameters could not be reprogrammed to achieve continued clinical improvement in voiding dysfunction[9]. Additionally, patients with an implanted neurostimulator cannot undergo magnetic resonance imaging (MRI) evaluation (with the exception of the head) due to the presence of metal in their body, which is the imaging modality of choice for evaluation of MS patients.

For dorsal penile or clitoral nerve stimulation electrodes are placed on dorsum of the penis or the clitoris and labia majora. In a 2006 study of eight patients with MS and detrusor overactivity, Fjorback *et al*[8] used simultaneous dorsal nerve stimulation and urodynamics to show suppression of bladder contractions and concomitant leakage in seven patients. These patients on average had fifteen minutes between the sensation of urgency and leakage with dorsal nerve stimulation, which the authors suggested was enough time to electively void. There was an increase in bladder capacity of up to 94% on average over this time period[8]. Additional studies have shown capacity to be increased by 55%[13] and conditional suppression of detrusor overactivity allowing for a delay of incontinence[14]. Although these results are promising, dorsal nerve stimulation is not available except at specialized centers and there is considerable hygienic concern with the chronic use of electrodes in the genital area.

An alternative form of neural stimulation that is gaining popularity due to its minimally invasive nature is posterior tibial nerve stimulation (PTNS). The posterior tibial nerve is a terminal branch of the sciatic nerve, which is derived from the L4 to S3 spinal nerves. The common origin of nerve fibers seems to allow for a simultaneous effect on the nerves to the bladder when the posterior tibial nerve is stimulated. In a 2008 study on acute urodynamic effects in patients with MS and overactivity treated with PTNS, Kabay *et al*[15] showed significant increases in volume at first involuntary contraction and maximum cystometric capacity (MCC) by performing urodynamics without stimulation followed by urodynamics with stimulation. Eighteen of the twenty-nine patients had a 50% increase in the bladder volume at first contraction and thirteen had a volume increase of at least 100 cc at first contraction. There was a 50% increase in MCC in 14 of 29 and 17 patients had a 100 cc increase in MCC Kabay *et al*[15]. A criticism of this evidence has been that there could be a conditioning effect of the bladder with the first filling allowing for delayed contraction even in the absence of tibial stimulation; a control group would have been helpful in this study[16].

Kabay *et al*[17] also published a study evaluating the effect of 12 wk of PTNS on 19 patients with MS and NDO utilizing repeat urodynamics and bladder diaries. They found complete clinical responses, which they defined as a 50% decrease below baseline findings, for urgency episodes in 33%, urinary incontinence episodes in 40%, daytime frequency episodes in 57%, nocturia in 75% and pad test in 90%. They also showed significant improvements in mean volume at first detrusor contraction and MCC, as well as decreases in detrusor pressure at first contraction and MCC, increase in max flow and decrease in post void residual (PVR)[17].

Gobbi *et al*[18] evaluated 21 patients with MS and overactivity that had failed anticholinergic therapy who were then treated with PTNS for 12 wk. They found significant reduction in daytime frequency, from 9 to 6

episodes, decreased PVR and increased mean voided volume. Eighty-nine percent of these patients reported a treatment satisfaction of 70%[18].

In a multicenter study, 70 patients with MS and NDO underwent 3 mo of daily 20-min sessions of PTNS. Primary outcomes were the effect on urgency and frequency and secondary outcomes were continence, quality of life, urodynamic changes and tolerance. At the end of 90 d, 83% of the patients had significant improvements in urgency and frequency. They also found a significant decrease in overactivity on urodynamics and a trend toward increased MCC and volume at first involuntary contraction. The treatment was well-tolerated and 70% of the patients wished to continue therapy at the end of the trial[19].

Finally, in 2013, Zecca *et al*[20] published a prospective study on 83 patients with MS who underwent 12 wk of PTNS and responders were then kept on maintenance therapy and followed for a total of 24 mo. 89% of the original treatment group were classified as responders as they had a greater than 50% improvement in their symptoms as measured by the perception of bladder condition questionnaire. Maintenance therapy was one day every four, three or two weeks, depending on patient response. Most patients required treatment every two weeks. 96% of the patients who underwent maintenance therapy were still classified as responders at the end of two years with a mean reduction of frequency episodes to 7 per day from 10, nocturia to 2 times per night from 4, increase in voided volume to 268 from 171 cc, decreased PVR to 52 from 101 cc and an increase in max flow to 25 from 15[20]. It seems that PTNS has the potential to be a lasting and well-tolerated treatment for patients with MS, however there is debate throughout the urologic community as to how real these effects are. Further investigation with randomized, blinded studies of PTNS *vs* sham stimulation is necessary before incorporating this treatment into a standardized algorithm.

Botulinum toxin injections

Botulinum Toxin injections have been adopted by the urologic community and approved by the FDA as a treatment for both idiopathic and neurogenic urinary incontinence secondary to detrusor overactivity. Additionally, it can be used in the MS population for detrusor-sphincter dyssynergia, which will be discussed in more detail later. For NDO in MS patients, *Botox* has been shown to effectively reduce daytime frequency, nocturia, pad use as well as improve urodynamic parameters. In 2006, in a trial in Berlin, 16 patients underwent injection of 300 U of *Botox* into 40 sites, including the bladder base and trigone. Fourteen of these patients also had 50-100 U of the original 300 injected into the external sphincter. At three months, day time frequency decreased from 12 to 6.75, nocturia from 2.16 to 0.61 and pad use from 1.75 to 0.63 daily. Additional significant changes were noted in volume at first contraction and MCC on urodynamics. The effects in this study lasted 3-6 mo and then patients' symptoms returned to baseline[21].

Using 300 U of *Botox*, while effective, does put patients at greater risk of urinary retention requiring clean intermittent catheterization. In 2011, a pilot study in 12 patients with MS and NDO was performed, where each patient was only injected with 100 U in 10 injection sites. All 12 had significant increases in MCC and volume at first desire to void. Voiding diaries showed significant decreases in frequency, urgency episodes and pad usage with a trend to toward a decrease in mean incontinence episodes. Mean maximum flow and voided volume decreased, but only two patients required intermittent catheterization. At 12 wk, patients were beginning to experience a loss of efficacy, but average time to request for re-injection was 8 mo[22].

In 2011, Cruz *et al*[23] published a randomized, double blinded, placebo trial in 154 patients with MS and 121 with spinal cord injuries and NDO. Patients were randomized to placebo, 200 U or 300 U of *Botox*, and 30 injections were administered. The primary endpoint was change in urge incontinence episodes six weeks after treatment, with secondary endpoints of change in MCC, maximal detrusor pressure during first involuntary contraction, and Incontinence Quality of Life score. They also assessed volume at first involuntary contraction, detrusor compliance and volume per void. At week six there were significant changes in weekly urge incontinence episodes in both treatment groups compared to placebo, but no difference between the treatment arms. MCC, volume at first involuntary contraction, and detrusor compliance were all significantly increased with treatment and pressure at first involuntary contraction was significantly decreased, with no differences noted between 200 or 300 U. Median duration of effect was 42 wk for both groups. Of note, there was a higher risk of UTIs in MS patients that received *Botox* treatment. Additionally, 30% of the patients who received 200 U and 42% of the patients who received 300 U began intermittent catheterization for residuals greater than 200 cc[23].

Botox injections can be another form of treatment of detrusor overactivity and urge incontinence in patients with MS. It significant reduces the lower urinary tract symptoms that plague this population, but it is not a perfect solution. First, it is only effective for a limited time, requiring readministration two to four times yearly. Second, it is not without risks, mostly of having to begin intermittent catheterization, but also an increased risk of urinary tract infections. However, for many patients, the potential for improvement in quality of life will likely outweigh the inconvenience of repeat procedures and the risk of catheterization.

TREATMENT OF INCOMPLETE BLADDER EMPTYING AND URINARY RETENTION

DSD is a common manifestation of disease in patients with MS[4]. In DSD the patient is unable to completely relax the detrusor sphincter in correct timing with contracting the bladder to allow normal voiding. This is one

of the most common urodynamic findings in MS. There are multiple treatment options available to patients for DSD. Pharmacologic options include antispasmodics and alpha blockers, but these come with their own side effects and therefore surgical options have also become available for these patients. The ideal solution would be a method of voiding that allows low pressure bladder emptying[24].

When behavioral modifications, bladder training, and alpha blocker medications have not helped the following treatments may be implemented. The first of these is the use of clean intermittent catheterization (CIC). This allows the patient or caregiver to use a disposable catheter to empty the bladder on a regular schedule[2]. There are convenient packs that contain the catheter lubricant and drainage bag all in one, this facilitates things for those that are wheelchair bound or have limited dexterity. It is the treatment of choice because it allows the bladder to fill and empty completely. Patients rarely have issues with urgency or incontinence using CIC because the bladder is emptied to completion each time. Most patients can empty with CIC every 4-6 h, if patients are finding that it needs to be done more often that should prompt urodynamic evaluation to determine the bladder capacity. This will determine a safe CIC frequency. The risk of infection is lowest with CIC because there is no indwelling catheter.

In patients where CIC isn't possible patients require indwelling catheters. Urethral foleys have significant complications including urethral erosion and chronic urinary tract infections. In these cases suprapubic catheters are the next best option. Suprapubic catheters allow constant bladder drainage without urethral damage. They also decrease urinary tract infections[2].

Urethral stenting

One surgical option available to patients with DSD is a urethral stent, temporary or permanent. This is rarely used today due to the permanent nature and potential for significant complication. There are several stents available; Urolume, Memotherm and Ultraflex. All of these can be temporary but the longer they stay in the more difficult it becomes to remove them[25]. This was first used as an option in 1990, and most studies have looked at permanent urethral stents[26]. Shaw *et al*[26] investigated this option in 9 patients with DSD to allow patients to easily pass a catheter for clean intermittent catheterization or wear a condom catheter. At the time this was a new technique that allowed the bladder to empty completely relieving some of the upper tract complications that can occur in patients with DSD. There are many risks associated with temporary or permanent stents including encrustation and migration[27]. Gamé *et al*[24] in 2008 presents the outcomes of temporary stents in 147 men who underwent the procedure for neurogenic DSD. In his study the patients that underwent temporary stenting were unable to self-catheterize. Over the long term the efficacy of stenting has not been shown to be satisfactory[28].

Sphincterotomy

Another option for these patients is a sphincterotomy or bladder neck incision[27]. The bladder neck incision technique was the first treatment used for patients with DSD, beginning in the 1940s. Emmet started with transurethral resection of the bladder neck, he was not very successful but it brought up the idea of having a more permanent lasting effect by doing an external sphincterotomy[29]. An external sphincterotomy became the mainstay of treatment of DSD, the goals of treatment were the resolution of hydronephrosis, reduction in frequency of or complete resolution of UTIs, reduction in frequency of autonomic dysreflexia, reduction in post-void residual urine volume, reduction in voiding pressure, reduction in leak point pressure[27]. A conventional sphincterotomy can result in significant blood loss, this was resolved by the ability to use a laser to perform the sphincterotomy resulting in almost immediate hemostasis[30]. This is considered a permanent procedure causing urethral incontinence; however, in some situations the incision needs to be performed repeatedly due to the development of scar tissue.

Botulinum toxin for DSD

More recently the most commonly used surgical technique for treatment of DSD is intrasphincteric injection of botulinum toxin A. Interestingly, the first use of Botulinum toxin in the urologic patient was for DSD. The injection acts on the sphincter as it does on the detrusor muscle in overactive bladder allowing the sphincter to relax. It does result in urethral incontinence, which limits its use. This is a viable option in patients with MS, there are no permanent changes made and the effects of the Botulinum injection wear off. Patients with MS have a constantly changing spectrum of urologic manifestations and systemic manifestations; a reversible agent is of interest to some patients at the time of MS flare.

In a French study by Gallien *et al*[31] 86 patients were randomized to receive an injection of Botulinum Toxin A (100 U) or a placebo (normal saline). He found no significant difference in the post void residual volume in patients with MS, but the procedure was well tolerated[31]. In 2005 Smith *et al*[32] investigated the reduction in post void residual volume with urethral injections of Botulinum toxin. Patients were treated with 100 to 200 U of Botulinum Toxin-A (BTX-A) in 4 mL divided in equal doses into the four quadrants of the external sphincter or by injection into the bladder base using 100 to 300 U of BTX-A diluted in approximately 10 to 30 mL of sterile saline. Sixty eight patients, thirty two of whom have MS, underwent the procedure, it was found that there was a statistically significant difference in the post void residual and the peak voiding pressure. Both values improved after the urethral injection[32]. The study also showed that the patients overall well-being improved and they had less urinary tract infections. In another study out of Germany it was found that the injection of botulinum toxin into the sphincter for patients with

MS decreases the incidence of urinary retention after intradetrusor Botulinum toxin injection. The authors of the study feel that this is due to a degree of DSD that is found in many patients with MS[21]. This study also found that with an injection of 300 U of *Botox-A* into the bladder and sphincter the incontinence rate decreased. They found that daytime frequency was reduced by 30% at 6 mo and the use of pads was reduced by 64% after 3 mo[21].

Neuromodulation for bladder emptying

There is only one study that reports neuromodulation for use in DSD. Hohenfellner *et al*[33] used sacral nerve stimulation on 11 patients with difficulty emptying. In 8 of the 11 patients symptoms of lower urinary tract dysfunction were decreased by 50%. After the time period of 56 mo all but one of the neurostimulators became ineffective[33].

Despite the success of these few patients it is still important to remind patients that CIC is still the best option and neuromodulation has a marginal place at best in urinary retention neurogenic bladder.

URINARY DIVERSION AND BLADDER AUGMENTATION

Urinary diversion is an option available to patients with MS who have failed all other therapies and remain symptomatic with either overactive bladder symptoms, emptying symptoms, or impaired compliance. When patients experience symptoms refractory to pharmacologic agents and conservative alternatives mentioned above, major surgery becomes the next option. There are several surgical options for patients with MS including bladder augmentation, continence or incontinent urinary diversion, and catheterizable channels. The exact procedure or combination of procedures depends on the underlying bladder dysfunction that needs to be addressed. When patients are unwilling or unable to catheterize an incontinence ileal loop urinary diversion is preferred. For patients willing to catheterize an augmentation cystoplasty is the simplest procedure to increase bladder capacity, improve compliance and improve overactive bladder symptoms. For patients who have an incompetent urethral sphincter or have trouble catheterizing through their urethra and they desire continence, bladder neck closure with creation of a catheterizable channel is possible. This generally utilizes a segment of small intestine and is brought up to the skin in the right lower quadrant or at the umbilicus. A concomitant bladder augmentation is generally performed. Urethral closure should accompany these procedures only if the patient has an incompetent sphincter. As with any surgery these procedures come with their own set of morbidities and these should be weighed carefully with the patient before proceeding.

Patients who undergo these operations are those that have run out of other options. They have usually tried multiple other modalities. In a study by Gudziak the average time from development of neurogenic bladder to ileovesicostomy was 147 mo. This is more than 10 years of suffering with urgency, incontinence, UTIs and multiple other problems that go along with neurogenic bladder[34]. The creation of an ileovesicostomy is a major surgery and there are multiple complications that can arise. Tan *et al*[35] looked at the adverse events of 50 patients who had undergone ileovesicostomy for neurogenic bladder, 19 of those 50 patients had MS. Complications included stomal complications, occurring in 38% of patients, mechanical complications occurring in 22% of patients and wound/bowel complications occurring in 54% of patients. Although the post-operative complication rate was high the urinary tract problems that these patients had preoperatively markedly decreased making ileovesicostomy a viable option for these patients[35].

Ileovesicostomy can also be done robotically, this has been shown to be equally effective in decreasing detrusor pressure while also being safe and effective with decreased hospital stays and less blood loss intraoperatively in one study[36]. In another study by Vanni *et al*[37] the only statistically significant difference between open and robotic ileovesicostomy was in the OR supply costs.

Bladder neck closure with suprapubic catheter placement is an option in patients who have urethral incontinence, decent bladder capacity and are poor surgical candidates. This procedure is simple and can be done without entering the peritoneum[38].

In a study of 9 patients with MS who underwent augmentation cystoplasty for their symptoms it was found that this procedure increases detrusor capacity and decreases intravesical pressure[39]. This is an ideal option in patients where the main problem of their voiding dysfunction is low bladder capacity, impaired compliance or detrusor overactivity with leakage. The patients who underwent the procedure all had preserved or improved renal function. An augmentation cystoplasty with a catheterizable stoma is another option that allows the patient to retain their own bladder while increasing its capacity and decreasing pressure. This is an ideal option in patients who have problems with bladder capacity as well as bladder emptying and prefer catheterization *via* a stoma. In a study by Khavari *et al*[40], 34 patients underwent the Indiana augmentation cystoplasty, 12 patients had MS as their cause for bladder dysfunction. At their final follow up all patients were continent. The patients were found to have increased bladder capacity from an average of 239.7 mL to 444.4 mL, this increase allows the patients to have more time between catheterizations, which leads to an improvement in quality of life. The rate of complications from the surgery was still high; 17% of patients had early post-operative complications including ileus, 44.1% had long-term complications including pyelonephritis and reoperations for stomal revisions[40].

The final option is an ileal conduit urinary diversion. This allows the bladder to be removed in its entirety, and diverting the ureters to a conduit made of small bowel. This is an option for MS patients with refractory neuro-

Table 1 Comparison of studies of urinary diversion for neurogenic bladder in patients with multiple sclerosis

Study	Year	Location	Patient	Outcome
Urinary diversion/reconstruction for cases of catheter intolerant secondary progressive multiple sclerosis with refractory urinary symptoms[43]	2011	Lahey Clinic	26 MS patients (22 female) 15 ileovesicostomy, 7 enterocystoplasty and 4 ileal loop procedures	Improved continence and fewer UTIs
Management of neurogenic bladder dysfunction with incontinent ileovesicostomy[34]	1999	Wayne State	Thirteen patients incontinent ileovesicostomy 8 SCI, 4 MS, 1 TB Meningitis	Safe and effective at providing low pressure urinary drainage
Augmentation cystoplasty in patients with multiple sclerosis[38]	2003	Czech Republic	9 MS patients (7 females, 2 males)	Increased detrusor capacity and decreased pressure
Bladder neck closure and suprapubic catheter placement as definitive management of neurogenic bladder[39]	2011	Tulane	35 patients, 11 male 24 female 27 SCI, 5 MS, 4 other	97% continent, 8 requiring reprocedure to achieve continence
Functional outcomes after management of end-stage neurological bladder dysfunction with ileal conduit in a multiple sclerosis population: A monocentric experience[42]	2011	France	53 MS patients, 6 men 47 women	Statistically significant improved QOL
Robotic-assisted ileovesicostomy: Initial results[36]	2009	Lahey	8 MS patients	Safe and effective with minimal blood loss and shorter LOS
Prospective evaluation of laparoscopic assisted cystectomy and ileal conduit in advanced multiple sclerosis[41]	2012	France	44 MS patients, 34 women 10 men	Decrease in limitations and constraint scores and an increase in autonomy scores
A modification to augmentation cystoplasty with catheterizable stoma for neurogenic patients: Technique and long-term results[40]	2012	Methodist Hospital	12 MS patients	Safe and effective, no ureteral reimplants, no need for cystectomy

MS: Multiple sclerosis; UTI: Urinary tract infection; SCI: Spinal cord injry; TB: Tuberculosis; QOL: Quality of life; LOS: Length of stay.

genic bladder symptoms and poor functional status and dexterity. The indications for an ileal conduit are recurrent febrile urinary tract infections in the setting of poor bladder emptying, chronic retention or an indwelling catheter, urinary incontinence refractory to conservative treatment affecting patients' quality of life (QOL), chronic renal failure secondary to poor bladder compliance, and recurrent urethral bleeding or erosion due to urethral trauma in patients with indwelling catheters[41]. One key aspect of this procedure is the necessity to do a cystectomy at the same time as the diversion due to the risk of pyocystitis[42]. A study was done to determine the potential benefit of laparoscopic-assisted surgery on the cystectomy and ileal conduit procedure. The prospective study by Guillotreau showed that patients had improved QOL scores by decrease in limitations and constraint scores and an increase in autonomy scores. The study was also important in showing that the morbidities were similar to open cystectomy in terms of long term complications but immediate complications were less, *i.e.*, less blood transfusions, shorter hospital stay. In addition, a finding of the study was that patients the longest length of disease duration had significantly more complications, therefore it may be important to consider ileal conduit surgery earlier on in the management of these patients. These studies are summarized in Table 1[41].

There are other surgical options that are available depending on the patients' age and symptoms but these are much less common, including ileal chimney, appendicovesicostomy, and Indiana Pouch. There is very little data to support the use of these options in MS. Augmentation has the benefit of preserving the native ureteral insertion over ileal loop diversion. The ureteral stricture rate is approximately 10%, making this a large consideration[2].

CONCLUSION

MS is a debilitating disease, which can have significant effects on patients' quality of life. Bladder dysfunction is a common symptom experienced by MS patients. There are multiple treatments available if pharmacologic treatments fail. They key is understanding what the underlying bladder dysfunction is, how it may impact the kidneys over time, and what the patients treatment goals are. Minimally invasive options such as clean intermittent catheterization, external condom catheters in men. Neuromodulation, botulinum toxin, and major bladder surgery are available to address patient symptomatology. Sacral nerve stimulation has become a proven technique to treat bladder overactivity in the setting of MS with the possibility of also improving emptying function; however, it requires implantation of metal precluding future MRI. *Botox* was FDA approved to treat neurogenic detrusor overactivity in 2011 and has had a major impact on the treatment algorithm of these patients; however, intermittent catheterization is generally needed for adequate bladder emptying. Major surgery is a final option for patients suffering from bladder dysfunction due to MS. It is usually reserved for patients with deteriorating upper urinary tract function due to impaired bladder compliance and those with intolerable incontinence.

With the many options available it is important to review all of the options with the patient so they are well prepared for the option they have chosen and understand all the risks and benefits.

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REVIEW

Acute management of symptomatic nephrolithiasis

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Abstract

Over half a million patients present to emergency departments and nearly 3 million patients visit healthcare providers annually due to problems associated with urolithiasis. Despite updated guidelines from the American Urological Association and European Association of Urology for the evaluation and management of nephrolithiasis, considerable variability still exists regarding treatment for acute symptomatic upper urinary tract stones. Therefore, this article will review the current evaluation and management of acute symptomatic nephrolithiasis. Initial management includes analgesia and antiemetics. Additionally, a urinalysis and creatinine are required laboratory evaluations. Acute imaging with a non-contrast computed tomography (CT) scan is the diagnostic imaging modality of choice. However, concerns over radiation exposure have led towards low-dose and even ultra-low-dose protocols for the detection of urinary calculi. Low-dose non-contrast CT scans are now standard of care for the initial diagnosis of renal colic in patients with a body mass index $\leqslant$ 30. Medical expulsive therapy is recommended for patients with a ureteral calculus < 10 mm and no signs of infection. Emergency urinary decompression is mandatory for a specific subset of patients, especially those with infection. Although limited data exists, emergency ureteroscopy or even shock wave lithotripsy may also be therapeutic options.

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Key words: Nephrolithiasis; Low-dose computed tomography scan; Medical expulsive therapy; Ureteroscopy; Extracorporeal shockwave lithotripsy

Core tip: Despite updated guidelines from the American Urological Association and European Association of Urology for the evaluation and management of nephrolithiasis, considerable variability still exists regarding treatment for acute symptomatic upper urinary tract stones, especially in regards to imaging modalities used in the emergency department. Acute imaging with a non-contrast computed tomography scan is the diagnostic imaging modality of choice. However, concerns over radiation exposure have led towards low-dose and even ultra-low-dose protocols for the detection of urinary calculi. Low-dose non-contrast computed tomography scans are now standard of care for the initial diagnosis of renal colic in patients with a body mass index $\leqslant$ 30.

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INTRODUCTION

Over half a million patients present to emergency departments (ED) and nearly 3 million patients visit healthcare providers annually due to problems associated with urolithiasis[1]. This has lead to nearly $5 billion spent annually in the United States for hospitalizations, procedures, and time lost from work associated with renal/ureteral stone disease[2]. Despite updated guidelines from the American Urological Association (AUA) and European Associa-

tion of Urology (EAU) for the management of ureteral calculi, considerable variability exists among practitioners. In this review, the acute management of nephrolithiasis will be discussed with a particular emphasis on diagnostic imaging choice, initial medical therapy, and acute surgical interventions.

PRESENTATION

Patients with nephrolithiasis typically present with acute flank pain with or without radiation to the groin. This is referred to as renal colic. The pain is described as colicky in nature because it is intermittent and associated with restlessness, differing from peritonitic pain where patients often remain still. The pain is thought to arise due to obstruction of the ureter with continued peristalsis or spasms of the ureter around the stone. Additionally, obstruction can lead to hydronephrosis and/or hydroureter with pain arising due to distention of the collecting system and renal capsule[3]. The level of the pain can often give a hint as to its location in the collecting system. For example, stones in the proximal ureter present with classic isolated flank pain, while ureterovesical junction (UVJ) stones often present with groin pain associated with frequency, urgency, and dysuria due to irritation of the bladder[3]. In addition to renal colic, patients often present with nausea and vomiting. Furthermore, microscopic or gross hematuria can be a presenting sign of nephrolithiasis due to irritation of the mucosa by the stone or a coexisting urinary tract infection (UTI). Close attention needs to be paid to patients presenting with suspected nephrolithiasis and signs and symptoms of a UTI or sepsis as these patients may require emergency surgical intervention.

INITIAL DIAGNOSTIC IMAGING

A thorough history and physical examination is the first step in the evaluation of suspected renal colic. This is particularly important given the often non-specific flank or groin pain associated with nephrolithiasis. Once renal colic is suspected, diagnostic imaging should be performed with the choice of modality selected based on patient type. In the adult, non-pregnant patient, the preferred initial imaging modality of choice is a non-contrast computed tomography (NCCT) due to its high sensitivity (Median 98%) and high specificity (Median 97%) for identifying urinary calculi[4]. This sensitivity and specificity compares favorably to other imaging modalities (Table 1). NCCT scans not only accurately report the presence of stones, but also the size, location, density *via* Hounsfield units, evidence of obstruction, and skin to stone distance, which all help to determine the need for surgical intervention[5]. Additionally, they also provide information on alternative diagnoses such as appendicitis and diverticulitis.

Although NCCT are valuable in diagnosing urinary calculi, one disadvantage is the delivery of ionizing radiation. Recent investigations have shown an increased risk of a secondary malignancy after just 2-3 computed tomography (CT) scans in a single year, with an estimated 1.5%-2.0% of all cancers in the United States being attributed to the radiation from CT scans[5-8]. Additionally, nearly 50% of all radiation received by the United States population is a direct result of medical imaging, much of which is related to CT usage[9]. Furthermore, it has been estimated that the risk of cancer is 1 in 200 for every 100 mSv of radiation received[10]. This information is critical given the effective dose of radiation for a standard dose NCCT scan is 10 mSv[11,12].

Table 1 Median sensitivity and specificity in detecting nephrolithiasis for various imaging modalities

Imaging modality	Sensitivity[1]	Specificity[1]
Non-contrast CT	98%	97%
Abdominal X-ray	57%	76%
Intravenous pyelogram	70%	95%
Renal/bladder ultrasound	61%	97%
MRI	82%	98%

[1]Information from this table obtained from Ref. [4]. MRI: Magnetic resonance imaging; CT: Computed tomography.

As a response to the risk of ionizing radiation delivered by standard dose NCCT, low-dose NCCT (LD-NCCT) protocols were developed. This imaging has an effective dose of radiation of 4 mSv[13] or less. A study by Poletti *et al*[14] assessed 125 consecutive patients admitted to the emergency department for renal colic with both a standard dose and LD-NCCT scan. LD-NCCT scans had a 97% sensitivity and 96% specificity for the diagnosing of renal colic based on either direct identification of a calculi or *via* indirect signs of a calculi (*i.e.*, hydroureteronephrosis, perinephric stranding, *etc.*)[14].

When stratified by stone size, a LD-NCCT scan was equivalent to a standard dose NCCT scan for stones $\leqslant$ 3 mm in patients with a body mass index (BMI) < 30[14]. In patients with stones < 3 mm, a LD-NCCT scan performed worse with a sensitivity of 83%[14]. Additionally, LD-NCCT scans resulted in ± 20% size variation as compared to standard dose NCCT scan[14]. Despite these limitations, the authors noted no change in their clinical decision-making[14]. Furthermore, the majority of stones < 3 mm rarely require urgent urologic procedures and often will pass spontaneously[14].

When examining the effectiveness of LD-NCCT, BMI also is important. For patients with a BMI $\geqslant$ 30, a standard dose NCCT is still preferred. Poletti *et al*[14] reported only 50% sensitivity and 89% specificity in patients with BMI $\geqslant$ 30 as opposed to 95% sensitivity and 97% specificity for patients with a BMI < 30. Similar investigations have demonstrated equivalent results[15,16].

Additional studies have suggested that ultra LD-NCCT scans can be used for patients with a BMI < 30 without significant loss of sensitivity or specificity[17]. Udayasankar *et al*[18] investigated the use of ultra-low dose CT scans (mean effective radiation dose of 2.10 mSv)

in 163 patients presenting to the ED with abdominal pain and found a high sensitivity (100%) and specificity (98.5%) for detection of free air, stones, and intestinal obstruction[18]. Additionally, overall there was a high sensitivity (86%) and specificity (96%) for identifying other sources of abdominal pain with the conclusion that ultra-low dose NCCT scans provide accurate diagnostic information and very low radiation doses[18] in patients presenting with acute abdominal pain.

The AUA guidelines recommend the use of a LD-NCCT as the preferred initial imaging modality for patients with a BMI $\leqslant$ 30 who are presenting with symptoms of renal colic or in those with a prior history of urinary stones. However, in those with a BMI $\geqslant$ 30, LD-NCCT may be used, although the preferred imaging modality would be a standard dose NCCT. At this time, there is not enough data available to recommend an ultra LD-NCCT scan.

FOLLOW-UP DIAGNOSTIC IMAGING

A recent review of the National Hospital Ambulatory Medical Care Survey estimated that approximately 5%-10% of visits to the ED for nephrolithiasis were return visits[19]. While LD-NCCT is the imaging modality of choice during the initial presentation, Goldstone and Bushnell reported that repeat CT imaging of known nephrolithiasis changed the diagnosis in only a small percentage of patients[11]. Therefore, the AUA recommends that initial imaging should include a renal ultrasound (RUS) and KUB in patients presenting with a known radio-opaque ureteral/kidney stone and persistent symptoms[4]. If no hydronephrosis or stone is identified on KUB or RUS and the patient is still symptomatic, then a LD-NCCT scan is recommended[4]. In those with radiolucent stones and persistent symptoms, RUS can be used to assess for hydronephrosis with a clinical decision made whether to repeat a NCCT based on the RUS results[4].

INITIAL MANAGEMENT

The initial management for renal colic is supportive care with analgesia and anti-emetics. The mainstay of pain control for renal colic includes non-steroidal anti-inflammation drugs (NSAIDs) and narcotic medications. NSAIDs have been shown to provide improved pain relief *vs* narcotics without the added side effects of nausea or vomiting[3]. Therefore, an oral or intravenous NSAID is first line therapy[3]. Narcotic medications can be added for additional relief. Furthermore, antiemetic medication can be utilized as needed for nausea and/or vomiting often associated with renal colic. There is no evidence supporting increased fluid intake for acutely symptomatic stones to help with spontaneous passage; however, increased fluid intake may help prevent future stones. Patients being treated conservatively should strain their urine for confirmation of passage and for analysis[3].

INITIAL LABORATORY AND URINE EVALUATION

According to the EAU guidelines, all patients presenting with acute symptomatic nephrolithiasis should have a urine dipstick to assess for blood in the urine, leukocytes for signs of inflammation, and nitrite to assess for specific bacteria and thus infected urine[20]. If the urine dipstick is suspicious for infection, a urine culture should be sent[20]. Additionally, all patients should have a creatinine level to assess for acute kidney injury and the possibility of an obstructive process[20]. In patients with a fever, evaluation should also include a complete blood count [for analysis of a patient's white blood cell (WBC) count for evidence of inflammation or infection] and C-reactive protein[20]. Additional studies can include a basic metabolic panel for analysis of sodium and potassium levels in those with nausea and vomiting[20].

INDICATIONS FOR CONSULTATION

Many patients with an acute episode of nephrolithiasis initially present to their primary care physician or the ED. At our institution we recommend consulting urology if pain is intractable, the patient is unable to tolerate an oral diet due to persistent nausea or vomiting, there is evidence of obstructive uropathy, concurrent UTI is suspected, or in any patient with a solitary or transplant kidney. If the patient is to be discharged from a primary care physician or ED, we generally recommend urology outpatient follow up in 1-2 wk in all cases of nephrolithiasis.

EMERGENCY DECOMPRESSION

The majority of patients (83%) presenting with nephrolithiasis will pass their stone without any need for intervention[21]. Furthermore, 95% of these patients will pass their stone within 6 wk[21]. While most patients will eventually pass small ureteral stones, clear indications for decompression in the acute management of ureteral stones includes the presence of infection, intractable pain or vomiting, obstruction in a solitary or transplant kidney, bilateral obstructing stones, or relief of ureteral calculi obstruction in pregnant females pending definitive management post-partum[20]. Randomized controlled trials (RCTs) have shown ureteral stenting and percutaneous nephrostomy (PCN) tubes are equally effective for emergency decompression of the urinary system[22]. A small RCT of 42 patients by Pearle *et al*[22] investigated ureteral stent *vs* PCN tube for obstructive ureteral stones and signs of infection, reporting equal times to normalization of fever and WBC count with a trend towards longer hospital stays in those following PCN placement. Another small trial assessed 40 patients with a ureteral stone and hydronephrosis, with or without signs of infection, and did not demonstrate a difference in outcomes between ureteral stent and PCN tube placement[23]. A recent retrospective study by Goldsmith

et al[24] investigated patients with obstructive stones identified on CT scan and systemic inflammatory response syndrome at the time of diagnosis to determine differences in outcomes between ureteral stent and PCN tube placement. A total of 130 patients met inclusion criteria. Patients selected for PCN tube placement had larger stones (10 mm *vs* 7 mm), were more ill based on their APACHE score, and had a higher proportion of surgically altered urinary tract anatomy[24]. After resolution of the patient's sepsis, those undergoing ureteral stent were more likely to be treated with ureteroscopy (65% *vs* 40%, $P = 0.004$) and those undergoing PCN tube placement were more likely to be treated with percutaneous nephrolithotomy (38% *vs* 6%, $P = 0.001$)[24]. Time from initial septic event to definitive treatment and rate of spontaneous stone passage was similar between the PCN tube and ureteral stent group[24]. Intensive care unit admission rates were higher for the PCN tube group (42% *vs* 20%, $P = 0.006$), likely due to more ill patients being selected for PCN tube placement[24].

In summary, indications for emergency urinary tract decompression include intractable pain, nausea/vomiting, evidence of obstructive uropathy, symptoms or signs of infection, and calculi in a solitary or transplant kidney. The preferred method of decompression (ureteral stent or PCN) is likely equivalent, and should therefore be based on stone size, stability of the patient, available hospital resources, and anticipated future method of definitive treatment.

URGENT URETEROSCOPY

In the acute management of stones, patients are typically discharged without the need for a procedure. If, however, a procedure is indicated, then palliation with a ureteral stent or PCN is often performed. Despite this common practice, recent investigations have assessed emergency ureteroscopy. Proponents of this practice cite that immediate stone removal can relieve pain, and prevent multiple trips to the operating or emergency room. Sarica *et al*[25] published a prospective study on 145 patients presenting to the ED with obstructing ureteral stones. Stones were located in the distal ureter in 67.6% and proximal ureter in 32%[25]. Patients were split into either ureteroscopy within 24 h of first colic attack or medical expulsive therapy (MET) for > 7 d followed by ureteroscopy within 7-21 d[25]. There was no difference in intraoperative complications or stone location[25]. Ureteral stents were placed in 24.6% of those on MET *vs* 0% in those undergoing immediately ureteroscopy ($P = 0.001$)[25]. There was no difference in the need for additional procedures[25]. Stone free rate was 87.9% in the MET first group and 90.8% in the emergency ureteroscopy group[25]. Readmission rates were higher in the MET first group, with 3.03 mean readmission to the ED[25].

Al-Ghazo *et al*[26] examined 244 patients treated with emergency ureteroscopy (within 24 h of admission) for acutely symptomatic ureteral stones. Overall success rate, defined as complete absence of stone fragments at 4 wk post-operatively, was 90.6%[26]. Proximal ureter, mid ureter, and distal ureter stones had 69.4%, 94.8%, and 96.6% success rates, respectively ($P < 0.001$)[26]. Overall complication rate was 13.1%, decreasing to 2.5% when excluding stones 10 mm or greater, consistent with prior studies[27-30]. The success rate of ureteroscopy is due in part to improving optics and advances in intracorporeal lithotripters such as the holmium yttrium-aluminum-garnet laser, allowing for safe and effective lithotripsy and stone removal[31]. Another advantage to ureteroscopy is that it does not require thromboprophylaxis following surgery, except in high risk patients[32].

Although limited data exists on this topic, ureteroscopy within 24 h of initial presentation may be a viable option, especially for patients with a symptomatic, obstructing mid to distal ureteral stone without evidence of infection. However, further investigation is necessary prior to widespread adoption.

EMERGENCY EXTRACORPOREAL SHOCKWAVE LITHOTRIPSY

Since its introduction in the 1980s, emergency extracorporeal shockwave lithotripsy (ESWL) is a minimally invasive method to treat both kidney and ureteral stones. According to the EAU guidelines, ESWL and ureteroscopy are both first line treatments for proximal ureteral stones[20]. A recent meta-analysis by Picozzi *et al*[33] assessed 7 studies with a total of 570 patients who underwent urgent ESWL for the treatment of a symptomatic stone. Stone free rates and complication rates did not differ statistically from those reported in the most recent AUA or EAU guidelines for elective ESWL; however, subsequent surgery was required in 15.8% of patients to completely remove the stone[33]. ESWL is thus an option to emergently treat stones, although further investigation is needed. One must be careful in performing ESWL in patients with a known bleeding diathesis or on blood thinning medications. Appropriate bridging therapy should be utilized in patients on warfarin and patients on antiplatelet therapy should discontinue these medications prior to ESWL as severe complications have been reported[32,34,35]. These patient's tend to undergo ureteroscopy as it is safer from a bleeding standpoint. This should be taken into account when deciding the best treatment method, especially if emergency surgery is being considered.

MEDICAL EXPULSIVE THERAPY

The majority of patients (83%) presenting with nephrolithiasis will pass their stone without any need for intervention[21]. Therefore, the EAU guidelines recommend for ureteral stones < 10 mm with minimal to moderate hydronephrosis and no evidence of renal damage, observation with or without MET is standard of care[20]. MET

has been shown to improve the rate of stone passage[20]. Calcium channel blockers, steroids, and alpha-blockers have all demonstrated improved stone passage rates[20]. Steroids are usually avoided because of the numerous systemic effects. In a meta-analysis of available RCTs comparing MET to placebo, calcium channel blockers showed an absolute increase in stone passage of 9% and alpha-blockers shown an absolute increase in stone passage of 29%[20]. Therefore, alpha-blockers are the preferred agent for MET.

Tamsulosin is the most widely studied alpha-blocker used for MET. Fan *et al*[36] performed a meta-analysis of 20 RCTs across 10 countries including 799 patients in the tamsulosin arm and 794 patients in the control arm. Expulsion rates for lower and upper ureteral stones were significantly higher in the tamsulosin arm (lower ureteral stones: RR = 1.55, $P < 0.00001$; upper ureteral stones: RR = 1.28; $P = 0.02$)[36]. Additionally, expulsion time was improved in the tamsulosin group by an average of 2.63 d[36]. These patients also had fewer colic episodes and underwent fewer auxiliary procedures. In a RCT, Al-Ansari *et al*[37] studied 100 patients with lower ureteral stones and compared placebo to tamsulosin and found spontaneous passage rate to be 82% in the tamsulosin group *vs* 61% in the placebo group. Expulsion time was also shorter[37]. Yencilek *et al*[38] showed improved passage rates in those receiving tamsulosin *vs* placebo for ureteral stones < 5 mm (passage rate 71.4% *vs* 50%).

In summary, for ureteral calculi < 10 mm without signs of infection or acute renal failure, a trial of MET should be initiated. Alpha-blockers are considered first line for MET due to the familiarity with the drugs, improved rates of spontaneous passage, decreased time to stone passage, and fewer colic episodes. While tamsulosin is the most studied medication for MET, other alpha-blockers should have similar outcomes. For those with documented spontaneous passage of their stone, repeat imaging is not necessary. If the patient is persistently symptomatic after passage, Fulgham *et al*[4] recommend a follow up RUS with a NCCT if the patient has hydronephrosis. While the optimal length of time of MET before intervention is controversial, common practice is for 4-6 wk[39].

PREGNANT PATIENTS

Nephrolithiasis affects about 1 in 500 pregnancies[40-42] and often becomes symptomatic in the second or third trimester[43-45]. Fortunately 70%-80% of these patients will pass their stone spontaneously with conservative management[45]. A RUS is universally accepted as the first line study in pregnant patients presenting with suspected nephrolithiasis with a sensitivity of 34% and specificity of 86%[46]. If a RUS fails to identify nephrolithiasis or alternative diagnoses, the EAU recommends either a transvaginal ultrasound to assess for UVJ or bladder stones or an MR Urography (MRU), which avoids ionizing radiation[20]. MRU has limited capacity to identify small calculi, is costly, and is often unavailable; however, it avoids ionizing radiation, which may increase the risk of secondary malignancies[47-49]. Additionally, MRU should not be used in the first trimester due to unknown risks to the developing fetus[47,50]. Some have advocated the use of LD-NCCT scans in complicated cases where no other diagnosis has been identified, but this requires ionizing radiation and patients must be counseled extensively about the risks and benefits to the mother and fetus. Most notably, a single pelvic CT may increase the risk of childhood cancer in the exposed fetus by 2 times; however, due to the low absolute risk of childhood cancer (1 in 2000), the increase in absolute risk is extremely low[51]. The American Congress of Obstetricians and Gynecologists guidelines for diagnostic imaging during pregnancy report that exposure to less than 5 rad (which is the case for a NCCT of the abdomen and pelvis) has not been associated with an increase in fetal anomalies or pregnancy loss and that a single diagnostic X-ray procedure does not result in harmful fetal effects[52].

Pregnant patients should be treated similarly to non-pregnant patients with fluids and analgesia[20]. Additionally, urinary diversion with a ureteral stent or PCN may be required in the emergent setting when meeting the same criteria as the non-pregnant patient. These should be placed using ultrasound guidance or with limited fluoroscopic radiation as possible. Ureteral stents or PCN tubes need to be exchanged every 4-8 wk during pregnancy *vs* every 3 mo in non-pregnant patients due to an increased risk of encrustation[20]. Ureteroscopy, using laser lithotripsy, is increasing being employed in this population as experience has increased[20].

CONCLUSION

Nephrolithiasis is common and is often treated by urologist and non-urologists alike. While the AUA and EAU currently have guidelines for the evaluation and management of nephrolithiasis, these are directed at urologists. To our knowledge no national or universal guidelines exist for the acute management of stone disease in the ED. Therefore, we hope that this review will assist physicians to evaluate and manage nephrolithiasis in the acute care setting.

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REVIEW

Metabolic syndrome in the development and progression of prostate cancer

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Abstract

Prostate cancer (PCa) is the most common noncutaneous malignancy and second leading cause of cancer-specific mortality for men in the United States. There is a wide spectrum of aggressiveness ranging from biologically significant to indolent disease, which has led to an interest in the identification of risk factors for its development and progression. Emerging evidence has suggested an association between metabolic syndrome (MetS) and PCa. MetS represents a cluster of metabolic derangements that confer an increased risk of cardiovascular disease and type 2 diabetes mellitus. Its individual components include obesity, dyslipidemias, high blood pressure, and high fasting glucose levels. MetS has become pervasive and is currently associated with a high socioeconomic cost in both industrialized and developing countries throughout the world. The relationship between MetS and PCa is complex and yet to be fully defined. A better understanding of this relationship will facilitate the development of novel therapeutic targets for the prevention of PCa and improvement of outcomes among diagnosed men in the future. In this review, we evaluate the current evidence on the role of MetS in the development and progression of PCa. We also discuss the clinical implications on the management of PCa and consider the future direction of this subject.

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Key words: Diabetes mellitus; Dyslipidemias; Humans; Hyperglycemia; Hypertension; Insulin resistance; Male; Metabolic syndrome X; Obesity; Prostatic neoplasms

Core tip: The current literature is conflicted on the association between metabolic syndrome (MetS) and prostate cancer (PCa), although several studies have demonstrated that men with MetS or its individual components may have an increased risk of more aggressive disease and mortality as well as a poorer outcome after their treatment for PCa. These men may benefit from weight loss, physical activity, and the addition of medications like statins for preventing PCa and improving their outcomes after treatment. A majority of the existing evidence is retrospective or observational in nature, which underscores the need for more randomized controlled trials in the future.

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INTRODUCTION

Prostate cancer (PCa) has been the most common noncutaneous malignancy diagnosed in men since 1984 and currently accounts for almost 30% of new cancer cases in the United States. Concurrent with the introduction of prostate-specific antigen (PSA)-based screening and development of effective treatments for PCa, a steady decline in its mortality rate has been observed since 1991.

Table 1 Joint criteria for clinical diagnosis of the metabolic syndrome[1]

Component	Threshold
Abdominal obesity	Sex- and population-specific waist circumference based on definitions established by IDF and AHA/NHLBI[8,9]
Dyslipidemias (or pharmacologic treatment)	
High triglycerides and/or	⩾ 150 mg/dL
Low high-density lipoprotein cholesterol	< 40 mg/dL in males, < 50 mg/dL in females
High blood pressure (or pharmacologic treatment)	Systolic ⩾ 130 mm Hg and/or Diastolic ⩾ 85 mm Hg
High fasting glucose (or pharmacologic treatment)	⩾ 100 mg/dL

[1]Must have 3 of the following 5 components. IDF: International Diabetes Federation; AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute.

Currently, only 16% of men diagnosed with PCa succumb to their disease[1]. This marked disparity between the incidence and mortality rates of PCa reflects a wide spectrum of aggressiveness. Differentiating between biologically significant and indolent disease, however, has proven to be difficult and led to an interest in the identification of risk factors for its development and progression.

Although the pathogenesis of PCa remains largely unknown, both genetic and environmental factors are thought to contribute to its development and progression. Significant geographic variations in the incidence and mortality rates of PCa indicate a possible role for dietary, lifestyle-related, and other environmental factors. Epidemiologic studies, for instance, have reported a 10- to 15-fold increased incidence of PCa in western compared to Asian countries and a rapidly rising incidence in Asian countries with the adoption of a more westernized lifestyle[2-4]. Migrant studies have also revealed that Asian men living in the United States have an increased risk of PCa compared to their counterparts living in their native countries[2,5]. However, it is unclear whether this increased incidence is related to the routine use of PSA-based screening in the United States.

The influence of westernization on the risk of PCa may be related to the pervasiveness of obesity and a sedentary lifestyle. A growing body of evidence has specifically identified an association between metabolic syndrome (MetS) and PCa. Their relationship is complex and yet to be fully defined. Developing a better understanding of this relationship may provide an opportunity for the prevention of PCa and improvement of outcomes among diagnosed men in the future. In this review, we evaluate the current evidence on the role of MetS in the development and progression of PCa. We also discuss the clinical implications on the management of PCa and consider the future direction of this subject.

REVIEW OF LITERATURE

A PubMed search was performed for relevant articles between 1966 and 2014. Terms for the search included MetS, obesity, dyslipidemias, hypertension, diabetes mellitus, hyperglycemia, and insulin resistance combined with PCa. Only articles published in the English language and limited to humans were considered. All titles and abstracts were reviewed for their relevance, after which the full texts of selected articles were reviewed. The full texts of additional articles were also reviewed based on the references of selected articles.

DEFINING THE METS

MetS represents a cluster of metabolic derangements that confer an increased risk of cardiovascular disease and type 2 diabetes mellitus (DM). Its individual components include obesity, dyslipidemias, high blood pressure (BP), and high fasting glucose levels. There often is an associated proinflammatory state and insulin resistance, both of which have been implicated in the pathophysiology of MetS.

Since the introduction of syndrome X by Gerald Reavan in 1988, a considerable amount of disagreement has emerged over the terminology and diagnostic criteria related to MetS. Various definitions have been proposed by multiple groups and international organizations over the past 15 years, beginning with the original definition by the World Health Organization in 1998[6-9]. The most recent recommendations by the International Diabetes Federation and American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) still differed on the importance of abdominal obesity and its definition being based upon waist circumference[8,9]. However, an attempt has currently been made to reconcile these differences and agree upon common criteria for the clinical diagnosis of MetS (Table 1)[10].

MetS has become a global epidemic and public health-related issue with a high socioeconomic cost. Based on the National Health and Nutrition Examination Survey from 2003 to 2006, approximately 34% of adults in the United States met the National Cholesterol Education Program adult treatment panel (ATP) Ⅲ criteria for MetS. The prevalence of MetS increased with an advancing age and obesity as measured by body mass index (BMI) in this population. It also varied by race, ethnicity, and gender[11]. Similarly, MetS is prevalent in other industrialized and developing countries throughout the world. Based on a meta-analysis, the prevalence of MetS varied from 10% (France) to 36.4% (India) for men in various populations aged from 20 to 25 years and older, as defined by ATP Ⅲ criteria[12].

THE METS AND RISK OF PCA

It is well-established that the development and progression of PCa is potentiated through the dysregulated stimulation of androgen receptor-mediated pathways in prostatic cells. Due to the identification of common putative pathways involving androgen synthesis and MetS, an increasing number of authors have investigated the association between MetS and development of PCa, but their findings have been equivocal and difficult to compare (Table 2). In 2004, Hammarsten *et al*[13] retrospectively analyzed 299 men diagnosed with PCa in Sweden and demonstrated that men with higher clinical stage and grade disease were more likely to have various components of MetS than men with lower clinical stage and grade disease. These findings were later supported by 3 cohort studies from Scandinavia, in which men with MetS or various components had an increased risk of developing PCa[14-16]. It should be noted that a majority of men did not participate in PSA-based screening in these studies.

Conversely, a large cohort study of a more diverse population from the United States observed an inverse association between MetS and development of PCa. Its authors reported a decreased risk of 23% (95%CI: 0.6-0.98) in men with ⩾ 3 components of MetS, which remained significant after excluding diabetic men (RR = 0.71; 95%CI: 0.54-0.94). Interestingly, non-diabetic men with 2 components had an increased risk of 37% (95%CI: 1.01-1.87), suggesting that the extent and duration of MetS as well as the presence of DM may affect the risk of PCa[17].

Other large cohort studies have also failed to demonstrate an association between MetS and development of PCa. In a cohort of 29364 men from Norway, Martin *et al*[18] observed that MetS and its individual components were not associated with the development of incident or fatal PCa, except for an increased risk of 8% (95%CI: 1%-17%) for each 12 mmHg increase in diastolic BP[18]. Another study followed 2445 men between 40 and 79 years of age participating in the Olmsted County Study over a period of 15 years and reported that multiple components of MetS each had a distinct association with the risk of developing PCa when considered individually and in various combinations[19]. Similarly, Häggström *et al*[20] demonstrated that MetS and its individual components were differently associated with the development of incident PCa in a cohort of 289866 men from Austria, Norway, and Sweden. These authors, however, observed an increased risk of PCa-specific mortality for men in the top quintile for BMI (RR = 1.36; 95%CI: 1.08-1.71) and systolic BP (RR = 1.62; 95%CI: 1.07-2.45) as well as for each 1-unit increase in the composite z score of all metabolic factors (RR = 1.13; 95%CI: 1.03-1.25)[20]. These are the only studies to consider the effect of MetS and its individual components on the risk of PCa. The individual components of MetS were differently associated with PCa in each study, emphasizing the importance of considering their separate and combined effects.

Given other evidence suggesting an association between vitamin D levels and PCa as well as the identification of common putative pathways involving vitamin D and lipid metabolism, Tuohimaa *et al*[21] investigated the combined influence of MetS and vitamin D levels on the development of PCa in a cohort of 588 men between 40 and 58 years of age participating in Helsinki Heart Study. Vitamin D levels were defined as low if <40 nmol/L, normal if 40-59 nmol/L, and high if ⩾ 60 nmol/L. These authors demonstrated an increased risk for men in the highest quartile for BMI (OR = 2.28; 95%CI: 1.22-4.25), systolic BP (OR = 3.33; 95%CI: 1.72-6.44), and diastolic BP (OR = 2.47; 95%CI: 1.3-4.69) only when they had low vitamin D levels as well. An increased risk of PCa was also observed when low vitamin D levels were simultaneously present with a high BMI and systolic BP (OR = 3.85; 95%CI: 1.57-9.41) as well as a high BMI, systolic BP, and low high-density lipoprotein (HDL) cholesterol levels (OR = 8.03; 95%CI: 1.89-34.09) but not when considered with normal or high vitamin D levels[21].

Several studies have been conducted outside of the United States or Scandinavian countries with conflicting results. In a case-control study of 2745 men less than 75 years of age from Italy, Pelucchi *et al*[22] reported an increased risk of PCa in those with MetS (OR = 1.66; 95%CI: 1.26-1.89). There was a dose-response relationship, with men having an increased risk of 12% (95%CI: 0.89-1.42) for any 2 components of MetS, 65% (95%CI: 1.15-2.36) for any 3 components, and 299% for any 4 components (95%CI: 1.03-15.4)[22]. Conversely, Russo *et al*[23] failed to demonstrate an increased incidence of PCa in a cohort of 16677 men greater than 40 years of age simultaneously prescribed with medications for hypertension, dyslipidemias, and DM in Italy[23]. Two large cohort studies from Japan have also failed to observe an association between MetS and development of PCa[24,25].

With the exception of the study by Tande *et al*[17], all of the previously discussed studies have primarily included Caucasian men. Due to the known increased risk of MetS and PCa in African-American men, Beebe-Dimmer *et al*[26] investigated the association between MetS and development of PCa in 498 African-American men between 40 and 79 years of age participating in the Flint Men's Health Study. These authors reported an increased risk of PCa in men with hypertension (OR = 2.4; 95%CI: 1.5-3.7) and a waist circumference > 102 cm (OR = 1.8; 95%CI: 1.2-2.9) individually. There was also an increased risk of PCa in men with any 2 components of MetS (OR = 1.76; 95%CI: 1.1-2.83)[26]. A subsequent study followed a diverse population of 881 men less than 75 years of age participating in the Genes Environment and PCa Study and sought to determine any racial differences in the association between MetS and development of PCa. Its authors demonstrated a marginal association between MetS and development of PCa among African-American men (OR = 1.71; 95%CI: 0.97-3.01) but not among Caucasian men (OR = 1.02; 95%CI: 0.64-1.62). MetS was further

Table 2 Summary of studies on the association between the metabolic syndrome and prostate cancer

Ref.	Design	Country	Population	Time period	Size of cohort	Number of PCa cases	Criteria for MetS	Findings	Association
Hammarsten *et al*[13]	Cross-sectional	Sweden	Referrals with PCa	1995-2002	299	299	N/A	Increased risk of clinical stage T3 and high-grade disease with various components	Positive
Laukkanen *et al*[14]	Longitudinal population-based cohort	Finland	Kuopio communities	1984-2001	1880	56	Modified WHO	Increased risk (RR = 1.9; 95%CI: 1.1-3.5)	Positive
Lund Håheim *et al*[15]	Longitudinal population-based cohort	Norway	Oslo study	1972-1998	15933	507	Modified ATP Ⅲ	Increased risk (RR = 1.56; 95%CI: 1.21-2.0)	Positive
Tande *et al*[17]	Longitudinal population-based cohort	United States	ARIC study	1987-2000	6429	385	ATP Ⅲ	Decreased risk (RR = 0.77; 95%CI: 0.6-0.98)	Inverse
Tuohimaa *et al*[21]	Longitudinal nested case-control	Finland	Helsinki heart study	1981-1997	588	132	N/A	Increased risk with high BMI, SBP, low HDL-C, vitamin D (OR = 8.03; 95%CI: 1.89-34.09)	Positive
Beebe-Dimmer *et al*[26]	Longitudinal case-control	United States	Flint Men's Health Study	1996-2002	498	139	Modified ATP Ⅲ	Increased risk in AA men with 2 components (OR = 1.76; 95%CI: 1.1-2.83)	Positive
Russo *et al*[23]	Longitudinal population-based cohort	Italy	Men treated for PCa	1999-2005	16677	94	Treated for MetS	No association (RR = 0.93; 95%CI: 0.75-1.14)	Null
Inoue *et al*[24]	Longitudinal population-based cohort	Japan	Japan Public Health Center-based Prospective Study	1993-2004	9548	119	Modified IDF	No association (HR = 0.76; 95%CI: 0.47-1.22)	Null
Beebe-Dimmer *et al*[27]	Longitudinal case-control	United States	GECAP study	2001-2004	881	637	Modified ATP Ⅲ	Increased risk of organ-confined disease in AA men (OR = 1.82; 95%CI: 1.02-3.23)	Positive
Martin *et al*[18]	Longitudinal population-based cohort	Norway	2nd Nord Trøndelag Health Study	1995-2005	29364	687	Modified ATP Ⅲ	No association (HR = 0.91; 95%CI: 0.77-1.09)	Null
Grundmark *et al*[16]	Longitudinal population-based cohort	Sweden	Uppsala Longitudinal Study of Adult Men	1970-2003	2322	237	ATP Ⅲ, modified IDF	Increased risk only under competing risk analysis	Positive
De Nunzio *et al*[28]	Cross-sectional	Italy	Men with PSA ≥ 4 or abnormal DRE	2009-2010	195	83	ATP Ⅲ	Increased risk of high-grade disease (OR = 3.82; 95%CI: 1.33-10.9)	Positive
Wallner *et al*[19]	Cross-sectional	United States	Olmsted county study	1990-2005	2445	206	Modified WHO	No association (HR = 0.81; 95%CI: 0.2-3.3)	Null
Pelucchi *et al*[22]	Longitudinal case-control	Italy	Men admitted to participating hospitals	1991-2002	289866	6673	Joint criteria	Increased risk (OR = 1.66; 95%CI: 1.22-2.28)	Positive
Osaki *et al*[25]	Longitudinal population-based cohort	Japan	General health examinees in Tottori Prefecture	1992-2007	8239	152	Modified WHO, ATP Ⅲ, IDF	No association based on any criteria	Null
Jeon *et al*[30]	Cross-sectional	South Korea	Men with PSA ≥ 4 or abnormal DRE	2003-2011	354	90	ATP Ⅲ	Increased risk of high-grade disease (OR = 0.101; 95%CI: 0.022-0.473)	Positive
Häggström *et al*[20]	Longitudinal population-based cohort	Norway, Sweden, Austria	Metabolic Syndrome and Cancer Project	1972-2006	289866	6673	Modified ATP Ⅲ	Increased risk of PCa-specific mortality with increased composite metabolic factors (RR = 1.13; 95%CI: 1.03-1.25)	Positive

Morote *et al*[29]	Cross-sectional	Spain	Men with PSA ⩾ 4 or abnormal DRE	2006-2010	2408	848	ATP Ⅲ	Increased risk of high-grade disease (OR = 1.75; 95%CI: 1.26-2.41)	Positive
Cicione *et al*[31]	Cross-sectional	Italy	Men with HGPIN	2004-2011	161	42	ATP Ⅲ	Increased risk with widespread HG-PIN (57.4% *vs* 23.5%)	Positive

PCa: Prostate cancer; MetS: Metabolic syndrome; N/A: Not applicable; WHO: World Health Organization; ATP: Adult treatment panel; ARIC: Atherosclerosis risk in communities; AA: African-American; GECAP: Genes environment and prostate cancer; IDF: International diabetes federation; PSA: Prostate-specific antigen; HGPIN: High-grade prostatic intraepithelial neoplasia.

associated with organ-confined disease (OR = 1.82; 95%CI: 1.02-3.23) but not advanced disease (OR = 0.93; 95%CI: 0.31-2.77) among African-American men. Interestingly, obese Caucasian men had a decreased risk of PCa (OR = 0.51; 95%CI: 0.33-0.8) and high-grade disease (OR = 0.30; 95%CI: 0.15-0.59), neither of which was observed among obese African-American men[27]. Whether this increased incidence is related to more aggressive screening practices in African-American men in the United States is unclear.

Several studies have investigated the association between MetS and development of PCa in a population at risk rather than the general population. In a cohort of 195 men with a median age of 69 years undergoing transrectal ultrasound-guided biopsies for PSA ⩾ 4 or an abnormal digital rectal exam (DRE), De Nunzio *et al*[28] reported an increased risk of high-grade disease in those with MetS (OR = 3.82; 95%CI: 1.33-10.9)[28]. A similar association between MetS and high-grade disease was demonstrated in another study of 2408 men with a median age of 68 years undergoing biopsies (OR = 1.75; 95%CI: 1.26-2.41)[29]. Conversely, Jeon *et al*[30] observed a decreased risk of Gleason grade ⩾ 7 (OR = 0.101; 95%CI: 0.022-0.473) as well as a lower Gleason grade of 6.63 ± 1.92 in men with MetS compared to 7.54 ± 1.71 in men without MetS[30]. Cicione *et al*[31] also reported that men with MetS and widespread high-grade prostatic intraepithelial neoplasia in ⩾ 4 cores had an increased risk of PCa on repeat biopsy in 6 mo (57.4% *vs* 23.5%). However, there is a potential for selection bias in these studies, as primary care providers and urologists are more likely to have a higher threshold for referral and biopsy, respectively, in men with multiple medical comorbidities.

A recent pooled analysis of studies between 2004 and 2007 demonstrated a 54% increased risk (95%CI: 1.23-1.94) of developing PCa in men with any 3 components of MetS[32]. A meta-analysis of 19 studies, though, did not confirm an association between MetS and overall risk of PCa (RR = 0.96; 95%CI: 0.85-1.09) but observed an increased risk of high-grade (RR = 1.44; 95%CI: 1.2-1.72) and advanced (RR = 1.37; 95%CI: 1.12-1.68) disease[33]. However, the findings of these studies are difficult to compare due to their different designs, particularly concerning the dissimilar populations with different rates of PCa, variable screening practices, use of various and modified criteria for MetS, and exclusion of certain risk factors or diabetic men. All of these studies are also retrospective or observational with inconsistent consideration of certain confounding variables. Lastly, only a few studies consider both MetS and its individual components, which appear to be differently associated with PCa. It is therefore difficult to conclude an association between MetS and development of PCa with any certainty.

COMPONENTS OF THE METS AND RISK OF PCA

Several authors have suggested that it may not be adequate to consider MetS as an individual entity but rather as a product of the separate and combined effects of its components[19].

Obesity

Several studies have reported an increased risk of PCa in obese men, while others have demonstrated either a null or even an inverse association between obesity and development of PCa. Due to these equivocal findings, MacInnis and English performed a meta-analysis of 31 cohort and 25 case-control studies and observed an increased risk of 5% (95%CI: 1.01-1.08) for each 5 kg/m^2 increase in BMI. A sub-group analysis of only studies reporting the stage of disease demonstrated a stronger association for advanced disease (RR = 1.12 per 5 kg/m^2 increment; 95%CI: 1.01-1.23) compared to localized disease (RR = 0.96 per 5 kg/m^2 increment; 95%CI: 0.89-1.03)[34]. Several large cohort studies and a more recent meta-analysis have subsequently confirmed these findings by observing an increased risk of high-grade and advanced disease as well as a decreased risk of low-grade and localized disease in obese men[35-38].

Various theories have been proposed for the differential influence of obesity on the risk of PCa. Some authors have suggested an inherent difference in the aggressiveness

of PCa due to lower testosterone levels in obese men[39]. Others have argued that a bias against the detection of PCa leads to its delayed diagnosis due to difficulty with DRE, lower serum PSA levels, and a larger prostatic size in obese men. Despite the anecdotal reports of difficulty with DRE in obese men, Price *et al*[40] failed to demonstrate any association between BMI and findings on DRE in men being screened for PCa. Alternatively, a number of studies have observed an inverse relationship between BMI and PSA, which may lead to delayed biopsies due to lower serum PSA levels in obese men[39]. This relationship is thought to be related to lower testosterone levels and/or a hemodilution effect on serum PSA concentrations from greater plasma volumes in obese men[41,42]. Two studies have also reported a larger prostatic size in obese men, which may decrease the likelihood of detection due to sampling error[43,44].

Dyslipidemias

Many studies have investigated the association between dyslipidemias and PCa since the initial finding that a cholesterol-lowering diet may increase the risk of various cancers and cancer-specific mortality in 1971[45]. The findings of earlier studies have largely been equivocal and unable to differentiate whether low cholesterol levels are the cause or effect of PCa. More recent evidence seems to favor an increased risk of PCa in men with various derangements of lipid metabolism[46].

In a nested case-control study of 698 men between 40 and 75 years of age participating in the Health Professionals Follow-up Study, Platz *et al*[47] demonstrated a decreased risk of high-grade PCa for men in the bottom quartile for total cholesterol (TC) level (OR = 0.61; 95%CI: 0.39-0.98). Several large cohort studies have subsequently confirmed these findings and further observed an increased risk of high-grade disease, advanced disease, and cancer-specific mortality in men with high TC levels[48-52]. Conversely, Van Hemelrijck *et al*[53] failed to report an association between TC levels and development of PCa in a cohort of 200660 men participating in the Swedish Apolipoprotein Mortality Risk Study. These authors noted that the associations between various components of MetS and PCa may be altered by non-cancer-related mortality due to the competing risk of premature cardiovascular death before the development of PCa. They suggested that the association between high glucose levels and decreased incidence of PCa may be overestimated, while the increased incidence of PCa in diabetic men with high triglyceride (TG) levels may be underestimated under a competing risk analysis[53].

Few studies have investigated the separate influence of HDL and low-density lipoprotein (LDL) cholesterol levels on the development of PCa. Van Hemelrijck *et al*[54] conducted a follow-up of their initial study and investigated the association between the individual components of the lipid profile and development of PCa. These authors demonstrated an increased risk for men in the lower quartile for HDL cholesterol and apolipoprotein A- I levels but no association between LDL cholesterol or apolipoprotein B levels and development of PCa[54]. In a cohort of 29093 men between 50 and 69 years of age participating in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, Mondul *et al*[51] observed a similar trend toward a decreased risk of PCa in men with higher HDL cholesterol levels, which persisted across all grades and stages[51]. A cohort study of 2842 Dutch men also reported an association between higher HDL cholesterol levels and non-aggressive PCa (HR = 4.28; 95%CI: 1.17-15.67) as well as an increased risk of PCa in men with higher LDL cholesterol levels (HR = 1.42; 95%CI: 1-2.02)[55]. Conversely, Jacobs *et al*[56] failed to demonstrate an association between HDL or LDL cholesterol levels and risk of aggressive PCa in a cohort of 14241 men between 50 and 79 years of age within the Cancer Prevention Study Ⅱ Nutrition Cohort. Only 6% of these men, however, met the ATP Ⅲ criteria for high LDL cholesterol levels. This proportion of men with high LDL cholesterol levels was much lower than in other studies, which may account for its conflicting results[56].

As previously discussed, Van Hemelrijck *et al*[53] reported that high TG levels were associated with an increased risk of PCa, but only in men with high glucose levels. Hayashi *et al*[57] also demonstrated an association between TG levels and development of PCa in a cohort of 905 men undergoing biopsies. This association was strengthened in men between 60-69 years of age (OR = 2.1; 95%CI: 1.31-3.37) and ≥ 70 years of age (OR = 1.91, 95%CI: 1.03-3.53), both of whom also had an increased risk of high-grade disease[57]. Several studies have supported these findings, while others have observed either a null or even an inverse association between TG levels and development of PCa but generally included a younger population of men ≤ 60 years of age[15,17,18,58-60]. In addition to age, the frequent co-occurrence of high TG levels and DM is thought to be a confounding factor that accounts for these conflicting results[17,32].

High blood pressure

The association between hypertension and development of PCa has not been as thoroughly investigated as other components of MetS but is thought to be related to the effect of sympathetic nervous activity on the androgen-mediated growth of prostatic tissue[61]. As previously discussed, several cohort studies have reported an increased risk of PCa in men with hypertension, including the 2nd Nord Trøndelag Health, Olmsted County, and Flint Men's Health Studies[18,19,26]. The latter 2 studies actually demonstrated that hypertension was the only component of MetS associated with an increased risk[18,19]. However, the remaining evidence is limited and warrants further investigation.

High fasting glucose

There is a large body of evidence supporting an inverse association between DM and development of PCa. Several meta-analyses have observed a decreased risk of

PCa in diabetic men with pooled RRs of 0.91 (95%CI: 0.86-0.96), 0.84 (95%CI: 0.76-0.93), and 0.86 (95%CI: 0.8-0.92)[62-64]. A more recent meta-analysis of 25 cohort and 12 case-control studies reported a similar association through a subgroup analysis of population-based studies (RR = 0.72; 95%CI: 0.64-0.81), cohort studies from the United States (RR = 0.79; 95%CI: 0.73-0.86), and studies with follow-up of greater than 5 years. Diabetic men on insulin were also noted to have a decreased risk of PCa in all studies included in this meta-analysis[65]. These findings suggest that the inverse relationship between DM and development of PCa is strengthened over time. There is a corresponding natural history for DM that begins with a rise in glucose and insulin levels followed by the development of insulin resistance and decline in insulin levels due to damaged pancreatic beta cells.

Various theories have been proposed for the inverse association between DM and development of PCa. Some authors have suggested a causal effect from the decreasing levels of hormones and other cancer-related mitogens such as insulin-like growth factor-1. Others have argued for a bias against the detection of PCa due to less health-care seeking behavior, lower serum PSA levels, and a larger prostatic size in diabetic men[66]. As previously discussed, a delayed diagnosis of PCa may lead to an increased risk of more aggressive disease in obese men. Several studies have indeed demonstrated an increased risk of high-grade and advanced disease in diabetic men undergoing biopsies and radical prostatectomy (RP)[67-73]. Interestingly, the association between DM and high-grade disease was only observed in obese Caucasian men in one of these studies and was strengthened in this population in another study[67,69].

THE METS AND PATTERNS OF TREATMENT FOR PCA

Men with MetS are often perceived as poor surgical candidates due to a concern for an increased risk of perioperative complications, increased technical difficulty with surgery, and poorer outcomes. These concerns may affect the counseling of these men and their resulting treatment, regardless of whether they are well-founded. There is only limited evidence on the influence of obesity on the patterns of treatment for PCa. When investigating the choice of treatment in men newly diagnosed with PCa, Davies *et al*[74] reported that obese men were more likely to receive a non-surgical therapy, such as active surveillance (AS), external-beam radiation therapy (EBRT), brachytherapy, or androgen deprivation therapy (ADT). Men with BMI $\geqslant$ 35 k/m^2, in particular, were more likely to receive brachytherapy (OR = 1.59; 95%CI: 1.01-2.52) or ADT (OR = 1.77; 95%CI: 1.12-2.81) alone[74].

THE METS AND ONCOLOGIC OUTCOMES FOR PCA

Emerging evidence has suggested that men with MetS or its individual components may have a poorer oncologic outcome after their treatment for PCa.

Radical prostatectomy

In a study of over 4000 men with a median age of 61 years undergoing robot-assisted laparoscopic radical prostatectomy (RALP), Kheterpal *et al*[75] demonstrated a higher pathologic Gleason grade and stage as well as a greater upgrading of Gleason grade 6 disease in men with MetS compared to best-matched controls. However, the prostatic volumes were not included in this study, and a larger prostatic size in obese men may account for these findings due to an increased sampling error at biopsy[75]. Another study of 261 men with a mean age of 64.5 years undergoing RP observed an increased tumor volume in those with MetS (6.6 ± 5.5 mL *vs* 5 ± 4.5 mL) but no differences in any other histopathologic features[76]. Castillejos-Molina *et al*[77] further reported that MetS was associated with an increased risk of biochemical recurrence (BCR) in men with a median age of 64.8 years undergoing RP. MetS was the strongest predictor of BCR on multivariate analysis (OR = 2.73; 95%CI: 1.65-4.5), although men with MetS had a significantly higher proportion with Gleason grade > 7 on biopsy and pathologic stage T3a-b. Therefore, the increased risk of BCR in men with MetS may have been due to selection bias with only those with high-risk disease undergoing RP. When confining their analysis to men with organ-confined disease, the 5- and 10-year BCR-free survival was 55% and 48% for those with MetS compared to 80% and 73% for those without MetS, respectively. There was still a strong association between MetS and BCR in this subgroup (OR = 3.42; 95%CI: 1.68-7.01)[77]. Post *et al*[78] also demonstrated a 50% increase in the rate of BCR after RP in men with MetS. This finding was primarily influenced by the effect of hypertension, which conferred an approximately 2-fold increased risk of BCR and was the only consistent association among all components of MetS[78]. A similar association for hypertension was observed in another study of 1428 men with a mean age of 59.1 years undergoing RP[79]. Most recently, Kwon *et al*[80] failed to report any differences in the operative parameters, histopathologic features, or functional outcomes of men with MetS undergoing RALP, except for an increased blood loss (OR = 1.592; 95%CI: 1.15-2.21)[80].

The oncologic outcomes after RP have been most thoroughly investigated in obese men. Several studies have demonstrated an increased risk of BCR after RP independent of adverse clinicopathologic features in obese men. Two recent meta-analyses confirmed these findings by observing a 25% (95%CI: 1.12-1.4) and 16% (95%CI: 1.08-1.24) increased risk of BCR for each 5 kg/m^2 increase in BMI[81,82]. Additional studies have also suggested an increased technical difficulty for all techniques of RP in obese men with increased operative times, estimated blood loss, complications, and positive surgical margins; while others have reported no differences in these operative parameters and demonstrated an increased risk of BCR independent of surgical margin status and in men

with organ-confined disease. It therefore remains unclear whether the increased risk of BCR after RP is related to an increased technical difficulty, inherently more aggressive disease, or both in obese men[39].

The oncologic outcomes after RP have also been investigated in diabetic men. Two studies have failed to observe an association between DM and risk of BCR after RP[67,83]. Interestingly, the latter study reported an increased risk of BCR after RP (HR = 2.52; 95%CI: 1.4-4.54) only in obese, Caucasian men with DM[67]. This group subsequently performed a study of 2083 United States veterans with a median age of 61 years and again demonstrated that DM was only associated with the development of metastatic disease after RP (HR = 2.8; 95%CI: 1.29-6.09) in obese men, despite receiving a more aggressive secondary treatment[84].

Radiation therapy

As with RP, a number of studies have observed that BMI is an independent predictor of BCR after EBRT and associated with a decreased PCa-specific survival. These findings are thought to be related to the greater daily variation in the location of the prostate and resulting loss of precision in the designated field of radiation in obese men[85-87]. One study also reported an increased risk of BCR in obese men with a median age of 61 years undergoing salvage EBRT therapy after RP[88]. In the absence of surgical pathology to confirm the grade and stage of disease, it is unclear whether a more aggressive disease accounts for these poorer outcomes in obese men undergoing EBRT. Furthermore, the demonstrated hemodilution of PSA in obese men may create the potential for these men being under risk-stratified and undergoing a less aggressive primary treatment with a shorter or absent regimen of ADT.

Conversely, brachytherapy appears to be feasible and effective in obese and diabetic men based on limited evidence. Several studies have failed to observe an association between BMI or DM and risk of BCR after brachytherapy[89-92].

Androgen deprivation therapy

While the development and exacerbation of MetS in men on ADT has been thoroughly investigated, the association between MetS and oncologic outcomes on ADT has not. Two studies have reported that obesity is an independent predictor of BCR and PCa-specific mortality after combined EBRT and ADT, while Keto demonstrated an increased risk of metastatic disease as well as a trend toward an increased risk of progression to castration-resistant disease and decreased cancer-specific survival in men undergoing ADT after RP[93-95]. Only 1 study has investigated the oncologic outcomes of men on primary ADT alone. Flanagan *et al*[96] observed a shorter time to PSA progression in men with MetS, who were treated with luteinizing hormone-releasing hormone agonists for BCR after a definitive local therapy or newly diagnosed metastatic PCa (16 mo *vs* 36 mo). These authors also reported a shorter time to PSA progression for each component of MetS except for TG levels as well as a decreased overall survival in men with hypertension[96]. These studies suggest that men with MetS may have a poorer response to ADT. The mechanism behind this relationship has not been elucidated but may be related to an excess level of estrogens, particularly in obese men. Estrone, estradiol, and free estradiol levels have all demonstrated a direct relationship with BMI, while lower testosterone levels have been observed in obese men[97]. Men with MetS may therefore be androgen-deprived at baseline and have an increased risk of progression to castration-resistant disease. Whether the levels of these hormones affect the progression to castration-resistant disease is unclear.

THE METS AND POST-TREATMENT QUALITY OF LIFE

Men with various components of MetS may have a worse quality of life (QoL) after their treatment for PCa.

The MetS

There is no evidence on the association between MetS and post-treatment QoL for men with PCa. However, 2 recent studies have investigated the combined influence of vascular risk factors on recovery of erectile function after RP, EBRT, and brachytherapy. In a study of 984 men with a mean age of 59.6 years undergoing RP, Teloken *et al*[98] investigated the effect of vascular risk factors (hypertension, hypercholesterolemia, DM, coronary artery disease, and history of smoking) on recovery of erectile function after RP. These authors reported a worse recovery in men with $\geqslant$ 3 compared to 1 or 2 vascular risk factors at 24 mo postoperatively (P = 0.02) independent of age, erectile function before RP, and nerve-sparing status[98]. Wang *et al*[99] conducted a study of 732 men with a mean age of 65.3 years undergoing EBRT and/or brachytherapy with or without ADT over 4 years and similarly demonstrated an increasing incidence of ED with an increasing number of vascular comorbidities (hypertension, DM, hyperlipidemia)[99]. Although men with MetS have many of the same risk factors, it is unclear whether the findings of these studies may be extrapolated to this population.

Obesity

The current evidence on the QoL for obese men after their treatment for PCa is equivocal. Two studies have observed a delayed return of bowel function and increased bother after RP as well as a worse hormonal function after RP and radiation therapy in obese men[100,101]. Others have failed to report a consistent difference between obese and non-obese men in any domains of QoL after RP[102,103]. Several recent studies have specifically addressed the recovery of urinary incontinence and erectile function after RP in obese men. Their conflicting results have been difficult to compare due to the different designs of these studies and use of variable surgical ap-

proaches. There was also an inconsistent use of validated questionnaires and lack of consideration for certain confounding variables[104-117]. These studies collectively suggest that obese men may have a slightly worse perioperative QoL. However, the major predictor of post-treatment QoL remains their QoL prior to the initiation of therapy. The current evidence is insufficient to recommend any particular treatment in obese men based on their post-treatment QoL.

High fasting glucose

The current evidence on the QoL for diabetic men after their treatment for PCa is much more robust. Latini *et al*[118] demonstrated a worse urinary function after RP in both diabetic and obese men. These authors further observed that the combined influence of DM and obesity may be greater than either alone[118]. Thong *et al*[119] also reported a worse urinary and sexual function as well as a worse general health-related QoL after all types of treatment in men with pre-existing DM compared to those with incident DM diagnosed after PCa and without DM[119]. Additional studies have demonstrated an association between DM and the development of ED after both EBRT and brachytherapy[120-122]. Most importantly, a growing body of evidence has established DM as a risk factor for the development of complications after radiation therapy. Several studies have observed an increased risk of $\geqslant$ grade 2 late gastrointestinal and genitourinary complications after EBRT in diabetic men based on the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer scale[123-127]. As with obesity, the current evidence is insufficient to recommend any particular treatment in diabetic men based on their post-treatment QoL. However, it is recommended that diabetic men be counseled about a potentially increased risk of complications after radiation therapy and be considered for the modification of its planning and delivery.

THE METS AND PCA-SPECIFIC MORTALITY

Emerging evidence has suggested an association between MetS and PCa-specific mortality. A recent meta-analysis pooled the findings of 3 cohort studies and reported an increased risk of PCa-specific mortality in men with MetS (RR = 1.12; 95%CI: 1.02-1.23)[33].

The risk of PCa-specific mortality has been most thoroughly investigated in obese men. Several studies have demonstrated that BMI is an independent predictor of PCa-specific mortality among obese men in a population-based cohort and those diagnosed with PCa. A recent meta-analysis of 12 studies confirmed these findings by observing a 16% increased risk of PCa-specific mortality in cohort studies (95%CI: 1.06-1.25) and a 20% increased risk in studies investigating the post-diagnosis survival (95%CI: 0.99-1.46) for each 5 kg/m^2 increase in BMI[81]. Although a bias against the detection of PCa may account for the increased risk of PCa-specific mortality in obese men, 2 studies reported a similar association before the introduction of PSA-based screening[128,129].

There is only limited evidence that men with other components of MetS have an increased risk of PCa-specific mortality. In a cohort of 17934 men between 40 and 69 years of age participating in the Whitehall study, Batty *et al*[50] demonstrated an increased risk of PCa-specific mortality for men in the upper tertile for TC levels over a period of 4 decades (HR = 1.35; 95%CI: 1.11-1.65)[50]. Only 1 study has similarly observed an increased risk of PCa-specific mortality in men with hypertension[20]. A recent meta-analysis also identified 4 studies investigating the association between DM and PCa-specific mortality, only 1 of which reported an increased risk. There was insufficient evidence to perform a formal meta-analysis of these studies. However, the authors identified 7 additional studies investigating the non-PCa or long-term, overall mortality and conducted a preliminary meta-analysis from 4 of these studies, which demonstrated an increased risk of overall mortality in diabetic men (HR = 1.57; 95%CI: 1.12-2.2)[130].

THE METS AND PREVENTION OF PCA

With the growing body of evidence on the association between MetS and PCa, the management of MetS has become a potential target for the prevention of PCa and improvement of outcomes among diagnosed men. Based on recommendations from the AHA and NHLBI, the primary emphasis on the management of MetS is to mitigate the modifiable risk factors of obesity and physical inactivity through dietary and lifestyle-related changes. The addition of pharmacologic treatment is a secondary consideration for patient at particularly high risk of cardiovascular disease and DM[9].

Weight loss

A majority of the evidence on the effect of weight loss and other dietary interventions on the risk of PCa is derived from animal studies. Several studies have observed a decreased risk in the development and progression of PCa in animals on a caloric restricted diet that is low in fats or carbohydrates[131].

Several studies have investigated the influence of weight change on the development and progression of PCa in humans, only a few of which focused on weight loss. The most intriguing of these studies is the PCa Lifestyle Trial, which is a randomized control trial (RCT) of men with PCa on AS. These men had a Gleason grade < 7 on biopsy, PSA between 4 and 10 ng/mL, and clinical stage T1-2 disease. They were randomly assigned to either a program that included a vegan diet, several nutritional supplements, moderate aerobic exercise (30 min of walking on 6 d per week), and various techniques for stress management or no intervention. Those in the experimental group reduced their weight by 4.5 kg and had a 4% decrease in serum PSA levels compared to a 6% increase

in the control group (P = 0.016) after 1 year. The growth of LNCaP cells was also inhibited by serum from the experimental group by almost 8-fold more than the control group[132]. At 2 years, a significantly fewer number of men pursued a conventional treatment for PCa in the experimental compared to the control groups (5% *vs* 27%)[133].

Several other trials have investigated the effect of various dietary and lifestyle-related interventions on a variety of biomarkers associated with PCa and its prevention. Freedland *et al*[131] recently published an excellent review on this subject. These trials generally reported that a low-fat and/or carbohydrate diet accompanied by weight loss may alter the tumor biology of PCa[131]. Larger studies with longer follow-up and assessment of clinical outcomes are necessary to determine the significance of these findings.

Physical activity

Physical activity has been increasingly recognized as a modifiable risk factor that may play a role in the prevention of many cancer, including PCa[134]. The mechanism behind this relationship remains unknown but is thought to be related to enhancing the immune system and altering the levels of various endogenous hormones associated with PCa, including androgens, insulin, insulin-like growth factors, and testosterone. Physical activity also assists in weight control and prevention of MetS, which may be associated with an increased risk of PCa. The findings of studies investigating the influence of physical activity on the risk of PCa have been equivocal. A recent meta-analysis of 19 cohort and 24 case-control studies demonstrated that total physical activity was associated with a small but significantly decreased risk of PCa (pooled RR = 0.9; 95%CI: 0.84-0.95). A sub-group analysis based on the type of physical activity observed a decreased risk of 19% (95%CI: 0.89-0.97) for occupational and 5% (95%CI: 0.89-1) for recreational physical activity. The risk reduction for total physical activity was reported in men between 20 and 45 years of age (pooled RR = 0.93; 95%CI: 0.89-0.97) as well as between 45 and 65 years of age (pooled RR = 0.91; 95%CI 0.86-0.97)[135]. The use of various methods to quantify the level of physical activity in these studies precluded the identification of a dose-response relationship or threshold of physical activity required for preventing PCa. There was also not any available data on the levels of various endogenous hormones associated with PCa.

Chemoprevention

The most thoroughly investigated and promising medication has been 3-hydroxyl-3-methylglutaryl-Coenzyme A reductase inhibitors (also known as statins). Our group recently published a review on this subject[136]. While there does not appear to be an association with the overall risk of PCa, several cohort studies and a meta-analysis have demonstrated a decreased risk of advanced disease in men taking statins[137-140]. Additional studies have investigated the oncologic outcomes of men taking statins after their treatment for PCa with conflicting results. Several recent meta-analyses have failed to observe an association between the use of statins and risk of BCR after RP with pooled RRs of 1.02 (95%CI: 0.8-1.29), 1 (95%CI: 0.8-1.19), and 1.05 (95%CI: 0.9-1.240)[141-143]. Studies of men undergoing EBRT were also included in the latter 2 meta-analyses. Scosyrev *et al*[142] failed to report an association with BCR after EBRT or any definitive local therapy, while Park *et al*[143] demonstrated an improved recurrence-free survival in their sub-group analysis of men undergoing EBRT (pooled HR = 0.68; 95%CI: 0.49-0.93). These findings may support the radiosensitizing effect of statins that has been observed in both *in vitro* and *in vivo* models[142,143].

Many other medications and dietary supplements targeting various components of MetS have been investigated. One particularly noteworthy medication is metformin, an oral biguanide medication used as a first-line treatment for type 2 DM. It is inexpensive, widely available, and thought to have an antineoplastic effect for various cancers. However, the current evidence on its association with the development and progression of PCa is equivocal. Several studies have reported a decreased risk of PCa and high-grade disease in diabetic men taking metformin as well as a decreased risk of progression, overall mortality, and PCa-specific mortality in those diagnosed with PCa[144-147]. In a study of 2901 men with a median age of 69 years undergoing EBRT, Spratt *et al*[148] also demonstrated a decreased risk of developing castration-resistant disease as well as an improved overall, BCR-free, distant metastases-free, and PCa-specific survival in diabetic men taking metformin[148]. Other studies have failed to observe an association between the use of metformin and development of PCa or BCR after RP in diabetic men[149-154]. Most recently, Rothermundt *et al*[155] performed a prospective clinical trial of 44 men with metastatic castration-resistant PCa on metformin and reported a stabilization of disease in 36% of men at 12 wk and 9.1% at 24 wk. These authors also demonstrated a prolongation of PSA doubling time in 52.3% of men after starting metformin[155].

Recommendations

The current evidence on the benefits of weight loss, physical activity, and medications like statins and metformin is encouraging but preliminary and requires further investigation before providing an specific recommendations. Nevertheless, it is important to recommend maintaining a desirable weight, engaging in regular exercise, and consuming a cardiovascular healthy diet to all patients. These interventions will improve their overall health and reduce their risk of cardiovascular disease, which is the primary cause of mortality among men in the United States[156].

CONCLUSION

Emerging evidence has suggested an association between

MetS and PCa. Many studies have observed that men with MetS or its individual components have an increased risk of more aggressive disease and mortality as well as a poorer outcome after their treatment for PCa, while others have not. These men may benefit from weight loss, physical activity, and the addition of medications like statins for preventing PCa as well as improving their oncologic outcomes and QoL after treatment. There is a paucity of RCTs with a majority of the existing evidence being retrospective or observational in nature. Potential biases such as screening practices, serum PSA level, or choice of treatment may therefore account for the findings of these studies. Lastly, only a few studies consider both MetS and its individual components. MetS is a complex disease with a poorly understood interplay among its individual components, which appear to be differently associated with PCa.

The association between MetS and PCa is a particularly attractive and fruitful area of research, given the increasingly aging population and epidemic proportions of both diseases. There is also a need for the identification of risk factors for the development and progression of PCa. Further research is necessary to corroborate the findings of earlier studies and to better define the separate and combined influence of the individual components of MetS on PCa. A majority of the existing evidence is retrospective or observational, which is subject to bias. More RCTs are needed to investigate the effect of dietary and lifestyle-related changes as well as chemopreventive medications on the risk of PCa and oncologic outcomes after treatment. Further research should also focus on the molecular pathways involved in MetS as well as the development and progression of PCa. A better understanding of these pathways will facilitate the development of novel therapeutic targets for the prevention and treatment of PCa.

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REVIEW

Preclinical therapy of benign prostatic hyperplasia with neuropeptide hormone antagonists

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Abstract

Benign prostatic hyperplasia (BPH) is a pathologic condition of the prostate described as a substantial increase in its number of epithelial and stromal cells. BPH may significantly reduce the quality of life due to the initiation of bladder outlet obstruction and lower urinary tract syndromes. Current medical therapies mostly consist of inhibitors of 5α-reductase or α1-adrenergic blockers; their efficacy is often insufficient. Antagonistic analogs of neuropeptide hormones are novel candidates for the management of BPH. At first, antagonists of luteinizing hormone-releasing hormone (LHRH) have been introduced to the therapy aimed to reduce serum testosterone levels. However, they have also been found to produce an inhibitory activity on local LHRH receptors in the prostate as well as impotence and other related side effects. Since then, several preclinical and clinical studies reported the favorable effects of LHRH antagonists in BPH. In contrast, antagonists of growth hormone-releasing hormone (GHRH) and gastrin-releasing peptide (GRP) have been tested only in preclinical settings and produce significant reduction in prostate size in experimental models of BPH. They act at least in part, by blocking the action of respective ligands produced locally on prostates through their respective receptors in the prostate, and by inhibition of autocrine insulin-like growth factors-Ⅰ/Ⅱ and epidermal growth factor production. GHRH and LHRH antagonists were also tested in combination resulting in a cumulative effect that was greater than that of each alone. This article will review the numerous studies that demonstrate the beneficial effects of antagonistic analogs of LHRH, GHRH and GRP in BPH, as well as suggesting a potential role for somatostatin analogs in experimental therapies.

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Key words: Benign prostatic hyperplasia; Luteinizing

hormone-releasing hormone; Growth hormone-releasing hormone; Gastrin-releasing peptide; Somatostatin; Targeted therapy

Core tip: A new, effective treatment for benign prostatic hyperplasia (BPH) is critically needed. Present side effects of therapy include impotence, decreased libido, abnormal ejaculation, dizziness, weakness, blurred vision and insomnia. Preclinical data suggest that antagonists of neuropeptides growth hormone-releasing hormone, luteinizing hormone-releasing hormone and gastrin-releasing peptide are effective in shrinking prostates in part by suppressing growth factors and inflammatory cytokines. Their effect is exerted through a decrease in levels of circulating hormones and also on a direct action on their respective prostatic receptors. These analogs seem to have the same clinical effects as the currently available BPH medical therapies but possess greater efficacy and have fewer or no side effects.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is an age-dependent condition which may start as early as 40 years of age and its prevalence increases to 50%-60% in men in their 60's[1,2]. The BPH-associated growth in prostatic volume arises from the increase in epithelial and stromal cell number occurring mainly in the transition zone of the prostate[3]. In some cases, histologic BPH remains asymptomatic and the patient does not require clinical treatment. However, the prostate gland frequently becomes substantially enlarged, resulting in compression of the diameter of the urethra thus leading to bladder outlet obstruction[1]. Lower urinary tract symptoms (LUTS) that are often associated with BPH are developed in response to the increased resistance of the urethra and the consequently elevated pressure in the bladder[4,5]. Unfortunately, the current medical modalities aimed at treating BPH are not completely effective[6]. These include therapies targeting 5α-reductase activity to inhibit the production of dihydrotestosterone as well as compounds that reduce the adrenergic tone at the bladder outlet, these collectively known as α_1-adrenergic blockers[7-9]. When an intervention is required, either a minimally invasive technique (such as transurethral needle ablation or microwave thermotherapy) or surgery (transurethral resection of the prostate or "open prostatectomy") is performed to reduce the volume of the prostate and its restriction in outlet flow[10-12].

The pathogenesis of BPH is not completely understood although it has been suggested that a decrease in the rate of cell death is more critical for the hyperplastic behavior than a rise in cell proliferation[13]. Various factors, such as a discrepancy in androgen and estrogen levels[14-17], altered autocrine regulation by growth factors [most importantly fibroblast growth factors-2 (FGF-2) and FGF-7][18] or cytokines released by infiltrated inflammatory cells[19] have been found to contribute to the development of BPH. It has also emerged that mesenchymal transition of epithelial and endothelial cells directed by the transforming growth factor (TGF)-β/Smad pathway may play a key role in the pathogenesis of BPH[20]. Most recently, neuropeptide hormones were also found to play a major role in this process, not only by indirectly controlling their classical hormonal targets but also as local regulators in the prostate[21-25]. Consequently, their receptors became potential targets for the development of new treatment strategies for BPH. These include the potential therapeutic utilization of antagonistic analogs of luteinizing hormone-releasing hormone (LHRH), growth hormone-releasing hormone (GHRH) and gastrin-releasing peptide (GRP). The utilization of these analogs in experimental BPH also improved our knowledge on the physiological role of neuropeptides and their receptors in the pathogenesis of BPH. The blockade of these receptors by specific antagonists inhibits the proliferation of stromal and epithelial cells and reduces the release of cytokines and growth factors[6,20,22,24,25] indicating the participation of the native neuropeptides in these processes. As new antagonistic analogs of neuropeptides have recently become available for clinical practice as well others are currently being developed for human trials, we felt that a review of recent findings related to their use in BPH is timely. This review therefore focuses exclusively on preclinical and clinical studies where neuropeptide antagonists were tested against BPH. Additionally, the use of somatostatin agonists is also suggested based on previous findings in prostate cancer with the hope it will facilitate their experimental and clinical testing.

ANTAGONISTS OF LHRH

Initially, LHRH antagonists were developed for the purpose of contraception using reduction of the mid-cycle pituitary follicule-stimulating hormone and luteinizing hormone (LH) release thus preventing ovulation[26,27]. Early antagonistic analogs of LHRH demonstrated low potency and significant side effects due to a substantial histamine release[28]. Since those first attempts, many antagonistic analogs of LHRH have been synthetized with higher potency and greatly decreased histamine-releasing activity[29,30]. Cetrorelix[29] was the first antagonistic analog of LHRH that was approved for use in clinical practice as part of the hormonal therapy of *in vitro* fertilization used to prevent premature LH surges[31]. Numerous clinical trials have been conducted with Cetrotide brand of cetrorelix for the treatment of ovarian cancer, endometriosis, ovarian hyperstimulation syndrome and uterine

leiyomyoma[32-35]. Cetrorelix was also tested in patients with prostate cancer[36,37]. The most advanced LHRH antagonist, degarelix, that has been approved for patients with advanced prostate cancer has an improved formula that allows the slow tonic release of the peptide, and moreover, has the lowest histamine-releasing activity among the LHRH antagonists[38,39].

The utilization of LHRH antagonists in the treatment of BPH is suggested by several previous findings. Hormonal therapy with the 5-α reductase inhibitors has long been used to treat BPH and has been shown to shrink prostate volume and improve urinary outflow[16]. This suggests a dihydrotestosterone-dependent pathology of the disease. However, only 30%-50% of patients respond to this treatment[40] highlighting the need for the development of a more effective intervention, such as a systematic suppression of testosterone levels. Cetrorelix (300 μg) was able to reduce serum testosterone levels by 80% at 12 h after administration in men with a mean age of 24[41]. This finding encouraged the clinical testing of cetrorelix in BPH.

In a study by Gonzalez-Barcena *et al*[36], 11 patients were recruited with symptomatic BPH. Subjects were treated with 500 μg cetrorelix every 12 h for 4 wk in an open label study. Improvements were seen in urinary flow just after the first week of treatment and it became normal after 4 wk. Also, the level of serum acid phosphatases reached normal levels at the end of treatment. Free testosterone levels either dropped immediately after the first cetrorelix injection or decreased gradually throughout the 4 wk, however, in 4 patients it remained similar to pretreatment values. In all cases, prostatic volume decreased significantly which suggests a testosterone-independent action of cetrorelix on the prostate in patients where testosterone level had not been reduced significantly[36]. In a subsequent Phase Ⅰ/Ⅱ clinical trial, 13 patients with moderate to severe BPH were treated with a loading dose of 5 mg cetrorelix twice daily for 2 d and then with 1 mg daily for two months[42]. In this study, testosterone fell to castrate levels during the initial high dose therapy and increased to approximately 30% of the normal serum level during the 2 mo of maintenance therapy. On week 8, the International Prostate Symptom Score (IPSS) was significantly reduced and there was a 27% decline in prostate volume.

A decade after these pilot studies, a placebo-controlled phase II trial explored the effects of a 4-wk treatment at 3 different dose levels of cetrorelix in 140 patients with symptomatic BPH[43]. LUTS were significantly improved in all treatment groups compared to placebo which effect occurred rapidly, by week 4 (time point of the first evaluation). Prostate size was also significantly reduced in two of the treatment groups and the overall reduction of symptoms lasted 16 wk after the termination of the treatment (time point of last evaluation). In a further study, cetrorelix pamoate was administered as a 60 mg sustained release formulation, in a double-blind, randomized, multicenter study[44]. One subsequent administration of cetrorelix (30 mg, sustained release) resulted in a 4-point improvement in IPSS and the significant advancement was sustained for 26 wk after the last dose was given. In these latter studies it was shown that the suppression of testosterone levels by cetrorelix was moderate and transient[43,44].

Despite the success of these studies, the phase III clinical trials conducted in the United States and in Europe by AEterna Zentaris[45,46] failed to confirm a significant improvement in IPSS in response to cetrorelix treatment compared to the placebo group. In the United States study, there were no significant changes after either 3 or 4 doses of cetrorelix administered during an 18-wk period, however, cetrorelix was beneficial in a subgroup of patients with substantially enlarged prostates[47]. Although the phase Ⅲ trial failed, all previous attempts were successful which encouraged the initiation of new clinical testing with the more potent LHRH antagonist, degarelix. This compound has greatly reduced histamine-releasing activity and upon subcutaneous administration it aggregates into a slow-release complex[38,39]. A Phase-Ⅱ study has been completed with this compound but results have not yet been released[48].

Initially, the concept of the management of prostate cancer and BPH by LHRH antagonists was based on their action on pituitary LHRH receptors (LHRHR) leading to suppression of gonadal testosterone production, however, there is a growing body of evidence that they also act directly in the prostate. This idea is supported by a number of studies showing the presence of LHRH receptor in the prostate[49-51]. In an early study by Kadar *et al*[49], a high affinity low capacity binding site for D-TRP-6-LHRH in prostate samples from patients with BPH and prostate cancer was found. A similar binding site and one with low affinity high capacity were also detected in Dunning prostate tumors[50]. In a more recent study, LHRHR was detected by reverse transcription polymerase chain reaction in 60% of patients with BPH[51].

A second line of evidence for the local action of LHRH antagonists in the prostate is derived from a number of studies where cetrorelix was tested *in vitro* on human BPH cell lines expressing LHRHR. Siejka *et al*[21] showed that cetrorelix inhibits proliferation of the immortalized human BPH cell line (BPH-1) and reduces the protein expression of proliferating cell nuclear antigen (PCNA), epidermal growth factor (EGF), EGF receptor, most abundant adrenergic receptor in the prostate (α_{1A}AR) and LHRHR in a concentration-dependent manner[21]. Proliferation was also inhibited by cetrorelix after cells were stimulated with growth factors insulin-like growth factors (IGF)-Ⅰ, IGF-Ⅱ or FGF-2. Additionally, the activation of signal transducer and activator of transcription 3 (STAT3) by phosphorylation, an event associated with increased proliferation in many cells[52], was suppressed by cetrorelix[21]. The downregulation of $_{\alpha 1A}$AR by cetrorelix might be of particular interest since an increase in $_{\alpha 1A}$AR expression induced by prolonged administration of α1A-adrenergic blockers might be responsible for development

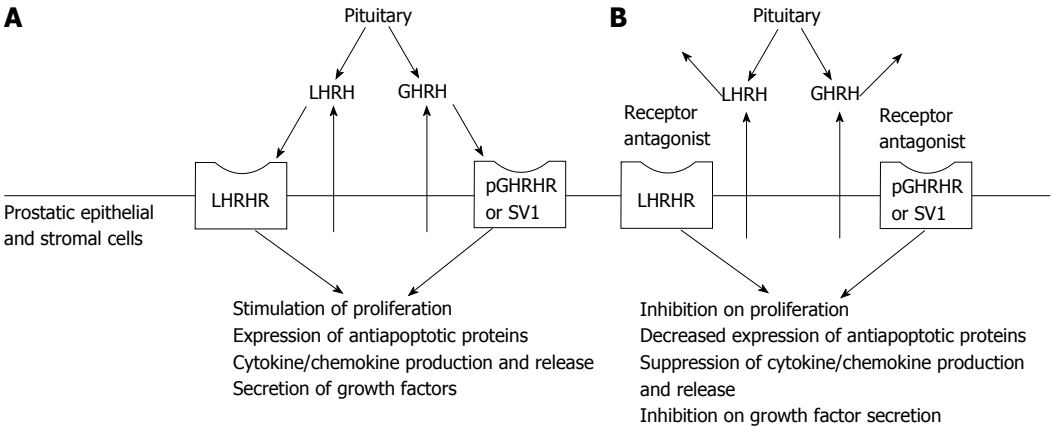


Figure 1 Local autoregulatory loop of luteinizing hormone-releasing hormone and growth hormone-releasing hormone in prostate cells and their blockade with antagonistic analogs: A novel strategy for the treatment of benign prostatic hyperplasia. A: Luteinizing hormone-releasing hormone (LHRH) secreted by pituitary somatotropes or by prostate cells activates local receptors (LHRHR). Similarly, pituitary-derived or paracrine/autocrine growth hormone-releasing hormone (GHRH) binds to pituitary type receptor (GHRHR) or its splice variant (SV1). These events lead to the changes in cellular homeostasis implicated in benign prostatic hyperplasia (BPH) pathophysiology; B: Disruption of the autoregulatory feedback loops by antagonists of LHRH and GHRH improves the condition of BPH by acting on these various cellular processes.

of the therapeutic tolerance to this treatment seen in clinical practice[53]. Rick *et al*[22] utilized a rat model of BPH in which the growth of prostate was induced by repeated administration of testosterone[54]. In this study, prostate size was reduced by cetrorelix in a dose-dependent manner compared to controls treated by testosterone only. In addition, the expression of various proinflammatory cytokines and growth factors that have been implicated in the pathogenesis of BPH were found to be reduced following cetrorelix treatment[22]. A significant reduction in serum levels of dihydrotestosterone and LH was also observed. Interestingly, cetrorelix treatment reversed testosterone-induced morphological changes to resemble the histology of the normal prostate, including a decrease in epithelial height[22]. In addition, AR and 5α-reductase levels were reduced by cetrorelix[22]. Unfortunately, the testosterone-induced BPH model has its limitations due to the complexity of the pathogenesis of BPH. Testosterone-induced hyperplasia selectively appears in the ventral prostate lobe in rats that might be the result of the distinct anatomy of this model from humans[55]. Also, the efficacy of testosterone to induce prostatic hyperplasia varies among different rat strains[56]. In addition to the noted disadvantages of the model, only the proliferation of epithelial cells is triggered by the addition of testosterone[56], whereas stromal-epithelial interactions are believed to be crucial in the pathogenesis of BPH[57,58]. Siejka *et al*[59] further examined this interaction using the BPH-1 cells and an immortalized stromal myofibroblast cell line, WPMY-1. Growth medium collected from either of the cell lines stimulated the growth of the other. This strongly supports the crucial role of epithelial-stromal cross-talk in the proliferative activity of these cells. This effect seemed to be directed through the mitogen-activated protein kinase (phosphorylation of ERK1/2) and STAT pathways. Cetrorelix inhibited the proliferation of both cell lines on its own and also after the BPH cells were stimulated with WPMY-1-conditioned medium[59].

The above mentioned studies shed light on the existence of a local autocrine/paracrine LHRH feedback in the prostate that might contribute to the pathogenesis of BPH (Figure 1). Since both LHRH ligand and its receptor are expressed in BPH[22] and the LHRH antagonist, cetrorelix, is able to decrease the proliferative activity of BPH cells *in vitro*[22], this feedback loop might act as a local stimulatory signal for cell proliferation or survival. It is also known that hormone-refractory prostate carcinomas express higher levels of LHRHR than BPH and hormone-dependent prostate cancer[60]. Consequently, LHRHR levels might gain prognostic value in the future.

GHRH ANTAGONISTS AND THEIR COMBINATION WITH LHRH ANALOGS

Antagonistic analogs of GHRH have been found to reduce the growth of various tumors[61-69] including prostate cancer in xenograft models of nude mice[70-74]. IGF-1 is a well-known growth-promoting factor for various tumors[70,75]. By blocking GHRH receptors on pituitary somatotropes, these antagonists suppress the production and secretion of growth hormone (GH) thereby decreasing circulating levels of IGF1. The full length pituitary type receptor (pGHRHR) and its main splice variant, splice variant 1, are expressed in various extrapituitary sites of normal and malignant tissues, including prostate[71,76,77]. GHRH is also secreted locally in normal and malignant prostate tissue, suggesting that it serves as an autocrine/paracrine regulator which process might be involved in the pathogenesis as well as the progression of prostate cancer[23,78,79]. Both *in vivo* tumor growth and *in vitro* cell proliferation are inhibited by GHRH antagonists in experimental androgen-dependent and-independent prostate cancers further indicating that, apart from their action in the pituitary, these peptides also function directly in the prostate[80].

Two studies have investigated the effect of GHRH antagonist monotherapy in experimental BPH models. They confirmed that both pGHRHR and GHRH are present in BPH-1 cells and in rat prostates[23,24]. Rick *et al*[23] reported also that the levels of pGHRHR and GHRH were increased following the induction of prostate growth by testosterone, indicating the importance of this autocrine/paracrine circuit in the pathogenesis of BPH (Figure 1). In the same study, GHRH antagonists were found to significantly reduce relative prostate weights better than finasteride, an 5-α reductase inhibitor. GHRH antagonists downregulated the mRNA and protein levels of various cytokines and growth factors that were elevated after testosterone treatment and also decreased proliferation and increased apoptosis in the prostate. GHRH antagonists decreased the transcriptional expression of growth hormones such as IGF-2, TGF-α, TGF-β1 and -β2, EGF, FGF-2, vascular endothelial growth factor (VEGF)-A, that have been found to contribute to the pathogenesis of BPH[81]. Cytokines interleukin (IL)-1α, IL-1β, IL-13, IL-15, and IL-17β, that have been downregulated by GHRH antagonists, otherwise promote T-lymphocyte infiltration and inflammation in BPH[82]. Interestingly, in this study, serum GH and IGF-1 levels were not affected significantly by the GHRH antagonist treatment, that might indicate the crucial role of their direct action in the prostate rather than through the pituitary axis. Intriguingly, prostates of testosterone-treated rats contained increased levels of the antiapoptotic molecule, B-cell lymphoma 2 (BCL-2), a process may explain the increased survival of cells implicated in the development of BPH[20]. Additionally, GHRH antagonist significantly dowregulated BCL-2 levels and simultaneously elevated the expression of the proapoptotic factor, BCL-2-associated X protein (BAX), and the tumor suppressor, p53, which events may underlie the strong apoptotic effect of these peptides.

In the study by Siejka *et al*[21] GHRH antagonists inhibited the proliferation of BPH-1 cells *in vitro*. The existence of the local GHRH/GHRHR loop was further supported by this study; incubation of the cells with GHRH resulted in an increased rate of proliferation which was then inhibited by the simultaneous addition of GHRH antagonist. Their study also revealed that GHRH triggers the phosphorylation of ERK 1/2, Janus kinase 2 (JAK2) and STAT3, signaling molecules that are known to be involved in the pathogenesis of BPH[83,84].

Since the existence of autocrine/paracrine systems of regulation by both LHRH and GHRH are strongly suggested in BPH, the simultaneous blockade of their receptors would be expected to result in a more effective therapy. Rick *et al*[85] studied the combination of cetrorelix plus a highly potent GHRH antagonist, JMR-132, in the testosterone-induced rat BPH model. They found that combination of LHRH and GHRH antagonists resulted in a greater decrease in prostate-specific antigen (PSA) and prostatic STEAP (six-transmembrane epithelial antigen of the prostate) protein levels than either of the peptides alone. Relative prostate weights were reduced to the control level by the combination therapy. Antagonists of GHRH and LHRH administered together were also more effective in inducing apoptosis as measured by changes in the levels of BCL-2, BAX, p53, nuclear factor (NF)-κB and cyclooxygenase-2 (COX-2). The combination therapy therefore has a great prospect in reducing hyperplastic prostate volume by triggering apoptotic cell death. In addition, chronic inflammation has been linked to the development and worsening of BPH; COX-2 has been proposed to play a key role in this process[86]. Hence, coadministration of GHRH and LHRH antagonists may also improve clinical outcome by reducing the expression of inflammation-related proteins such as NF-κB and COX-2[87]. In a subsequent study[88], the cumulative effect of cetrorelix plus JMR-132 was also superior to their individual inhibition on the proliferation of BPH-1 and WPMY-1 cells *in vitro*. Only the combination of JMR-132 and cetrorelix increased the proportion of cells in the S-phase significantly with a simultaneous decrease in the number of cells in G0/G1 and G2/M phases in BPH-1 cells. A decrease in the expression of several genes was detected in response to the combination treatment in the rat testosterone-induced BPH model; these included growth factors (EGF, FGF-1, -2, -7, -8 and -14, IGF-1 and-2, BMP5 and -7, VEGF-A, *etc*.), genes implicated in inflammatory response (chemokines, chemokine receptors, cytokines and cytokine receptors), and members of the Wnt, Hedgehog, PI3-kinase/AKT, JAK-STAT, Phospholipase C and low-density lipoprotein (LDL) pathways. According to the authors, among these changes, the downregulation of IGF-1 is of particular interest, since it has been linked to the development of BPH in diabetic men[89]. Also, inflammation-related chemokine/cytokine release has been shown to trigger the production of growth factors leading to the hyperplastic behavior of prostatic cells[90]. We therefore believe that combination therapy with antagonists of GHRH and LHRH might provide a highly beneficial approach to the management of BPH.

GRP

GRP is a bombesin-related hormone first isolated from porcine stomach and named for its ability to trigger the secretion of gastrin[91,92]. Among the three receptor subtypes that had been described for bombesin-like peptides, GRP binds to the first type (GRPR) with high affinity, and to the second type (neuromedin-B receptor) with a relatively low activity[93]. GRPR expression has been found in a variety of tissues where it regulates the secretion of gastric acid and stimulates exocrine function of the pancreas as well as triggering smooth muscle contraction in the stomach, gall bladder and urinary bladder[94,95]. In the prostate, GRP and bombesin have been shown to display mitogenic activity, affect cell migration and induce contraction in bladder and left ventral prostate[95,96]. In addition, GRPR has been implicated in the neurophysiology

of memory and fear-related behavior, and the processing of pruritus and penile reflexes[97]. In small-cell lung carcinoma xenografted into nude mice, an antibody against the GRPR receptor significantly inhibited tumor growth suggesting the crucial role of a GRP/GRPR autocrine/paracrine loop[98]. Soon after, the existence of this feedback regulation was demonstrated in various tumors, such as glioblastoma, colon cancer, hepatic cancer, prostate and gynecologic cancers[99-104]. Several antagonistic analogs that target GRPR have been synthetized by our group; among these RC-3940-Ⅱ possesses the highest affinity for GRPR combined with an increased antitumor efficacy[105].

GRPR is expressed in prostates from healthy patients as well as in those diagnosed with BPH and malignant prostate[106,107]. A study by Rick *et al*[25] using the testosterone-induced rat model, investigated the role of GRP/GRPR in BPH in greater depth. They demonstrated that GRPR and its ligand are expressed in prostates of normal as well as testosterone-induced rats and also in the human BPH-1 and WPMY-1 cell lines. A single high-affinity binding site was also identified, in control rat prostates and human cell lines, with a radioligand binding assay using ^{125}I-labeled [Tyr4]bombesin. In this study, the GRP antagonist, RC-3940-Ⅱ, inhibited the proliferation of BPH-1 and WPMY-1 cells *in vitro*. It also significantly decreased cell volume and triggered S-phase cell cycle arrest in these cells. The GRP antagonist dose-dependently decreased prostate size *in vivo* in testosterone-treated rats. The proteomic analysis of rat prostates revealed that treatment with RC-3940-Ⅱ reversed the testosterone-induced elevation in NF-κB phosphorylation and expression of androgen receptor and PCNA. Also, it decreased the mean epithelial area and induced apoptosis in testosterone-treated prostates. Analysis of the transcriptional changes in the different treatment groups identified several genes responsible for the beneficial effects of RC-3940-Ⅱ. Changes were found in the levels of growth factors, inflammatory chemokines, cytokines and their receptors; attempts to identify key signaling pathways for this process resulted in the implication of the Wnt, Hedgehog, TGF-β, NF-κB, JAK-STAT and LDL pathways. Accordingly, GRP antagonists may represent an important tool for the management of BPH, either alone or in combination with LHRH and/or GHRH antagonists.

POTENTIAL USE OF SOMATOSTATIN ANALOGS

Somatostatin inhibits the release of GH from the pituitary and also possesses inhibitory action in the gastrointestinal-tract and pancreas as shown by suppression of secretion of gastrin and glucagon, respectively[108,109]. There is much evidence that analogs of somatostatin can inhibit growth of various experimental tumors including prostate cancer[110]. Kadar *et al*[111] identified a single binding site for somatostatin using somatostatin analog RC-160 in rat prostate adenocarcinoma. In normal and pathologic prostate, findings deciphering the expression pattern of somatostatin receptors are contradictory. According to Dizeyi *et al*[112], among the five somatostatin subtypes (SSTRs), SSTR1-3 is expressed in the epithelium of normal and malignant prostate cancer, whereas SSTR4 was found only in epithelial cells. Specific neuroendocrine cells expressing SSTRs have also been identified. In a study by Tatoud *et al*[113], SSTR1 was found in most of the epithelial and stromal cell lines tested whereas SSTR2 was only detected in one BPH stromal cell line. By using fluorescent *in situ* hybridization techniques, SSTR4 mRNA expression was found only in the epithelium whereas SSTR2 was mainly detected in stromal cells of BPH and carcinoma[114]. Nevertheless, the expression of SSTRs in the prostate suggested that the use of somatostatin analogs in pathologic conditions of the prostate by inhibiting the autoregulatory loop of GHRH/GHRHR might be beneficial. In accord with this hypothesis, somatostatin analogs were shown to decrease the proliferation of androgen sensitive and androgen independent prostate cancer cells by elevating p27 and p21 protein levels, decreasing cyclin E expression and ERK1/2 phosphorylation and the secretion of IGF-1 and IGF-2[113,115,116]. The inhibitory activity of somatostatin analog on the production of growth factors, IGF-1 and IGF-2, is of particular interest since these powerful octapeptides have been linked to the pathogenesis of BPH[90].

Somatostatin analogs have also been tested clinically in patients with androgen-independent prostate cancer. A study by Maulard *et al*[117] showed improvement in PSA levels and achieved a reduction in bone pain. A Phase-I study demonstrated the favorable toxicity profile of somatostatin analog lanreotide, and showed its inhibitory effect on plasma IGF-1 levels. In contrast, no clinical improvement has been noted with this analog in advanced metastatic androgen-independent prostate cancer[118]. In a study by Berruti *et al*[119], lanreotide was also able to decrease plasma levels of IGF-1 and of the prognostic marker, chromogranin-A, but had no effect on serum PSA levels in patients with advanced prostate cancer. The poor or no inhibition of tumor growth to somatostatin analogs found in these clinical trials is thought to be due to differences in the receptor subtype-specific binding of the analogs. Consequently, the utilization of a non-receptor selective somatostatin analog has been suggested[120]. According to Cariaga-Martinez *et al*[121], whereas SSTR2 is expressed in benign prostatic hyperplasia, in most cases, it is repressed or absent in malignant prostate tissue. Conversely, the profound expression of somatostatin receptors in non-malignant prostate tissue indicates the need for preclinical and clinical testing of its analogs in BPH. This suggests that monotherapy with a somatostatin analog or a combination treatment with antagonists of GHRH and/or LHRH might represent a promising strategy for the treatment of BPH which should be investigated in the future.

CONCLUSION

The development of novel therapies for BPH is undoubtedly required. Whereas the beneficial effects of LHRH antagonists in pathological conditions of the prostate are already confirmed in clinical setting, other peptide analogs (antagonists of GHRH and GRP) have only been tested in experimental BPH models. We hope that the present review of findings on this topic will accelerate the further experimental and clinical investigation of these compounds. It appears that the local actions of various analogs in the prostate are more crucial for their beneficial influence on BPH than are their systemic effects on hormonal levels. By affecting the activation of multiple signaling pathways, LHRH, GHRH and GRP regulate cell cycle, apoptosis, cytokine and chemokine release as well as local immune response. Monotherapy or combination therapy with antagonists of LHRH, GHRH and GRP are suggested to represent an improved treatment compared to the currently available medical modalities.

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REVIEW

From the battlefield to the bladder: The development of thioTEPA

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Abstract

Effective medications for the treatment of cancer were nonexistent in the early twentieth century. Ironically the widespread use of toxic chemical weapons, chlorine and sulfur mustard gas, during the "Great War" led to the first successful chemotherapeutic treatment of cancer patients. Soon after the introduction of poisonous gas on the battlefield, reports of the resulting pancytopenia in exposed combatants appeared in the medical literature. The biologic effect of chemical weaponry on rapidly dividing cells eventually was recognized for its salutary potential in the treatment of cancer. Once this potential was appreciated, hundreds of similar compounds were synthesized and evaluated as chemotherapeutic agents. One such compound, thioTEPA, would eventually open the era of intravesical treatment of urothelial cancer.

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Key words: ThioTEPA; Bladder cancer; Urothelial cancer; Intravesical chemotherapy; Mustard gas; Mitomycin C

Core tip: We attempt to outline the lineage of intravesical chemotherapy. Specifically, we look at its relationship to poisonous gas used in wartime and chronicle its journey to the bedside.

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GAS! GAS! GAS!

While both the Allies and the Central Powers engaged in chemical warfare in World War I , it was the Germans who first developed an effective chemical weapon of mass destruction. The French tried tear gas, originally intended for riot control, but found it ineffective[1]. The Germans also deployed tear gas and additionally experimented with sneezing powder. These early forays in gas warfare had little strategic effect[2]. However, rumors of Allied research into more potent chemical weapons (subsequently proved untrue) spurred the Germans to develop more sinister chemical agents. Nobel laureate Fritz Haber would mobilize Germany's scientific community and spearhead the development of new chemical weapons for the Central Powers[3]. Of note, most of the belligerents in World War I had signed the Hague Peace Conference of 1899 that forbade "use of projectiles the sole object of which is the diffusion of asphyxiating or deleterious gases[4]". The Germans circumvented this clause by developing and then deploying chlorine gas batteries that were installed in front of their trenches, to be opened when a favorable wind could carry the gas towards the enemy. This system did not rely on "projectiles" and thus was not a violation of the Peace Conference[2]. Such chlorine gas batteries were installed near the Belgian



Figure 1 A group of British soldiers blinded by a gas attack on April, 10 1918-From the collections of the Imperial War Museum in London, England.



Figure 2 A Canadian soldier after exposure to mustard gas. Large bullae are present, especially where the fabric of his uniform doubled back on itself, allowing increased soaking of the compound into his clothing (at the armpit, shirt collar)-Obtained from Library and Archives Canada.

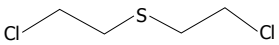


Figure 3 Bis(2-chloroethyl)sulfide, Sulfur Mustard.

town of Ypres and were "fired" on April 22, 1915[1-3,5]. The weapon system's code name was "Disinfection" and its use proved effective as it tore holes in the allied lines threatening total collapse. Within days of the attack, however, allied forces distributed gas masks neutralizing their toxic effects[4].

Gas masks proved insufficient protection against sulfur mustard gas (Figure 1), first used against British soldiers in July of 1917, as exposure damaged more than the respiratory tract[5]. Mustard gas needed only to penetrate the combatant's clothing for once in contact with the skin, it created chemical burns which blistered, ulcerated, and were slow to heal (Figures 1 and 2). Inhalation led to sloughing of the respiratory epithelia and pseudomembranous ulceration. Exposure often led to months of hospitalization[6]. To this day, no widely effective antidote exists.

Sulfur mustard gas, bis (2-chloroethyl)sulfide (Figure 3), was originally synthesized in the mid 1800's by the French chemists Cesar-Mansuete Despretz and Alfred Riche. In 1860, the German chemist Alfred Nielmann first described its toxicity and in the same year the British investigator Frederick Guthrie remarked that "its smell...resembling that of the oil of mustard…" resulting in its eponym. In 1886, the German chemist Victor Meyer described a more effective synthesis. Meyer noted that "the resulting oil is intensely poisonous, producing wounds that heal with great difficulty." In 1913, British chemist Hans T Clarke improved upon Meyer's synthesis. In 1915, while working in the laboratory of famed German chemist Emil Fischer, a flask of the compound inadvertently shattered resulting in Clarke sustaining chemical burns. The injury required two months of inpatient treatment to heal. Emil Fischer would subsequently propose sulfur mustard as a possible agent of warfare to Fritz Haber, a fact which Clarke attributed to his horrific accident[7].

Soon after chlorine and mustard gas' tactical introduction, military physicians began publishing reports on the injuries resulting from exposure. Combatants exposed to both gases often developed leukocytosis early in their clinical course[8-10]. The leukocytosis of sulfur mustard victims was characterized by immature white blood cell precursors and eventually developed into leukopenia[10,11]. The degree of leukopenia seemed to correlate with the severity of gas exposure and subsequent morbidity and mortality. Anemia developed concurrently with the leukopenia. Autopsies showed bone marrow depleted of white and red cell precursors[11,12]. Increased coagulation times indicated thrombocytopenia. It was clear from these findings that the bone marrow was particularly vulnerable[11].

A generation later, in 1941, through their studies on chemically induced gene mutations in fruit flies, British geneticists Charlotte Auerbach and John Michael Robson demonstrated that mustard gas was directly mutagenic[13]. Medical research into mustard gas was also underway in the United States during World War Ⅱ. In 1946, Alfred Gilman and Frederick S Philips published a review article exploring the physiological effects of mustard exposure. In their review, the pancytopenia sustained by those suffering exposure was highlighted. The authors noted the chemical's affinity for more mitotically active tissues[14]. This paper was a preamble to another paper Gilman was soon to publish with colleague Louis Goodman. This subsequent study was to detail the first clinical use of the alkylating agents in the treatment of human cancer.

As the name suggests, alkylating agents donate alkyl groups to other molecules, changing their structure and in the case of certain biologic molecules potentially disrupting their function. While sulfur mustard was the chemical weapon widely used in World War Ⅰ, it would be nitrogen-based mustards (Figures 4 and 5) that would see clinical use. By covalently binding to DNA, the sulfur/nitrogen mustards are cytotoxic. Rapidly dividing cells are especially vulnerable[15,16]. Nitrogen mustard would also be shown to induce metabolic derangements at the cellular level, inducing lymphocytes to convert from aerobic to anaerobic metabolism[17].

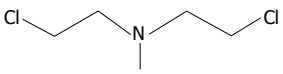


Figure 4 Bis(2-chloroethyl)methylamine, HN2.

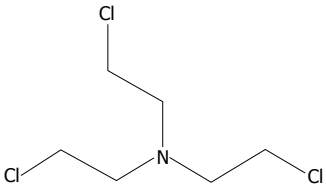


Figure 5 Bris(2-chloroethyl)amine, HN3.

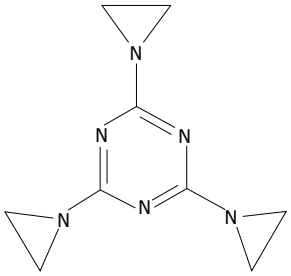


Figure 6 Triethylenemelamine, 2,4,6-tris(aziridin-1-yl)-1,3,5-triazine, triethylenemelamine.

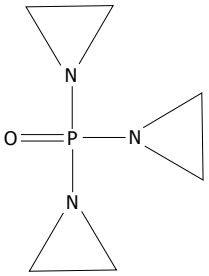


Figure 7 Triethylene phosphoramide, 1-[bis(aziridin-1-yl)phosphoryl]-aziridine, triethylene phosphoramide .

Translating use of nitrogen mustard to a clinical trial was the result of an interdisciplinary effort of the faculty at Yale in the early 1940s. Members of the departments of biochemistry, anatomy, zoology, and medicine all participated in the historic undertaking. The group had been commissioned by the federal government to find an antidote to mustard gas, research efforts for which provided a segue way to the use of nitrogen mustard for chemotherapy[18]. The collaborative report was released in 1946 and detailed the first successful application of anticancer chemotherapy. Prior to this landmark study, endeavors in the field of cancer chemotherapeutics were best characterized by the sign hanging in chemist Paul Ehrlich's lab "Give up all hope oh ye who enter[19]." In 1963, Gilman reminisced "in the minds of most physicians the administration of drugs, other than analgesic, in the treatment of malignant disease was the act of a charlatan[20]."

The specific mustards tested were bis(2-chloroethyl)-methylamine (HN2), and tris(2-chloroethyl)amine (HN3). Both are hydrophilic and were dissolved in water for intravenous injection. Because the trial was conducted during wartime, the clandestine nature of the "medicine" resulted in designating the treatment agents as "compound X". Patients had no understanding of the chemicals they were receiving. They were, however, desperate for treatment as their disease was refractory to the only treatment at the time, radiation. Yale surgeon Gustav Lindskog injected the first human with mustard chemotherapy in the early winter of 1942. In 1943 there followed a trial in which an additional 76 patients were entered.

After the drug's administration, patient's symptoms resembled those of the exposed doughboys. They developed debilitating nausea and vomiting. Thrombophlebitis occurred at the drug injection sites. All experienced profound bone marrow suppression and several developed life-threatening pancytopenia, this occurring in a dose-dependent fashion. As the drugs suppressed the marrow, they also affected the malignancies that resided within. Patients with Hodgkin's lymphoma, non-Hodgkin's lymphoma, and assorted leukemias saw their tumors recede dramatically. Unfortunately malignancies recurred with resistance to the mustard alkylating agents. This trial's success resulted in the establishment of chemotherapy as a viable treatment for human malignancy. However it was clear that toxicity and recurrence limited efficacy[21].

ROAD TO THIOTEPA

Goodman and Gilman's publication led to a flurry of research into HN2 and HN3 and stimulated a search for new alkylating agents. Molecules that bore structural resemblance to the mustards were explored. Triethylenemelamine (TEM) (Figure 6) was one such compound. It contains aziridine groups, structures similar to the 3 membered rings that HN2 and HN3 display when they react. Two or more aziridine moieties were necessary for a drug to be biologically active[22,23]. Possessing three of these moieties, TEM was found to be efficacious against various mouse cancers[24-26]. TEM was shown to induce less nausea and vomiting than HN2 and patients tolerated higher doses[27]. Like HN2 and HN3 before it, TEM went to human trial. Unlike HN2/HN3, intravenous administration of TEM caused no thrombophlebitis. TEM was also effective orally, making it possible to treat on an outpatient basis. Like HN2 and HN3 however, TEM was limited by its toxic effect on the bone marrow[26].

Other alkylating agents were examined, including triethylene phosphoramide (TEPA) (Figure 7). Like TEM before it, it was first found to be effective in treating cancer in various animal models[28-30]. Also like its cousin TEM, TEPA was tested in humans. Administration was possible intramuscularly[31]. However, its clinical efficacy did not exceed that of HN2 or TEM[31,32].

THIOTEPA

1,1',1"-phosphorothioyltriaziridine (thioTEPA) (Figure 8),

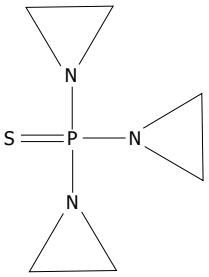


Figure 8 1,1',1''-phosphorothioyltriaziridine, thioTEPA.

and its synthesis, were patented in 1952 by the American Cyanamid Company. It was intended for use in the textile industry and in the production of plastics[33]. However, it went on to see heavy use in the medical field. It disrupts the functionality of nucleic acids[34] like HN2 and HN3 before it. Similar to TEM and TEPA, thioTEPA contains aziridine moieties.

ThioTEPA was entered into human trials in 1953 and was found effective against acute myeloid leukemia, chronic myelogenous leukemia (CML), and Hodgkin's lymphoma, much like HN2, HN3, and TEM. Additional effectiveness was found in the treatment of HN2/TEM-refractory CML. Furthermore, it showed promising results in its use in two cases of adenocarcinoma of the breast. ThioTEPA produced no nausea or vomiting and was not associated with thrombophlebitis when injected intravenously. It was efficacious when injected intramuscularly and yielded no pain at the injection site. The first clinical trial noted a "reasonable margin for safety" between the apparent effective dose and undesired bone marrow suppression[35].

Following the 1953 clinical trial of thioTEPA, Jeanne Bateman explored other routes of administration. He injected the drug into the various cavities of the body with the goal of concentrating it at disease sites. He noted improvement in patients suffering from breast and ovarian cancer. ThioTEPA proved useful in managing the masses, lesions, and effusions associated with these disease processes. Bateman concluded that thioTEPA could be useful in a palliative role for these patients, for which it is still used today[36-39]. All his patients were treated on an outpatient basis and he wrote that "clinical side effects after administration of thioTEPA were rare[40]."

Bateman's intracavitary instillation of thioTEPA led to further studies using this technique. In 1957, HN2 was instilled into the urinary bladder as an adjuvant to surgical urothelial tumor removal[41]. Another study would show that thioTEPA had little effect on surgical site healing[42]. Since healing seemed to be unaffected, thioTEPA was thought to be ideal as an adjuvant to endoscopic bladder tumor removal.

This tactic was first described in an article published in 1961 by HC Jones and John Swinney. At that time, these cases were typically managed solely endoscopically. The investigators described intravesical thioTEPA's efficacy in treating smaller papillary tumors. In cases where multiple tumors would have precluded meaningful surgical management, lesion regression was sufficient such that endoscopic management of the disease became possible[43]. Jones would publish another trial in 1963 that showed similar results. He would note that there were few side effects of intravesical thioTEPA treatment[44].

Clinical use by Veenema demonstrated that bladder carcinomas of lower grade responded better to intravesical thioTEPA and that patients could be treated on an outpatient basis[45]. He too coupled thioTEPA to surgical therapy and concluded that thioTEPA was especially useful perioperatively in cases in which the tumor hadn't grown into the muscular layers of the bladder[46]. He would later state that "The only real indication for topical agents is in the treatment of multiple superficial papillary tumors and for an attempt at prevention of recurrences[47]." Randomized control trials would confirm Veenema's findings. ThioTEPA was clinically beneficial in reducing short-term recurrence in patients with stage I bladder carcinoma that were managed endoscopically[48-50].

CARCINOMA OF THE BLADDER TODAY

Other intravesical agents are available today of which mitomycin C, an intercalating compound, is widely utilized. It was tested in Japan in the mid-70s and showed efficacy similar to that of intravesical thioTEPA[51]. Following initial trials, some reports suggested that mitomycin C was more effective than thioTEPA[52,53]. But, the proposed increased efficacy would turn out to be minimal[54]. Much of the later, definitive research showed the two to be generally equally efficacious in preventing short term recurrence[55]. Combined with its increased cost, the Urologic community had little reason to switch to mitomycin C in the early years[56]. It was, however, useful in treating patients with carcinoma refractory to thioTEPA[57].

Today, mitomycin C is often used instead of thioTEPA because it undergoes less systemic absorption from the bladder. thioTEPA's smaller size allows for potential, significant leukopenia following its introduction. Because of these concerns, Mitomycin C has by-and-large replaced thioTEPA as the preferred intravesical chemotherapeutic[58].

Of all the available intravesical chemotherapeutics, no single agent has clearly demonstrated increased efficacy[58-60]. These agents are primarily used in cases of low-grade disease in conjunction with endoscopic ablation because all have been found to be ineffective in preventing long-term recurrence[61] and are thus most appropriate in treating lesions that are at low risk of progressing[59].

Intravesical immunotherapy followed the chemotherapeutics. The anti-tuberculosis vaccine agent Bacillus Calmette-Guérin (BCG) has been found to be the most effective of these agents. Its mechanism of action is believed to work by inducing a T1 cellular response in the bladder wall, in which recruited immune cells target the neoplastic tissue[60]. Maintenance BCG has been demonstrated to prevent long-term disease recurrence and additionally is effective against higher grade disease. Because it is an attenuated mycobacterium, it can be life-threaten-

ing in immunocompromised patients. It can induce intense hypersensitivity responses in the immunocompetent patient. Currently, intravesical chemotherapeutic agents maintain their role in the management of carcinoma of the bladder, specifically in treating low-grade disease that has little risk of progression[59,60].

Since their introduction as weapons of mass destruction, alkylating agents have transformed the prognosis for patients suffering from a number of malignancies including bladder cancer. In particular they led to the clinical use of chemical agents for intravesical chemotherapy. The history of their development from poisons to potent antineoplastic agents fulfills the biblical dictum "...and they shall beat their swords into plowshares and their spears into pruning hooks....".

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REVIEW

Spontaneous regression of renal cell carcinoma: Reality or myth?

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Abstract

Spontaneous regression of a malignant tumor is a very rare phenomenon. Renal cell carcinoma (RCC) is an aggressive malignancy with an often unpredictable behaviour. The incidence of spontaneous regression in metastatic RCC has been estimated to lie between < 1% and 7%. The spontaneous regression of a primary RCC has been reported much less commonly. Our literature review assesses the published literature concerning spontaneous regression of either primary or metastatic RCC. In order to examine this phenomenon in more detail we performed a literature search in the PubMed Database using the Keywords "renal cell carcinoma", "metastatic disease", and "spontaneous regression" and included reports from the last 100 years. The incidence of spontaneous regressions in RCC has always been considered a special feature of RCC compared to other solid malignancies. The majority of case reports of spontaneously regressed RCC describe the regression of metastases after nephrectomy rather than the spontaneous regression of a primary tumor. In cases of reported regression of metastatic RCC, this mostly applied to pulmonary lesions. As possible reasons for spontaneous regressions host immune defense mechanisms against metastatic RCC tissue following nephrectomy are discussed as important factor. RCC is known to be highly immunogenic and the possible existence of cytotoxic serum factors and tumor-specific surface antigens may trigger a cell-mediated cytotoxicity as an immunological basis for regression. Histological verification of supposed regression of a primary tumor may cause diagnostic difficulties, since large central areas of necrosis and cystic lesions of the tumor can occur simultaneously. The well-known phenomenon of necrosis in a fast growing RCC at the time of nephrectomy must not be confused with true spontaneous regression. Therefore, in our opinion such reported cases of supposed partial spontaneous regressions of primary RCCs are highly questionable. Most cases of spontaneous regression of RCC metastases have been reported after nephrectomy as the only treatment. Debulking by tumor nephrectomy then gives the immune system the chance to cope effectively with the remaining much lower quantity of tumour antigens. However, the mechanisms leading to spontaneous regression of metastatic lesions after cytoreductive nephrectomy are still poorly understood.

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Key words: Renal cell carcinoma; Spontaneous regression; Primary renal cell carcinoma; Metastatic renal cell carcinoma lesions; Cytoreductive nephrectomy

Core tip: Renal cell carcinoma (RCC) is an aggressive malignancy, which, from an immunological point of view, is highly variable. In the era of immunotherapy for metastatic RCC with interferon or interleukin it was always emphasized that spontaneous remissions of RCC, although comparatively rare, do occur and support the use of immunological therapies in metastatic disease. However, we suspected that this frequently cited occurrence of spontaneous remissions is more

legend than reality. We therefore undertook an extensive literature search and included reports from the last 100 years in order to evaluate the scientific evidence describing spontaneous regressions of primary or metastatic RCC.

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INTRODUCTION

Spontaneous regression of a malignant tumor must by necessity be considered a very rare phenomenon. One of the first reported cases was that of a soft tissue sarcoma described by Coley[1] in 1893. In a historic study from 1918, 302 cases of spontaneous tumor regressions were described with only one case of a renal tumour among them[2].

Renal cell carcinoma (RCC) is an aggressive malignancy with an often unpredictable behaviour. At the time of diagnosis, about one third of all patients will already have metastases and another third will develop metachronous metastatic disease after surgery[3].

As early as 1928, Bumpus[4] reported the first case of a spontaneous regression of metastatic RCC. Further reports of spontaneous regressions of metastatic RCC have mostly been those of pulmonary RCC metastases, which supposedly regressed after radical nephrectomy. The incidence of spontaneous regression in metastatic RCC has been estimated to lie between < 1% and 7%[5,6]. However, the spontaneous regression of metastatic RCC lesions has also been reported for brain, bone, adrenal and liver metastases[7].

The spontaneous regression of a primary RCC has been reported much less commonly. However, underlying hypothetical mechanisms for spontaneous regression, which have been mentioned in the literature include humoral, immunological and vascular factors, *e.g.*, autoinfarction[8,9].

In the reported cases of spontaneous regression, the duration of disease remission is either relatively short or has not been reported at all. The longest reliably observed durations of regression of metastatic RCC lasted for 10 years[10] and for over 20 years[5]. Our literature review assesses the published literature concerning spontaneous regression of either primary or metastatic RCC.

LITERATURE SEARCH

A literature search was performed in the PubMed Database using the Keywords "renal cell carcinoma", "metastatic disease", and "spontaneous regression" and included reports from the last 100 years.

RESEARCH

Spontaneous regression of a malignant tumor or its metastases has been defined as a partial or total disappearance of disease without any treatment or induced by local treatments or interventions like embolisation of the primary tumor. The incidence of spontaneous regressions in RCC has usually been reported to be about 1% and this has always been considered a special feature of RCC compared to other solid malignancies. However, the natural course of RCC is not always predictable and includes spontaneous regression of pulmonary metastases following nephrectomy, prolonged survival and stable disease, late relapse after nephrectomy and poor long term outcome despite spontaneous regression[11]. Spontaneous regression is not synonymous with cure, as later recurrences have been reported[12,13]. Thus, a patient cannot be considered cured even if spontaneous regression is suspected if an RCC has been diagnosed[14].

Possible causes of spontaneous regression

Following nephrectomy, it is conceivable that host immune defense mechanisms against metastatic RCC tissue may be activated. Everson *et al*[12] in 1966 postulated such an immune mechanism as the most important factor for spontaneous regression of cancer.

Clinical observations seem to support this hypothesis and several examples for the relationship between neoplastic disease and the function of the immune system exist such as the incidence of lymphomas in patients with AIDS or after organ transplantation, the regression of Kaposi's sarcoma after withdrawal of immunosuppressive therapy[15-18] and the generally increased risk of cancer development with immunosuppression after organ transplantation[19]. As a possible reason some authors postulated a lack of immuno-surveillance of virus-transformed cells by strong immunosuppression. That may lead to an increased frequency of viral infections and/or virus-induced malignancies. Nevertheless, other types of malignant tumors, which are not associated with viral infections, are frequently increased in transplant recipients, too, in dependence of the duration of exposure to immunosuppression[19].

RCC is known to be highly immunogenic and the possible existence of cytotoxic serum factors and tumor-specific surface antigens may trigger a cell mediated cytotoxicity as an immunological basis for regression[20,21]. The majority of case reports of spontaneously regressed RCC describe the regression of metastases after nephrectomy rather than the spontaneous regression of a primary tumor. In malignant melanoma, studies analyzing the T-cell response in regressive primary melanoma in comparison to the metastatic lesions have found a major difference in the number of T-cells in the regressed primary and in metastatic lesions[22].

In cases of reported regression of metastatic RCC, this mostly applied to pulmonary lesions. The constant antigenic stimulation to which the lungs are exposed and

the high quantities of macrophages, lymphocytes and immunoglobulin IgA present in pulmonary tissue have been discussed as possible factors explaining such a phenomenon[23-25]. In contrast, the spontaneous regression of brain metastases has rarely been reported and this has been explained with the blood-brain-barrier limiting an immune response because of a lack of lymphocytes infiltrating the brain tissues compared to other organs and tissues[26]. Thus, hypotheses explaining observed immunological responses against malignant lesions in different sites are available.

This theory of an underlying immune mechanism has been proposed by several authors[27,28]. A remarkable report is that by Horn *et al*[26] (1971) about the induction of an RCC regression in a patient with metastatic disease after the transfusion plasma from another patient of the same family who had experienced spontaneous regression. The authors suggested "some sort of host resistance" in this case, mainly a plasma-related transfer factor, an interferon-like agent or a kind of cytotoxic antibody or a substance mediating cellular immunity[27].

In contrast, the generally poor response of metastatic RCC to immunotherapy is perhaps an argument not supporting the general importance of immunological mechanisms.

From reported experience with other malignancies which have undergone spontaneous regression (neuroblastoma, malignant melanoma, malignant lymphoma and leukemias), several other factors have been proposed as underlying mechanisms such as growth factors and/or cytokines, the induction of differentiation, endocrine mechanisms, the elimination of a carcinogen, tumor necrosis, apoptosis and/or the inhibition of angiogenesis and epigenetic mechanisms[29]. This number of proposed mechanisms just underscores the fact very little is actually known about spontaneous regression and/or that different mechanisms may be of importance in different cases[10,29,30]. For example, cytokine production by the tumor itself or by host tissue has been postulated to be involved in regressions of RCC because in one reported case of regressed intrathoracic metastases elevated serum levels of interleukin 2 receptor were reported[31].

Necrosis and apoptosis both occur in RCC and result in cell death. Gross central tumour necrosis is often clinically and pathologically seen in large and rapidly growing RCCs. This is usually considered as indicating that the rapid growth outgrowths the tumour's blood supply. Interestingly, synchronous necrosis within the primary tumor and in the metastatic lesions of RCC seems to be very rare. Boasberg *et al*[25] (1996) reported such a case of RCC with a caval thrombus and the spontaneous regression of pulmonary metastases. After resection of the primary tumor and the thrombus, histological examination verified necrosis at both sites.

Apoptosis, programmed cell death, has also been suggested to be an underlying mechanism of spontaneous regression in RCC. Pansera[31] (1992) postulated spontaneous RCC regression to be a re-expression of cell death programs typical for renal tissues since pronephros and mesonephros undergo complete regression during embryogenic renal development. Such an embryological cell death program could hypothetically be reactivated in immature RCC tissue. Indeed, the manifestation of embryological cell characteristics does occur in many neoplasms[32]. This phenomenon of morphological similarities between growing tissues, like embryological and cancer cells has its reasoning in a common origin from a precursor stem cell. Thus, spontaneous regression of RCC may be explained as a kind of re-expression of embryonic features by adult carcinoma[32].

Histological verification of regression

In a primary RCC: Histological verification of supposed regression of a primary tumor may cause diagnostic difficulties, since large central areas of necrosis and cystic lesions of the tumor can occur simultaneously. Therefore, the differential diagnosis of a spontaneously regressed RCC should include inflammatory lesions of the kidney, *e.g.*, xanthogranulomatous pyelonephritis, sinus histiocytosis and tuberculosis or malakoplakia. This requires an extensive tissue sampling by the pathologist to confirm or refute the diagnosis of spontaneous regression of an RCC[33].

In our review of the literature we found only 7 reported cases of partial or total spontaneous regression of primary RCCs (Table 1). However, in most of these seven cases the regressions were not unequivocally confirmed.

The first documented case of a total spontaneous regression of a primary RCC was reported by Choi *et al*[33] in 1986. The authors diagnosed a left sided renal tumor and performed a nephrectomy. Histologic examination revealed a cyst-like capsule with coagulated blood, necrotic tissue, calcifications and a cluster of tumor cells, which were classified as a spontaneously regressed primary RCC.

Hamid *et al*[32] in 1998 described two similar cases, one with a cystic cavity of the kidney "containing necrotic debris and brown fluid occupying virtually the whole of the specimen" and "occasional foci of viable renal cell carcinoma …seen in the capsular area". These findings were deemed to represent spontaneous regression by the authors. However, the differential diagnosis must include a developing RCC within a cystic renal lesion with previous hemorrhage. The second case reported by Hamid *et al*[32] should also be viewed critically. There they found "an extensively involuted/hyalinised lesion with extensive metaplastic ossification and also foci of dystrophic calcification" and, again, "occasional foci of cells with clear cytoplasm". Neither of these two case reports included any follow-up information at all[33].

More stringently, spontaneous regression of an RCC or its metastases should be defined as a partial or complete regression of a renal neoplasm which has been histologically confirmed first and then regressed either without treatment or sometimes following an intervention, *e.g.*, cytoreductive nephrectomy. It is important to note that it

Table 1 Regression of primary renal cell carcinoma

Case	Year	Type of regression	Histology documented	Follow up (interval)
Hall[53]	1908	Total regression (?)	Entirely necrotic tumor	Not given
Choi *et al*[33]	1986	Total regression (?)	Cyst-like capsule with necrotic tissue Calcifications and a cluster of tumor cells…	Not given
Edwards *et al*[34]	1996	Partial regression	Residual RCC with marked fibrosis And calcification	36 mo
Hamid *et al*[32]	1998	"Extensive regression"	Extensively hyalinised lesion, also foci Of cells with clear cytoplasm	Not given
			Cystic cavity containing necrotic Debris, occasional foci of viable RCC	Not given
Lacquaniti *et al*[35]	1999	Partial regression	Fibrotic involution….. With few central areas of RCC	7 mo
Kobayashi *et al*[8]	2002	Partial regression of primary RCC with inferior V. cava Tumor thrombus	No	2 yr

RCC: Renal cell carcinoma.

is possible to find necrosis in a fast growing RCC at the time of nephrectomy and this well-known phenomenon must not be confused with true spontaneous regression. However, some authors do consider such a necrosis as a partial regression[34-36]. Thus, in our opinion such reported cases of supposed spontaneous regressions of primary RCCs are highly questionable (Table 1).

In metastatic RCC lesions: In all, we found 94 reported cases of spontaneous regression of metastatic lesions in patients with an RCC (Table 2). Most of these reports concern pulmonary metastases (75 cases) and only a few other sites: pleura and mediastinum (3), liver (4), pancreas (1), brain (3), bone (5), eyes (2) and skin (1) (Table 2). Most of these case reports give no histological verification of the supposedly metastatic lesions. Thus, the diagnosis of spontaneous regression was based on changes in size on imaging which therefore cannot be considered as a proof beyond doubt.

Kavoussi *et al*[36] reported a rate of regression of 20% based on diagnosis by cytology. However, Davis *et al*[37] found a regression of pulmonary RCC metastases in only 3/14 documented cases, *i.e.*, unrelated to any kind of treatment including nephrectomy[38].

In evaluating spontaneous regression of a primary tumor as well as metastatic lesions, histological verification can be a diagnostic challenge. Patients with advanced metastatic disease are often not in good general condition for any surgical or interventional procedures. Edwards *et al*[34] (1996) pointed out that fine needle biopsy carries a risk of gross bleeding due to blood coagulation potentially affected by paraneoplastic mechanisms. Furthermore, computed tomography (CT)-guided biopsies may be unsuccessful in rendering good histologic reports because of insufficient sample size.

In case of eye or brain lesions, cytological or histological verification is even more difficult and dangerous. Thus, in clinical practice such procedures are usually avoided when the primary tumor has been histologically confirmed as an RCC[39-41].

Thus, in cases without convincing histological evidence of pulmonary RCC metastases, several other benign conditions must be considered in the differential diagnosis, such as fungal or mycobacterial infections, sarcoidosis, Wegener granulomatosis and vasculitic lesions which can all appear as pulmonary lesions and regress later[38]. In the pre-CT era, the misinterpretation of radiological findings in conventional chest X-ray studies may have been more common than appreciated at the time. Thus, cases diagnosed as spontaneous regression of RCC metastases in the lungs by chest x-ray only should also be regarded with caution. Furthermore, even cases of pulmonary RCC lesions by cytology or even histology which then had spontaneous regression diagnosed by chest X-ray only must be questioned in retrospect[42]. Embolisation of the lung or a pulmonary segment by tumor thrombi from the renal vein may cause regional pulmonary infarction, which may have the radiological appearance of metastatic lesions. The disappearance of these findings after improvement of inflammatory lesions close to such an embolus could also be misinterpreted as a spontaneous regression[43,44].

Cytoreductive nephrectomy

More than 40 years ago, Markewitz *et al*[44] advocated a palliative nephrectomy only to be considered in individually selected cases with careful evaluation of the potential benefit. However, since then a markedly longer survival has been shown in patients with RCC after nephrectomy and metastasectomy[45,46]. Therefore, the concept of cytoreductive nephrectomy should today be taken into consideration in all patients with metastatic RCC when the short-term outcome of the surgical procedure can be predicted to be acceptable[47].

Indeed, most cases of spontaneous regression of RCC metastases have been reported after nephrectomy as the only treatment. Two possible hypotheses have been put forward as explanations for this phenomenon:

First, a dissemination of tumor cells into the systemic circulation and the lymphatic system induced by the surgical procedure results in a large and ubiquitous presentation of tumor antigen and this may initiate a strong anti-

Table 2 Regression of metastases from renal cell carcinoma

Site of metastases	Source	Number of cases	Histological documentation	Follow up (interval)
Lung	Meinders[54]	1	No	3 yr
	Boasberg *et al*[25]	1	No	2.5 yr
	Cited by Freed *et al*[23]	45 (from 1928-1976)	13/45	
	Vizel *et al*[55]	1	No	11 mo
	Mohr *et al*[56]	1	No	22 mo
	Snow *et al*[5]	1	Yes	6.5 yr
	Nakano *et al*[57]	1	No	8 yr
	Barré *et al*[58]	2	Yes	5 yr
	Kavoussi *et al*[36]	1	Yes (cytologically)	6 yr
	Eissler[59]	1	Yes	7 yr
	Omland *et al*[60]	1	No	14 mo
	Davis *et al*[37]	1	Cytologically	18 mo
	de Riese *et al*[14]	2	No	5.5-11 yr
	Vogelzang *et al*[61]	1	Yes (cytologically)	5 yr
	Palmer *et al*[62]	1	No	15 mo
	Garcia-Del-Muro *et al*[63]	1	No	1 yr
	Czaplicki *et al*[64]	1	Not given	16 yr
	Marcus *et al*[65]	4	No	1-4.5 yr
	MacManus *et al*[30]	1	Yes	9 mo
	Bos *et al*[24]	1	No	1 yr
	Edwards *et al*[34]	1	No	36 mo
	Lokich[7]	1	No	2 yr
	Rauh *et al*[66]	1	Yes	8 mo
	Chang *et al*[11]	1	Yes	16 mo
	Sánchez-Ortiz *et al*[67]	1	Yes	10 mo
	Lekanidi *et al*[29]	1	No	5 mo
Bone	Mims *et al*[68]	1	Yes	1 yr
	Doolittle[69]	1	Yes	4 yr
	Freed *et al*[23]	1	Yes	10 yr
	Kerbl *et al*[70]	1	Yes	13 mo
	Nakajima *et al*[71]	1	Yes	8 mo
Pleural/mediastinal	Kallmeyer *et al*[72]	1	Yes	3 mo
	Abubakr *et al*[27]	1	Yes	1.5 yr
	Thoroddsen *et al*[49]	1	Yes	9 yr
Liver	Deweerd *et al*[73]	1	Yes	6 mo
	Ritchie *et al*[74]	1	Yes	9 mo
	Wyczólkowski *et al*[75]	1	Yes	12 mo
	Christophersen *et al*[10]	1	Yes	5 yr
Pancreatic choroidal	Altschuler *et al*[76]	1	Yes	2.5 yr
	Langmann *et al*[38]	1	No	6 mo
	Hammad *et al*[40]	1	No	
Brain	Omland *et al*[60]	1	No	14 mo
	Guthbjartsson *et al*[39]	1	No	9 yr
	Hensiek *et al*[77]	1	No	4 yr
Skin	Chang *et al*[11]	1	No	16 mo

tumoral immune response by the host. Secondly, because of the mass of tumour antigen is located in the primary tumor, debulking by tumor nephrectomy then gives the immune system the chance to cope effectively with the remaining much lower quantity of tumour antigens and thus to mount an effective antineoplastic response[13,14].

Although the morbidity and mortality of nephrectomy should always be taken into consideration, a palliative cytoreductive nephrectomy in metastatic RCC may also be beneficial for other reasons: in terms of the prevention of tumor toxicity, for the correction of hypercalcaemia and for the improvement of local symptoms, such as pain or hematuria[40,48-53].

Regression has also been described to occur after other local treatments such as radiotherapy or embolisation of the primary tumor[7,37,38,48-50].

CONCLUSION

Spontaneous regression in renal cell carcinoma is very rare and there probably has been an overreporting in the literature. However, it has been described plausibly in metastatic RCC sites-mostly pulmonary-and then mostly after nephrectomy, thus supporting the concept of cytoreductive nephrectomy. Despite several plausible hypotheses, the mechanisms leading to spontaneous regression of metastatic lesions after cytoreductive nephrectomy are still poorly understood.

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REVIEW

Injectable treatments for female stress urinary incontinence

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Core tip: While there are different types of injection materials available, it is unknown which one is superior as few head to head studies have been performed between the newer agents. It is important to inform patients that treatment with injectable agents is not as effective as surgical treatment, and that such agents might necessitate additional and repeated administrations in order to achieve the desired therapeutic effect.

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Abstract

The use of injectable agents for the treatment of stress urinary incontinence (SUI) is an option for female patients who are unwilling to undergo surgery, or have concurrent conditions or diseases that render surgical treatment unsuitable. To be effective for SUI, an injectable agent must be nonimmunogenic, hypoallergenic, biocompatible, permanent, nonerosive, nonmigratory and painless. It must also heal with minimal fibrosis, possess a long-term bulking effect, and be easily stored and handled. Glutaraldehyde cross-linked bovine collagen (Contigen), silicone polymers (Macroplastique), Durasphere, calcium hydroxyapatite (Coaptite), polyacrylamide hydrogel (Aquamid, Bulkamid), Permacol, and stem cell therapy have been used as injectable agents. Patients must be informed that treatment with injectable agents is not as effective as surgical treatment, and that such agents might necessitate additional and repeated administrations in order to achieve the desired therapeutic effect.

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Key words: Stress urinary incontinence; Injectable treatment; Bulking agent; Outcomes; Adverse events

INTRODUCTION

Injection treatment for female stress urinary incontinence (SUI) is not new. Murless[1] reported the first results in 1938, followed by Quackles[2] in 1955 and by Sachse[3] in 1963. In the 1970s and 1980s, polytetrafluoroethylene (Teflon) was used extensively. However, safety concerns that included distant particulate migration, foreign body reaction, severe granulomatosis reaction and possibly carcinogenic effects inhibited its usage[4,5]. In 2001, autologous fat was studied in women with urethral hypermobility but serious adverse events including systemic embolization and death were reported[6-8]. Dextranomer/hyaluronic acid copolymer (Deflux) was another agent used as a bulking agent, and while short term results were satisfactory, long term durability was poor[9,10]. Ethylene vinyl alcohol copolymer (Tegress), a permanent, hypoallergenic, nonimmunogenic implant demonstrated equivalence in outcomes with collagen and was approved by the Food and Drug Administration (FDA) in 2004[11,12]. However, a high rate of urethral erosion was noted and as a result, the manufacturer voluntarily withdrew Tegress from the market in 2007[13].

After glutaraldehyde cross-linked bovine collagen

(GAX-collagen) was introduced in 1993, it was reported as a safe and reliable endoscopic treatment option of SUI. Currently, collagen is the gold standard method in SUI treatment modalities[14]. To be effective against SUI, an injectable agent must be nonimmunogenic, hypoallergenic, biocompatible, permanent, nonerosive, nonmigratory and painless. It must also heal with minimal fibrosis, possess a long-term bulking effect, and be easily stored and handled[15-17]. It is important that the components of these agents remain structurally stable following administration. In addition, to preclude any significant risk of migration, the microcrystalline or micro polymer particles of such agents must have nearly spherical shapes, with diameters greater than 110 micrometers[15]. No agent to date has satisfied all these requirements and research continues on additional bulking materials and modes of delivery.

RESEARCH

We searched the Medline database for published articles, reviews and case reports between 2000 and 2013. The key words "stress urinary incontinence", "injectable treatment" and "bulking agent" were used for literature research. Three hundred and ninety nine abstracts were viewed, and many of these articles were chosen for review and inclusion.

MECHANISM OF ACTION

Although the exact mechanism is not understood, it is thought to improve the urethral sphincter function. Urethral mucosal surface expands with injection, augments urethral coaptation and consequently post injection abdominal leak point pressure (ALPP) increases[17]. Though originally thought to be primarily an obstructive effect, Monga *et al*[18] showed that the mechanism of action might be related to an expanded urethral area and better pressure passage on the proximal urethra. The bladder neck opening during stress maneuvers could be prevented by injection of the proximal urethra and bladder neck[19]. Klarskov *et al*[20] introduced urethral pressure reflectometry, a technique for measuring pressure and cross sectional area in the urethra, which provides parameters useful for studying the mechanism of action of urethral injection therapy. The authors found that the group who showed subjective improvement after injection therapy had significantly higher squeezing opening pressure compared with the group that didn't improve. They concluded the bulking material might function as additional central filler volume which increase the length of the muscle fiber and thereby the power of the sphincter[20].

PATIENT SELECTION CRITERIA

Patients normally considered for intraurethral bulking include females with SUI attributed to intrinsic sphincter deficiency (ISD) and a urethral mobility below 30 degrees. However, various studies have demonstrated that intraurethral bulking is also effective for patients affected by urethral hypermobility[21]. Herschorn *et al*[22] previously demonstrated the absence of any significance difference between patients with urethral hypermobility and patients without urethral hypermobility. Overall, the dry rate at 1 year, 2 years and 3 years were 72%, 57% and 45%, respectively. Others have demonstrated similar results[23-25].

Intraurethral bulking agents are a good choice for patients who do not desire to undergo more invasive surgery and who comprehend that efficacy and durability are inferior to surgery[26]. Bulking agents should not be offered to patients who are seeking a permanent therapy for SUI[27]. Potential candidates for bulking agents are: the elderly; those who are a high anesthetic risk; those who are at an increased risk of urinary retention after a sling operation; those who are on anticoagulation therapy; those who desire to have children; those who have mild persistent SUI after an incontinence surgery; those who have SUI and insufficient bladder emptying; those who have mild SUI after exercise; those who don't want more invasive procedures; and those who are content with improvement instead of a cure[16].

For intraurethral bulking, exclusion criteria include urinary tract infections, hypersensitivity reactions to the bulking agent, and urinary incontinence caused by abnormal detrusor contractions[28].

INJECTION TECHNIQUES

Bulking agents are generally administered with cystoscopic assistance under local anesthesia. The implant can be administered *via* the urethral submucosa or lamina propria by transvaginal, transurethral or periurethral approaches. Currently the transurethral approach is the most preferred technique[28]. During implantation, it is very important to place the bulking material into the proximal urethral wall near the bladder neck. The amount of material needed for injection is the volume that achieves complete coaptation.

Before commencing the injection procedure, the patients are positioned in dorsal lithotomy, and prepared in accordance to the applicable sterile procedures. To prevent any infections, antibiotics are usually administered both before and after the procedure. During the procedure, a 20% benzocaine ointment can be applied to the vulvar vestibule as a local anesthetic. A 2% lidocaine jelly can also be administered intraurethrally. Perimeatal blebs are raised with 1% or 2% lidocaine at the 3- and 9-o'clock or 4- and 8-o'clock positions, 3 to 4 mm lateral to the urethral meatus using a 25-gauge needle, followed by 4 mL of 1% or 2% lidocaine injected periurethrally. The cystourethroscope is inserted and the bladder is inspected. After the bladder is emptied, the endoscope is backed to the mid-urethra with an irrigation rate just enough for visibility. Generally the 3- and 9-o'clock or 4- and 8-o'clock positions are preferred area for injection[29]. For the initial injection, the 6-o'clock position is often an ideal starting

Table 1 Level of evidence and grade of recommendation for injectable agents (b)

Evidence summary	LE
Periurethral injection of bulking agent may provide short-term improvement in symptoms (3 mo), but not cure, in women with SUI	2a
Repeat injections to achieve therapeutic effect are often required	2a
Bulking agents are less effective than colposuspension or autologous sling for cure of SUI	2a
Adverse effect rates are lower compared to open surgery	2a
There is no evidence that one type of bulking agent is better than another type	1b
Transperineal route of injection may be associated with a higher risk of urinary retention compared to the transurethral route	2b
Recommendations	GR
Do not offer bulking agents to women who are seeking a permanent cure for stress urinary incontinence	A

LE: Level of evidence; GR: Grade of recommendation; SUI: Stress urinary incontinence.

point[28]. The needle diameter is dependent on the viscosity of the injected material. The patient can be instructed to perform a valsalva maneuver after injection and if urine leakage is still present, additional material may be injected[28-30].

Faerber *et al*[31] previously performed a review of the transurethral and periurethral techniques for ISD treatment and found both techniques were similar with respect to treatment outcome and the occurrence of adverse events. It was noted, however, that the transurethral technique involved the injection of smaller amounts of material[31]. Likewise, in a prospective and randomized study performed by Schulz *et al*[32], it was observed that the transurethral and periurethral techniques were similar with respect to efficacy however, it was also observed that the periurethral group required the injection of larger volumes of material as well as exhibited a higher rate of urinary retention[32]. Thus, the periurethral technique appears to require higher volumes of material than the transurethral technique, while also requiring more time to be learned effectively. This is important as higher volumes are associated with higher costs, and may increase the potential for postoperative complications.

INJECTABLE AGENTS

Many different agents have been studied for use as bulking material with varying success and adverse events. Patients should be informed that surgery is associated with greater improvement of symptoms, but at the expense of higher risks. Patients should also be informed that treatment with injectable agents is less durable and repeated injections will likely be needed to maintain effect. Cochrane systemic review did not show significant differences in clinical outcomes and complications between different injectable agents[33,34]. Table 1 list the level of evidence and grade of recommendation for injectable therapies for SUI in women by European Association of Urology guidelines[27].

Biomaterials

GAX-collagen: GAX-collagen is produced by cross-linking bovine collagen with glutaraldehyde. The resulting substance is a highly stable collagen complex with a fibrillar structure. This structure confers GAX-collagen resistance to enzymatic breakdown by collagenases, thus increasing the durability of the implant[35]. Contigen is composed of 1%-5% collagen type Ⅲ, and approximately 95% of collagen type Ⅰ [36]. It was available as pre-filled syringes containing 2.5 mL of agent and which needed storage in the refrigerator. Injection was performed with 23 G injection needle through the cystoscope. A volume of 30 mL injection material may be necessary to ensure enough urethral coaptation during the operation[29]. Since GAX-collagen is both biocompatible and biodegradable, only minimal inflammatory changes occur[37]. It is not known to migrate. Although the collagen in GAX-collagen begins to denature by the 12th week of application, it can persist for up to 19 mo[38].

GAX-collagen is a widely used injectable agent and there are numerous studies pertaining to its efficacy and safety[39,40]. It has been considered the gold standard of urethral bulking material such that the FDA required direct comparison to collagen for any new bulking agents in clinical trials[14]. In the North American study group, 382 patients were followed for 2 years, with improvement and cure rates determined as 45% and 33% respectively. The dryness rate at the end of the first-year was 52% but dropped to 38% in the second year[41]. Many patients required repeat injections to maintain efficacy[42]. In a study by Winters *et al*[17], it was observed that 45% of the treated elderly women showed discernible improvement up to 24.4 mo post treatment; however, more injections were required to maintain efficacy in 40% of the patients after an average of 7.9 mo. Corcos *et al*[43] reported that GAX-collagen cured approximately 30% of the women, while improving the condition of 40% of the women in a study group of 40 women at 50 mo of follow up. However, they also reported that four of the women who were cured and five of the women who showed improvement later required "maintenance" injections.

Only a limited number of side effects are associated with GAX-collagen treatment[44]. Clinical trials in the United States have reported transient urinary retention among 15% of the patients, urinary tract infections (UTIs) among 5% of the patients, and irritative voiding syndrome among 1% of the patients[45]. *De novo* urgency as high as 10% has also been reported in certain studies[43]. Due to the minute amounts of GAX-collagen injected to the patients during treatment, the substance is not associated with any signifi-

cant immunoreactivity and cytotoxicity[46]. For this reason, no previous cases of migration or foreign-body responses have been reported with the GAX-collagen.

Importantly, GAX-collagen has the potential to trigger an allergic reaction to bovine protein in patients. For this reason, all patients are required to undergo an allergy skin test 30 d prior to treatment. In general, 3% of the patients are expected to exhibit a positive allergy result. Nearly 70% of such patients will display an allergic reaction within 3 d following the test, which indicates that their allergy to bovine collagen is pre-existing through dietary exposure. The other 30% of the patients will take longer to display any reaction, which may take up to 4 wk. Patients who display a positive allergy test result will not be able to receive GAX-collagen treatment. However, it is still possibly for patients with a negative allergy test result to later display an allergic reaction during and after GAX-collagen administration[47].

Owing to its lack of migration, its degradability and its limited allergenic potential, GAX-collagen is the most popular and widely used intraurethral bulking agent for the treatment of urinary incontinence[14]. However, despite its long track record, although with modest results, the manufacturer ceased production in 2011 and the implant is no longer available.

Synthetic materials

Silicone polymers (macroplastique): Macroplastique (Uroplasty Inc, Minneapolis, MN, United States) is composed of vulcanized polydimethylsiloxane macroparticles suspended in a hydrogel of polyvinylpyrrolidone (povidone). This hydrogel also functions as a lubricant during injection[48]. It has been in use in Europe for SUI since 1996. The material consists of particulates of various shapes and sizes with marked variability but 99% of the particles are greater than 100 μm. However, the potential for migration is present, though remote. Henly *et al*[49] identified small macroplastique particles within the lymph nodes, kidneys, lungs and brain of dogs within 4 mo following administration. Large particles, on the other hand, were identified in the lungs of only one case but without associated reaction[49].

Marcoplastique can be injected with standard cystoscopic equipment but since the substance is fairly viscous, it requires a high pressure 18-gauge Uroplasty needle gun for administration. Alternatively, non-endoscopic transurethral injection device, the Macroplastique Implantation System (MIS) can be used. The MIS consists of a multichannel needle positioning device that is oriented at the 2-, 6-, and 10-o'clock positions for injection. Henalla *et al*[50] noted successful results (74.3%) at the three months follow-up. Tamanini *et al*[51] published the results of 21 patients treated with MIS and at the 12 mo follow-up, an improvement was reported in 57% of the patients, but 23% of patients were deemed failures. ter Meulen *et al*[52] analyzed the efficacy of MIS in women with SUI and urethral hypermobility after an unsuccessful conservative treatment. Twenty-four women received Macroplastique *via* MIS compared with 21 patients who underwent a pelvic floor muscle exercise program. Decreased pad usage and improved questionnaire scores were seen in the MIS group ($P = 0.017$, $P = 0.015$, respectively)[52].

A recent North American multicenter trial randomized 247 patients with ISD to transurethral injection of either Macroplastique or Contigen. After 12 mo, there were significant clinical improvement and dry/cure rates in 61.5% and 36.9% of patients treated with macroplastique *vs* 48% and 24.8% of patients treated with Contigen, respectively. This indicated that macroplastique was non-inferior to Contigen ($P < 0.001$)[53]. In a randomized study by Maher *et al*[54], macroplastique was compared with pubovaginal sling. At the 12 mo follow-up, subjective success rates were 90% in the sling group and 77% in Macroplastique group ($P = 0.41$), and there was no differences between the two groups in patient satisfaction. The authors emphasized the obvious advantages of macroplastique in terms of shorter operative time, less blood loss and shorter hospitalization time.

Complications associated with macroplastique use include urinary retention (5.9%-17.5% of cases), urinary frequency (0%-72.4%, of cases), dysuria (0%-100% of cases), and UTI (0%-6.25% of cases)[55].

Durasphere

Durasphere consists of nonabsorbable zirconium oxide beads coated with pyrolytic carbon, and suspended in a water-gel with 2.8% beta-glucan. It gained FDA approval for use in patients with ISD in 1999[28]. During administration, the zirconium oxide beads are encapsulated by the periurethral tissues[56], which allows it to have a long-lasting bulking effect. In contrast to GAX-collagen, Durasphere is nonimmunogenic and inert. Thus, skin testing with this agent is not necessary prior to administration. Durasphere was designed with larger caliber particles (> 80 μm) to prevent migration. The size of the beads varies between 212-500 μm which contributes to its higher viscosity. As a result, the injection of Durasphere is often more difficult. To overcome this difficulty, an alternative injection method was developed for Durasphere, which involves the injection of local anesthetic to raise a circumferential bleb into which agent was injected[57]. Additionally, as a way to remedy this, the manufacturer introduced Durasphere EXP (Injectable Bulking Agent from Carbon Medical Technologies Inc.) in 2006, whose smaller bead size (range 90 to 212 μm), facilitated injection. However, the size of these particles is still greater than the threshold for migration. As of late 2013, no clinical results have been published with this new formulation.

In a multicenter, randomized, controlled, double-blind study consisting of 355 patients, Lightner *et al*[56] reported that Durasphere was as effective as GAX-collagen. The authors emphasized that the Durasphere group required significantly less volume of injected material to obtain comparable clinical results (4.83 mL *vs* 6.23 mL, $P < 0.001$), and that the probability of achieving successful treatment

with a single injection was higher for the Durasphere group. At the one year follow-up, the ratio of patients that demonstrated an improvement of one Stamey grade or more in the Durasphere and the GAX-collagen groups were 80% and 69%, respectively[56]. Long-term data have been reported by Chrouser *et al*[58], and they observed a decrease in clinical success over time. For the Durasphere group, the success rate for treatment was 63% at one year, 33% in the second year, and 21% in the third year follow-up. For the collagen group, the success rate was 19% in the second year, and 9% in the third year follow-up[58].

In the clinical studies that served as the basis for the FDA approval, the most commonly observed adverse effects included acute retention for ≤ 7 (13% of subjects), dysuria (12% of subjects), UTI (9% of subjects), hematuria (6% of subjects), and retention for > 7 d (6% of subjects)[59]. In a randomized multicentre clinical study comparing Durasphere and GAX-collagen, adverse events were similar between the two groups, except for a higher incidence of urgency and acute retention in the Durasphere group (24.7% and 16.9%, respectively) than in the collagen group (11.9% and 3.4%, respectively)[56]. There have also been case reports of pseudo abscess formation and urethral prolapse[60,61]. Distant particle migration has been reported with the original Durasphere formulation, which raised concern since its bead sizes ranged from 212 to 500 μm, higher than the threshold thought to be critical for migration. With its smaller bead sizes (range 90 to 212 μm), it is unknown whether the risk for migration is increased with Durasphere EXP[62].

Calcium hydroxyapatite (Coaptite)

Calcium hydroxyapatite received its first approval for the treatment of SUI in women in 2005. Calcium hydroxyapatite is a synthetic substance composed of glycerin and carboxymethylcellulose, and which structurally forms an aqueous gel. The spherical calcium hydroxyapatite particles have an average diameter of 100 μm (75-125 μm), which is above the threshold for migration[28]. The gel facilitates injection and provides the initial bulking effect but is designed to degrade over time, and allowing for ingrowth of tissue around the particles. Calcium hydroxyapatite is a natural component of teeth and bones; it has hence been used in orthopedic and dental applications, and also in the ureteral orifice for vesicoureteral reflux, with excellent biocompatibility[63,64]. It is not antigenic, immunogenic or toxigenic and thus a pre-procedure skin test for hypersensitivity is not needed. Refrigeration or special handling are not required[63,64]. Because the material is not viscous, it can be performed with a 21 G injection needle. Since calcium hydroxyapatite is radiopaque, it can be seen on ultrasonography or fluoroscopy, which may aid in accurate localization and placement[14].

In the first pilot study with calcium hydroxyapatite, Mayer *et al*[65] reported a reduction in average pad weight, a reduction of 45% in pad usage, and an increase in average ALPP from 39 to 46 cm H_2O at the 12 mo follow-up. Seven patients needed a second injection after 8.4 mo[65]. In the data leading up to FDA approval, there was no significant difference in quality of life, pad weight or Stamey grade change between coaptite and collagen at a mean follow-up of 11.2 mo[66]. In a large multicenter randomized clinical study, Mayer *et al*[65] compared Coaptite *vs* collagen in 296 women with ISD and demonstrated, at the 12 mo follow-up, an improvement of one or more Stamey grade in 63.4% of the Coaptite group and 57% of the collagen group (P = 0.34). There was no difference in cure rates (39% Coaptite *vs* 37% collagen). More patients in the Coaptite group required only one injection (38%) compared with the collagen group (26.1%) (P = 0.03) and the mean injected volume was lower (4.0 mL *vs* 6.6 mL, $P < 0.0001$)[67].

Common adverse associated with calcium hydroxyapatite are urinary retention (41% of patients), hematuria (19.6% of patients), dysuria (15.2% of patients), UTI (8.3% of patients), urinary urgency (7.6% of patients), urinary frequency (7.0% of patients), and urge incontinence (5.7% of patients). The overall erosion rate was 1.3%[49]. Palma *et al*[68] published a case with urethral prolapse 3 mo after calcium hydroxyapatite injection therapy. The prolapsed nodule was surgically extracted and the resulting pathology reported lymphocytic infiltration, giant cells and granulomatous reaction characterized with macrophages[68].

Polyacrylamide hydrogel (Aquamid, Bulkamid)

Aquamid and Bulkamid are polyacrylamide hydrogels composed of 2.5% polyacrylamide that are nontoxigenic. They possess a homogeneous composition and texture, presenting elasticity and viscosity properties similar to tissue. They contain no solid particles, thereby eliminating any risk of particle migration[28]. Aquamid is used clinically as soft tissue filler in plastic surgery and reconstructive procedures[69]. Bulkamid is the corresponding product for the SUI indication. It does not need refrigeration or any special handling[16]. Bulkamid and Aquamid are currently not approved for use in the United States; however, randomized clinical studies involving numerous centers are currently being conducted in both Europe and North America. Toozs-Hobson *et al*[70] reported a noncomparative, multicenter case series of 135 women in 2010. Half the patients had mixed urinary incontinence. At 12 mo, two-thirds of the women reported being cured or improved, and there were significant reductions in incontinence episodes per 24 h and pad weight testing. Efficacy was similar between patients with pure SUI and those with mixed incontinence. There was a 35% reinjection rate. Minor adverse events were noted and included UTI (5%), transient urinary retention (3%), hematuria (1%), transient *de novo* urgency and urge incontinence (1%)[65]. In the 2 years follow up analysis of this cohort, there was durability of success with 64% of women cure/improved, which was not significantly different compared with the 12 mo data[70].

In a recent prospective, multicenter study Sokol *et al*[71] randomized 345 women with SUI or stress predominate

mixed incontinence to Bulkamid or Contigen and a > 50% reduction in leakage and incontinence episodes was seen in 53.2% of the Bulkamid group *vs* 55.4% of the Contigen group at 12 mo. Additionally, at 12 mo, 47.2% of the Bulkamid patients and 50% of the Contigen patients reported no SUI episodes. Seventy seven percent of the Bulkamid patients and 70% of the Collagen patients considered themselves cured or improved. The authors concluded that Bulkamid is not inferior to Contigen and is a promising new simple office based bulking agent for women with SUI, particularly since Contigen is no longer commercially available[71].

Permacol

Permacol is a dermal implant made from non-reconstituted collagen obtained from porcine skin. Until now, it has been mainly used for pelvic reconstruction and hernia repair. Non-collagenous material, except elastin, is removed and a cross linking process is performed. It maintains its 3-dimensional structure and when implanted, it allows for in-growth of new tissue, which can potentially be permanent. Permacol is non-allergenic, obviating the need for skin hypersensitivity testing[72].

Bano *et al*[73] compared Permacol and Macroplastique in a randomized controlled trial that included 50 women with urodynamic SUI. A great majority of the injections (84%) were performed peri-urethrally in the Permacol group. At 6 mo, 62.5% in the Permacol group were dry *vs* 37.5% in the Macroplastique group but no statistical analysis was reported. Rates of adverse events were similar between both groups[73].

Future directions

Stem cell therapy: Recently, within the context of tissue engineering strategies, stem cell therapy was evaluated for the experimental treatment of SUI[74,75]. The first report evaluating the safety and efficacy of cell therapy in humans yielded an 81.3% cure/improved rate at 12 mo[76]. Multiple small series have been published since but none generated as much excitement as the study by Strasser *et al*[77], which demonstrated very impressive outcomes from sphincteric injection of autologous myoblasts compared with collagen in a randomized trial[77]. This article was later retracted due to ethical violations and investigators question whether this trial as described ever existed. Most recently, Stangel-Wojcikiewicz *et al*[78] reported a case series of 16 women who had sphincteric injection of muscle derived stem cells with a cure/improved rate of 75% at 2 years of follow-up. There were no adverse events from either the deltoid muscle biopsy or from the injection[78]. Cook MyoSite, a part of Cook Medical, has two active clinical trials underway with intrasphincteric injections of autologous muscle-derived cells for the treatment of SUI. While still considered experimental, stem cell therapy holds great promise and in the near future may very well be an additional option in therapy for the treatment of SUI[79]. The clinical trials on injectable treatments for female SUI is shown on Table 2.

POSTOPERATIVE PERIOD

It is rare to observe postoperative complications immediately following the administration of injectable treatments. Once the injection procedure is completed, patients must be capable of voiding without difficulty. In the unlikely case of post operative urinary retention, a Foley catheter should be avoided, as is possible for the bulking agent to become molded around the catheter, and to thus lose its effectiveness. However, it should be noted that there is currently no data or findings indicating that short-term use of catheters might adversely affect intraurethral bulking. For patients that require catheterization for longer periods, it is preferable to use suprapubic catheters in order to prevent the bulking agent's position or efficacy from being affected.

For most patients, multiple treatments with injectable agents will be necessary before achieving the desired therapeutic effect. Each bulking agent requires different time intervals between successive treatments. For instance, GAX-collagen can be administered once every 7 d (although a 4 wk time interval was used in the initial clinical studies with GAX-collagen); however, in order to better evaluate the patient response to the injections, many physicians prefer to wait for 4 or more weeks before continuing with the next injection[23]. Teflon requires a 4-mo waiting period, while Macroplastique injections can be performed at 12-wk intervals. Durasphere injections require a minimum time interval of 7 d, while Coaptite can be injected once every month. In case erosion is observed within the bladder during repeat injections, the eroded side should not be reused for injection purposes until the epithelium recovers[23].

CONCLUSION

Injectable agents represent an effective and safe approach for treating SUI in women. They should especially be considered for female patients who are unwilling to undergo surgery, or have concurrent conditions or diseases that render surgical treatment unsuitable. It must be remembered that injection treatments will mainly serve to improve the patients' symptoms, and that they will not provide a definite cure for SUI. It is also important to inform patients that treatment with injectable agents is not as effective as surgical treatment, and that such agents might necessitate additional and repeated administrations in order to achieve the desired therapeutic effect. Although there are different types of injection materials with different properties, the superiority of one agent over another is not known. There is thus a continuous search for effective, inert, nonmigratory and nonimmunogenic materials that can be readily injected and incorporated into the patient's tissues, and that can maintain their structure for extended periods of time once injected.

Table 2 The clinical trials on injectable treatments for female stress urinary incontinence

Ref.	Bulking agents	Number of patients	Assessment methods	Outcomes
Lightner *et al*[56]	Durasphere *vs* Collagen	*n* = 61 (Durasphere) *n* = 68 (Collagen)	SUIS Standardized pad test	At the one year follow up, the Durasphere group achieved improvement in one Stamey grade or more in 80.3% of patients compared to 69.1% of patients in the Collagen group (P = 0.162)
Chrouser *et al*[58]	Durasphere *vs* Contigen	*n* = 43 (Durasphere) *n* = 43 (Contigen)	Patient satisfaction and continence were subjectively evaluated *via* telephone interview	Success rateswere reported in 33% of Durasphere group and 19% in Contigen at 24 mo; at 36 mo, 21% in Durasphere, 9% in Contigen No significant difference was observed in time to failure between the injection groups (P = 0.25)
Bano *et al*[73]	Permacol *vs* Macroplastique	*n* = 25 (Permacol) *n* = 25 (Macroplastique)	1-h pad test SUIS KCQ	At 6 mo, 62.5% in the Permacol group were dry *vs* 37.5% in the Macroplastique group but no statistical analysis was reported
Hurtado *et al*[13]	Tegress	*n* = 19	Physical exam Urodynamic findings Complications	A 58% of the patients had a complication related to the procedure with 37% experiencing urethral erosion 10.5% of the patients reported at least a 50% subjective improvement
Mayer *et al*[67]	Coaptite *vs* Collagen	*n* = 131 (Coaptite) *n* = 100 (Collagen)	SUIS	Improvement of one or more Stamey grade was showed 63.4% in Coaptite group and 57% in the Collagen group, at 12 mo follow-up (P = 0.34) More patients in the Coaptite group required only one injection (38%) compared with the Collagen group (26.1%) (P = 0.03)
Ghoniem *et al*[53]	Macroplastique *vs* Contigen	*n* = 122 (Macroplastique) *n* = 125 (Contigen)	SUIS 1-h pad test Urinary Incontinence QoL Scale scores	After 12 mo, improved 1 or more Stamey grade and dry/cure rates were determined in 61.5% and 36.9% of patients treated with Macroplastique, *vs* 48% and 24.8% of patients treated with Contigen, respectively (P < 0.05)
Toozs-Hobson *et al*[70]	Bulkamid	*n* = 135	24-h pad weighting test 3-d micturition diary ICIQ score QoL score VAS score	There was durability of success with 64% of women cure/improved, which was not significantly different compared with the 12 mo data
Sokol *et al*[71]	Bulkamid *vs* Contigen	*n* = 229 (Bulkamid) *n* = 116 (Contigen)	Bladder diaries QoL questionnaire Pad weight testing VLPP	47.2% of Bulkamid patients and 50% of Contigen patients reported no SUI episodes 77.1% of Bulkamid patients and 70% of Collagen patients reported improvement or cure

SUIS: Stamey urinary incontinence scale; KCQ: Kings college hospital quality of health questionnaire; QoL: Quality of life; SUI: Stress urinary incontinence; ICIQ: International consultation on incontinence questionnaire; VAS: Visual analogue scale; VLPP: Valsalva leak point pressure.

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REVIEW

Adenine phosphoribosyltransferase deficiency: Leave no stone unturned

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Abstract

Adenine phosphoribosyltransferase (APRT) deficiency is a rare autosomal recessive disease leading to generation of large amounts of 2,8-dihydroxyadenine (DHA). DHA is excreted in urine, where it precipitates into crystals due to its low solubility. DHA crystals can aggregate into stones or cause injury to the renal parenchyma (DHA nephropathy). Recurrent urolithiasis and DHA nephropathy are the two clinical manifestations of APRT deficiency. Diagnosis of APRT deficiency can be made during childhood as well as adulthood. Diagnosis mainly relies on the recognition of DHA in stones or urine crystals. Measurement of APRT activity and genetic testing are useful for confirmation of diagnosis, for family screening and should be considered in difficult cases of urolithiasis or crystalline nephropathy. Allopurinol therapy is the cornerstone of treatment and is highly effective in preventing recurrence of stones and kidney disease. High fluid intake and dietary modifications are also recommended. Early diagnosis and treatment are of paramount importance to prevent renal damage. Unfortunately, diagnosis of APRT deficiency is often overlooked and irreversible renal failure still occurs in a substantial proportion of patients. Clinicians must be alert to the possibility of APRT deficiency and consider the appropriate diagnostic tests in certain cases. This review discusses the genetic and biochemical mechanisms of APRT deficiency, and the issues of diagnosis and management.

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Key words: Adenine phosphoribosyltransferase; Dihydroxyadenine; Urolithiasis; Crystalline nephropathy; 2,8-dihydroxyadenine nephropathy

Core tip: Adenine phosphoribosyltransferase (APRT) deficiency is a rare but underrecognized genetic disease causing recurrent dihydroxyadenine urolithiasis and crystalline nephropathy. Clinical presentation is variable and diagnosis can be made at any age. Treatment with a xanthine dehydrogenase inhibitor is highly effective in preventing recurrence of stones and kidney disease. Unfortunately, diagnosis of APRT deficiency is often overlooked and irreversible renal failure still occurs in a substantial proportion of affected individuals. Early diagnosis is of paramount importance to prevent long term complications.

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INTRODUCTION

In 1968, Kelley *et al*[1] identified partial adenine phosphoribosyltransferase (APRT) deficiency of autosomal inheritance in otherwise healthy individuals and reported mutant forms of the enzyme. In 1974, Cartier *et al*[2] described one child with 2,8-dihydroxyadenine (DHA) urolithiasis secondary to complete APRT deficiency inherited in an autosomal recessive manner. In 1977, Van Acker *et al*[3] described one family with APRT deficiency. Later, most cases were reported in Japan, where the prevalence of DHA stones appeared to be especially important[4]. Clinical presentation of APRT deficiency is not restricted to urolithiasis. DHA can cause renal failure by precipitating in the renal tubules and interstitium[5-7]. We previously proposed the denomination "DHA nephropathy" for the kidney disease caused by the precipitation of DHA crystals into the renal parenchyma[8]. Unfortunately, APRT deficiency is often recognized late after recurrent stone episodes or once irreversible renal insufficiency has occurred[7,9-11]. Given that the disease is easily treatable, clinicians must be alert to the possibility of APRT deficiency and consider ordering the diagnostic tests in appropriate cases. This review discusses the genetic and biochemical mechanisms of APRT deficiency, and the issues of diagnosis and management. Unless otherwise indicated, the term "APRT deficiency" refers to complete APRT deficiency in this review.

MECHANISMS OF THE DISEASE

APRT is an ubiquitously expressed enzyme that catalyzes the reaction in which 5'-adenosine monophosphate and inorganic pyrophosphate are synthesized from adenine and phosphoribosyl pyrophosphate. APRT provides the only pathway for the metabolism of adenine[12]. As a result of APRT activity, adenine is present only at low levels in blood and urine[13]. In the absence of functional APRT, adenine is metabolized to 8-hydroxyadenine, which is then converted to 2,8-dihydroxyadenine (DHA) by the xanthine dehydrogenase enzyme (XDH)[14]. DHA is eliminated in urine through and tubular secretion[15]. APRT deficiency thereby leads to excretion of large amounts of DHA in urine[3]. Due to the very low solubility of DHA, this causes the formation of DHA crystals, which can aggregate into stones[3,16], or precipitate into tubular lumens, inside renal epithelial cells, and in the interstitium, thereby causing crystalline DHA nephropathy (Figure 1)[4,7,9,11,17-19].

Two types of APRT deficiency are recognized based on the level of enzyme activity in cell extracts[13]. APRT activity is null in type Ⅰ, whereas it is about 15% to 30% of the normal activity in type Ⅱ[4]. It has to be stressed that this classification has no relevance *in vivo* or in intact cells, where enzyme activity is less than 1% in types Ⅰ and Ⅱ[20,21]. The clinical presentation is similar in both types of deficiency[4,7,10,22]. However, it is still important for clinicians to be aware of this classification when it comes to interpreting the results of APRT activity measurement (see below Diagnostic tests). Type Ⅰ APRT deficiency has been mostly reported in Caucasian individuals, but also in diverse ethnic groups[7,10,23]. Type Ⅱ has been almost exclusively described in Japanese patients, where it accounts for 70% of cases of APRT deficiency[4].

PREVALENCE OF APRT DEFICIENCY

Although APRT deficiency is often viewed as a very rare condition, its prevalence worldwide remains unknown and the number of reported cases are increasing each year. The vast majority of cases and studies published came from Japan, France and Iceland[4,7,10]. One explanation to this may be that certain mutations are frequent in these countries (Met136Thr in Japan, c.400 + 2dup in France and Asp65Val in Iceland). However, the variability in number of cases identified and reported among different countries may also reflect variability in awareness of APRT deficiency and availability of diagnostic tests. In our series, several affected families originated from places outside Europe, including African countries, Turkey, Martinique, Lebanon and Canada[7]. This suggests that APRT deficiency is a ubiquitous disease. The increase in the number of identified cases in some countries, like France, probably reflects improved recognition and diagnosis of the disease[8].

The prevalence of complete APRT deficiency was estimated to be 1/27000 in the Japanese population, corresponding to a heterozygote frequency of 1.2%[4]. The heterozygote frequency in caucasian populations, estimated from measurements of enzyme activity in healthy subjects, ranges from 0.4% to 1.2%, suggesting that the prevalence of homozygosity is higher than 1/100000[24,25]. If this holds true, more than 60000-80000 individuals may be affected worldwide. The limited number of cases recorded in most countries suggests that many individuals with APRT deficiency are currently unrecognized[26]. APRT deficiency may be a seriously underestimated cause of urolithiasis and chronic kidney disease, progressing over time to end stage renal disease (ESRD) in a non-negligible proportion of cases when left untreated[7,10].

CLINICAL PRESENTATION AND NATURAL HISTORY

The age at diagnosis of APRT deficiency varies widely, ranging from infancy to more than 70 years of age[4,7,10,22]. In our series, diagnosis was made before the age of 16 in only 37% of patients[7]. In an Icelandic series, 47% of affected individuals were diagnosed before the age of 18[10]. In some instances, APRT deficiency is diagnosed late because some patients present with symptoms late in their adulthood, while in other instances, despite early onset symptoms, recurrent urolithiasis and kidney disease, diagnosis is often delayed due to low index of suspicion[7,8,10,22]. Urolithiasis is the most common manifestation of APRT deficiency in both children and adults[10,27]. The first urolithiasis episode may occur during infancy as

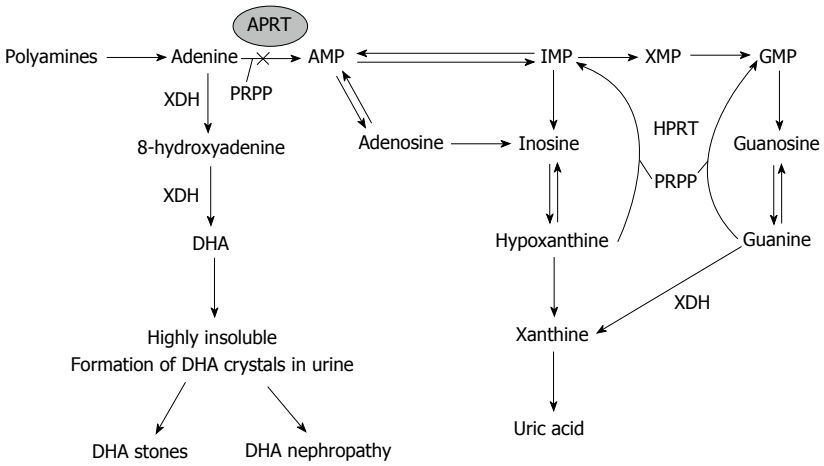


Figure 1 Biochemical pathway of purine metabolism and mechanisms of adenine phosphoribosyltransferase deficiency. DHA: 2,8-dihydroxyadenine; APRT: Adenine phosphoribosyltransferase; HPRT: Hypoxanthine phosphoribosyltransferase; PRPP: Phosphoribosyl-pyrophosphate; IMP: Inosine monophosphate; XDH: Xanthine dehydrogenase enzyme; AMP: Adenosine monophosphate; GMP: Guanosine monophosphate; XMP: Xanthosine monophosphate.

well as later than age 40[4,7,8,22]. In affected infants, reddish-brown diaper stains related to DHA crystalluria can be observed[10]. In some instances, bilateral DHA stones can cause urinary tract obstruction and acute renal failure, especially in children[10,28,29].

DHA stones are usually radiolucent and thus can be detected only by imaging techniques able to detect radiolucent stones, such as ultrasonograpy or computed tomography. However, DHA stones may sometimes appear as radiopaque when containing calcium salts[4,30]. Due to their radiolucent character, DHA stones are often mistaken for uric acid stones. Differential diagnosis for radiolucent stones also includes cystine and xanthine.

DHA nephropathy represents the second manifestation of APRT deficiency. It is commonly observed in adults but rarely in children[10,27]. DHA nephropathy typically occurs in patients who have remained undiagnosed and untreated despite a history of recurrent urolithiasis. However, it should be emphasized that DHA nephropathy can also occur in patients who experienced just a few episodes of stones or even in patients with no history of urolithiasis[31]. Imaging studies demonstrating the absence of stone do not definitively rule out the possibility of DHA nephropathy. DHA nephropathy usually develops insidiously and cause chronic kidney disease progressing over a period of years[31]. Less commonly, the presentation can be acute or subacute. Massive precipitation of DHA into the kidney can sometimes be triggered by urine concentration and supersaturation of DHA in the context of dehydration. In Japanese and European studies, nearly 30% of patients had decreased renal function and 10% had ESRD when APRT deficiency was diagnosed[4,7,10]. In our French cohort, among patients who were diagnosed and treated later than 40 years of age (n = 14), 6 patients (42.8%) had glomerular filtration rate (GFR) > 60 mL/min per 1.73 m^2, 3 patients (21.4%) had GFR of 30 to 60 mL/min per 1.73 m^2 and 5 patients (35.8%) had GFR < 15 mL/min per 1.73 m^2[7].

In some patients, APRT deficiency is not diagnosed until after kidney transplantation, which can have disastrous consequences. These patients are at high risk of losing their transplant in the absence of appropriate therapy. Several cases of DHA nephropathy recurring after kidney transplantation and rapidly leading to transplant failure have been reported[9,11,18,19,32,33]. In most tragic cases, several kidney transplantations failed before APRT deficiency was properly recognized and treated[19,32].

Nearly 15% of individuals with APRT deficiency may be asymptomatic[7,10], but are at risk of developing complications if left untreated. The factors underlying the variability of clinical presentation are unknown. There is no phenotype-genotype correlation, which is explained by the fact that biallelic mutations in *APRT* lead to null enzyme activity in all cases, whatever the mutations maybe. Inter-individual differences in water intake and consumption of foods high in purines may be involved in the variability of the clinical presentation. The potential influence of modifying genes has not been reported in humans. It is unknown whether osteopontin is a modifier of APRT deficiency severity in humans, as demonstrated in mice[34].

APRT deficiency is not known to cause extrarenal manifestation. Although clinical observations in some heterozygotes suggested that APRT deficiency may contribute to hyperuricemia and gout[35-37], patients with APRT deficiency usually show serum uric acid within the normal range. Rare cases of eye discomfort and corneal involvement were reported in APRT deficiency, but the significance of this association remains undetermined[38,39]. It is unknown whether long-term exposure to high systemic levels of DHA could have deleterious consequences. This issue may be of particular relevance for patients with APRT deficiency and ESRD, who might be subjected to constant DHA exposure due to the loss of renal clearance of DHA.

Heterozygotes are asymptomatic and usually have normal excretion of DHA and no DHA crystals in their urine, despite the fact that they have partial APRT defi-

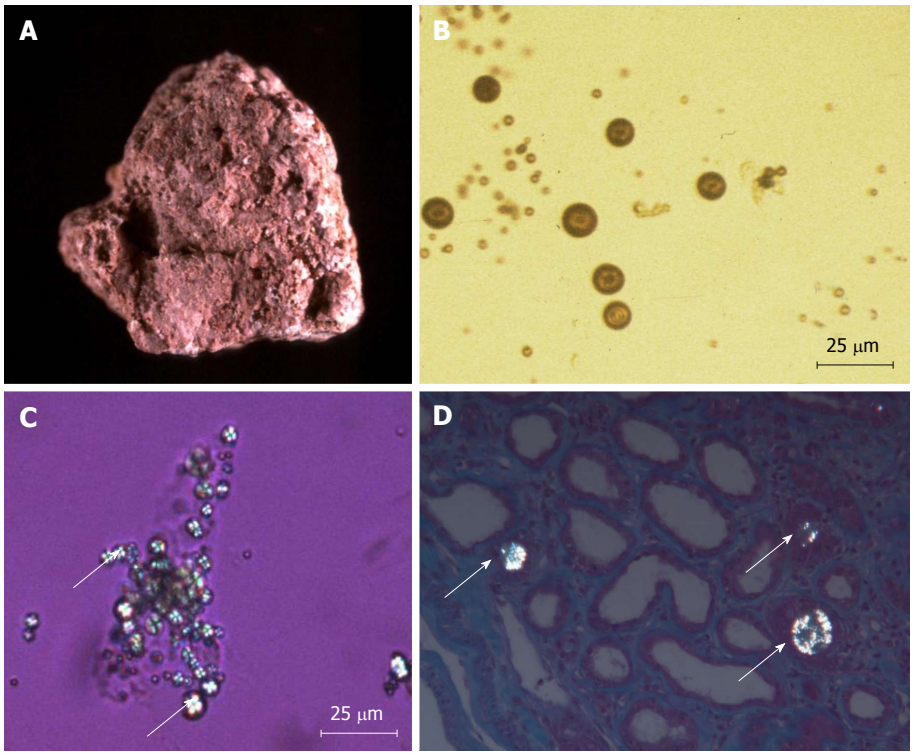


Figure 2 Stones and crystals of 2,8-dihydroxyadenine. A: Typical aspect of a 2,8-dihydroxyadenine (DHA) stone, showing a rough and humpy surface and a reddish-brown color that turns grey when drying. The stone is friable and sections show porosities and beige to brown color; B: Light microscopy aspect of DHA crystals in urine (non-polarized image), showing a round shape and a reddish-brown color; C: Urine DHA crystals in polarized light, showing typical central Maltese cross pattern (arrows); D: Periodic acid-Schiff of kidney biopsy under polarized light in a patient with adenine phosphoribosyltransferase deficiency, showing crystals within the renal tubules (arrows) (× 400). Fourier transform infrared microscopy study of the biopsy demonstrated that crystals were composed of DHA.

ciency[3,38]. However, one case of DHA urolithiasis was reported in an individual with a mutation (*APRT*J*) in only one of the two alleles[40]. To the best of our knowledge, no other case of symptomatic heterozygote has been reported to date and the mechanisms that might cause urolithiasis in some heterozygotes remain unknown.

GENETICS

APRT deficiency is caused by biallelic mutations (homozygous or compound heterozygous) in *APRT* gene, which is over 2.8 kb long. The gene contains five exons and encodes a 540 bp mRNA[5]. Over 40 mutations in the coding region of *APRT* gene have been identified in more than 300 affected individuals worldwide, with the majority arising from Japan[38]. Mutations causing type I APRT deficiency are referred to as *APRT*Q0*, which includes a wide variety of mutations in the coding region, including missense[5,41-43], non-sense[5-7], insertion and deletion[5,7,44,45], and splice-site mutations[5,7,44,46]. Type I deficiency is caused by homozygous or compound heterozygous *APRT*Q0* mutations. Certain mutations are more common in some populations. The c.400 + 2dup mutation (previously named IVS4 + 2insT)[44], appears to be the most frequent mutation in the European population[5,13,44,47,48]. and account for 40% of mutations in France[7]. Another mutation, Asp65Val , is highly prevalent in Iceland[10] and has been also reported in British and Spanish families[7,41,47].

Type Ⅱ APRT deficiency has been observed almost exclusively in Japan and is due to a missense mutation (*Met136Thr*) called APRT*J[4,42]. Patients with type Ⅱ deficiency have two APRT*J alleles, or less frequently one APRT*J and one APRT*Q0 allele[45]. The only known exception to this is a peculiar mutation (*V150F*) that was shown to cause type Ⅱ deficiency in a Polish patient[49].

DIAGNOSTIC TESTS

The identification of DHA in stone or urine is pathognomonic of APRT deficiency. Tests available for diagnosis of APRT deficiency include stone analysis, urine microscopy, renal biopsy, APRT activity and molecular genetic testing.

Stone analysis

Stone analysis should combine morphological examination by stereomicroscopy (Figure 2A) and analysis using infrared spectroscopy or X-ray crystallography, which will unambiguously demonstrate the DHA nature of the stone[50-52]. Standard biochemical methods to evaluate the composition of urinary stones can mistake DHA for uric acid and other purines, and are no longer recommended.

Urine microscopy

Urine examination by light and polarized microscopy can

detect DHA crystals, which have a characteristic appearance (Figure 2B and C)[50,51]. In general, the first morning urine specimen is particularly valuable for crystalluria study, because it is more concentrated. The amount of crystals is high in the urine of untreated affected individuals[7]. Infrared spectrophotometry (IRS) provides characterization of the composition of crystals and confirms diagnosis. In our experience, urine microscopy has an excellent sensitivity and DHA crystals can be detected in the urine of nearly all affected individuals. Rarely, false-negative may be observed[10].

Renal biopsy

Renal biopsy is, at least in theory, not necessary for diagnosis, given that DHA crystals can be identified in urine. In some instances where crystalline nephropathy was not expected, however, renal biopsy can demonstrate the presence of DHA crystals into the renal parenchyma and lead to diagnosis of APRT deficiency (Figure 2D). DHA crystals are mainly observed within tubules and in the renal interstitium. It must be emphasized that DHA crystals in renal biopsy often lack the characteristic morphology of crystals that can be observed in urine. We strongly recommend that crystals seen in renal biopsy specimen be fully characterized in order to avoid confusion with other crystalline deposits, especially uric acid and calcium oxalate. Whenever available, the combination of polarizing microscopy and Fourier transform infrared microscopy (FTIRM) is a reliable method for characterizing crystals in renal biopsy[53,54].

APRT activity

Measurement of enzyme activity in cell lysates is a useful tool for diagnosis of APRT deficiency[55,56]. Unfortunately, the availability of this test is limited in most countries. As discussed above, APRT activity is null in almost all non-Japanese patients with APRT deficiency (type I APRT deficiency). The only exception known to this is a patient of Polish origin with type Ⅱ deficiency related to a peculiar mutation[49]. Complex *in vivo* assays, such as uptake of adenine by intact cells, may rarely be used to assess the functional significance of such new mutations associated with residual activity in cell extracts. In type Ⅱ APRT deficiency, which is observed in patients of Japanese origin, APRT activity is usually less than 30% of normal level[4]. Therefore, a detectable APRT activity in cell extracts does not rule out the possibility of APRT deficiency, although this possibility is an exception in non-Japanese patients.

In heterozygous individuals with one APRT*Q0 and one non-mutated allele, APRT activity is decreased but still detectable (in our experience 5% to 60% of normal value)[8]. In heterozygotes carrying the APRT*J allele, the enzyme activity is usually higher than 50%, which tends to overlap with the values observed in normal individuals[21,22]. To put it in a nutshell, APRT activity assay demonstrates abnormal values in virtually all individuals with APRT deficiency (0% in type I and less than 30% in type Ⅱ) but is not a reliable technique to identify heterozygotes.

Molecular genetic testing

Mutation screening of the *APRT* gene can be relatively easily performed by sequencing of exons and flanking intronic sequences[5]. The diagnosis is confirmed if genetic testing shows functionally significant mutations in both alleles (see Genetics). In our experience, approximately 10% of mutations are not unidentified by *APRT* sequencing[7]. This may be due to large allelic deletions or mutations in promoter region.

Others

Measurement of purine metabolites in urine, as performed by certain laboratories, may reveal increased levels of adenine, suggesting a diagnosis of APRT deficiency. An assay for measurement of DHA would be more desirable but is not currently available. Developing a urinary DHA assay for screening and monitoring of treatment in clinical laboratories is one of the objectives of the APRT Deficiency Research Program, which is a part of the international Rare Kidney Stone Consortium (rarekidneystones.org)[38].

TESTING STRATEGY

Testing strategy may vary depending on the local availability of diagnostic tests. It may also depend on whether the aim is to establish diagnosis in a proband (individual without a family history of APRT deficiency) or to screen relatives of an affected individual. An algorithm for the diagnosis and treatment of APRT deficiency is provided in Figure 3. Key points are also summarized in Table 1.

Strategy for diagnosis in a proband

We recommend screening for APRT deficiency in all cases of urinary stones in children, recurrent urinary stones (especially if stones are radiolucent) and history of urinary stones associated with acute or chronic kidney disease of uncertain cause (including ESRD patients and renal transplant recipients).

Diagnosis primarily relies on the recognition of DHA in stones or crystals. Whenever a stone is available, it should be analyzed, even if it was passed a long time ago. Urine microscopy examination should be systematically done. IRS analysis of crystalluria is recommended when DHA is suspected or, more broadly, when crystals of uncertain composition are observed.

As discussed above, renal biopsy is not necessary for the diagnosis of DHA nephropathy, given that DHA crystals can be detected in urine in almost all affected individuals. Whenever histopathological findings consistent with crystalline nephropathy are observed, full characterization of the nature of crystals is mandatory. Such findings should prompt clinicians to search for crystals in urine. Crystals can be characterized in renal biopsy specimen using FTIRM. However, this technique is restricted to a few laboratories and crystalluria study is often an

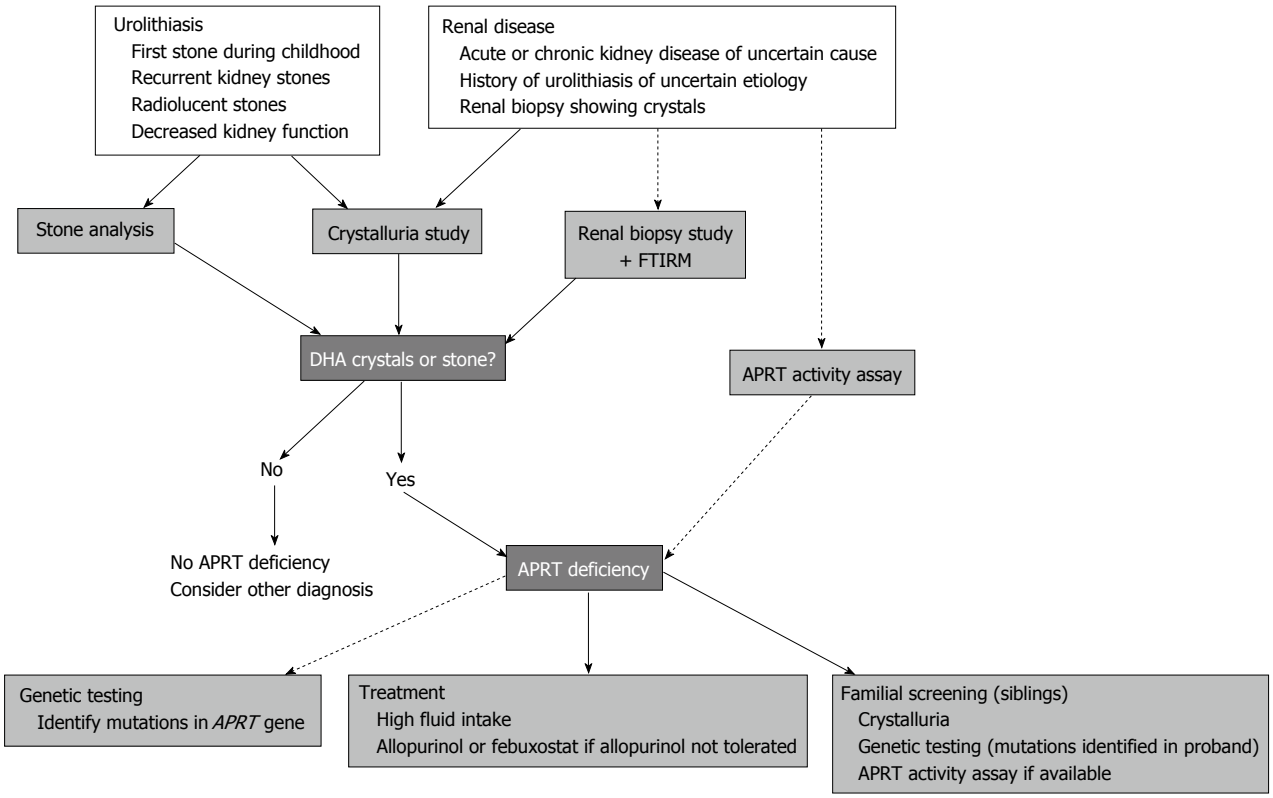


Figure 3 Algorithm for diagnosis and management of adenine phosphoribosyltransferase deficiency. This clinical algorithm summarizes situations where adenine phosphoribosyltransferase (APRT) deficiency should be suspected, testing strategies and management of the disease. FTIRM: Fourier transform infrared microscopy; DHA: 2,8-dihydroxyadenine.

Table 1 Key points about the diagnosis and treatment of adenine phosphoribosyltransferase deficiency

Key points
APRT deficiency is a rare but underrecognized genetic disease
Recurrent urolithiasis and DHA nephropathy are the two clinical manifestations of APRT deficiency and diagnosis can be made at any age
DHA nephropathy can relapse after renal transplantation
In most cases, urine microscopy and stone analysis will lead to diagnosis
APRT activity assay and genetic testing are useful for confirmation of diagnosis, for family screening and in difficult cases of urolithias or crystalline nephropathy
Allopurinol is the cornerstone of preventing recurrence of kidney stones and DHA nephropathy

APRT: Adenine phosphoribosyltransferase; DHA: 2,8-dihydroxyadenine.

easier way to identify DHA crystals.

If adequate stone and urine analysis exclude the presence of DHA, APRT deficiency is very unlikely and no other tests are usually needed. However, an exception can be made when the index of suspicion is high, especially in cases of crystalline nephropathy of uncertain cause.

Although the presence of DHA in crystals or stones is pathognomonic of APRT deficiency, APRT activity assessment and/or genetic testing are recommended to confirm diagnosis[38]. Measurement of enzyme activity is particularly helpful when stone or urine analysis is not feasible (*e.g.*, anuric patient). We strongly recommend APRT activity measurement in ESRD patients awaiting a kidney transplant who have a history of urolithiasis, when the cause of the kidney disease and the composition of the stones are uncertain.

Genetic testing is useful as a confirmatory test but is not intended as a primary screening procedure. Identifying the disease-causing mutations is of great relevance for familial screening once diagnosis has been confirmed in the proband.

Strategy for familial screening

Each sibling of a proband with APRT deficiency has a 25% chance of carrying two mutations and being affected. It is important to keep in mind that affected individuals may be asymptomatic but is still at risk for developing complications if the disease remains undiagnosed and untreated. All siblings, symptomatic or not, should therefore be investigated for APRT deficiency.

Once the mutations causing APRT deficiency have been identified in the proband, it is recommended that

the siblings undergo genetic testing. APRT activity measurement may also be useful, especially in the case where causative mutations could not be found in the proband. Urine microscopy examination should also be performed. Considering the risk, although very small, of false negative[10], urine microscopy should not be solely used to screen at-risk relatives. Further investigations, including assessment of renal function and imaging studies, are warranted in individuals with biallelic mutations, decreased APRT activity, or DHA crystals in urine. As discussed above, DHA crystals are usually absent in the urine of heterozygotes. APRT activity may be decreased but not null in heterozygotes.

TREATMENT AND SURVEILLANCE OF APRT DEFICIENCY

Treatment

No treatment is known to increase APRT activity. However, the disease can be efficiently treated with allopurinol, which inhibits XDH, thereby blocking the formation of DHA from adenine. Allopurinol is the cornerstone of treatment for APRT deficiency. Allopurinol therapy usually leads to a rapid reduction of DHA crystalluria and stone formation[7,27]. Allopurinol efficiently prevents the occurrence or progression of DHA nephropathy in most patients[7,10]. However, kidney disease can be irreversible, especially if tubulointerstitial lesions are advanced. The usual daily dose of allopurinol is 300 to 600 mg (maximum dose 800 mg) in adults and 5 to 10 mg/kg in children. In adults, we recommend initiating allopurinol therapy at a dose of 300 mg/d, which is sufficient to achieve good control of the disease in most patients. The dose should be increased in patients with persistent crystalluria and must be adapted when renal function is impaired. Allopurinol is well-tolerated by most patients, including children[27]. Febuxostat, a specific inhibitor of XDH[57], may be used in patients who do not tolerate allopurinol. However, the benefit and safety of febuxostat in APRT deficiency patients has not been evaluated. All patients with APRT deficiency, symptomatic or not, must receive life-long therapy with a XDH inhibitor. Patients and their families should be educated on the importance of life-long therapy and the risk of developing urolithiasis and DHA nephropathy if the treatment is stopped. Treatment with allopurinol is of paramount importance in patients undergoing kidney transplantation in order to prevent recurrence of DHA nephropathy, which can lead to transplant failure[9,11,18,19,32,33].

Whether patients with APRT deficiency on dialysis benefit from allopurinol is unknown. One may be concerned about the long-term impact of chronic exposure to high systemic levels of DHA. Although no deleterious effects have been reported to date, existing data on APRT deficiency patients undergoing chronic dialysis are very limited. In dialysis patients awaiting a kidney transplant, it seems preferable to initiate allopurinol therapy and achieve stable metabolic control prior to transplantation rather than initiate treatment only after a new kidney has been implanted.

Along with XDH-inhibiting drugs, high fluid intake achieving a urine volume of 2.5 liters daily (in adults) should be advised. It is usually recommended to avoid foods high in purines, although the impact of this diet on DHA excretion has not been established. Urinary alkalinization is not recommended, as DHA has very low solubility at pH values lower than 8.5[13].

Available data are limited regarding urological management of DHA stones. In our experience, patients can benefit from various procedures, including extracorporeal shock-wave lithotripsy, endoscopy and surgery, as for the treatment of other types of stones.

Surveillance

For the surveillance of patients with APRT deficiency, we recommend monitoring their renal function, performing quantitative analysis of crystalluria, and renal ultrasound every 6 to 12 mo in stable patients. The treatment usually leads to the disappearance or at least a drastic reduction of the number of DHA crystals[7,27]. A minority of patients treated experience stone recurrence[7,27]. Non-compliance or an insufficient dose of XDH inhibitor should be suspected in these patients or if there is no marked reduction in crystalluria.

CONCLUSION

APRT deficiency is a potentially severe condition that tends to be overlooked, especially in adults. A high index of suspicion for APRT deficiency and performing the appropriate investigations are mandatory in patients with recurrent urolithiasis and decreased renal function. There are few examples of diseases that can lead to complications as severe as irreversible renal failure but that can be efficiently treated with one pill a day. No stone should be left unturned in the effort to better recognize APRT deficiency and thereby enabling early and effective therapeutic intervention.

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REVIEW

Functional and metabolic complications of androgen deprivation therapy

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Abstract

Prostate cancer is the most common non-cutaneous cancer in men worldwide. Several different treatment strategies are available including minimally invasive procedures for localized tumors such as radical prostatectomy, radiotherapy, and androgen deprivation therapy, among others. All these strategies can be given as mono-therapy or as combination therapy. For this review, we will focus on the side effects of androgen deprivation therapy, independent of the other treatment modalities. Some of the most common affections are loss of bone mineral density, weight gain and obesity, myocardial infarction and sudden death, metabolic syndrome and insulin resistance, dyslipidemia, loss of libido and erectile dysfunction, fatigue, cognitive decline, vasomotor flushing, to mention a few. All these alterations can have an impact on quality of life and even lead to more serious complications such as fractures and cardiovascular complications. We present recommendations for prevention, early recognition and treatment. The different modalities for androgen deprivation therapy have particular side-effects profiles and indications should be made in an individualized manner. Androgen deprivation therapy is a useful tool for some patients with prostate cancer but every effort should be made to avoid related complications. The use of guidelines and educational programs for both, patients and urologists, are extremely useful strategies.

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Key words: Prostate cancer; Metabolic syndrome; Frailty syndrome; Complications; Sarcopeny; Androgen deprivation therapy

Core tip: The article will review the most common complications related to the androgen deprivation therapy. It includes the most relevant and up-to-date information, aiming to provide a reliable and concise review of the side effects of such therapy. Recommendations are made for the prevention, early detection and early treatment of patients.

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INTRODUCTION

Overall, the highest incidence of tumors is from the genital system (almost 338450 new cases expect to be diagnosed in 2013). Out of these, prostate cancer (PCa) is the most common accounting for more than 233000 new cases[1]. Anyhow, incidence of mortality from prostate cancer remains lower than that of lung cancer[1].

Patient prognosis depends widely on the risk classification[2]. With radical prostatectomy as the standard treatment for localized PCa, 5-year recurrence rates go from 6%-45%. Death rates from PCa also vary, going

from 65% survival at 5 years to a mortality of 1%-8% by year 10[3]. Considering these facts PCa can be considered a chronic disease, with chronic complications.

With an increasing survival expectancy, quality of life and functionality become a more relevant problem. In the general population, by age 70 about 20%-30% of patients present some form of disability [mobility, Instrumental or in activities of daily living (ADL)][4]. In patients with PCa, good oncologic outcome can be achieved with several treatment options. Each treatment modality has its own set of side effects that can interfere with patients' performance.

The following review will focus on the functional and metabolic complications that PCa patients develop during the follow-up of the disease.

EVIDENCE ACQUISITION

Original and review articles addressing complications of androgen deprivation therapy were obtained by a systematic search of PubMed. Keywords included "prostate cancer", "androgen deprivation therapy", "hormonal therapy", "frailty syndrome", "sarcopeny", "obesity", "osteoporosis", "metabolic syndrome", "arterial stiffness", "cardiovascular", "fatigue", "cognitive impairment", "loss of libido", "erectile dysfunction", "adverse effects" and "complications". We selected the most recent articles with a good level of evidence to be examined and included in our review. Prospective, randomized trials were prioritized. The review will focus on androgen deprivation therapy complications, in terms of related metabolic and functional impairment.

INTRODUCTION-ANDROGEN DEPRIVATION THERAPY

After the description of Huggins *et al*[5] about the hormonal dependency of PCa, androgen deprivation therapy (ADT) has been widely used for its treatment. There are several indications for ADT including metastatic and locally advanced tumors, concomitant administration with radiotherapy, recurrence treatment, primary treatment for localized tumors. It has also been proposed for all high-risk patients after radical prostatectomy[6,7]. Average survival of patients using ADT, with recurrence after primary treatment is 84 mo[8].

Physicians must be aware of the potential benefits and side effects associated to ADT. Such therapy should be used exclusively when there is a known benefit. The benefit must outweigh the affection in Quality of Life (QoL). Once the decision to start ADT is made, actions must be taken to reduce adverse side effects. Patients should start an exercise program[9]; early detection strategies and adequate treatments could improve outcomes.

In an interesting prospective randomized trial by Salonen *et al*[10], they treated patients with advanced PCa for 6 mo with goserelin acetate. After the initial 6 mo, patients were randomized to receive either continuous or intermittent ADT (given for at least 6 mo when PSA reached levels > 20 ng/mL). Adverse effects (cardiovascular complications and cardiovascular deaths, bone fractures and hot flushes) were similar among groups. Nevertheless, the primary outcome was QoL (activity limitation, physical activity and sexual function), which was significantly better in the group of intermittent therapy. Erectile dysfunction (ED) (15.7% *vs* 7.9%) and depressed mood (2.2% *vs* 0%) were more frequent in the intermitted ADT group. Hot flushes were the most frequent adverse events (47.1% *vs* 50.4%)[10]. Other studies have also shown a benefit of intermittent treatment in terms of QoL, fatigue, hot flashes, sexual desire and urinary symptoms in patients with biochemical recurrence after radiotherapy[11].

In a recent systematic review by Botrel *et al*[12] including 13 trials and 6419 patients under continuous or intermittent ADT, there was no benefit in overall QoL. Except for the presence of hot flushes and sexuality scores, there were no differences between the two treatment strategies. A benefit in survival could not be demonstrated[12]. A Cochrane Database Systematic Review obtained similar conclusions[13].

ADT can have several side effects including new onset diabetes mellitus, osteoporosis, decreased libido, ED, hot flushes, acute kidney injury and cognitive decline, among others[14]. These side effects can occur both with short- and long-term ADT[7]. For these reasons, urologists and oncologists should discuss the possible treatment strategies with the patients. The patient with PCa must be well aware of the possible complications before initializing either therapeutic option. They must know that even though intermittent ADT might have a slight benefit in terms of QoL or sexual function, such effects do not seem to last long, and there is no proven benefit in terms of survival[15,16]. Particularly concerning ADT, the patient must be aware that even though ADT may be regarded as a "non-invasive treatment" the side effects can be very deleterious and have a significant impact in QoL, or even life expectancy due to cardiovascular implications[17]. Urologists should consider the evidence based benefits and side effects before initiation of therapy[7].

The following review will focus on the adverse effects of ADT, as well as the management strategies of these complications. True benefits and complications should be analyzed before indicating ADT.

ANDROGEN DEFICIENCY IN THE AGING MALE

Testosterone deficiency is a common condition in aging men, with a decline rate of 0.8%/year in total testosterone levels. Such decline is greater after the age of 60 and in patients with chronic illness, including obesity[18]. Symptoms are non-specific and frequently go unrecognized. Several terms have been used for the condition encompassing a low testosterone level and compatible symptoms in a male patient, usually older than 40 years

old. The most accurate term is "Androgen Deficiency in the Aging Male" (ADAM)[19].

ADAM symptoms are divided in physical (decreased bone mineral density, decreased muscle mass and strength, increased body fat and body mass index, gynecomastia, anemia and fatigue), psychological (depressed mood, diminished energy, diminished sense of vitality or well-being, impaired cognition and memory) and sexual (diminished libido, ED, difficulty achieving orgasm, decreased morning erections, decreased performance)[20].

Testosterone replacement therapy (TRT) can improve many of the associated symptoms (mostly low libido, energy, mood, low muscle mass, osteoporosis and hot flushes). TRT is only indicated when the patient has symptoms and a corroborated low testosterone level[19].

In patients with PCa or at risk of PCa [first degree relatives with PCa, prostate specific antigen (PSA) > 4 ng/mL, palpable prostate nodules, PSA dynamics], treatment is controversial and assessment by an urologist is recommended[21]. Several studies have questioned the possible deleterious effects of TRT in patients with PCa. Recently, a meta-analysis showed no statistically significant differences in terms of progression or development of PCa, particularly with short term use (less than 12 mo)[22]. Also, in a study using the Surveillance, Epidemiology and End Results Program (SEER) database, Kaplan *et al*[23] showed that 0.79% of the patients who were diagnosed with PCa from 1992-2007 received TRT. In their analysis, they found a statistically significant higher overall and cancer specific mortality in patients not receiving TRT in comparison to those who did receive TRT. The need of salvage ADT was not different in both groups, reflecting similar cancer control.

Testosterone replacement in patients with diagnosis or high risk factors for PCa is still very controversial and no formal recommendations can be made in favor of such therapy.

OSTEOPOROSIS

After age 45, about 25% of male patients suffer some degree of osteoporosis. Risk factors are hypogonadism, alcohol abuse, smoking, sedentary lifestyle and calcium and vitamin D deficiency[7]. Osteopenia is defined as a T score between -1.5 and -2.4, and osteoporosis as a T score greater than -2.5, according to the World Health Organization[24]. It is recommended that all men over 50 years of age take calcium (1200 mg/d) and vitamin D supplements (800-1200 IU/d) along with exercise, quit smoking and limit alcohol drinking, regardless of ADT[25]. In men with prostate cancer, dose of supplementation has not been well established[26]. It is recommended that patients should be evaluated with a dual-energy X-ray absorptiometry (DXA) before initiation of ADT[27]. Further DXA should be done according to the results of the basal exam. Osteoporotic patients should have a DXA done every 6 mo, osteopenic patients every year and patients with a normal DXA can do the next exam up to 24 mo after the previous, provided they do not have high risk factors[28].

ADT has been proven to cause a decreased bone mineral density (BMD), independent of the modality used (either pharmacological or surgical blockade)[24]. During ADT, bone remodeling takes place, osteoclast activity seems to be increased whereas osteoblast repair is insufficient[29]. Such phenomenon causes decreased BMD and increases bone fracture risk and clinical fractures[30,31]. In a study by Shahinian *et al*[32] using the SEER database, the fracture rate for patients using gonadotropin releasing hormone (GNRH) agonists was 19.4% *vs* 12.6% in the non-ADT group. The risk was higher for patients receiving ≥ 9 doses of medication and orchiectomized[32]. The rate of decrease in BMD is 3%-5.6% during the first year of ADT and 1.1%-2.3% a year from the second year on[33]. The longer the time of ADT, the patients have a greater risk of having clinically significant fractures[34]. Also, in patients with osteoporosis the standardized mortality ratio after fractures increased according to the site of fracture (1.45 for minor fractures and 3.17 for proximal femur fractures)[35]. The use of calcium and vitamin D supplements is a possible treatment. Nevertheless, calcium supplementation has been associated with aggressive prostate tumors and increased cardiovascular disease[26].

It has been suggested that ADT should be avoided in patients with a high fracture-risk (age ≥ 80 years old, diabetes mellitus, alcoholism, cigarette smoking, rheumatoid disease, moderate-severe liver disease, paralysis and/or history of osteoporosis and fractures)[36]. The use of estrogens [diethylstilbestrol 1 mg/d orally (PO) or polyestradiol phosphate] for ADT may be the better option for high-risk patients. The cardiovascular effects of the latter medication have been minimized according to recent studies, and the repercussion on skeletal-related events seems less than using other ADT strategies[37].

Patients can be initially treated in a conservative fashion with smoking[38] cessation, controlled exercise, adequate calcium (1200 mg/d) and vitamin D (400-800 IU/d) intake as the first line of treatment. The initiation of exercise within 10 d from the first dose of ADT improves BMD compared to usual care. For more advanced cases the use of bisphosphonates is advised. The above mentioned therapies have shown benefits in prostate cancer patients receiving ADT[39], or even before initiation of ADT[27]. The use of alendronate (70 mg PO weekly) has showed benefits in patients with prostate cancer and osteoporosis or severe osteopenia[40,41]. For patients with non-metastatic disease, bisphosphonates can keep the BMD stable[42].

Denosumab, a human monoclonal antibody against the receptor of the nuclear factor-κβ ligand, has proven better in terms of risk of fracture and BMD increase on patients with ADT, compared to placebo[43,44]. Also, in a randomized study with 1904 patients with metastasis and castration resistant disease, denosumab 120 mg subcutaneously was compared against zoledronic acid 4 mg IV.

Denosumab was better for prevention of skeletal-events; adverse events were similar for both groups[45]. Both, denosumab and zolendronic acid can cause osteonecrosis of the jaw and hypocalcemia[45]. Bisphosphonates can cause nephrotoxicity, particularly in patients with chronic kidney failure and also a flu-like condition during the first doses[28].

Even though several studies have pointed to the benefits of exercise in different aspects of PCa and ADT related adverse effects, such strategy is still poorly applied. Osteoporosis and fracture prevention strategies are unknown to many patients and therefore underutilized[46].

OBESITY

Other side effects of ADT are a loss of lean muscle mass and a gain in body fat[47], particularly subcutaneous fat[48]. It causes a weight gain of about 1.8%-2.4% and an increase in fat body mass by 9.4%-11%[48,49]. The term used to describe the increased body fat along with decreased lean muscle mass that characterizes ADT-induced obesity is "Sarcopenic Obesity"[50]. Sarcopenia is reported in about 20% of patients with an average lean muscle mass loss of 2.8%[51].

Obesity and increased insulin secretion have been related to an increased incidence and more aggressive PCa[52,53]. Obese patients have increased progression to castration-resistant disease; increased rate of metastasis development and some authors have proposed a relationship between obesity and larger cancer specific mortality[54]. Obese patients have an increased oxidative stress, predisposing them to the development of several cancers such as endometrial, bladder, breast and prostate[55].

A structured exercise program, including both resistance and aerobic exercises helps against the metabolic complications of ADT. In a randomized trial by Cormie *et al*[38] the initiation of such exercise program at the beginning of ADT has improved outcomes in terms of lean muscle mass and less fat body mass. Such improvement is evident within the first 3 mo of treatment[38].

CARDIOVASCULAR COMPLICATIONS

These have been described in about 30% of patients undergoing ADT (intermittent and continuous). Also, cardiovascular death events happen in around 8% of such patients[10]. In a large study, comparing PCa patients without ADT with patients using GNRH agonists and surgically castrated patients, increased coronary heart disease (CHD), myocardial infarctions (MI) and sudden cardiac death (SCD) were seen in both ADT modalities[17]. Nevertheless, other large studies have not shown such findings in orchidectomized patients, only showing them in the group receiving GNRH agonists[56]. Estimated increased risk using ADT is 16% for CHD, 11% for MI and 16% for SCD[50]. In a comparative study of abiraterone *vs* placebo in patients with metastatic PCa, cardiovascular events occurred in 13% of patients in the abiraterone group *vs* 11% in the placebo group[57]. Recently, an observational study found that risk factors for developing cardiovascular diseases using ADT are the same than those for patients not receiving such therapy[58]. Screening strategies for cardiovascular disease should not change for patients whether or not they receive ADT.

In a comparative analysis of patients receiving GNRH agonists *vs* antagonists, interesting differences were found in terms of cardiovascular complications. There were no differences among men without preexisting cardiovascular disease. On the other hand, when analyzing patients with a previous history of cardiovascular disease, there was a 56% less chance of cardiovascular events or death within the first year of treatment in the GNRH antagonist group compared to the group receiving GNRH agonists (6.5% *vs* 14.7%)[59].

In a multicenter study by Galvão *et al*[60], they compared the benefit of 6 mo of supervised exercise training (resistance and aerobic training) followed by 6 mo of home training routines *vs* 12 mo of handouts of printed educational material of physical activity. Self-reported physical functioning, and objective measurements of muscle strength were better for the supervised group at 6 and 12 mo. Blood values showed little change between groups[60]. This should be taken into account when recommending exercise to patients; supervised training should be encouraged.

In conclusion, there is no definitive evidence about an increased cardiovascular death in patients using ADT, but they do seem to have more cardiovascular events. This is something physicians must keep in mind and make patients aware of when starting ADT. In selected patients with known cardiovascular disease, revascularization previous to starting ADT can improve survival[61].

METABOLIC SYNDROME

An increased incidence of metabolic syndrome (MetS) has been described, particularly in the group of PCa patients receiving ADT (50% *vs* 20% of naive patients)[62]. When patients with MetS are analyzed, they appear to have an increased risk for PCa (particularly clinically significant PCa, intermediate/high-risk tumors, progression and upgrading after surgery)[63-66]. The more elements of MetS they have, the greater the risk of PCa[67]. Specifically, high blood pressure and high body mass index are the two most relevant factors in terms of PCa death[68]. The possible explanations for this increased aggressiveness are the state of chronic inflammatory, high insulin levels, increased leptin and low adiponectin, and increased estrogen levels[63].

Saylor *et al*[50] recently reviewed the effect of ADT on the different MetS components. Patients showed a weight gain of 2% in the first year, 4%-8% fat body weight increase by the third month and 10% after 12 mo, lean muscle mass decrease of 3% by 12 mo, 26% increase in triglycerides by 6 mo, 8%-20% HDL increase by a year, 7% LDL increase by the third month, 26%-65% increase

in fasting plasma insulin and a 13% decrease in insulin sensitivity index. ADT confers a 44% risk of developing diabetes mellitus.

It is important to mention that unlike the "regular MetS", when patients receive ADT they gain mostly subcutaneous fat, not visceral fat; HDL increases instead of decreasing; blood pressure, waist-hip ratio and inflammatory markers such as C-reactive protein remain unchanged[49,69]. Also, patients on ADT do not develop non-alcoholic steatohepatitis[70]. These different features of the "ADT-related" MetS, perhaps, should be considered and treated independently as it is not a systemic inflammatory condition as opposed to the "regular MetS".

A supervised exercise program twice a week including aerobic and resistance training at the initiation of ADT offers benefits by reducing changes in body composition, physical function, lipid profile, sexual function and psychological distress[38]. The use of metformin along with an exercise program have shown improved outcomes in terms of blood pressure and body weight[62]. Metformin can control MetS, but also has an anti-proliferative effect by inhibiting the anabolic stimulation of insulin and activating the 5'-adenosine monophosphate-activation protein kinase[70].

Finally, the relation between MetS and PCa appears to be bidirectional. MetS could increase the risk of PCa, specifically aggressive and clinically significant tumors. On the other hand, patients with PCa and ADT have a high incidence of a "MetS-like" condition. Metformin, exercise, and other alternatives like statins and orlistat, can play an important role in treatment of patients with ADT and MetS.

INSULIN RESISTANCE AND LIPID ALTERATIONS

An increase in triglycerides and cholesterol levels is seen after ADT[49]. These side effects are more significant within the first 12 wk of therapy[71].

The incidence of diabetes mellitus increases with both, pharmacological and surgical ADT[17]. In only 12 wk, glycosylated hemoglobin and fasting plasma insulin levels increase significantly. By the same time, insulin sensitivity decreases[71]. There is an increase in glycosylated hemoglobin (HbA1c) of 0.13% after two years of treatment in non-previously diabetic patients[42].

It has been proposed, that the insulin increase in patients after ADT causes an increase in insulin-like growth factor (IGF-1). While this last situation could control the metabolic effects of ADT, it might also favor the development of castrate-resistant prostate cancer (CRPC). The insulin increase appears to stimulate intra-tumoral androgen synthesis, leading to CRPC development[72]. It seems that IGF-1 proteins can be involved in the change from benign cells to malignant prostate cancer cells. This is a hypothesis that explains why diabetics, obese individuals and patients with insulin resistance develop more PCa and more aggressive variants of PCa. Specifically, these patients have a shorter time between biochemical recurrence to the development of CRPC. Perhaps, diabetes treatment in patients with ADT is beneficial not only against the hyperglycemic state, but also to control IGF-1 and progression to CRPC[73].

Interestingly, some diabetic and weight control medication such as metformin, orlistat, statins and thiazolidinediones, could control cancer progression promoting apoptosis, decreasing cell mitosis and increasing sensitivity to chemotherapeutic agents[55]. Among these, metformin is the most studied medication; with a good safety profile it improves the lipid results, normalizes insulin levels and does not cause dysglycemia in non-diabetic patients.

As ADT increases insulin and causes insulin resistance, the development of diabetes is not the only complication we should worry about. The relationship between insulin rises and disease progression to CRPC is something we should be aware of. Perhaps, metformin should become an imperative companion to ADT. We recommend performing a metabolic assessment including fasting plasma glucose, HbA1c and a lipid profile every six months during the first year of initiating ADT, followed by yearly assessment thereafter; even in patients on intermittent ADT.

FRAILTY SYNDROME

With the growing age of the population and the survival rate of patients with PCa, disability has become a growing issue that should be taken into consideration by the treating physician. Disability, referred to as the dependency of another person to perform ADL, can happen due to several causes including weakness, comorbidities and aging[4]. Similarly, inflammatory markers such as IGF-1 (which is frequently elevated during ADT), predispose patients to the development of frailty syndrome[74,75].

In a large prospective cohort, Rockwood *et al*[76] developed a scale known as "Frailty Index". After a 5-year follow-up, such scale correlated with the risk of death and the risk of entry to an institution. This is a simple tool that can be applied to patients with PCa. In general, Frailty Syndrome (FS) is characterized by the presence of $\geqslant$ 3 of the following conditions: weight loss, weakness, fatigue, low activity and slow motion performance with balance and gait abnormalities[77,78]. Also, a "Pre-Frailty" condition has been described in which patients can either develop a full-blown FS or recover[4,77]. Early detection is a major intervention for such patients. Pre-Frailty patients must be advised of the increased risk of a worsening condition.

ADT can cause FS. Sarcopenia in patients with ADT can have the same impact as weight loss[77]. The "weight loss" definition for FS is recognized as an unintentional loss of > 10 pounds (4.5 kg) in the last year. Furthermore, obesity is recognized as a risk factor for the development of FS[79]. Weakness is defined as low grip strength measured with a hand-held dynamometer. Weakness can also

be caused by ADT[80]. A 15 feet (4.6 m) walk (speed less than 0.8-1 m/s) evaluates motion performance, which can be affected by a hypogonadal state[74,81]. Fatigue, is a well known side effect of ADT that will be discussed later in this review. Low physical activity can be caused by sarcopenia as well. Finally, as most patients with PCa are older than 65 years old, this is also a contributing factor for the development of FS[82].

FS has been associated with increased mortality, hospitalizations and worsening daily functions. Risk of falls and dependency are also increased in patients receiving ADT[77,83].

We advise physicians to perform scrutiny of FS before starting ADT. Patients with Pre-Frailty should be recognized and conditions optimized (weight loss, exercise routine, dietary advice) before ADT initiation.

LOSS OF LIBIDO AND ERECTILE DYSFUNCTION

Loss of libido is quite frequent in patients with ADT. Within the first year of ADT, 80% of patients without previous erectile dysfunction refer to having impotence[84]. The reason is a lack of testosterone stimuli. Patients often stop having sexual impulse and difficulty achieving an erection good enough for sexual intercourse. This leads to and avoidance of sexual contact because they might feel ashamed. Evaluation consists on interrogation of the patient and partner. Questionnaires such as the International Index of Erectile Function can be used to standardize results.

First line of treatment is the use of phosphodiesterase inhibitors, although the benefit is not always as good as with patients without ADT. Contraindications such as severe coronary artery disease, severe liver failure, nitrate therapy, *etc*. should be considered before initiation of therapy. Other options are penile prosthesis, vacuum devices and intracavernosal injections of prostaglandins[28].

FATIGUE

Fatigue in prostate cancer may be related to the loss of lean muscle mass, gain of body fat, and/or emotional distress in patients undergoing ADT[85,86]. Fatigue is described in about 40% of these patients[87]. With the use of enzalutamide fatigue was reported in 34% of patients (*vs* 29% in the placebo group)[88], and with abiraterone 44% (*vs* 43% in the placebo group)[57].

Cancer-related fatigue can be improved by aerobic exercise[89]. Better outcomes have been shown in group-exercise programs and in such programs including resistance training. Exercise should be recommended under supervision whenever it is possible[90,91]. Increased muscle mass and muscle strength can be obtained along with improved quality of life and fatigue[91,92].

In conclusion, supervised resistance and aerobic exercise is recommended two to three times a day in order to improve muscle mass, strength and fatigue.

COGNITIVE DECLINE

Even though there is not much evidence regarding cognitive decline associated with ADT, both transdermal estrogen therapy and exercise programs can improve this possible side effect[87]. Cognitive decline happens in up to 48% of patients on ADT[93]. Most studies assessing cognitive affection include a small number of patients. The most affected areas of cognition are executive, verbal and spatial functioning[28]. Mini-Mental Exam can be used as a standardized evaluation.

The reason for the mental decline during ADT might be an affection of the sex-steroid receptors in prefrontal cortex and hypothalamus. Gonadectomy in animals causes a decrease in 40% of synaptic unions that can be restored by androgen replacement. Transdermal estradiol (0.6 mg/24 h) applied every 7 d can improve memory loss[94].

Because of the safety profile, the most recommended strategy is a resistance and aerobic exercise program. Mental health and psychological distress benefits are evident after 3 mo of supervised exercise[38].

In a study including patients receiving ADT for causes other than PCa, ADT receiving patients had better scores of "agreeableness" (kind, cooperative and considerate personality) compared to patients not on ADT. This was the only significantly affected element of the "Big Five Personality Traits"[95].

Depression diagnosis can be made in a quarter of patients. Nevertheless, such diagnosis cannot be completely attributed to ADT[51]. According to a SEER study, ADT by itself does not increase the possibilities of developing depression[96]. Either way, PCa patients have a raised incidence of depression (seems to be multifactorial). Periodic evaluation of depressive symptoms and psychiatric attention to those with positive results should be part of the multidisciplinary approach. Interrogation of the patient's partner can be useful for gathering information.

VASOMOTOR FLUSHING

This is the most frequently found side effect of ADT (about 75% of patients with ADT). Interestingly, with the use of enzalutamide the reported incidence is much lower (20%)[88]. It is referred to as sweating, flushing of the upper body and a feeling of anxiety that lasts for about 3-10 min. Mean time of appearance is 2.7 mo after the start of ADT[97]. The physiopathologic explanation for such phenomenon seems to be an affection of the hypothalamic thermoregulatory center due to sex hormones, affecting serotonin and norepinephrine amounts at this level[98]. The psychological affection in these patients is quite relevant, feeling an impact in masculinity, powerlessness against such symptoms and social embarrassment[99]. A classification of the severity of hot flashes is used frequently[100].

Behavioral modifications oriented to keeping a low body temperature (dressing with cool cloth, drinking cold beverages, using a fan, avoiding spicy food, *etc*.) are

the first line of therapy when facing a patient with hot flushes[101,102].

Several strategies have been attempted in order to improve hot flushes. Estrogen and progesterone supplements are effective in about 85%-91% of patients (medroxyprogesterone acetate 5 mg twice daily[11] PO or 400 mg intramuscular, and megestrol acetate 20-40 mg bid PO)[97,103]. This seems to be the most effective therapy[98]. One should be aware of case-reports about a decrease in PSA levels when megestrol acetate is discontinued, considering it a possible tumor growth-stimulant when patients' PSA levels increase under such treatment. This last phenomenon may require suspension of megestrol acetate[104].

One-milligram diethylstilbestrol has shown effectiveness in 70% of patients, with no increase in thromboembolic or cardiovascular complications. There is concern about the increased risk of breast cancer in women, although it has not been well studied in men with ADT.

Gabapentin has been used in both men and women experiencing hot flushes. In men, it has an efficacy of up to 49% with a 300-900 mg/d dose. Side effects include nausea, loss of appetite, vomiting, dizziness and somnolence[105,106].

Anti-depressive medications such as Venlafaxine (serotonin and norepinephrine reuptake inhibitor) and paroxetine have achieved symptomatic reduction (about 55% with Venlafaxine) with few side effects (dry mouth, weight gain, nausea, headache and decreased appetite[87]) and improvement in fatigue, diaphoresis and sleeping trouble[107]. Unfortunately, these are mostly from small trials as well as evidence derived from menopausal women[108]. Caution should be exercised when using abiraterone acetate since this medication could narrow therapeutic index[104].

A large randomized trial, including 919 patients comparing vanlafaxine 75 mg/d, cyproterone acetate and medroxyprogesterone acetate 20 mg/d proved a significant benefit from all therapies (-47.2%, -94.5% and -83.7% after 4 wk, and -56.7%, -100% and -97.3% after 8 wk from randomization, respectively). No statistically significant difference was found in cyproterone and medroxyprogesterone groups[109].

Some medications such as vitamin E and Clonidine have been used in women, although benefit has not been proven in men and they have significant side effects, therefore are not recommended. Acupuncture electrostimulated and traditional[110], has also been studied in small trials. One systematic review assessed the utility of acupuncture in patients with PCa and was unable to recommend such therapy[111].

With such high incidence, vasomotor flushing risk should be commented with the patients and behavioral recommendations should be given to every patient from the beginning. If conservative treatment is not enough, the other available strategies can be considered according to the benefits and specific side effects.

OTHER COMPLICATIONS

Other complications such as gynecomastia can develop with ADT, interfering with patients social performance in up to 28% of cases[51]. Penile length decrease of > 1 cm was reported by 93% of patients in one study[112]. A hemoglobin decline of -1.11 g/dL (normocytic normochromic) has been reported, and most patients are asymptomatic[113]. We recommend an assessment prior to initiation of ADT and a complete evaluation if positive for anemia. Further testing every 3-6 mo should be done depending on hemoglobin values.

CONCLUSION

Prostate cancer and ADT is nowadays a common combination. ADT represents one of the most utilized therapies for PCa and can be implemented in nearly every stage of the disease. Side effects are frequent and can have serious implications in quality of life or even mortality. All types of ADT are prone to present side effects, with certain differences among the different modalities. Adherence to guidelines has great implications on patients. Individual patient selection, surveillance of complications and educational strategies (for both patient and urologists) are important cues in treatment.

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REVIEW

MiRNA in bladder carcinogenesis: A review

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Abstract

Bladder cancer (BC) is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. In the world, BC is responsible by about 386000 new cases and 150000 deaths annually with considerable economic impact and high costs for health systems. After its discovery more than 20 years, micro RNAs (miRNAs) have been recognized as molecules that work specifically in post-transcriptional control in majority of eukaryote genomes. MiRNAs are a family of small non-coding RNAs of 19-25 nucleotides in length, expressed in a wide variety of organisms, comprising plants, worms and mammals, including humans. They have a fundamental role in physiological and pathological processes in organs and tissues in a context-dependent manner. This review brings new roles of protective and oncogenic miRNAs linked to carcinogenesis of urothelial carcinoma of the bladder, and associated with behavior of disease. Many studies have demonstrated promising roles of miRNAs working as diagnostic and prognostic biomarkers or involved in target therapies, consolidating miRNAs as crucial players in human cancer. This review allowed a reflection about the true functions of miRNAs in bladder carcinogenesis. Not only by their wide capacities of action, but also by abilities in define the cell date. The future of anti-tumor target therapies will be based not in one, but in groups of miRNAs working together in several steps of carcinogenic process, being able to identify the disease, predicting behavior and effectively treat bladder cancer.

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Key words: Bladder cancer; Urothelial carcinoma; MiRNA; Biomarkers

Core tip: Bladder cancer is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. Micro RNAs (miRNAs) are a class of small non-coding RNAs that play roles in many physiological and pathological processes, including cancer. This review brings new roles of protective and oncogenic miRNAs linked to carcinogenesis of urothelial carcinoma of the bladder, and associated with behavior of disease. Most importantly, we provided a reflection about the true functions of miRNAs in bladder carcinogenesis, not only by their wide capacities of action, but also by abilities in define the cell date.

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INTRODUCTION

Bladder cancer (BC) is the second urological malignancy in incidence, currently being one of the most neoplasms studied with profile and biology poorly defined. BC development is related to environmental exposures that by genetic and epigenetic mechanisms can modify the cellular machinery and trigger the carcinogenic process. In the world, BC is responsible by about 386000 new cases and 150000 deaths annually[1] with considerable economic impact and high costs for health systems[2].

Conventional clinical and pathological parameters are used for BC histological graduation and stage and they are still the only tools now available employed to predict the prognosis of disease. However, this ability is limited and lacking data allowing a prospective analysis of risk of progression and behavior of BC.

Scientific evidences support the concept that BC is a many phases disease, and several alterations are needed until clinical presentation of BC. Thus, BC has been used as a main information source about mutational events that trigger carcinogenic pathways of solid human tumors[3,4]. Although genetic and molecular pathways are relatively well demonstrated and some biomarkers established, there are many questions to be answered about biological behavior or BC and novel methods that more specifically predict BC behavior are necessary.

The most common histological type of BC is bladder urothelial carcinoma (UC), occurring in 80%-90% of cases. UC can present in some different forms, from a small low-grade non-invasive tumor to advanced disease invading bladder wall and adjacent organs, with grade and stage established as main prognostic factors in bladder UC[4]. At diagnosis, 70% are low-grade non-invasive tumors evolving with optimal survival rates, while 10%-20% is high-grade invasive and aggressive disease with poor prognosis and increased index of mortality.

MOLECULAR BIOLOGY OF UC

A wide number of genetic events are involved in etiology, progression and treatment responses of UC[5]. The light of the molecular pathways related to UC carcinogenic process is crucial to know its etiopathogenesis and behavior. Biological variations such as both carcinogens conversion and detoxification and DNA repair can modify the expression and action of related genes in different phases of UC carcinogenesis.

Genetic events in non-invasive UC

Carcinogenic pathways that trigger low-grade non-invasive and high-grade invasive tumors are specific and mutually exclusives, and are showed in Figure 1[6-8]. The most of bladder malignancies is non-muscle invasive in initial presentation and its main tumorigenic route is mediated by fibroblastic growth factor receptor 3 (*FGFR3*) gene. Nevertheless, less common mutations in *RAS* gene have been described.

FGFR3 has 18 exons, it is located in 4p16.3 chromosomal region and belongs to tyrosine kinase growth factors receptors family, involved in functions related to embryogenesis and tissue homeostasis[8,9], regulating several biological processes, including proliferation, differentiation, migration and apoptosis[10]. Point mutations or other alterations which lead to *FGFR3* over-activity can alter cellular proliferation and trigger low-grade well differentiated UC, having little effect in cellular differentiation and apoptosis. These influences propitiate advantage to cell proliferation, but they do not change the genomic stability. *FGFR3* point mutations were described for the first time by Cappellen *et al*[11] (1999) that identified mutations in 35% of tumors. Point mutations in codons 248, 249 and 375 comprise more than 95% of *FGFR3* mutations.

Mutations in *RAS* oncogene has also been associated with UC non-invasive tumors and can be responsible by 30% of human cancers[12]. RAS works in regulation of cellular functions as proliferation, differentiation, motility and apoptosis in response to extracellular signals. In bladder UC, RAS seems to act through both mitogenic-activated protein kinase (MAPK) and AKT/STAT pathways[13]. Interestingly, *RAS* and *FGFR3* mutations do not occur at the same time, being considered mutually exclusive events indicating biological equivalence between these two types of point mutations[13].

Genetic events in invasive UC

The most of known genetic events in bladder UC is described in high-grade invasive tumors and many of them, as mutations and loss of function in the protective genes *p53*, retinoblastoma (RB1) and phosphatase and tensin homolog gene (*PTEN*) are associated with poor prognosis and high genetic instability[3].

p53 gene product is a tumor suppressor protein that is activated in response to signals of cellular stress, promoting transcriptional regulation of genes that induce cell cycle arrest, apoptosis, senescence, DNA repair and alterations in metabolism of the cell. Somatic mutations in *p53* are described in more than 50% of human tumors and germinal mutations can promote the tumor development in some hereditary syndromes. Unlike FGFR3, loss of function of p53 lead to important genomic instability associated with high-grade and stage tumors (Figure 1)[14-16].

RB1 suscetibility, a prototype of suppressor tumor gene, has been associated with UC progression and development. This phosphoprotein is a negative regulator of cell cycle and promotes chromatin stabilization allowing maintenance of its structure. RB1 mutations are strongly related to infant retinoblastoma, osteogenic sarcoma and bladder cancer (www.ncbi.nlm.nih.gov/gene). RB1 inac-

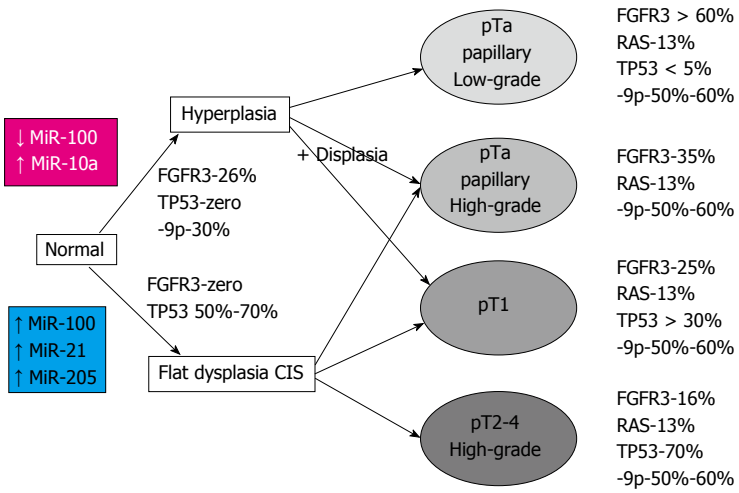


Figure 1 Genetic carcinogenic pathways of bladder urothelial carcinomas incorporating micro RNA. FGFR3: Fibroblastic growth factor receptor 3.

tivation is linked to UC, more specifically to invasive and aggressive disease[17,18].

PTEN is located in chromosome 10 (10q23) and works like a traditional tumor suppressor, acting in proliferation control, migration and cellular invasion by PI3K/AKT/mTOR pathway[4,19]. Despite its influence in non-invasive bladder UC, PTEN is more associated with invasive carcinogenic pathways. Although there are evidences that show its role in initiation and neoplastic progression, PTEN is not able to trigger them alone[20]. Recent data show that, when loss of function of PTEN is associated with *p53* mutations, invasive UC progresses more quickly, demonstrating worst prognosis and lower survival rates[21].

Associations of genetic alterations into UC high-grade invasive carcinogenic pathway seem to be the key event leading to initiation and progression of bladder urothelial carcinomas.

Epigenetic events in UC

Genetic alterations only are not able to explain cancer molecular diversity. Other mechanisms can also affect gene expression and signal pathways. Epigenetic changes, such as DNA methylation and histones deacetylation, can occur without changing DNA structure and seem contribute to malignant transformation and UC progression[22,23]. They can be promoted by external agents, including smoke, diet and carcinogens exposure.

A wide variety of important genes in several cellular processes could present DNA methylation oscillating from 1% to 98%, and appear in initial stages of disease[24-30]. Another epigenetic mechanism is the transcription repression through interaction between micro RNA (miRNA) and specific sequences in messenger RNA (mRNA), as discussed below.

MiRNA

After its discovery more than 20 years[31], miRNAs have been recognized as molecules that work specifically in post-transcriptional control in majority of eukaryote genomes. miRNAs are a family of small non-coding RNAs of 19-25 nucleotides in length, expressed in a wide variety of organisms, comprising plants, worms and mammals, including humans[32]. They have a fundamental role in physiological and pathological processes in organs and tissues in a context-dependent manner. Many miRNAs are highly conserved between species and the machinery of its biogenesis can be found in archaebacteria and eubacterias, establishing its ancestral characteristic. Currently, there are more than 2500 miRNAs with specific biogenesis (Figure 2) and related to control of more than 30% of human genes (www.mirnabodymap.org)[33] involved in multiple processes of development and cell differentiation, apoptosis, homeostasis and metabolic pathways[34-36]. In oncologic research, miRNAs work tumor suppressors or oncogenic (oncomiR), showing specific profiles that could characterize different types of cancer[36,37]. Albeit there are studies exploring miRNA expression profile in bladder UC, data are still scarce and biological field so vast[38-41].

MiRNA in bladder UC

MiR-100: MiR-100 is a protective miRNA in human cells[42], acting in a context-dependent manner[43]. Under-expression profiles has been found in non-invasive bladder UC[44,45], ovarian carcinoma[46], oral cavity carcinoma[47], osteosarcoma[48], vulvar carcinoma[49], lymphoblastic leukaemia[50], gastric cancer[51] and several other types of human cancer.

In non-invasive bladder UC, miR-100 has as main target the *FGFR3* gene, whose mutation and over-activity is related to this neoplasm[44]. In physiological conditions, miR-100 exerts negative control over FGFR3 decreasing their post-transcriptional expression levels (Figure 3). As Blick *et al*[52], we also suggest that there might be an alternative pathway triggering UC non-invasive carcinogenesis not associated with *FGFR3* activating point mutations[53]. Under-expression of miR-100 could be responsible by lack of negative control and FGFR3 over-expression, promoting non-invasive UC

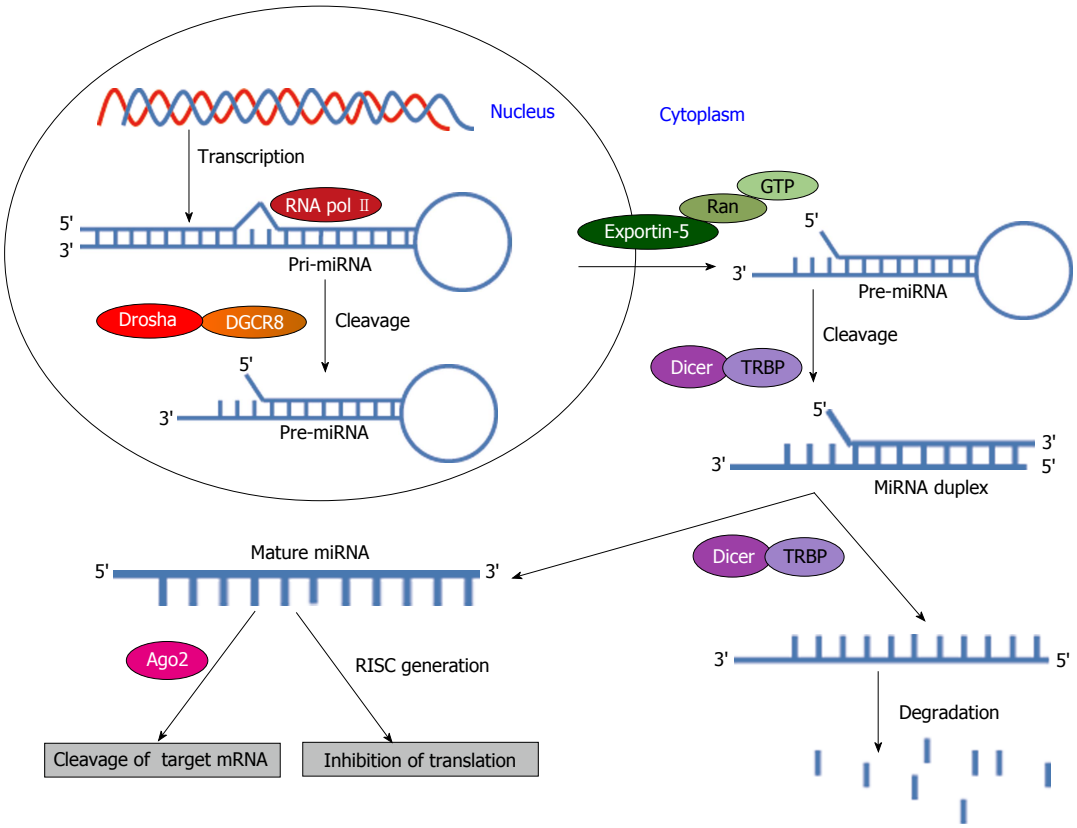


Figure 2 Micro RNA biogenesis in human cells. After transcription by RNA polymerase Ⅱ, the primary micro RNA (miRNA) precursor (Pri-miRNA) is cleaved by Drosha microprocessor complex and converted in Pre-miRNA, a 60-70 nt double-strand molecule. The Pre-miRNA is transported from nucleus to the cytoplasm by Exportin-5 and then it is cleaved by Dicer to generate the miRNA duplex. Again, Dicer enzyme acts over miRNA duplex and produces single-strand mature miRNA that, in turn, is incorporated into RNA-Induced Silencing Complex (RISC). RISC drivers mature miRNA to the target messenger RNA (mRNA), triggering mRNA cleavage (Slicer activity) or inhibition of translation by complete or incomplete complementarity, respectively.

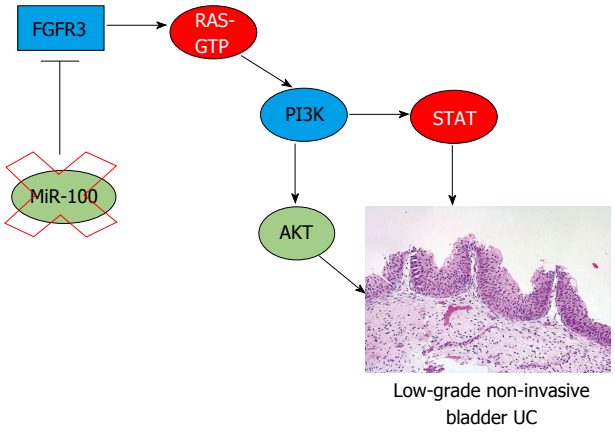


Figure 3 MiR-100 in low-grade non-invasive urothelial carcinoma carcinogenesis. Under-expression of miR-100 leads to *FGFR3* gene over-expression, stimulus to PI3K/AKT/STAT pathway and low-grade non-invasive tumor development. Adapted by Wu[19], 2009. FGFR3: Fibroblastic growth factor receptor 3; UC: Urothelial carcinoma.

carcinogenesis and low-grade tumor development (Figure 3). Catto *et al*[44] found an inverse ratio between miR-100 and *FGFR3*, where the under-expression of miR-100 led to increased gene activity before the occurrence of point mutation, suggesting that increased levels of *FGFR3* could facilitate the mutational event through increased cellular turnover or natural selection of mutant cells. Maybe, miR-100 loss of expression can be the first trigger event of disease and could occur before clinical presentation of the tumor. This fact is important because this molecular characteristic could be used for initial diagnostic and predicts disease behavior, allowing a conservative treatment due to rare chance of progression and excellent survival. We speculate that miR-100 will be used in clinical practice as a diagnostic and prognostic biomarker and employed in target therapies.

On the other hand, we showed an over-expression of miR-100 in high-grade invasive UC[45]. We suggest that miR-100 acts as a negative controller of *THAP-2* gene, directly involved in proliferation control through modulation of proteins that control cell cycle such as pRB and E2F[54]. Loss of function of RB1, p53 and PTEN is involved in carcinogenic route of invasive UC, promoting genomic instability and facilitating tumor progression. MiR-100 over-expression could trigger THAP-2 silencing and, consequently, RB1 inactivity (Figure 4). *BAZ2A* and *SMARCA5* genes are also targets of miR-100 and are associated with DNA transcription repression and chromosomal instability[55,56]. Recently we demonstrated in cell cultures of BC that miR-100 has a role over BAZ2A and SMARCA5 activity (data submitted for publication), but better investigation are needed to establish the role of

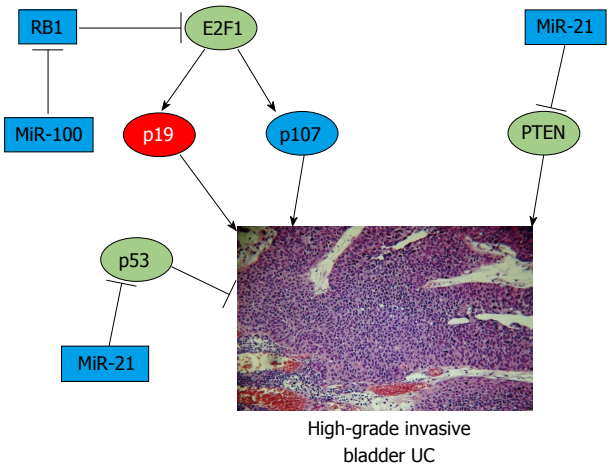


Figure 4 MiR-100 and miR-21 in high-grade invasive urothelial carcinoma carcinogenesis. High levels of miR-100 inhibit retinoblastoma (RB1) and over-expression of miR-21 suppresses p53 and phosphatase and tensin homolog gene (*PTEN*), three crucial protective genes. Inactivity of RB1 associated with loss of function of p53 and *PTEN* trigger high-grade urothelial carcinoma (UC) carcinogenesis. Adapted by Wu[19], 2009.

miR-100 in invasive UC carcinogenesis.

MiR-10a: MiR-10a comprises 23 nitrogenous bases and is located in chromosome 17q21.23, within *HOXB* gene cluster, upstream of *HOXB4* (www.mirbase.org). A number of *HOX* genes have been found to be regulated by miR-10. These genes encode mainly transcription factors which have crucial roles in embryonic development and cell differentiation. In humans, miR-10a exerts a negative control over *HOXA1* and *HOXA3* genes supporting that this miRNA can play fundamental roles in physiological activities of the cell[57,58].

MiR-10a has two different mechanisms of action over control of gene expression. The first one, extensively demonstrated by literature, is the canonical inhibition of protein product by miR-10a binding in target 3'UTR mRNA. On the other hand, Ørom *et al*[59] have described the second mechanism of action, and demonstrated a positive effect of miR-10a through its complementary interaction with mRNA 5´UTR, allowing a translational stimulus of proteins associated to the ribosomal machinery, increasing global cellular activities[59]. Although miR-10a is linked to carcinogenesis of several human tumors, it is believed that its functions are involved in physiological situations of the cell. Nowadays it is well established the crucial role of miR-10a in physiological process of cellular differentiation. Even being able to act through mechanisms described above, translational inhibition by negative control of miR-10a on its target genes highlights as the most active mechanism in the differentiation process.

Recently, we have demonstrated a miRNA expression profile in bladder urothelial carcinoma and we found that miR-10a over-expression was one of the most evident changes. MiR-10a is able to effectively separate two genetically distinct tumor groups, which are, low-grade non-invasive pTa from high-grade invasive pT2-3 UC, with over-expression in first and under-expression in the second tumor group[45]. Moreover, miR-10a expression profile was associated with disease-free and cancer-specific survivals between groups[45].

Regarding low-grade non-invasive pathway, FGFR3 over-activity triggered by down-regulation of miR-100 could be corroborated through up-regulation of miR-10a, both promoting higher cell proliferation rates. Maybe this mechanism miR-10a-mediated is constituted in a negative control of physiological inhibitor of FGFR3 through its canonical activity, increasing cellular proliferation without modify the genetic stability of the neoplastic cell. About high-grade invasive tumors, sharing close homology with miR-100, miR-10a maybe could be continuously blocking *HOX* genes, promoting poor differentiation, enhanced aggressiveness and worse tumor behavior.

MiR-21: Corroborating with findings published by Neely *et al*[39], we recently have demonstrated that miR-21 presented strong over-expression (17-fold higher) in high-grade invasive UC[39,45]. Recent evidences show that miR-21 is a truly oncogenic miRNA, presenting over-expressed in wide majority of human tumors. miR-21 can promote tumorigenesis by inducing cell proliferation and blocking of apoptotic control mechanisms[60], thus triggering more aggressive disease and poor responses to treatments[61,62].

p53 is considered the most important gene involved in invasive UC carcinogenesis[14]. p53 is responsible for control of global activities of the cell by cell cycle arrest, stimulus of apoptosis and DNA repair. Catto *et al*[44] have observed miR-21 over-expression associated with p53 inactivation, invasiveness and tumor progression. Another important protective gene related to UC carcinogenesis is PTEN, also being target of miR-21[4,19,63]. PTEN is a lipid phosphatase that inhibits PI3K/AKT pathway, blocking cell proliferation. In an actual review about genetic and molecular mechanisms involved in initiation and progression of UC, McConkey *et al*[4] have suggested that loss of function of PTEN is much more common in invasive disease related to PTEN/PI3K/AKT/mTOR characterizing a worse prognostic factor. Figure 4 shows a schematic flowchart regarding miR-21 roles in high-grade invasive bladder UC.

We showed that miR-21 under-expression was associated with better disease-free survival in non-invasive UC, consolidating oncogenic behavior for miR-21[45].

MiR-205: MiR-205 has been defined as a tumor suppressor miRNA involved in epithelial-to-mesenchymal transition (EMT), a process provided by malignancies to perform a fundamental step to tumor progression and systemic dissemination. Like miR-200 family, miR-205 acts negatively over ZEB-1 and ZEB-2 that, in turn, suppress E-cadherin, an adhesion molecule responsible for physiological conditions of bladder epithelia[64,65]. Under-expression of miR-205 leads to ZEB-1 and ZEB-2 over-

activity and sequential E-cadherin inhibition, facilitating the metastasizing process. EMT is crucial to success and maintenance of bladder carcinogenesis, being directly associated with worse behavior, high tumor aggressiveness, poor prognosis and shorter survival. The role of miR-205 is similar in several human tumor types, blocking tumor progression and dissemination. Some authors have already demonstrated a miR-205 under-expression in lung, breast and prostate cancer[66-68].

In bladder UC, evidences has been suggested aberrant methylation in chromosome 1q32.2 where is located the *miR-205* gene[69]. Neely *et al*[39], in 2008, established that miR-21:miR-205 ratio is progressively increased according tumor progression. Interesting data are shown by Brabletz *et al*[70], demonstrating that ZEB/miR-200 feedback loop is crucial to define cell status. Thus, an invasive and progressive tumor status occurs when the loop is favoring ZEB, leading tumor dissemination beyond epithelial barriers. On the other hand, a proliferative environment predominates when the loop tend to miR-200 family over-expression, allowing tumor growth. Second authors, both states are needed to carcinogenic process, a first early proliferative promoting tumor growth and a second late progressive triggering tumor dissemination[70]. A very interesting fact is that miR-200 over-expression could favor the metastasizing process but, after metastasis sites are defined, the proliferation occur again, being necessary the re-expression of miR-200. In 2012, we published data supporting this idea, where miR-205 was under-expressed in 100% of low-grade non-invasive pTa UC, according with early carcinogenic state, while there was a re-expression of miR-205 in a third of cases of high-grade invasive UC[45].

MiR-let7c: The literature consolidates the suppressor role of miR-let7c in almost all human cancers, reflecting its important suppressive action against malignant events. MiR-let7c targets RAS and c-MYC oncogenes and under-expression of this miRNA is related to neoplasm development[71,72]. RAS is the second most important oncogene in low-grade non-invasive tumorigenesis[73]. Point mutations can lead to genetic alterations that, in turn, promote a strong stimulus of AKT and STAT pathways, triggering high rates of cellular proliferation. c-MYC is a prototype of oncogene promoting RB1 inhibition in high-grade invasive UC[71]. c-MYC is able to stimulate cyclins CDK4, CDK6 and D1 leading to phosphorylation and inactivation of RB1 and increased mitogenic and proliferative processes. Otherwise, c-MYC can stimulate p53 over-activity *via* MDM2 inhibition, favoring cell cycle arrest and apoptosis[3]. Moreover, p53 can enhance expression levels of miR-let7 promoting a cumulative protective effect against carcinogenesis[74].

MiR-125b: Evidences are controversial regarding role of miR-125b in cancer. While some studies define miR-125b as a tumor suppressor, other suggest its oncogenic functions[40,75-78]. However, miR-125b seems to have a protective role in bladder UC. Four main authors demonstrate the suppressive role of miR-125b in bladder cancer, acting over transcription factors, oncogenes and metallopeptidases[40,79-81]. In agreement to these authors, we have also verified an under-expression profile for miR-125b in almost all cases of low-grade non-invasive and high-grade invasive UC, suggesting its suppressive function in bladder UC[45].

MiR-143 and miR-145: MiR-143 and miR-145 are closely located in 5q32 chromosome and share similar suppressive tumor functions, including bladder cancer[45,82-85]. Evidences demonstrate RAS as a target gene of miR-143. Mutations in RAS lead to stimulus of MAPK/AKT/STAT pathway, triggering low-grade non-invasive UC[19], but RAS may also be involved in high-grade tumors[4]. Lin *et al*[86] showed by studies in malignant tissues and cell cultures that under-expression of miR-143 is the rule in UC. In tumor tissues, miR-143 presented 13.7 fold-changes down-regulated in comparison to normal bladder tissues, while in EJ and T24 cell lines it was not identified. When transfected in cell lines, miR-143 significantly inhibited cellular proliferation[86]. Noguchi *et al*[87] also showed the suppressive role of miR-143 in bladder cancer cell lines, and replacement treatment with miR-143 and miR-145 induced synergistic inhibition of tumor by regulating PI3K/AKT/MAPK signaling pathways. Moreover, miR-143 can regulate other target genes involved in UC carcinogenesis. For example, Song *et al*[88] established an inverse correlation between miR-143 and COX-2, an oncogene associated with grade, prognosis and recurrence of bladder UC. Furthermore, the authors verified in T24 cell line that restoration of miR-143 by transfection decreased COX-2 expression and reduced proliferation and motility of tumor cells.

Many studies have validated miR-145 as an inhibitor of cell cycle and tumor growth, promoting induction of apoptosis and lower progression of disease. MiR-145 is a protective miRNA presenting under-expressed in many human tumors, such as colorectal, lung, breast, prostate and renal cancer, and in non-malignant disease such as benign prostatic hyperplasia[89-93]. The first report of miR-145 under-expression was performed by Michael *et al*[94] in a study suggesting that these alterations could be involved in the initiation of colorectal cancer. These findings were confirmed by Shi *et al*[95] who showed that miR-145 under-expression was associated with malignant tumors. Sachdeva *et al*[96] showed in breast cancer cell lines that *MUC-1* gene was associated with tumor onset, invasion and dissemination, where miR-145 was able to inhibit these factors controlling tumor development[96]. Another target of miR-145 is *c-MYC* gene, an oncogene implicated in carcinogenic process of high-grade invasive UC[3,91]. Furthermore, miR-145 is induced by p53 activity, being directly related to invasive UC tumorigenesis. In 2009, Sachdeva *et al*[91] observed that, in physiological conditions, cellular stress promoted higher levels of p53 that, in turn, increased concentrations of miR-145 through

p53 response element. Higher levels of miR-145 inhibit c-MYC activity, allowing normal function of p21 and cell cycle arrest[91]. Under-expression of miR-145 could promote fail in c-MYC control, decreasing p21 levels and stimulates cell proliferation. These complex mechanisms could lead, or at least initiate, UC carcinogenesis[97]. Following the same idea, Spizzo *et al*[98] analyzing breast cancer cell lines demonstrated the crucial suppressor role of miR-145, and its transfection inhibited growth and cell proliferation, inducing p53-mediated apoptosis. Chiyomaru *et al*[99] demonstrated an association between FSCN-1 oncogene and miR-145 under-expression in UC, suggesting that loss of expression of miR-145 and consequent FSCN-1 up-regulation may be associated with bladder tumors in all stages, promoting more aggressive and invasive tumors. Last year we published a work demonstrating that miR-145 is a well-characterized tumor suppressor miRNA in UC. We hypothesize that lack of protector role promoted by miR-145 over probable target genes PI3K/AKT, FSCN-1, MDM-2, c-MYC and MUC-1 could be involved in carcinogenic process of low-grade, non-invasive and high-grade invasive urothelial carcinomas, being an interesting candidate diagnostic biomarker[100].

MiR-199a: Low levels of miR-199a have already found in ovarian and endometrial cancer, testicular tumors, hepatocarcinoma (HCC) and osteosarcoma[101-105] and it seems to be directly involved in tumor progression and worse prognosis. Fornari *et al*[102] studying HCC suggest that miR-199a targets mTOR, demonstrating an inverse relationship between them. Similarly to HCC, PI3K/AKT/mTOR pathway is also involved in high-grade UC carcinogenesis and miR-199a under-expression could explain mTOR over-activity and, at least partially, the tumor onset and progression of disease.

In bladder UC, even though we have shown miR-199a under-expression in most of both low-grade non-invasive and high-grade invasive tumors, statistical differences were not observed[45]. On the other hand, Ratert *et al*[106] found miR-199a down-regulated in malignant bladder samples compared to healthy tissue. Also, the authors demonstrated that miR-199a was differentially expressed between non-invasive and invasive bladder cancer, underling together miR-205 and miR-141, the potential to work as biomarkers of diagnosis and prognosis in bladder UC[106].

MiR-452: There are few and controversial studies concerning miR-452 in human cancer. Some works demonstrate an under-expression of miR-452 in squamous lung tumors, gliomas, prostate and breast cancer[107-110], suggesting a protective role of miR-452 in these assessed tumors.

In bladder cancer, Veerla *et al*[41] and Puerta-Gil *et al*[111] showed that higher levels of miR-452 are the rule in UC and associated with high-grade and invasive disease, poor behavior and metastatic process of disease. In disagreement with the former two researchers, but in concordance with other several studies in human cancer, we verified a strong under-expression profile for miR-452 in both low-grade non-invasive and high-grade invasive UC, suggesting a tumor suppressor role for this miRNA[45]. Moreover, we observed increased under-expression levels according higher grade and stage, reinforcing the protective function of miR-452. Human miR-452 has approximately 220 target genes and we speculate that loss of control over genes involved in cell growth and proliferation as E2F3 and MEF2C could explain the involvement of miR-452 in bladder carcinogenesis. However, complementary mechanistic studies are needed to consolidate this hypothesis.

Final considerations

Many studies have demonstrated promising roles of miRNAs working as diagnostic and prognostic biomarkers or involved in target therapies, consolidating miRNAs as crucial players in human cancer. This review allows a reflection about the true functions of miRNAs in bladder carcinogenesis, not only by their wide capacities of action, but also by abilities in define the cell date. MiRNAs characterization in plasma and urine, representing tissue levels, are potential non-invasive methods that could assist diagnosis, treatment and evaluation of bladder cancer improving management of this prevalent disease.

Finally, the future of anti-tumor target therapies will be based not in one, but in groups of miRNAs working together in several steps of carcinogenic process, being able to identify the disease, predicting behavior and effectively treat bladder cancer.

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REVIEW

Phosphodiesterase inhibitors for treatment of voiding dysfunction: An overview of experimental and clinical evidence

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Abstract

Recently, the focus of the origin of lower urinary tract symptoms (LUTS) has change from the prostate to the bladder. Regardless of the underlying mechanism associated with the origin of LUTS, alpha-blockers continue to be the most common medicine prescribed to treat LUTS due to benign prostatic obstruction (BPO). The newest class of drug introduced to treat LUTS/BPO is phosphodiesterase inhibitors (PDEi) and the aim of this study was to review the role of PDEi in the treatment of LUTS/BPO. In this review, the first evidence was evaluated based on epidemiological studies followed by randomized clinical trials which provide evidence on the administration of PDEi in patients with LUTS/BPO. Experimental studies were also assessed to tentatively elucidate the association between LUTS and erectile dysfunction, and to elucidate the underlying mechanism. There is still controversy regarding the administration of PDEi due to the fear of detrusor impairment, response to acute administration, and the effects of PDEi combined with alpha-blockers. Following this review, we conclude that treatment of BPO/LUTS with PDEi is beneficial, based on experimental studies, strong evidence and the large number of randomized clinical trials confirming their efficiency.

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Key words: Benign prostatic hyperplasia; Lower urinary tract symptoms; Phosphodiesterase inhibitor; Mechanism of action; Urodynamics

Core tip: In this study, an extensive review was performed on the use of phosphodiesterase inhibitors to treat lower urinary symptoms due to benign prostatic obstruction. This study explored experimental and recent clinical evidence in order to assist in the decision-making process in daily practice.

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INTRODUCTION

The population is ageing worldwide and consequently the prevalence of lower urinary tract symptoms (LUTS) is increasing and becoming a public health problem. As men grow older the prevalence of histologic benign prostatic hyperplasia (BPH) also increases. BPH is observed in approximately 8% of men aged 31-40 years, 42% of men aged 51-60 years, 71% of men aged 61-70

Table 1 Initial evidence based in epidemiological studies of a common pathophysiology between lower urinary tract symptoms and erectile dysfunction

Ref.	Number of participants	Major conclusion
Cologne Male Survey Braun *et al*[6]	4000	72.2% of patients with ED had concomitant LUTS Only 37.7% had LUTS without ED
Population-based cohort study in Brazil Moreira *et al*[7]	602	Incidence of ED was 65.5 cases per 1000 person-years Relative risk of ED was 1.8-7.5 in patients with LUTS
Sexual dysfunction in European men Vallancien *et al*[8]	1274	Prevalence ED-Mild (55%), severe (70%) LUTS Prevalence of ED was 55% in men with mild LUTS and increased to 70% in severe LUTS
Association of LUTS in Japanese men with erectile dysfunction Terai *et al*[9]	3189	Severity of ED was significantly associated with moderate to severe IPSS, RR = 1.5 which persisted after adjustment for age
Boston Area Community Health survey Brookes *et al*[10]	2301	Strong association was observed between the AUA-SI associated to ED and ED after adjusting for age

ED: Erectile dysfunction; LUTS: Lower urinary tract symptoms; RR: Relative risk; IPSS: International Prostatic Score Symptoms; AUA-SI: American Urological Association Symptom Index.

years, and 88% of men aged 81 years and older[1]. BPH may result in enlargement of the prostate, also defined as benign prostatic enlargement and may be associated with bladder outlet obstruction (BOO). BOO in this case is defined as a benign prostatic obstruction (BPO). Permanent BPO may result in adaptive changes of the detrusor muscle causing storage LUTS, if BPO progresses and persists this may lead to a failure of the detrusor resulting in emptying LUTS. Due to these observations the focus of the origins of LUTS has changed from the prostate to the bladder. As the pathophysiology of LUTS is not totally understood it has been hypothesized that possible LUTS/BPO arises due to local alterations in detrusor smooth muscle cells, local receptors, neural signalization, blood flow and changes in the extracellular matrix. Regardless of the underlying mechanism associated with the origin of LUTS, alpha-blockers are the most common medicine prescribed to treat LUTS/BPO[2]. Alpha-blockers decrease urethral resistance and improve the urinary flow by relaxation of smooth muscle of the prostate and bladder neck.

Other drugs have been used to treat LUTS related to BPO such as 5-alpha-reductase inhibitors. These are taken alone or with alpha-blockers to decrease progression of the disease or to avoid urinary retention[3,4]. Anticholinergics have also been administered to patients with predominant storage LUTS/BPO with a low risk of urinary retention regardless of obstruction[5]. The newest class of drug introduced to treat BPO is phosphodiesterase inhibitors (PDEi) and the aim of this study was to review the role of PDEi in the treatment of LUTS/BPO.

INITIAL EVIDENCE

The use of PDEi in patients with LUTS/BPO was proposed initially based on observational epidemiological studies specially designed to evaluate erectile dysfunction (ED). It was observed in these studies that demographic data showed a similar prevalence of ED and LUTS/BPO in men as they aged, raising the possibility of a common underlying mechanism contributing to both conditions.

The pioneering work carried out in 2000 to study the prevalence of ED in Germany in the Cologne Male Survey evaluated 4000 patients[6]. LUTS/BPO was present in 72.2% of patients with ED, however, only 37.7% had LUTS/BPO without ED. It was also observed in a Brazilian Cohort Study that an epidemiological association existed between LUTS/BPO and ED. In this particular study, the relative risk of ED was 1.8-7.5 in patients complaining of LUTS and this risk was greater than smoking or cardiac symptoms[7]. In Europe a demographic study evaluating 1274 European men showed that 55% of patients with mild LUTS/BPO had ED, however, the prevalence of ED increased to 70% in patients with severe LUTS/BPO[8]. In a Japanese Cross-Sectional Survey, a correlation between ED and LUTS/BPO was observed and the relative risk was 1.5 which persisted after adjustment for age[9]. In the United States of America, multivariate regression of the Boston Area Community Health Survey data found an association between the American Urological Association Symptom Index and ED without differences in race or ethnicity[10]. Therefore, in different parts of the world several studies showed an epidemiological correlation between ED and BPO/LUTS (Table 1).

Clinical use of phosphodiesterase inhibitors to treat BPO/LUTS

Following and during these observational studies, a proof-of-concept clinical study to evaluate improvement in BPO/LUTS in men taking sildenafil for ED was performed in 2002[11]. Patients taking sildenafil were evaluated using the International Index of Erectile Function (IIEF) and International Prostate Symptoms Score (IPSS) instruments at baseline, one and three months. During

the treatment period, an improvement in the IPSS and quality of life (QoL) was observed. An inverse relationship between IPSS and IIEF during treatment with sildenafil was also noted. The major limitations of this study were its open label and uncontrolled design. In other uncontrolled studies, a similar impact of sildenafil in BPO/LUTS and ED was observed[12,13]. Different from the uncontrolled design of the papers reported above, the next generation of studies included randomized and placebo-controlled trials.

In 2007, the first multicenter, randomized, placebo-controlled, double-blind trial was reported[14]. The end point was defined as change from baseline of erectile function assessed with the IIEF instrument. Secondary end points were changes in LUTS from baseline evaluated with the IPSS, QoL question of the IPSS, Benign Prostatic Hyperplasia Impact Index (BPHII), peak flow rate (Qmax), Self-Esteem And Relationship (SEAR) scores and end of treatment satisfaction using Erectile Dysfunction Inventory of Treatment Satisfaction Index Score. Compared with placebo, sildenafil significantly improved the IIEF, IPSS, BPHII, IPSS QoL and SEAR score. Significant improvement in Qmax was not observed in the sildenafil group compared with placebo. The limitations of this study were lack of a placebo run-in period and determination of correlations between LUTS and ED improvements.

In 2007, another multicenter, randomized, placebo-controlled, double-blind trial assessed the efficacy of tadalafil once daily for BPO/LUTS[15]. Inclusion criteria were age greater than 45 years and IPSS > 12 for at least six months. Exclusion criteria were elevated prostatic score antigen (PSA), recent use of 5∝-reductase inhibitors, use of BPH medication during study, history of pelvic surgery, liver failure, other causes of LUTS, uncontrolled diabetes, and nitrate use or chemotherapy. Different from previous studies, a placebo run-in period was included in the study design. After a four-week placebo run-in period, 281 men with BPO/LUTS were randomized to 5 mg tadalafil daily for six weeks, followed by dose escalation to 20 mg for six weeks or placebo for a total of 12 wk. Tadalafil significantly improved the mean change from baseline IPSS compared with placebo. Improvement was also seen in the IPSS QoL, BPHII and IIEF. No significant change was observed in Qmax. Based on these results, the authors concluded that daily tadalafil caused a significant improvement in BPO/LUTS and ED.

In 2008, in an 8-wk randomized, double-blind, placebo-controlled study, vardenafil 10 mg was administered to 222 men with BPO/LUTS with or without ED. Inclusion criteria were age 45-64 years and IPSS score ⩾ 12[16]. Exclusion criteria were vardenafil contraindications, spinal cord injury, prostatitis, urethral stricture, urinary retention, bladder or prostate cancer, past cancer with low life expectancy, use of androgens, anticoagulants, ED treatments, or alpha-blockers during the treatment period. The IPSS score, Qmax, postvoid residual urine volume (PVR), and the erectile dysfunction domain of the IIEF were assessed. Vardenafil significantly improved the mean change in the IPSS from baseline compared with placebo. It also improved ED and QoL. However, no changes in Qmax or PVR were noted. A weak point of this study was the lack of a placebo run-in phase.

A dose-finding study was reported in 2008[17]. In this study, after a 4-wk placebo run-in period, 1058 men with BPO/LUTS were randomized to receive daily tadalafil (2.5, 5, 10 or 20 mg) or placebo. Inclusion criteria were age greater than 45 years, IPSS score ⩾ 12 for at least 6 mo, and Qmax between 4 and 15 mL/s. Exclusion criteria were elevated PSA, recent use of 5∝-reductase inhibitors, use of BPH medication during study, history of pelvic surgery, liver failure, other causes of LUTS, uncontrolled diabetes, and nitrate use or chemotherapy. IPSS change from baseline to endpoint was improved with all tadalafil doses compared with placebo. In the Global Assessment Questionnaire, LUTS also improved at all doses, however, doses greater than 5 mg had minimal improvement with more side effects. As a consequence, this improvement demonstrated a dose-response relationship and 5 mg tadalafil once daily had a positive risk-benefit profile. No significant change in Qmax was observed.

It is possible to conclude with a high level of evidence that PDEi clearly improves BPO/LUTS based on the results presented in these four clinical trials.

Following these randomized controlled trials (RCTs), more recent systematic reviews and meta-analyses have emerged[18-20].

In 2013, a study that aimed to evaluate the efficacy and safety of tadalafil 5 mg once daily compared to placebo over 12 wk for the treatment of both LUTS/BPO and ED in sexually active men was performed. The data were pooled from four multinational, randomized studies of men ⩾ 45 years with LUTS/BPO. The randomization and placebo run-in period were strong points in this study. Principal end-points were change in the IPSS, QoL, BPHII, and IIEF. Tadalafil (n = 505) significantly improved total IPSS *vs* placebo (n = 521); mean changes from baseline were -6.0 and -3.6, respectively ($P < 0.001$). Improvements in the IIEF Domain score (tadalafil, 6.4; placebo, 1.4) were also significant *vs* placebo, as were the IPSS, IPSS QoL, and BPHII (all $P < 0.001$). The authors concluded that tadalafil was efficacious and well tolerated in the treatment of ED and LUTS/BPO[20].

In one of these meta-analyses, the use of PDEi alone or in combination with alpha-blockers was summarized to identify the best candidates for this treatment based on clinical features and LUTS severity[18]. Trials included in this review were selected using the following inclusion criteria: (1) They were RCTs; (2) The subject of the study was a PDEi for LUTS/BPO; (3) Control groups received placebo for PDEi alone or alpha-blockers alone and PDEi plus alpha-blockers; and (4) The primary outcomes were the IPSS, IIEF, and Qmax. Of 508 retrieved studies, 497 articles were excluded; leaving only 11 studies. More than 6000 men evaluated in these 11 studies were includ-

Table 2 Randomized clinical trials and meta-analyses with strong evidence for the use of phosphodiesterase inhibitors in patients with lower urinary tract symptoms due to benign prostatic obstruction

Ref.	Design of study	Placebo run-in	Participant/inclusion criteria	End point	Major conclusion
Sairam *et al*[11]	Not RCT	No	112 male patients All taking sildenafil Inclusion criteria was presence ED	Assess relationship between ED and LUTS; if sildenafil influences LUTS in patients with ED	No relation between ED score and LUTS before treat ED Sildenafil improves ED and LUTS
McVary *et al*[14]	Open-label, randomized, double-blind, placebo-controlled	No	369 patients were randomized to sildenafil 100 mg (n = 189) or placebo (n = 180) during 12 wk/Men with ED and LUTS	Change IPSS, QoL,BPHII, Qmax, SEAR, and EDITS	Sildenafil improve IIEF, IPSS, BPHII, IPSS QoL and SEAR score Qmax not altered
McVary *et al*[15]	Randomized, double-blind, placebo-controlled	Yes	281 men randomized to tadalafil 5 mg daily, followed by dose escalation to 20 mg/Men aged 45 yr or higher and IPSS > 12	Change IPSS, QoL, BPHII, Qmax, and IIEF	Tadalafil improve IPSS, QoL, BPHII, and IIEF Qmax not altered
Stief *et al*[16]	Randomized, double-blind, placebo-controlled	No	222 men were randomized to vardenafil 10 mg twice daily or placebo/age 45-64 yr, IPSS ⩾ 12, with or without ED	Change in IPSS, Qmac, PVR, and IIEF	Vardenafil improve IPSS, IIEF, and QoL Qmax and PVR not altered
Roehrborn *et al*[17]	Randomized, double-blind, placebo-controlled	Yes	1058 men were randomized to receive daily tadalafil 2.5, 5, 10 or 20 mg/age greater than 45 yr, IPSS ⩾ 12, and Qmax between 4-15 mL/s	Change in IPSS, IIEF, QoL, BPHII, GAQ, and Qmax	Tadalafil improve IPSS and GAQ in all doses But, dose higher than 5 mg had minimal improvement with higher side effects Qmax not altered
Porst *et al*[20]	Meta-analysis		1026 men, tadalafil (n = 505) compared to placebo (n = 521). Data pooled from four multinational study/age ⩾ 45 yr, presence of LUTS/BPO	Change in IPSS, QoL, BPHII, and IIEF	Tadalafil improve IPSS, QoL, BPHII, and IIEF compared with placebo
Gacci *et al*[18]	Meta-analysis		Twelve studies, been seven studies (n = 3214) comparing PDEi *vs* placebo, and five (n = 216) on the combination of PDEi with ∝-blockers *vs* ∝-blockers alone/Men with LUTS/BPO	Change in IPSS, IIEF, and Qmax Identify best candidates for treatment with PDEi based on clinical features	PDEi alone improve IPSS, IIEF, but not Qmax Association of PDEi with ∝-blockers improve IPSS, IIEF, and Qmax
Yan *et al*[19]	Meta-analysis		515 patients (seven studies)/ patients with LUTS/BPO and ED	Compare combination of PDEi with ∝-blockers *vs* ∝-blockers alone. Change IPSS, QoL, BPHII, Qmax, and IIEF	Combination of PDEi with ∝-blockers has additive favorable effects compared with PDEi monotherapy

RCT: Randomized control trial; ED: Erectile dysfunction; LUTS: Lower urinary tract symptoms; IPSS: International prostatic symptoms score; QoL: Quality of life; BPHII: Benign Prostatic Hyperplasia Impact Index; Qmax: Peak flow rate; SEAR: Self-esteem and relationship; EDITS: Erectile Dysfunction Inventory of Treatment Satisfaction Index Score; IIEF: International Index of Erectile Function; PVR: Postvoid residual urine volume; GAQ: Global Assessment Question; BPO: Benign prostatic obstruction; PDEi: Phosphodiesterase inhibitors.

ed in this meta-analysis, with seven evaluating PDEi *vs* placebo in 3214 men, and five evaluating the combination of PDEi with alpha-blockers *vs* alpha-blockers alone in 216 men. Median follow-up in all RCTs was 12 wk. The IIEF score (5.5; $P < 0.0001$) and IPSS (-2.8; $P < 0.0001$) were significantly different, but not the Qmax (-0.00; P = not significant) at the end of the study as compared with placebo. The association of PDEi and alpha-adrenergic blockers improved the IIEF score (3.6; P <0.0001), IPSS score (-1.8; $P = 0.05$), and Qmax (1.5; $P < 0.0001$) at the end of the study as compared with a-blockers alone. Therefore, the meta-analysis suggested that PDEi can significantly improve LUTS and EF in men with BPO.

A recent meta-analysis was carried out to evaluate the efficacy of PDEi alone or in combination with alpha-blockers for the treatment of ED and LUTS/BPO. The databases MEDLINE, EMBASE, PubMed, the Cochrane Controlled Trial Register of Controlled Trials, and the Chinese Biological Medical Database were searched to identify RCTs that referred to the use of a combination of PDE5 inhibitors and alpha-blockers for the treatment of ED and LUTS associated with BPH. The principal objectives were to evaluate the IPSS, Qmax, and IIEF. Seven publications involving 515 patients were included in the meta-analysis. PDE5 inhibitors and alpha-blockers significantly improved the IIEF, IPSS, and Qmax values compared with PDE5 inhibitors alone ($P = 0.04$, 0.004, 0.007, respectively). The major conclusion was that the combined use of PDEi and alpha-blockers results in additive favorable effects in men with ED and LUTS/BPO compared with PDEi monotherapy[19] (Table 2).

It is important to note that although many of these studies evaluated men with ED and LUTS/BPO, some studies reported an improvement in LUTS/BPO inde-

pendently of ED[21,22].

Experimental studies

Due to the particular course presented above, the clinical use of PDEi to treat BPO/LUTS began before the mechanism of action of these drugs was known. Therefore, because the mechanism of action was not understood, a common pathophysiological link between ED and BPH was investigated, and an increasing number of experimental studies have emerged in subsequent years[23,24].

To explain the mechanism involved, several theories have been proposed. The four principal hypotheses are: ischemia due to pelvic atherosclerosis, autonomic hyperactivity, a calcium-independent Rho-kinase activation pathway, and reduced nitric oxide (NO) levels[25,26].

The ischemia hypothesis is based on blood flow to the lower urinary tract (LUT) being affected by smooth muscle cell (SMC) contraction, thus decreasing oxygenation leading to chronic ischemia of LUT tissue and contributing to LUTS. Atherosclerosis is associated with remodeling of SMCs in the pelvic vasculature[27,28], penis[29,30], and bladder[28] also associated with LUTS. Therefore PDEi may act by increasing perfusion of the bladder through relaxation of SMCs resulting in increased oxygenation.

It has also been postulated in experimental studies that overactivity of terminal afferent nerves (autonomic) within LUT may be associated with contraction of SMCs[31-33]. Again PDEi might be associated with relaxation of SMCs thus improving LUTS.

Rho-kinase/RhoA activation has been shown to mediate detumescence and maintain flaccidity. Rho kinase inhibits the regulatory subunit of myosin phosphatase within SMCs and maintains contractile tone under low-cytosolic calcium concentration. Upregulated Rho-kinase activity has been reported in ED, as a consequence Rho-kinase inhibitors have been examined to treat ED[34].

Despite the candidate mechanisms mentioned above, it is likely that there is an overlap between the roles of these mechanisms. The reduced NO hypothesis seems to be the best one.

The cornerstone of the process seems to be cyclic nucleotide monophosphate, cyclic adenosine monophosphate and cyclic guanosine monophosphate (cGMP). Cyclic nucleotides are synthesized from the corresponding nucleoside triphosphates by the activity of adenylyl and guanylyl cyclases. Soluble guanylyl cyclase is a widely distributed signal transduction enzyme that, under activation by NO, converts GTP into the second messenger, cGMP, which exerts its effect by activating cyclic guanylyl kinase I (cGK Ⅰ) and cGK Ⅱ, cGMP-gated ion channels, and/or cGMP-regulated phosphodiesterases (PDE). The accumulation of intracellular cGMP triggers a cascade, leading to decreased intracellular calcium level and subsequent relaxation of SMCs[35,36]. The amount of cGMP results from the balance between production (NO) and degradation due to PDE isoenzymes which can hydrolyze and inactivate cyclic nucleotides[24]. Therefore, increased smooth muscle tension may play a central role in the pathophysiology of LUTS.

An *in vitro* study revealed that 4 wk of treatment with the NO synthase (NOS) blocker, N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), caused *in vitro* detrusor muscle supersensitivity to muscarinic agonists *via* increases in the levels of [H^3]-inositol-phosphate[23]. This finding was corroborated by in vivo experimental studies which showed that administration of L-NAME resulted in a significant increase in non-voiding contractions (NVC) in rats[24].

Based on experimental studies which have shown that rat PDE expression is highest in the bladder, approximately 10-fold higher than in rat corpora cavernosa followed in decreasing prevalence by vas deferens, prostate, kidney, testis, and epididymis[37], further experimental studies evaluated the action of PDEi on LUT. One of these studies demonstrated that administration of sildenafil in rats improved detrusor overactivity and bladder outlet obstruction (lack of urethral relaxation) caused by the NOS inhibitor, L-NAME, in a urodynamic study (UDS)[24]. In another study, with similar methodology, it was also demonstrated that tadalafil decreased NVC and frequency of micturition (FM) in rats in a UDS[38].

As a consequence of these experimental studies, there is good support for the use of PDEi in the treatment of BPO/LUTS.

CONTROVERSIES IN ADMINISTRATION OF PHOSPHODIESTERASE INHIBITORS TO TREAT BPO/LUTS

Impairment detrusor

It has been observed in several clinical trials that PDEi improved the IPSS without changing Qmax in uroflowmetry[14,16,17,39]. If a PDEi caused relaxation of the bladder neck, urethra, and prostatic relaxation in human and animals[24,37,40], it was expected to increase Qmax. Thus, these findings have raised the theoretical possibility that administration of PDEi cause impairment in detrusor function with unknown long-term effects[41].

As a consequence, an experimental study was performed with the endpoint of determining whether tadalafil caused detrusor muscle impairment. In this study, it was reported that chronic depletion of NO caused an increase in NVC, volume threshold (VT) and FM in rats and treatment with tadalafil reduced VT and FM. However, tadalafil did not decrease threshold pressure or peak pressure (PP) in rats with chronic NO deficiency. Tadalafil which increased cGMP probably explains the reduction in VT (decrease in urethral resistance) and MC (relaxation of detrusor) observed in this study. As tadalafil did not decrease detrusor pressure (threshold pressure or PP) it is evident that PDE5i do not cause impairment in detrusor muscle[38].

A clinical trial assessed the impact of tadalafil treatment (20 mg once daily) compared to placebo on detru-

sor pressure and maximum flow (pdetQmax) in men with BPO/LUTS with or without bladder outlet obstruction at baseline. In this study, tadalafil was not associated with a negative impact on detrusor function as the change of pdetQmax was not significant compared with the placebo arm[42].

Acute effects of phosphodiesterase inhibitors on BPO/LUTS

There are few reports of the acute effects of PDEi on BPO/LUTS. The vast majority of studies have evaluated chronic administration of PDEi.

In one clinical trial, 68 patients were randomized to the placebo (n = 32) or sildenafil arm (n = 36). All patients were evaluated at baseline with free uroflowmetry. Uroflowmetry was repeated two hours after administration of placebo or sildenafil. The authors concluded that sildenafil caused a significant improvement in Qmax 15.6 ± 6.8 mL/s from baseline to end point 19.3 ± 7.2 mL/s (P < 0.001), and compared with the placebo arm 15.8 mL/s (P < 0.0001). The increase in Qmax was attributed to urethral relaxation[43].

An experimental study was performed to observe the effect of acute infusion of sildenafil in rats with detrusor overactivity. It was observed that sildenafil decreased the number of micturition cycles from baseline to end point (-0.93 ± 0.34, P = 0.031)[24].

Combination of phosphodiesterase inhibitors with alpha-blockers

According to the American Urological Association guidelines, a1-adrenergic blockers are considered to be the most effective monotherapy for the treatment of LUTS secondary to BPH[2]. PDE5 inhibitors are the first-line treatment for erectile ED. Due to the strong association between BPO/LUTS and ED, the coprescription of PDE5 inhibitors and a1-adrenergic blockers is likely to increase. Thus, one of the most frequently asked questions by physicians is whether to combine or replace alpha-blockers with PDEi in patients with BPO/LUTS and ED.

To address this issue a randomized, double-blind, placebo- and active-controlled (tamsulosin 0.4 mg), and parallel design trial was carried out to compare the effects of daily tadalafil 5 mg in patients with LUTS/BPO. The IPSS, BPH Impact Index, and IIEF-Erectile Function Domain (IIEF-EF) were assessed at baseline and end point (12 wk or end of therapy). The Patient and Clinician Global Impression of Improvement (PGI-I and CGI-I, respectively) instruments and the subject-rated Treatment Satisfaction Scale-BPH (TSS-BPH), evaluated from 0% (greater) to 100% (lower) satisfaction, were administered at end point. Uroflowmetry and postvoid residual were also assessed at screening, baseline, and end of visits. Tadalafil and tamsulosin caused an improvement in the IPSS from baseline to endpoint. However, for the IPSS QoL a significant improvement compared with placebo was only reported in the tadalafil arm, but not the tamsulosin arm. The TSS-BPH overall satisfaction score at endpoint was significantly lower (indicating higher satisfaction) in the tadalafil group compared with placebo, driven by greater satisfaction with efficacy. There was no significant difference between tamsulosin and placebo in TSS-BPH overall satisfaction or satisfaction with efficacy. Tadalafil resulted in an improvement in IIEF-EF, but tamsulosin did not change this index. Tadalafil and tamsulosin caused a significant increase in Qmax. For PVR, both active treatments caused reductions, but these were not statistically significant. The strong point of this study was the wash-out and placebo run-in periods. The principal limitation was that it was not powered to assess noninferiority or superiority between tadalafil and tamsulosin. The author concluded that tadalafil or tamsulosin resulted in significant improvements in IPSS and Qmax related to BPO/LUTS. However, only tadalafil had a significant impact on the IPSS QoL and erectile function[44].

A randomized, double-blind, placebo-controlled study was performed to compare the effect of tamsulosin 0.4 mg/daily and tadalafil 5 mg daily with tamsulosin 0.4 mg/placebo on the lower urinary tract in a UDS. All patients underwent a baseline UDS before randomization to tamsulosin 0.4 mg/tadalafil 5 mg or tamsulosin 0.4 mg/placebo once daily for 30 d. An end of study UDS was performed on completion of treatment. The UDS assessed pdetQmax, Qmax during voiding, bladder outlet obstruction index calculated as pdetQmax-2Qmax, and detrusor overactivity (assessed as incidence). The primary end points were a change in urodynamic variables in the voiding phase, pdetQmax and Qmax, from baseline to week four. The secondary endpoint of this study was improvement in the IPSS. A total of 40 men were randomized to receive tamsulosin 0.4 mg/tadalafil 5 mg (n = 20) or tamsulosin 0.4 mg/placebo (n = 20) once daily for four weeks. When the groups were compared, pdetQmax decreased significantly in the tamsulosin/tadalafil group compared with the tamsulosin/placebo group. In both groups, Qmax increased from baseline to endpoint, however, the difference in Qmax at endpoint was not significant between the groups. Significant improvements were observed in total IPSS, IPSS filling and voiding subscore in the tamsulosin/tadalafil group compared with the tamsulosin/placebo group. The limitations of this study were lack of placebo run-in period and the small number of participants. A strong point was that this was the first study to define the action of tadalafil in LUT using a computerized UDS. The principal conclusion of this study was that only the combination of tamsulosin/tadalafil decreased after-load (pdetQmax) and had the potential to protect detrusor smooth muscle. In addition, the combination significantly improved the IPSS compared with tamsulosin/placebo[45].

In another clinical trial similar to the above study, tadalafil 20 mg in combination with tamsulosin 0.4 mg was compared to tamsulosin 0.4 mg in patients with LUTS. Improvement in the IPSS was greater with the combination treatment. No difference was observed in

Table 3 Studies evaluating the combination of phosphodiesterase inhibitors and alpha-blockers in patients with lower urinary tract symptoms

Ref.	Design of study	Placebo run-in	Participant /Inclusion criteria	End point	Major conclusion
Oelke *et al*[44]	Randomized, multicentric, placebo controlled, and parallel-group	Yes	Men ⩾ 45 yr of age with BPO/LUTS, IPSS ⩾ 13, and Qmax between 4-15 mL/s 512 men were randomized to placebo (n = 172), tadalafil 5 mg (n = 171) or tamsulosin 0.4 mg (n = 168); Men ⩾ 45 yr of age with BPO/LUTS, IPSS ⩾ 13, and Qmax between 4-15 mL/s	Compare effect of tadalafil 5 mg once daily with placebo on BPO/LUTS	Tadalafil 5 mg and tamsulosin 0.4 mg had similar improvement in BPO/LUTS and Qmax compared with placebo However, only tadalafil caused a significant improvement in QoL, treatment satisfaction, and erectile function
Regadas *et al*[45]	Randomized, double-blind, and placebo controlled	No	Men ⩾ 45 yr of age, BOOI ⩾ 20 and IPSS ⩾ 14 A total of 40 men were randomized to tadalafil 5 mg/tamsulosin 0.4 mg (n = 20) or tamsulosin 0.4 mg /placebo (n = 20) Men ⩾ 45 yr of age, BOOI ⩾ 20 and IPSS ⩾ 14	Observe changes in urodynamic variables (Qmax and PdetQmax)	Combination of tamsulosin/tadalafil decrease after-load (pdetQmax) and has potential to protect detrusor smooth muscle Additionally, the combination resulted in a significant improvement in IPSS compared with tamsulosin/placebo
Bechara *et al*[46]	Randomized, double-blind, and crossover study	No	History of LUTS/BPO of at least six months Thirty men were randomized to tamsulosin 0.4 mg or tamsulosin 0.4 mg/tadalafil 20 mg daily History of LUTS/BPO of at least six months	Acess efficacy and safety of combination of tamsulosin with tadalafil compared with tamsulosin alone	Combination therapy had more significative impact in IPSS and ED compared with tamsulosin alone
Kaplan *et al*[39]	Randomized, double-blind study	No	Men aged 50-76 yr with untreated LUTS and ED Sixty two patients were randomized to receive alfuzosin 10 mg (n = 20), sildenafil 25 mg (n = 21), or a combination of both (n = 20) Men aged 50-76 yr with untreated LUTS and ED	Acess efficacy and safety of combination of alfuzosin with sildenafil	Only sildenafil or combination improve ED Improvement in IPSS was observed with three treatments
Gacci *et al*[47]	Randomized, double-blind placebo-controlled trial	Yes	Men with persistent storage LUTS Sixty men were randomized to tamsulosin 0.4 mg or tamsulosin 0.4 mg/vardenafil 10 mg Men with persistent storage LUTS	Acess efficacy and safety of combination of tamsulosin with vardenafil	Combination therapy had more significative impact in IPSS and ED compared with tamsulosin alone

BPO/LUTS: Benign prostatic hyperplasia/lower urinary tract symptoms; Qmax: Peak flow rate; QoL: Quality of life; IPSS: International Prostatic Symptoms Score; PdetQmax: Detrusor pressure at maximum flow; ED: Erectile dysfunction; BOOI: Bladder outlet obstruction index.

Qmax or PVR between the groups. The IIEF improved in the combination group, but not in the tamsulosin group. The authors concluded that tamsulosin with tadalafil was more effective than tamsulosin alone in improving LUTS and ED. Small sample size was the principal limitation of this study[46].

In 2007, a pilot study was carried out to assess the efficacy of alfuzosin 10 mg, sildenafil 25 mg daily, and the combination of both on LUTS and ED. Improvements in the IPSS and IIEF were significant with the three treatments, but were greatest with the combination of alfuzosin and sildenafil. An improvement in the IIEF was only observed in the sildenafil and combination groups. The authors concluded that the combination of sildenafil and alfuzosin was more effective than monotherapy in improving both voiding and sexual dysfunction in men. Weak points of this study were no placebo arm in the study design, small sample size, and the experimental dose of sildenafil (25 mg once daily)[39].

Tamsulosin 0.4 mg/d was compared with the combination of tamsulosin 0.4 mg/d plus vardenafil 10 mg/d. After a 2-wk run-in period, tamsulosin patients were randomized to a 12-wk treatment period. The IPSS, IPSS-bother, IIEF-5 and Over Active Bladder questionnaire scores, uroflowmetry data (Qmax, Qave), and postvoiding residual urine were recorded after run-in (baseline), and 2 and 12 wk after treatment. Significant differences from baseline to 12 wk were observed in the following: Qmax (placebo: 0.07, vardenafil: 2.56, P = 0.034); Qave (placebo: -0.15, vardenafil: 1.02, P = 0.031); filling-IPSS subscores (placebo: -1.67, vardenafil: -3.11, P = 0.039); and IIEF (placebo: 0.06, vardenafil: 2.61, P = 0.030). The authors concluded that the combination of tamsulosin and vardenafil was more effective in improving both LUTS and erectile function, as compared with tamsulosin alone[47] (Table 3).

CONCLUSION

Treatment of BPO/LUTS with phosphodiesterase inhibitors is beneficial, based on experimental studies, strong evidence and a large number of randomized clinical trials confirming their efficiency.

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REVIEW

Benefits and risks of erythrocyte-stimulating agents

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Core tip: Renal anemia is a common clinical problem in patients with severe chronic kidney disease. To overcome the shortage of endogenous erythropoietin (EPO), administration of exogenous EPO is an effective treatment. The advent of recombinant human erythropoietin (rHu-EPO) products has dramatically changed the therapeutic strategy and has shown outstanding effectiveness in patients with renal anemia. Here we discuss the treatment of renal anemia and the adverse effects of rHu-EPO.

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Abstract

Chronic kidney disease (CKD) is a common and serious clinical problem. Anemia in patients with advanced CKD, frequently called renal anemia, causes disabling fatigue and diminishes patients' quality of life. Frequent and excess transfusions or iron supplementation are potentially hazardous. Although it remains unclear whether the main factor in the development of renal anemia is the failure of erythropoietin (EPO) production in the kidney or a dysfunction in oxygen sensing exogenous EPO administration is considered a rational treatment. The advent of recombinant human erythropoietin (rHu-EPO) products has dramatically changed the therapeutic strategy for renal anemia. Although rHu-EPO therapy has improved patients' quality of life and decreased the need for blood transfusions, some potential adverse effects have been reported till date. This brief review discusses the treatment of renal anemia with regard to the following: (1) historical background; (2) effectiveness of rHu-EPO; (3) some topics regarding the treatment of anemia, including EPO resistance, hemoglobin (Hb) cycling, and adequate Hb levels; (4) major adverse effects of rHu-EPO, including hypertension, thrombotic complications, and pure red cell aplasia; and (5) future problems to be resolved.

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HISTORICAL BACKGROUND

Serious anemia requiring transfusion and/or iron supplementation is one of the frequent complications in patients with progressed chronic kidney disease (CKD)[1]. This type of anemia is also known as renal anemia, and it causes disabling fatigue and diminishes the quality of life (QOL) in patients with CKD. Excessive blood transfusions and/or iron supplementation are potentially hazardous. Rare but serious risks associated with transfusion include procedural complications, pulmonary congestion, electrolyte imbalance, metabolic alkalosis, hypocalcemia, and severe lung injury (Table 1)[1]. Moreover, the great risk of transfusion-related infections, such as hepatitis virus, West Nile virus, and human immunodeficiency virus infections, cannot be ignored. The other important and common therapy for renal anemia is iron supplementation. Iron deficiency is a serious clinical problem in patients with chronic heart failure[2,3]. However, iron overload has potentially adverse outcomes, including hy-

Table 1 Risks associated with blood transfusions[11]

Adverse effects
Fever/allergic reactions
Hemolytic reaction
TRALI
Anaphylaxis
Fatal hemolysis
GVHD
Thrombotic complications
Mistransfusion

TRALI: Transfusion-related acute lung injury; GVHD: Graft-versus-host disease.

potension and dyspnea[4,5], transfusional hemosiderosis, and increased risk of infection[6-8].

The advent of recombinant human erythropoietin (rHu-EPO) was a major breakthrough in the treatment of renal anemia. Although it remains unclear whether the leading factor in the development of renal anemia is the failure to produce EPO in the kidney or a dysfunction in oxygen sensing[9], exogenous EPO administration is considered a rational method to treat anemia in patients with CKD. Human EPO, which is derived from the urine of patients with aplastic anemia (AA), is purified to apparent homogeneity. In 1977, Miyake *et al*[10] first purified human EPO in an amount sufficient for chemical characterization. Recombinant human EPO was subsequently produced and became available for clinical use more than two decades ago. Consequently, it completely altered the medical management of renal anemia.

THE EFFECTIVENESS OF ERYTHROCYTE-STIMULATING AGENTS

The introduction of exogenous rHu-EPO into clinical practice dramatically altered the treatment of renal anemia in patients with CKD[11]. Some of the beneficial effects of rHu-EPO therapy include elevated hemoglobin (Hb) levels, improved QOL and cognitive function[12-14], and decreased left ventricular mass, among others[15]. In 1990, Evans *et al*[16] reported that rHu-EPO therapy led to significant improvements in personal activity levels, functional ability, appetite, sleeping hours, condition or satisfaction with health, and happiness. However, no significant differences were observed in patients' working capacity or job status[16]. Overall, the spread of exogenous rHu-EPO treatment diminished the need for transfusion[17] and the risk of transfusion-related complications. Roth *et al*[18] reported that rHu-EPO therapy improves anemia in patients with stage 3 or 4 CKD [glomerular filtration rate (GFR) = 15-59 mL/min per 1.73 m^2] and does not increase the severity of CKD[18]. The administration of rHu-EPO is definitely regarded as an effective therapy for transfusion-dependent long-term dialysis patients (GFR < 15 mL/min per 1.73 m^2 and use of maintenance renal replacement therapy; CKD 5D) with extremely low Hb levels.

EPO-RELATED TOPICS

EPO resistance

Erythrocyte-stimulating agents (ESA) resistance is a serious clinical problem that physicians often encounter in patients with renal anemia. It has been reported as the most predominant predictor of cardiovascular events and fatality[19]. The Kidney Disease Improving Global Outcomes (KDIGO) guidelines describe the evaluation and management of ESA resistance (Table 2)[20]. Table 3 presents a clinical approach to manage ESA resistance. Among many factors associated with ESA resistance, including hyperparathyroidism, inflammation, and underdialysis, iron deficiency is the leading cause. Iron supplementation often results in the improvement of anemia[1].

Hb cycling

Fishbane *et al*[21] previously defined Hb cycling as cycles with > 1.5 g/dL and > 8 wk and excursions as half of a cycle. In their report, more than 90% hemodialysis (HD) patients were reported to experience Hb cycling. The mean duration of Hb excursions was 10.3 ± 5.1 wk. An increase in the rHu-EPO dose, intravenous iron treatment, and post-hospital discharge were the factors associated with upward excursions[21]. Yang *et al*[22] reported that Hb variability is related to severe mortality in CKD 5D patients in the United States. However, this trend was negated in a subsequent study conducted in Europe[23] and remains to be verified in future research.

Adequate Hb levels

According to recent major randomized controlled trials (RCTs), more harm than benefit may be caused by higher Hb levels. Therefore, setting the Hb level at < 11.5 g/dL in adult patients with CKD is suggested by the KDIGO 2012 guidelines[20]. In the Canadian Cardiac Randomized Evaluation of Antidepressant and Psychotherapy Efficacy trial[24], 603 CKD 3-5 patients (GFR < 59 mL/min per 1.73 m^2) treated with rHu-EPO were evaluated. The number of patients who required HD was greater in the high Hb group (13.0-15.0 g/dL) than in the low Hb group (10.5-11.5 g/dL). The rate of decrease in GFR between the two groups was similar. On the other hand, 1432 CKD patients (GFR, 15-59 mL/min per 1.73 m^2) were studied in the Correction of Hemoglobin and Outcomes in Renal Insufficiency trial[25]. Patients receiving strong treatment for anemia (aiming at 13.5 g/dL) experienced a greater incidence of combined cardiovascular adverse events compared with those receiving standard treatment (11.3 g/dL). Considering these findings, the adequate and appropriate range of Hb levels that should be achieved with rHu-EPO therapy remains to be determined. Intentionally increasing Hb levels to > 13 g/dL using erythrocyte-stimulating agents (ESAs) is not recommended for all adult patients according to the KDIGO guidelines[1,20]. In addition, an excessively high dose of ESA is reported to be potentially harmful for patients according to the results of post-hoc analysis of RCTs[26,27],

Table 2 Erythrocyte-stimulating agents hyposensitivy (Kidney Disease Improving Global Outcomes Guideline 2012)[20]

Initial ESA hyporesponsiveness
Classify patients as having ESA hyporesponsiveness if they have no increase in Hb concentration from baseline after the first month of ESA treatment on appropriate weight-based dosing
In patients with ESA hyporesponsiveness, avoid repeated escalations of the ESA dose beyond double the initial weight-based dose
Subsequent ESA hyporesponsiveness
Classify patients as having acquired ESA hyporesponsiveness if after treatment with stable doses of ESA, they require two increases in ESA doses up to 50% beyond the dose at which they had been stable in an effort to maintain a stable Hb concentration
In patients with acquired ESA hyporesponsiveness, avoid repeated escalations in ESA dose beyond double the dose at which they had been stable
Management of poor ESA responsiveness
Evaluate patients with either initial or acquired ESA hyporesponsiveness and treat for specific causes of poor ESA response
For patients who remain hyporesponsive despite the correction of treatable causes, accounting for relative risks and benefits: decline in Hb concentration; continuing ESA if needed to maintain Hb concentration, with due consideration of the doses required; blood transfusions

ESA: Erythrocyte-stimulating agent; Hb: Hemoglobin.

Table 3 Practical approach in the presence of erythrocyte-stimulating agents resintance (Kidney Disease Improving Global Outcomes Guideline 2012)[20]

Tests	Finding and action
Check adherence	If poor, attempt to improve (if self-injection)
Reticulocyte count	If > 130000/μL, look for blood loss or hemolysis: endoscopy, colonoscopy, hemolysis screen
Serum vitamin B_{12}, folate	If low, replenish
Iron status	If low, replenish iron
Serum PTH	If elevated, manage hyperparathyroidism
Serum CRP	If elevated, check for and treat infection or inflammation
Underdialysis	If underdialyzed, improve dialysis efficiency
ACEi/ARB use	If yes, consider reducing dose or discontinuing drug
Bone marrow biopsy	Manage condition diagnosed, *e.g.*, dyscrasia, infiltration, fibrosis

ACEi: Angiotensin-converting enzyme inhibitor; ARB: Angiotensin-receptor blocker; CRP: C-reactive protein; PTH: Parathyroid hormone.

Table 4 Nonhematologic complications associated with erythropoietin therapy[11]

Adverse effects
Hypertension
Injection site pain
Seizure
Pure red cell aplasia
Liver dysfunction
Shock, anaphylaxis
Thrombotic complications

notably in concurrence with high Hb levels[28].

ADVERSE EFFECTS OF ESA

Table 4 shows the major complications associated with EPO therapy[11].

Hypertension

Hypertension has been considered a common complication of ESA therapy, particularly in the early phase of indication for rHu-EPO therapy[29]. Previous studies have noted that rHu-EPO is the leading factor in approximately 20% patients with clinically important increases in blood pressure during amelioration of anemia. Hb levels, history of hypertension, and previous antihypertensive drug use were not confirmed as risks[30,31]. Similar to the observation in essential hypertension and CKD-associated hypertension, in which an increase in peripheral vascular resistance is the main cause, rHu-EPO-induced hypertension manifests hemodynamic changes[11]. An approximately 30% increase in systemic vascular resistance has been reported[32,33]. Management of this adverse event includes the adjustment of dry weight or limiting the rate of Hb increase and prescription of antihypertensive medication.

Thrombotic complications

The increased incidence of vascular access thrombosis or serious cardiovascular events associated with rHu-EPO therapy is multifactorial and controversial[34,35]. Churchill *et al*[36] reported an insignificant difference between patients treated with rHu-EPO and comparison groups in the time to development of the first thrombosis of fistula. However, rHu-EPO treatment increased the frequency of graft thrombosis[36]. Tang *et al*[37] confirmed that the occurrence of thrombosis in rHu-EPO-treated patients was not related to patients' hematological responses to the drug; rather, it depends on the integrity of patients' vasculature and the type of vascular access used.

Pure red cell aplasia

Normocytic anemia with decreased reticulocytes and

Table 5 Evaluation for pure red cell aplasia (Kidney Disease Improving Global Outcomes Guideline 2012)[20]

Investigate for possible antibody-mediated PRCA when a patient receiving ESA therapy for more than 8 wk develops the following:
Sudden rapid decrease in Hb concentration at the rate of 0.5 to 1.0 g/dL (5 to 10 g/L) per week OR requirement of transfusions at the rate of approximately 1 to 2 per week
Normal platelet and white cell counts
Absolute reticulocyte count less than 10000/mL
ESA therapy should be stopped in patients who develop antibody-mediated PRCA
Peginesatide should be used to treat patients with antibody-mediated PRCA

PRCA: Pure red cell aplasia; ESA: Erythrocyte-stimulating agents; Hb: Hemoglobin.

absence of erythroblasts is the main feature of Pure red cell aplasia (PRCA). The onset of secondary PRCA may follow parvovirus infection, leukemia, lymphoma, collagen disease, or rHu-EPO treatment. In Europe, the frequency of rHu-EPO-associated PRCA reached a peak in 2001-2002, in connection with Eprex (Johnson and Johnson, New Brunswick, NJ, United States)[38]. It was suggested that subepidermal immune reactions play an essential role in PRCA induced by rHu-EPO, as the condition was mostly induced by subcutaneous administration[39]. To guide the close examination and therapy of patients with a strong possibility of developing antibody-associated PRCA, recommendations based on expert opinions have been published[40,41]. The two major clinical features of antibody-mediated PRCA are as follows: (1) An associated decline in blood Hb levels of 4 g/dL per month; and (2) A decrease in the number of reticulocytes to < 10000/mL (Table 5)[20].

Currently, the incidence rate of PRCA is very low. However, PRCA may still occur even though rHu-EPO is thought to be well preserved under good storage conditions. This is because rHu-EPO has been more popular for the treatment of renal anemia[42,43].

We previously reported an elderly patient with PRCA who was positive for anti-erythropoietin (anti-rHu-EPO) antibodies[43]. Transfusions for symptomatic anemia and discontinuation of rHu-EPO treatment are important for the initial management of anti-EPO antibody-mediated PRCA[44]. Subsequent immunosuppressive therapy should be considered because PRCA in this setting is immune-mediated and because spontaneous remission is rare. In a previous study related to anti-EPO antibody-mediated PRCA (N = 47), 78% (N = 29/37) patients who received immunosuppressive therapy recovered[45], whereas all patients without immunosuppressive drug therapy (N = 9) did not recover from PRCA. According to another report of 62 PRCA patients who did not receive immunosuppressive therapy, only one patient showed spontaneous recovery[46].

Fisch *et al*[47] reviewed serum antibodies and natural killer cells or T-lymphocyte associated mechanisms of erythropoiesis. Rituximab (an anti-CD20 monoclonal antibody)[48] and alemtuzumab (an anti-CD52 monoclonal antibody)[49] are expected to represent an alternative therapeutic strategy for patients with refractory PRCA. However, the patient in our presented case report refused immunosuppressive treatment, and successful remission was achieved with cessation of rHu-EPO treatment alone. The patient's severe anemia gradually ameliorated along with a decrease in antibody titer[43]. In 1997, Prabhakar *et al*[50] reported the first case of a patient with PRCA caused by rHu-EPO who recovered after the discontinuation of ESA therapy. In 1996, Casadevall *et al*[51] reported a patients with PRCA and spontaneously decreasing anti-EPO antibodies. It should be emphasized that spontaneous PRCA remission following the cessation of rHu-EPO therapy is extremely unlikely. Early recognition as well as appropriate and prompt management is important for managing the consequences of this antibody-related PRCA.

FUTURE PROBLEMS TO RESOLVE

Peginesatide is an rHu-EPO receptor agonist without cross-reactivity with anti-EPO antibodies[52]. Peginesatide is expected to be a potential alternative therapy for managing patients with anti-EPO antibody-associated PRCA[53]. In February 2013, the drug industry recalled peginesatide because the Food and Drug Administration received reports of anaphylactic reactions following peginesatide administration, some of which resulted in death[54].

As an alternative to the administration of exogenous rHu-EPO or its mimetics, induction of endogenous EPO production by several mechanisms, including prolyl hydroxylase domain protein inhibitors and GATA-binding protein inhibitors, is considered to have potential advantages of better availability and lower immunogenicity[55].

Further research in this field is required to answer the following clinical questions[20]: (1) Is there any difference in outcomes between intravenous and subcutaneous ESA administration? (2) Can any vascular complications occur in association with normalization of Hb levels using ESA therapy? and (3) Are there any potential risks associated with ESA use in patients with a history of cancer?

CONCLUSION

rHu-EPO therapy is undoubtedly an effective strategy for renal anemia. However, clinicians must balance the benefits, such as a decrease in the requirement of transfusions and amelioration of anemia-related symptoms, with the potential side effects of rHu-EPO administration. Further research is expected to answer some interesting

clinical questions related to the use of rHu-EPO therapy for renal anemia.

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REVIEW

Review of novel therapeutic medicines targeting androgen signaling in castration-resistant prostate cancer

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Abstract

Prostate cancer is the most common male malignant neoplasm. Androgens and the androgen receptor (AR) play a key role in the onset and progression of prostate cancer. The expression of the AR is still preserved in the majority of patients with castration-resistant prostate cancer (CRPC). CRPC is considered to be induced by the following mechanisms: (1) sustained AR activation by enhancing intracellular conversion of adrenal androgens to dehydrotestosterone *via* a *de novo* route; (2) AR hypersensitivity; (3) promiscuous activation of AR signaling; and (4) outlaw pathways. Recent advances in the treatment of CRPC include novel medicines targeting AR signaling pathways. In addition, functional molecular studies have shown that some of the AR-regulated genes and AR coregulators are prognostic markers and potential therapeutic targets for prostate cancer, particularly in the castration-resistant state. Therefore, identification of the AR signaling pathways responsible for establishment of CRPC is critical for developing new strategies for the treatment of CRPC.

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Key words: Androgen receptor; Prostate cancer; Pyrrole-imidazole polyamide

Core tip: Prostate cancer is the most common male malignant neoplasm. Androgens and the androgen receptor (AR) play a key role in the onset and progression of prostate cancer. The expression of the AR is still preserved in the majority of patients with castration-resistant prostate cancer (CRPC). Therefore, identification of the AR signaling pathways responsible for establishment of CRPC is critical for developing new strategies for the treatment of CRPC.

Obinata D, Fujiwara K, Yamaguchi K, Takayama K, Urano T, Nagase H, Inoue S, Takahashi S, Fukuda N. Review of novel therapeutic medicines targeting androgen signaling in castration-resistant prostate cancer. *World J Clin Urol* 2014; 3(3): 264-271 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/264.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.264

INTRODUCTION

Prostate cancer has been the most common male malignant neoplasm for more than 30 years and is the second leading cause of cancer-related death of men in the United States[1]. In Japan, partially because the diet seems to

be becoming Westernized, the incidence of prostate cancer has been increasing. The population of older males is also becoming larger and may also be a contributor.

Androgens and the androgen receptor (AR) play a key role in the onset and progression of prostate cancer. Functional ARs are expressed during various stages of prostate carcinogenesis, from prostate intraepithelial neoplasia to locally advanced primary tumors. Approximately 80%-90% of prostate cancers are androgen-dependent at the time of diagnosis[2-4].

Since the discovery that the progression of prostate cancer could be inhibited by castration in the 1940s[5,6], hormonal therapy that specifically inhibits AR activity by using a luteinizing hormone-releasing hormone analog/antagonist, with/without anti-androgens [androgen deprivation therapy (ADT)] has become the most effective and widely used palliative method for advanced and/or metastatic prostate cancer[7-9]. In the majority of patients, although ADT leads to a biochemical response for up to 3 years, prostate cancer eventually continues to progress through cell transformation. Previously, these conditions were known by various names over the years, including hormone-resistant prostate cancer and androgen-insensitive prostate cancer. However, most recent reports indicate that AR is still expressed in the majority of ADT resistant cases, and expression of AR target genes, such as prostate-specific antigen (PSA), remains persistently high despite serum testosterone in the castrated range after surgical castration or ADT[9-11]. This condition is called castration-resistant prostate cancer (CRPC)[12]. Patients with CRPC demonstrate poor prognosis associated with a deterioration in the quality of life, and few therapeutic options are currently available[13]. Therefore, it is important to understand the AR signaling pathway to develop an effective treatment for CRPC. In this review, we summarize the roles of the AR signaling pathway and novel therapeutic medicines that target this pathway in prostate cancer. We focus in particular on functional analyses of AR targets and indicate future directions for their therapeutic use.

AR STRUCTURE

The *AR* gene is a member of the steroid hormone receptor superfamily, which includes genes encoding receptors for estrogen, progesterone, glucocorticoids, mineralocorticoids, vitamin D, retinoic acid, and the retinoid X receptor. Similar to many other steroid receptors, the AR is characterized by a modular structure consisting of distinct functional domains: a poorly conserved N-terminal domain (NTD; 555 amino acids coded by exon 1), a highly conserved DNA-binding domain (DBD; 68-amino acid coded by exon 2 and 3), a hinge region, and a moderately conserved ligand binding domain (LBD; 295 amino acids coded by exons 4-8)[14]. The AR NTD contains the major transactivation function of the AR, which is known as activation function (AF)-1, and consists of two transcriptional activation units (TAU): TAU-1 and TAU-5[15]. AF-1 interacts with the LBD, the basal transcription factors transcription factor ⅡF (TFⅡF) and TFⅡH, members of the p160 family of nuclear receptor coactivator proteins and the general coactivator cAMP response-binding protein-binding protein[16-28]. These reports also indicate that AF-1 is one of the major domains responsible for mediating AR transcriptional activity.

The DBD has important roles in mediating AR nuclear localization, homodimer formation, and specific DNA binding. The activated AR binds as a homodimer to specific DNA sequences called androgen response elements (AREs) located around the target genes[29]. These AREs can be classified into two types: canonical and non-canonical AREs[29]. Canonical AREs consist of an inverted repeat of hexameric half-sites (5'-TGTTCT-3') with a 3-bp spacer[30]. The non-canonical AREs have an atypical motif, and their binding specificity to steroid receptors is relatively weak, resulting in their need for coregulators[31,32]. Data indicate that some important androgen-regulated genes in prostate cancer are regulated by atypical AREs. The DBD contains two zinc finger domains. The first zinc finger contains a conserved P-box motif that binds the half site of the classical ARE. The second zinc finger contains a conserved D-box motif that functions to stabilize the DNA-receptor complex and to produce a receptor homodimer[33].

The LBD is located in the C-terminal region and comprises 12 helices. The LBD assists in the binding of dihydrotestosterone (DHT), which is the first step in the androgen signaling pathway. Like the AF-1 region in the NTD, the LBD contains an AF2 that interacts with coregulators [the steroid receptor coactivator (SRC)/p160 family] to bind to the NTD[34,35]. Because AF-2 shows a higher affinity for the NTD than the coactivator, interaction with the NTD is the primary role for AF-2 rather than direct transcriptional activation. The LBD plays a key role in current ADT for prostate cancer. Although anti-androgens used in ADT, such as bicalutamide, block the activity of AF-2 by binding to the LBD[36], point mutations in the AR in prostate cancer primarily occur in the regions of the LBD that include amino acids 670-676, 701-730, and 874-919, causing resistance to anti-androgens binding and proliferation in the presence of the anti-androgens[37-39].

THE AR SIGNALING PATHWAY

Androgens, the male sex steroids, regulate numerous physiological responses ranging from male sexual differentiation to the development of bone and muscle. The biological action of androgens is mediated through the AR. In prostate tissue, DHT is the primary ligand for the AR and is synthesized from testosterone by 5-reductase. The ligand-unbound AR is present primarily in the cytoplasm, where it interacts with heat shock proteins (Hsp)-90, -70, -56, cytoskeletal proteins, and other co-chaperones[40]. The AR-Hsp90 interaction is necessary to maintain the AR in a high-affinity ligand-binding confor-

mation, suggesting that Hsp90 plays a key role in the activation of agonist-bound AR regulation of nuclear transfer, nuclear matrix binding, and transcriptional activity. Following DHT binding to the AR, the AR translocates into the nucleus, and binds to the ARE in the promoter and enhancer regions of target genes. For example, PSA is a typical product of the AR-dependent gene and an important biomarker for prostate cancer.

The AR transcriptional complex is completed by recruitment of coregulators, which ultimately results in regulation of gene expression[36]. After the discovery of SRC-1, more than 200 nuclear receptor coregulators have been identified[41,42]. Coregulators were previously classified as either enhancing (coactivators) or repressing (corepressors) AR activity, and the requirements for coregulators vary among genes[43].

The AR is also regulated by post-translational modifications generated by signal transduction pathways. These modifications can be further divided into two categories: (1) reversible modifications of specific amino acid residues of target proteins (phosphorylation and acetylation); and (2) modifications involving addition of other proteins or polypeptides (ubiquitination and sumoylation). These changes have the potential to affect AR stability, subcellular localization, and interaction with other proteins, including coregulators.

MECHANISMS OF CASTRATION RESISTANCE

Castration resistance has been reported to be induced by: (1) sustained AR activation by enhancing intracellular conversion of adrenal androgens to DHT *via* a *de novo* route[44]; (2) AR hypersensitivity[45]; (3) promiscuous activation of AR signaling; and (4) outlaw pathways[9]. AR hypersensitivity results in the facilitation of a susceptibility to androgen by variation of a coregulator or cytokine activity and overexpression of the AR. In addition, Cai *et al*[46] reported that prostate cancer cells incubated with levels of androgen comparable to those seen in castration decreased AR-induced lysine-specific demethylase 1 levels, which negatively regulates AR signaling, resulting in an increase in the expression of AR and of multiple genes that contribute to increased androgen synthesis and sensitivity in CRPC[46].

Promiscuous activation of the AR signaling pathway occurs in cases of AR structural change and when the AR combines with ligands other than androgen. This phenomenon induces the anti-androgen withdrawal syndrome, *i.e.*, anti-androgen itself serves as an accelerator of progression. Outlaw pathways include AR structural changes when androgen is absent or when cytokines other than androgen bind to the AR to activate a signaling pathway, resulting in a facilitation of an androgenic response in gene expression. The extragonadal androgen synthesized in adrenal or prostate cancer cells plays a key role in the occurrence of sustained AR activation. Androgen is a metabolite of cholesterol in the testis and adrenal gland. Most of its synthetases belong to the cytochrome P450 (CYP) family. CYP17 in particular has both 17-hydroxylase and 17,20-lyase activity that plays an important role in the synthesis of adrenal androgen. Castration does not influence the synthesis of adrenal androgen. Adrenal androgen is converted into DHT by 5-reductase in prostate cancer cells. The affinity of DHT for the ligand-binding domain on the AR is higher than testosterone as a main ligand in prostate cells. Furthermore, the CRPC cells contain increased CYP17 activity with a new metabolic pathway that converts cholesterol to androgen. Thus, the expression of androgen-dependent genes is achieved by a very small amount of testosterone under castration[47].

NEW THERAPEUTIC DRUGS TARGETING THE AR SIGNALING PATHWAY

Standard therapeutic strategies for prostate cancers have recently been changed to utilize newly developed medicines for CRPCs. As described above, because androgens and the ARs remaining in CRPC cells are important for cancer progression, novel medicines should be designed to target the androgen synthesis pathway or the AR signaling pathway (Figure 1). Here, we introduce these new medicines, including compounds currently being developed in Japan.

Abiraterone

Abiraterone is a dual inhibitor of the 17-hydroxylase and 17,20-lyase expressed in testicular, adrenal, and prostatic tumor tissues. Ketoconazole, an azole antifungal medicine that has a similar target as abiraterone, initially showed a preferable outcome in patients with CRPC[48]. Ketoconazole inhibits both testicular and adrenal androgen biosynthesis by targeting cytochrome P450 isozyme 3A4 and 17,20-lyase. However ketoconazole results in severe toxicities because it has low specificity for the CYP17 family. Based on these pieces of evidence, abiraterone was selected for development at the Institute of Cancer Research in the UK as a selective inhibitor of CYP17. The multicenter phase Ⅲ randomized placebo-controlled trial COU-AA-301 evaluated the efficacy of abiraterone compared with docetaxel for men with progressive CRPC[49]. The overall survival in the abiraterone arm was significantly longer than in the docetaxel arm (14.8 *vs* 10.9 mo). However, abiraterone induces hyperaldosteronism by reduction of glucocorticoid following secondary adrenocorticotropic hormone overexpression. Although steroids are useful to protect against such adverse effects, the combination of steroids with abiraterone could limit its use for patients with early-stage disease and longer life expectancies.

TAK-700

TAK-700 is a next-generation CYP17 inhibitor that requires no steroid administration. Because TAK-700 selectively targets 17,20-lyase, and inhibition of 17α-hydroxylase

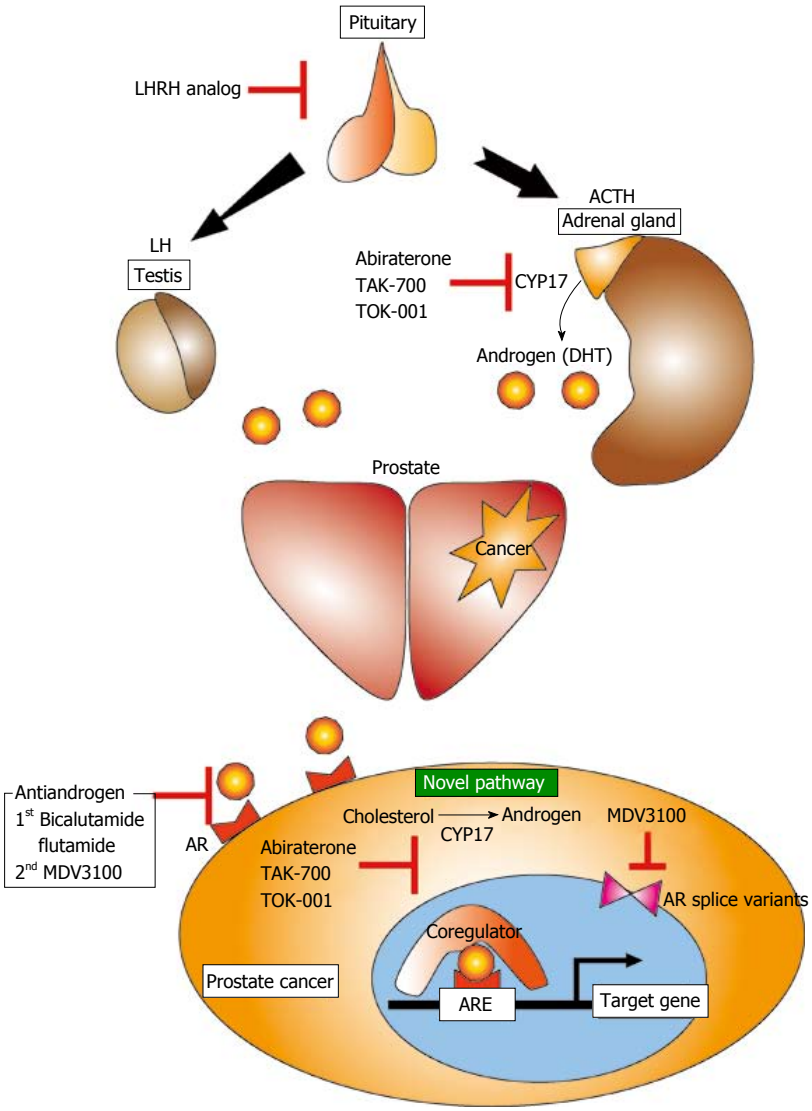


Figure 1 Schematic view of the therapeutic medicines targeting androgen receptor signaling pathways. ACTH: Adrenocorticotropic hormone; AR: Androgen receptor; ARE: Androgen response element; LH: Luteinizing hormone; LHRH: Luteinizing hormone-releasing hormone; DHT: Dihydrotestosterone; CYP: Cytochrome P450.

and reduction of glucocorticoids were smaller than those seen in patients treated with abiraterone[47,50]. A phase Ⅲ clinical trial of TAK-700 for metastatic CRPC (Evaluation of the Lyase inhibitor orteronel in Metastatic Prostate Cancer 5) is ongoing. The results of the interim analysis show that although overall survival was not significantly improved as a primary endpoint, progression-free survival was improved in the TAK-700 arm compared with the placebo arm (HR = 0.755).

TOK-001

The chemical composition of TOK-001 is similar to that of abiraterone. In addition to 17-hydroxylase inhibitory activity, TOK-001 also has AR antagonistic action[51-53]. Phase Ⅰ / Ⅱ clinical trials for CRPC are ongoing.

MDV3100

MDV3100 binds to the AR directly and targets multiple steps in the AR signaling pathway, including translocation to the nucleus and binding of the AR to the ARE and coregulators. Because MDV3100 differs from an agonistic anti-androgen, this compound does not induce anti-androgen withdrawal syndrome. MDV3100 induces the same action as the structural variant AR, which causes castration resistance; therefore, it is considered to be a second generation anti-androgenic agent[54]. In a phase Ⅲ double-blind, placebo-controlled trial (AFFIRM Clinical Trials), MDV3100 was superior in the proportion of patients with a reduction in PSA level by 50% or more, the quality-of-life response rate, progression-free survival, and overall survival of men with metastatic CRPC after chemotherapy[55]. Based on these results, MDV3100 obtained approval for treatment of metastatic prostate cancer in Europe. In addition, recent clinical trial (PREVAIL Clinical Trials) showed that MDV3100 significantly decreased the risk of radiographic progression and prolonged overall survival in men with metastatic CRPC who have not received chemotherapy[56].

DEVELOPMENT OF THERAPEUTIC DRUGS THAT TARGET THE ANDROGEN RESPONSIVE GENES

The goal of the AR signaling pathway is the transcriptional activation of target genes (androgen-responsive genes). The overexpression of the androgen-responsive genes is the main cause of cancer progression regardless of the presence or absence of castration resistance. We have reported the results of the functional analysis of novel androgen responsive genes and AR coregulators that influence the progression of prostate cancer[57-62]. Here, we introduce a part of these studies, including therapeutic medicines.

ADP ribosylation factor GTPase-activating protein 3

ADP ribosylation factor GTPase-activating protein 3 (*ARFGAP3*) is a novel androgen-regulated gene that is considered to be associated with regulation of the vesicular transport of the Golgi apparatus. In androgen-sensitive prostate cancer LNCaP cells, we observed induction of ARFGAP3 expression at the mRNA and protein levels in response to stimulation with 100 nmol/L DHT[59]. In functional analyses using LNCaP cells, the increased expression of ARFGAP3 was associated with cell growth, the G1/S cell cycle progression and cell migration. In addition, we found that ARFGAP3 interacted with paxillin as an AR coactivator, and enhanced migration activity and AR activity in LNCaP cells[59]. These findings suggest that *ARFGAP3* is a novel androgen-regulated gene that can promote prostate cancer cell proliferation and migration in collaboration with paxillin.

Octamer transcription factor 1

Octamer transcription factor 1 (Oct1) is a ubiquitous member of the Pit-Oct-Unc-homeodomain family. Although Oct1 does not demonstrate androgenic responsiveness, it works as a coregulator of the AR, binding to neighbors of the ARE and regulating AR activity[29]. We found that Oct1 is expressed in the nuclei of LNCaP cells using immunocytochemistry. SiRNA silencing of Oct1 inhibited the proliferation in LNCaP cells[60]. In addition, using surgical specimens, we found a positive correlation between Oct1 immunoreactivity in samples with a high Gleason score and AR immunoreactivity. Moreover, patients with high immunoreactivities of both Oct1 and AR exhibited poorer cancer-specific survival[60]. These results demonstrate that Oct1 may be a prognostic factor for prostate cancer and a contributing factor for increased AR sensitivity and castration resistance.

Pyrrole-imidazole polyamides

Pyrrole-imidazole (PI) polyamides are small synthetic molecules that recognize and attach to the minor groove of DNA to inhibit DNA-transcription factor interactions with high affinity and sequence specificity[63-65]. DNA recognition by PI polyamides depends on a code of side-by-side pairing of pyrrole and imidazole in the hairpin structure. Various types of sequence-specific PI polyamides have recently been developed to control gene expression[66-69]. One of the most important advantages of PI polyamide is their resistance to biological degradation by nucleases and proteases compared to nucleic acid medicines, including siRNA. In addition, PI polyamides could be efficiently delivered to cell nuclei without any specific drug delivery system. Another important advantage is the safety of PI polyamides when they are injected intravenously or *via* peritoneal to mice or rats[66,67]. PI polyamide that recognizes AREs suppresses DHT-dependent gene expression in LNCaP cells[70]. In addition, this polyamide was reported to inhibit the binding of RNA polymerase Ⅱ to the transcription start site of AR-driving genes[71]. These reports indicate that PI polyamides could be a powerful tool in the development of molecularly targeted therapeutics for androgen responsive genes/AR coregulators in prostate cancer.

CONCLUSION

The clinical challenges in prostate cancer are currently focused on controlling the action of the AR, which plays an important role in the development of hormone therapy naïve prostate cancer and also CRPC. Recent evidence shows that CRPC cells are still dependent on AR activity after ADT. Selective inhibition of the AR signaling pathway by typical ADT induced a bypass mechanism to activate the AR in low dose or in the absence of DHT, and thereby restore AR-dependent cellular proliferation. Thus, blocking the AR with second-generation AR antagonists has the potential to treat CRPC because of stronger and more durable inhibition of transcriptional activity than previous compounds. Various functional studies, including our reports, have cited androgen-regulated genes and AR collaborating factors, such as Oct1, as preferable candidates for biomarkers and therapeutic targets for CRPC. We believe future investigations of the AR signaling pathway and novel therapeutics targeting this pathway are mainstays for considering new strategies to treat CRPC.

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REVIEW

Stem cell therapy for erectile dysfunction

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Abstract

Erectile dysfunction (ED) is an important health problem that has commonly been clinically treated using phosphodiesterase type 5 inhibitors (PDE5Is). However, PDE5Is are less effective when the structure of the cavernous body has been severely injured, and thus regeneration is required. Stem cell therapy has been investigated as a possible means for regenerating the injured cavernous body. Stem cells are classified into embryonic stem cells and adult stem cells (ASCs), and the intracavernous injection of ASCs has been explored as a therapy in animal ED models. Bone marrow-derived mesenchymal stem cells and adipose tissue-derived stem cells are major sources of ASCs used for the treatment of ED, and accumulated evidence now suggests that ASCs are useful in the restoration of erectile function and the regeneration of the cavernous body. However, the mechanisms by which ASCs recover erectile function remain controversial. Some studies indicated that ASCs were differentiated into the vascular endothelial cells, vascular smooth muscle cells, and nerve cells that originally resided in the cavernous body, whereas other studies have suggested that ASCs improved erectile function *via* the secretion of anti-apoptotic and/or proangiogenic cytokines rather than differentiation into other cell types. In this paper, we reviewed the characteristics of stem cells used for the treatment of ED, and the possible mechanisms by which these cells exert their effects. We also discussed the problems to be solved before implementation in the clinical setting.

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Key words: Erectile dysfunction; Stem cell therapy; Bone marrow-derived mesenchymal stem cells; Adipose tissue-derived stem cells; Endothelial progenitor cells; Adrenomedullin; Angiopoietin-1

Core tip: Adult stem cells (ASCs) have been used for the treatment of erectile dysfunction. Although previous studies reported that ASCs differentiated into cells that originally resided in the cavernous body, recent studies indicate that the major, if not all, effects of ASCs on erectile function are achieved through the secretion of paracrine factors rather than their direct differentiation into the cells in the cavernous body. Among various cytokines that ASCs produce, we have recently identified adrenomedullin as a candidate peptide that is implicated in the restoration of erectile function. We introduced these data in this review.

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INTRODUCTION

Erectile dysfunction (ED) is a worldwide health problem. Although psychogenic factors are a major cause of ED, other factors such as age, diabetes, total prostatectomy and radiation in the pelvis also contribute to the occurrence of ED. These factors cause structural changes as well as functional abnormalities in the cavernous body,

and therefore selective phosphodiesterase type 5 inhibitors (PDE5Is) are not so effective in the treatment of these diseases. For the recovery of erectile function in these patients, the regeneration of the cavernous body is necessary. In this regard, much attention has recently been placed on gene therapy and stem cell therapy.

Stem cells are defined as being capable of self-renewal and of differentiation into a variety of phenotypes[1]. There are two categories of stem cells: embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs were originally isolated from the inner cell mass of blastocysts[2]. ESCs are pluripotent stem cells that can give rise to the three germ layers. However, harvesting ESCs requires the destruction of human embryos and has therefore raised ethical concerns. To overcome this limitation, induced pluripotent stem (iPS) cells have been produced. Adult fibroblasts were reprogrammed by introducing four factors, Oct3/4, Sox2, c-Myc and Klf4 under ESCs culture conditions[3,4]. iPS cells are pluripotent stem cells with very similar characteristics to ESCs. Furthermore, many groups have now succeeded in reprograming somatic cells to create iPS cells by overexpression of variable sets of several transcription factors in cells without employing viruses or vectors[5-7]. Therefore, iPS cells are a promising option for regenerative medicine in the near future. A further option for avoiding the ethical problems of ESCs is the use of ASCs, which are basically multipotent stem cells that reside in various tissues, including the brain, skeletal muscle, bone marrow, adipose tissue, and dental pulp[8-11]. Besides having the potential to differentiate into various cell types, ASCs produce a broad range of cytokines that exert their effects in a paracrine and/or autocrine manner. Among ASCs, bone marrow-derived mesenchymal stem cells (BMMSCs) are the most commonly studied. BMMSCs reportedly have a potential to differentiate into various cell types including bone, cartilage, cardiac muscle, skeletal muscle, vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs)[12,13]. Recently, adipose tissue-derived stem cells (ADSCs) have gained much attention because of the simplicity of harvesting hundreds of grams of subcutaneous adipose tissue without using invasive procedures, whereas painful bone marrow aspiration is required to collect just grams of bone marrow. Similar to BMMSCs, ADSCs have the potential to differentiate into various cell types[14]. ADSCs strongly resemble BMMSCs in that they share similar expression patterns of cell surface markers and similar gene expression profiles[15,16].

TREATMENT OF ED USING ASCs

BMMSCs

Many studies have demonstrated the efficacy of BMMSCs in the treatment of ED, and their efficacy seems to be reliable[17-27]. However, the mechanism by which BMMSCs restore erectile function remains controversial. BMMSCs were originally believed to home in on damaged tissues efficiently and differentiate into various cells at the target site. Genetically manipulated BMMSCs were also used expecting that these cells remain in the tissues and express a specific gene over a long period. Bivalacqua *et al*[17] infected BMMSCs with an adenovirus expressing endothelial nitric oxide synthase (eNOS) and injected these cells into the penis of aged rats. The eNOS-modified BMMSCs restored erectile function, with the recovery being associated with increased eNOS protein expression, NOS activity, and cyclic guanosine monophosphate levels. Furthermore, the authors demonstrated that the injected BMMSCs survived for at least 21 d in the cavernous body and expressed markers for VECs and VSMCs. Song *et al*[18] used immortalized human BMMSCs and injected them into rat penises, where the cells expressed markers for VECs and VSMCs in the cavernous body, although the authors did not examine whether erectile function was restored or not. Qiu *et al*[21] injected BMMSCs into the penis of streptozotocin (STZ)-induced diabetic rats and found that the BMMSCs injection restored erectile function. The authors also demonstrated that injected cells remained in the cavernous body for at least 4 wk and some expressed markers for VECs and VSMCs. Although the injection of BMMSCs restored erectile function in these studies, there are several problems. First, these studies did not clearly calculate the percentage of injected BMMSCs that remained in the cavernous body and obtained markers for VECs and VSMCs, or the percentage mentioned in these studies was not sufficient to explain the recovery of erectile function. Second, there was a possibility that the injected BMMSCs fused with the residing cells in the cavernous body and acquired the phenotype of the residing cells. Terada *et al*[28] used BMMSCs obtained from female transgenic mice expressing green fluorescent protein (GFP) and puromycin resistance gene. The BMMSCs were cocultured with a male embryonic stem cell line and then puromycin was added to remove the embryonic stem cells. The remaining cells were GFP positive and puromycin resistant, and morphologically similar to embryonic stem cells. These cells could be induced to differentiate into cardiac myocytes and neuronal cells, suggesting that embryonic stem cell-like pluripotent stem cells were established from BMMSCs. However, following DNA ploidy (the number of DNA copies) analysis using fluorescence-activated cell sorting, the cells were found to be tetraploid (4n) or hexaploid (6n), suggesting that they had developed from spontaneous fusion between the BMMSCs and the embryonic stem cells. The possibility of cell fusion *in vivo* was also reported. Alvarez-Dolado *et al*[29] used transgenic mice that contain the *lacZ* reporter gene downstream of a stop codon flanked by loxP sites (floxed). Therefore, the *lacZ* reporter gene was only expressed when the loxP-flanked stop codon was excised by Cre recombinase. The authors lethally irradiated these mice and intraperitoneally injected BMMSCs from mice that ubiquitously express Cre recombinase and GFP. If cells from the donor and recipient fused, the Cre enzyme would excise the Lox P-flanked stop codon, thereby allowing expression of

the *lacZ* gene. Results revealed that β-gal+ (fused) and GFP+ cells were found in the brain, heart, and liver of recipients, 2 and 4 mo post-transplantation[29]. Thus, BMMSCs potentially fuse with other cell types *in vivo* and it appears that BMMSCs are differentiated into other cell types because of this phenomenon. In contrast, the paracrine effects of BMMSCs have been reported. Kendirci *et al*[20] isolated BMMSCs positive for p75 low-affinity nerve growth factor receptor using magnetic-activated cell sorting, and injected these cells into the penis of rats following bilateral cavernous nerve crush injury. The injection of these cells restored erectile function[20]. The engraftment of these cells in the cavernous tissue occurred very rarely, and the engrafted cells appeared fibroblastic. Furthermore, these cells secreted fibroblast growth factor 2 (FGF2), which suggested that FGF2 might protect the cavernous nerve after crush injury. More direct evidence of the paracrine effects of BMMSCs was reported by Yeghiazarians *et al*[30] who injected BMMSCs extracts into infarcted hearts and found that the procedure effectively improved cardiac function, suggesting that BMMSCs *per se* were not required for their tissue protective effects. Although this scenario is attractive, no cytokines that are implicated in the recovery of erectile function have been specifically identified.

ADSCs

The efficacy of ADSCs in the treatment of ED seems to be reliable from the results of many previous reports[31-45]. However, the mechanisms by which ADSCs restore erectile function remain controversial. Ning *et al*[46] reported that ADSCs could differentiate into VECs. ADSCs injected into the penis obtained a marker for VECs. FGF2 appeared to be necessary for the differentiation of ADSCs into VECs *in vitro*, although the functional role of FGF2 in the differentiation of ADSCs into VECs *in vivo* was not studied[46]. Ryu *et al*[40] demonstrated that the injection of ADSCs into the penis of STZ-induced diabetic mice restored erectile function. They also found that some injected ADSCs became CD31 positive, suggesting that these cells differentiated into VECs[40]. However, ADSCs injected into the penis disappeared within 14 d. Kim *et al*[43] applied human ADSCs and nerve growth factor-incorporated hyaluronic acid-based hydrogel to the cavernous nerve of rats following bilateral cavernous nerve crush injury. The authors demonstrated that this treatment restored erectile function. They also showed that some ADSCs were engrafted into the cavernous nerve 4 wk after treatment, suggesting that ADSCs could differentiate into nerve tissue[43]. Although these studies showed that ADSCs have the ability to differentiate into the cells located in the cavernous body, the possibility of cell-cell fusion was not excluded in these studies. Alternatively, paracrine effects of ADSCs have been suggested. Several reports suggested the possibility of paracrine effects of ADSCs because ADSCs did not remain in the cavernous body for a long period[32,33]. Albersen *et al*[31] injected both ADSCs and the lysate of ADSCs into the penis of rats that were subjected to bilateral cavernous nerve crush injury. They demonstrated that the injection of ADSCs lysate significantly restored erectile function[31]. These results suggested that most, if not all, of the effects of ADSCs were mediated through their production of cytokines and/or immune modulators, although the authors did not identify any molecules that are functionally implicated in the restoration of erectile function. Zhang *et al*[47] reported that ADSCs produced chemokine (C-X-C motif) ligand 5 (CXCL5) and that CXCL5 was implicated in the neurotrophic effects of ADSCs *in vitro*, although they did not confirm this finding *in vivo*. We recently reported that adrenomedullin (AM) is implicated in ADSCs-induced restoration of erectile function in diabetic rats[36]. AM was originally isolated from human pheochromocytoma tissue, and has potent vasorelaxant and diuretic effects[48]. In addition, AM is also produced by VECs, VSMCs and macrophages[49-51] and has the ability to stimulate angiogenesis[52-54]. When rat ADSCs were cultured in a medium containing growth factors for VECs, ADSCs produced significant amounts of AM (Figure 1A). The injection of ADSCs into the penis of diabetic rats restored erectile function (Figure1B), the morphology of the cavernous body (Figure 1C), and the expression of vascular endothelial (VE)-cadherin, a marker for VECs. However, when the expression of AM was knocked down using a small interfering RNA for AM, the favorable effects of ADSCs disappeared (Figure 1B and C). Furthermore, when AM was overexpressed in the penis using an adenovirus expressing AM, erectile function and the morphology of the cavernous body were restored in diabetic rats (Figure 1B and C). We also demonstrated that ADSCs produce angiopoietin-1 (Ang-1) and Ang-1 secreted from ADSCs are implicated in ADSCs-induced suppression of neointimal formation and stimulation of reendothelialization in a wire injury model of the rat femoral artery[55]. Furthermore, we reported that overexpression of both AM and Ang-1 using adenoviruses expressing those proteins restored erectile function in diabetic rats to the same level as that observed in age-matched positive control rats (Figure 2)[44]. Therefore, it seems obvious that ADSCs produce various cytokines that potentially restore erectile function. The limitation is that ADSCs do not remain in the cavernous body for a long period, usually disappearing within a month[32,33,36]. Where do they go? Do they die or migrate to other tissues? Several interesting papers have been published regarding these points. Lin *et al*[56] injected rat ADSCs and traced their locations after 2 and 7 d. ADSCs remained not only in the penis but also in the major pelvic ganglia (MPG), spleen and bone marrow. ADSCs preferentially remained in the bone marrow and the number of ADSCs remaining in the bone marrow was much larger than that remaining in the penis. Because ADSCs secrete various cytokines, this result suggests that ADSCs remaining outside the penis may affect the restoration of erectile function, although the survival of ADSCs for long periods was not examined in the study. Several

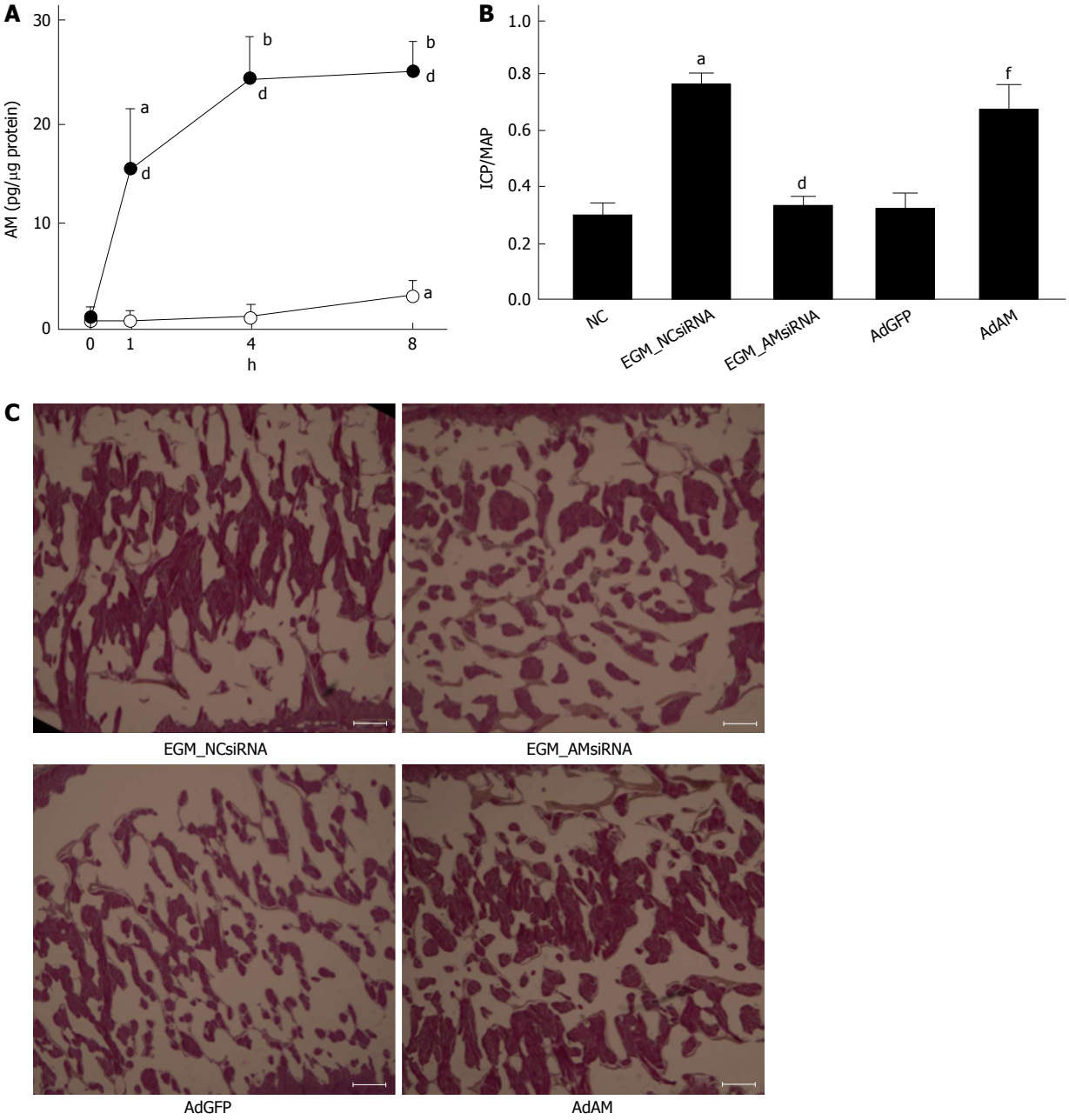


Figure 1 Production of adrenomedullin by adipose tissue-derived stem cells, and effects of knockdown and overexpression of adrenomedullin on the function and histology of the penis. A: Adipose tissue-derived stem cells produce adrenomedullin especially when they were cultured in a medium containing growth factors for vascular endothelial cells (VECs); adipose tissue-derived stem cells (ADSCs) were cultured in endothelial basal medium (EBM: white circles) or endothelial growth medium (EGM: black circles) that contains growth factors for VECs. Medium was replaced with serum-free medium and incubated for the indicated periods. Adrenomedullin (AM) accumulated in the medium was measured. $^{a}P < 0.05$ *vs* 0 h, $^{b}P < 0.01$ *vs* 0 h and $^{d}P < 0.01$ *vs* EBM culture at each time point ($n = 6$ per group); B: Effect of knockdown and overexpression of AM on erectile function. ADSCs were infected with lentivirus expressing negative control siRNA (LV_NCsiRNA) that is predicted not to target any known vertebrate gene or lentivirus expressing AM siRNA (LV_AMsiRNA). ADSCs were cultured in EGM for 1 wk, and those LV_NCsiRNA-infected ADSCs (EGM_NCsiRNA) and LV_AMsiRNA-infected ADSCs (EGM_AMsiRNA) were injected in the cavernous body of STZ-induced diabetic rats. ICP was measured 4 wk after the ADSCs injection. An adenovirus expressing AM (AdAM) or adenovirus expressing green fluorescent protein (AdGFP) was also injected into the cavernous body of STZ-induced diabetic rats, and ICP was measured 4 wk after the infection. Nontreated STZ-injected diabetic rats were used as the negative control (NC). Bar graphs show ICP/MAP ($n = 5$ per group). $^{a}P < 0.001$ *vs* NC, $^{d}P < 0.001$ *vs* EGM_NCsiRNA injection and $^{f}P < 0.001$ *vs* AdGFP infection; C: Effect of knockdown and overexpression of AM on the morphology of the cavernous body. Experiments were performed in the same way as described in the legend for Figure 1B. The cavernous body was stained by the Elastica van Gieson method. The histology of the root portion of the penis (longitudinal section) is shown. Bars are 200 μm. Note that the size of trabeculae of the cavernous body is smaller when EGM_AMsiRNA or AdGFP was injected into the penis of diabetic rats, compared with when EGM_NCsiRNA or AdAM was injected into the penis. ICP: Intracavernous pressure; STZ: Streptozotocin; MAP: Mean arterial pressure.

studies have reported that ADSCs injected into the penis or placed around the prostate gland migrated to the MPG[35,39,45]. Fandel *et al*[35] demonstrated that ADSCs injected into the penis migrated to the MPG, although ADSCs were not engrafted in the nerve tissue. Interestingly, the expression of stromal cell derived factor-1 (SDF-1)

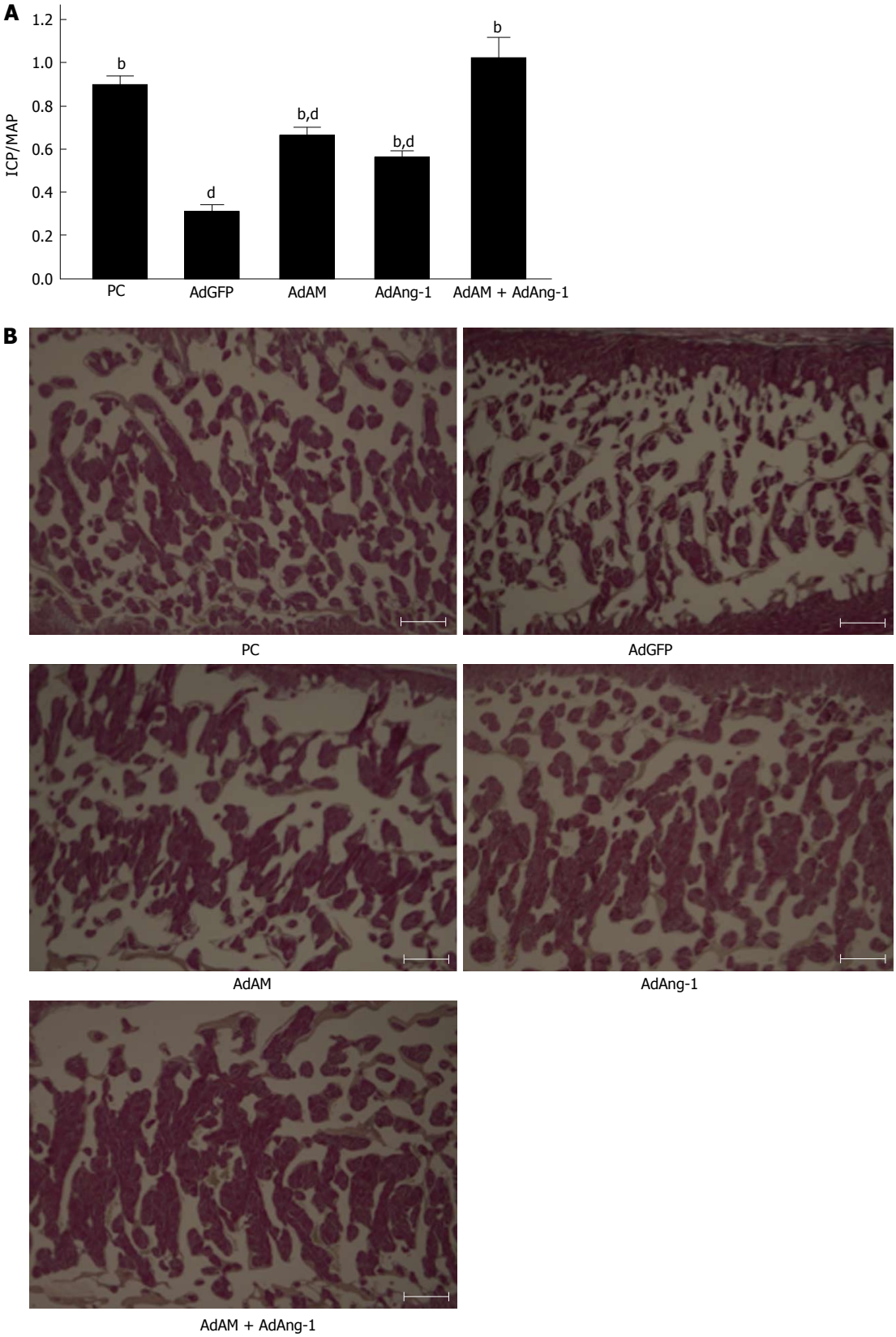


Figure 2 Effects of adrenomedullin and angiopoietin-1 on the function and morphology of the penis. A: Effects of adenovirus expressing adrenomedullin (AdAM), adenovirus expressing angiopoietin-1 (AdAng-1) and AdAM plus AdAng-1 infection on erectile function. These adenoviruses were infected into the cavernous body of STZ-induced diabetic rats. Adenovirus expressing green fluorescent protein (AdGFP) was also infected as the negative control. Age-matched Wistar rats were used as the positive control (PC). Bar graphs demonstrate ICP/MAP (n = 6 per group). $^{b}P < 0.001$ *vs* AdGFP infection and $^{d}P < 0.001$ *vs* PC; B: Histological analysis of the cavernous body after adenoviral infection. Elastica van Gieson staining of the cavernous body isolated from age-matched Wistar rats (PC), and STZ-induced diabetic rats infected with AdGFP, AdAM, AdAng-1, and AdAM plus AdAng-1. The histology of the root portion of the penis (longitudinal section) is shown. Bars are 300 μm. Note that the size of trabeculae of the cavernous body is small when AdGFP is injected into the penis of diabetic rats, and that the size is restored to a similar level as observed in the age-matched control group when AdAM and/or AdAng-1 are injected. ICP: Intracavernous pressure; STZ: Streptozotocin; MAP: Mean arterial pressure.

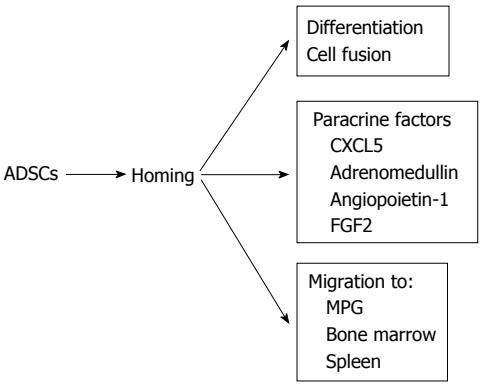


Figure 3 Possible mechanisms by which adipose tissue-derived stem cells stimulate the recovery of erectile function. FGF2: Fibroblast growth factor 2; MPG: Major pelvic ganglia; ADSCs: Adipose tissue-derived stem cells; CXCL5: Chemokine (C-X-C motif) ligand 5.

increased in MPG, suggesting that ADSCs preferentially migrated to the site of SDF-1 production. Qiu *et al*[39] also reported that intracavernously injected ADSCs migrated to the MPG, where they remained 17 wk after injection, although the number of ADSCs remaining in the MPG was quite low. You *et al*[45] showed that periprostatic implantation of ADSCs, but not their intracavernous injection, resulted in the migration of ADSCs to MPG. In summary, some ADSCs injected into the penis migrated to the tissues such as the bone marrow and MPG, and these cells may be implicated in the restoration of erectile function. The possible mechanisms by which ADSCs restore erectile function are summarized in Figure 3.

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) were originally isolated from human peripheral blood by Asahara *et al*[57]. They isolated CD34-positive mononuclear blood cells and demonstrated that these cells obtained the characteristics of VECs when cultured on fibronectin-coated dishes. They also demonstrated that these cells were incorporated in ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model. Furthermore, the authors showed that Flk-1-positive mononuclear blood cells were also integrated in the capillaries and small arteries when the hindlimb ischemia model was used. These cells were designated as EPCs. EPCs are progenitor cells whose differentiation potential is restricted to one lineage (VECs); therefore, they are not multipotent stem cells. Results of subsequent studies revealed that EPCs express three cell surface markers; CD133 (termed originally AC133), CD34, and Flk-1[58-60]. Premature EPCs either in the bone marrow or immediately after entering into the systemic circulation are positive for CD133/CD34/Flk-1. However, when EPCs become more mature, they lose the expression of CD133 and begin to express CD31 and VE-cadherin. An attempt to use EPCs for the treatment of ED has been reported by Gou *et al*[61]. They transfected EPCs with the vascular endothelial growth factor gene and injected the transfected cells into the penis of diabetic rats. The authors found that this treatment restored erectile function and the injected cells were integrated into the sites of neovascularization in the cavernous body[61]. However, most researchers in this field regard EPCs as a marker for ED rather than a therapeutic tool for ED. Factors affecting the number of circulating EPCs or their functions have been reported. The number of circulating EPCs and their migratory activity are reportedly reduced in patients with coronary risk factors[62,63]. EPCs isolated from type 2 diabetes patients have a decreased capacity for proliferation and the formation of capillary tubes *in vitro*[64]. In contrast, the number of circulating EPCs rapidly increases after limb ischemia and acute myocardial infarction[65,66]. Because atherosclerosis and ED share a common feature, *i.e.*, endothelial dysfunction, it has been speculated that the dynamics of EPCs might change in ED. Indeed, several studies have reported that the number of circulating EPCs decreased in ED patients[67-69], suggesting that the decrease in the number of circulating EPCs can predict the presence of ED as well as cardiovascular diseases. Interestingly, the number of circulating EPCs increased when patients were administered statins or PDE5Is[70-73] probably *via* the mobilization of EPCs from the bone marrow. Therefore, these drugs may improve erectile function *via* the mobilization of EPCs to the cavernous body.

Muscle-derived stem cells

Muscle-derived stem cells (MDSCs) are ASCs that exist in skeletal muscle. MDSCs can be obtained from autologous muscle biopsies and have been used for the treatment of ED in several studies. Nolazco *et al*[74] injected MDSCs into the penis of aged rats and found that MDSCs differentiated into VSMCs in the cavernous body, resulting in the recovery of erectile function. Woo *et al*[75] used a bilateral cavernous nerve injury model in rats and examined the effects of the injection of MDSCs into the penis on erectile function. They demonstrated that MDSCs remained in the cavernous body 4 wk after injection, and erectile function was significantly restored. Kovanecz *et al*[76] used a bilateral cavernosal nerve resection model of rats and, following the injection of MDSCs into the cavernous body, found that erectile function was restored and α-smooth muscle actin expression was increased. The injection of MDSCs also increased the expression of neural nitric oxide synthase and brain-derived neurotrophic factor[76]. In summary, MDSCs seem to be useful for the treatment of ED. However, because harvesting MDSCs from the skeletal muscle is relatively more invasive than the collection of ADSCs, the beneficial characteristics of MDSCs compared with ADSCs should be clarified before introducing them to clinical application.

Umbilical cord blood stem cells

Umbilical cord blood stem cells (UCBSCs) are an attractive type of stem cells in that they are youngest stem cells among a variety of ASCs. Because they are young, they have less possibility to have DNA damage than other ASCs[77,78]. Bahk *et al*[79] used human UCBSCs to treat

diabetic patients with ED, and demonstrated that erectile function was restored and that blood glucose levels decreased in these patients, although the mechanisms remain unknown.

Brain-derived stem cells

Brain-derived stem cells (BDSCs) reportedly have capacity to differentiate into VSMCs[80]. Song *et al*[81] isolated fetal BDSCs from embryonal 12-d rats and injected them into the penis. They demonstrated that injected BDSCs obtained characteristics of VSMCs *in vivo* 6 wk after injection[81], although they did not examine their effect on erectile function. Considering the source of BDSCs, it will be difficult to use them in clinical settings.

Neural crest stem cells

Neural crest stem cells (NCSCs) are the progenitor cells of several cell types that constitute the peripheral nervous system, including neurons, Schwann cells, adrenal chromaffin cells and smooth muscle cells. Transplantation of NCSCs could reportedly induce the regeneration of connective tissues, VSMCs, skeletal muscle and VECs[82,83]. Song *et al*[84] injected NCSCs into the penis of rats and demonstrated that they obtained markers for VECs and VSMCs 2 wk after injection, although their effects on erectile function was not analyzed[84]. Clinical application of NCSCs may also be difficult considering the source of these cells.

Treatment of ED using ESCs and iPS cells

Because iPS cells have been created, clinical application of pluripotent stem cells will be intensively explored in the future. To our knowledge, no studies have been published in which iPS cells or iPS cells-derived cells were used to treat ED. Bochinski *et al*[85] used ESCs that had differentiated into the neural cell line, and injected them into the MPG or cavernous body using a bilateral cavernous nerve injury model. They found that the injection into the both MPG and cavernous body restored erectile function. They also found that neurofilament staining was recovered in the ESCs-injected group[85]. Therefore, ESCs and iPS cells may be useful for treatment of ED. However, these cells may not efficiently home and survive for a long period under persistent inflammation. For example, in diabetic conditions hyperglycemia and adipocytokines induce persistent inflammation in the tissues. Implanted cells may not home and survive under these conditions unless such an inflammation is sufficiently controlled.

FUTURE DIRECTIONS

As mentioned above, stem cell therapy for ED appears to be a promising strategy. However, several problems should be solved before moving to clinical application.

Tumorigenesis

It is well known that ESCs and iPS cells easily form tumors, because these cells are pluripotent. Although ASCs seem to be less prone to forming tumors, ASCs can form malignant tumors when transplanted *in vivo*[86]. Jeong *et al*[86] injected BMMSCs into the peri-infarct area of myocardial infaction (MI) model of mice and hindlimb muscle of mice with diabetic neuropathy. They found sarcoma formation in 30% of hearts in the MI model and in 46% of hindlimbs in the diabetic neuropathy model[86]. Therefore, it will be necessary to sufficiently investigate the malignant potential of stem cells prior to their clinical use and establish methodology to select "healthy" stem cells that will not form tumors.

Fate of injected cells

As described above, some (most) of stem cells injected into the penis do not remain in the penis and migrate to other tissues such as the bone marrow and spleen. Little is known about the fate of these cells that have migrated to non-diseased organs. Detailed examinations will be necessary to detect the fate of these cells before moving to clinical applications.

How to improve homing and survival of stem cells

Most studies suggested that injected stem cells disappeared from the penis in one month. It is crucially important to explore methods to improve homing and survival of stem cells. It was reported that expression of SDF-1 was increased in the MPG and SDF-1 might stimulate migration of ADSCs to the MPG[35]. Therefore, SDF-1 is a candidate that stimulates migration and homing of stem cells into injured sites. Intensive studies will be necessary to identify molecules that are implicated in migration, homing and survival of stem cells.

Activation and mobilization of endogenous stem cells

It is suggested from EPC study that statins or PDE5Is can stimulate mobilization of EPCs from the bone marrow[70-73]. Therefore, it may be possible to activate and/or mobilize tissue-residual endogenous stem cells by some drugs. If endogenous stem cells residing in the penis can be efficiently activated, it will help to regenerate the cavernous body. This possibility should be examined in the future.

Identification of paracrine factors

ASCs produce a variety of paracrine factors that potentially regenerate the cavernous body. However, paracrine factors that are implicated in the regeneration of the cavernous body have not been sufficiently identified. Furthermore, it remains unknown what combinations of these paracrine factors are most suitable to stimulate the regeneration of the cavernous body. If these problems are solved, administration of cytokines cocktail may be more effective to treat ED than ASCs injection.

CONCLUSION

Stem cells especially ASCs have been used in the treatment of ED, and stem cell therapy seems to be effective

at least in animal modes. Major, if not all, effects of ASCs on erectile function appear to be achieved by secretion of paracrine factors rather than their direct differentiation into cells residing in the cavernous body. Before moving to clinical application, malignant potential of stem cells should be carefully considered. It is also necessary to explore methods to improve homing and survival of stem cells.

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REVIEW

Postnatal management of antenatally detected hydronephrosis

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Abstract

With the increasing use of ultrasonography, congenital anomalies are often picked *in utero*. Antenatally detected hydronephrosis is amongst the most commonly detected abnormality. The management of this condition has raised considerable debate amongst clinicians dealing with it. This article is written with an idea to provide comprehensive information regarding the postnatal management of antenatally detected hydronephrosis. A detailed review of the current literature on this topic is provided. Also, guidelines have been given to facilitate the management of this condition.

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Key words: Antenatal hydronephrosis; Ultrasonography; Pelvi ureteric junction obstruction; Megaureter; Hydronephrosis; Multicystic dysplastic kidney

Core tip: This article provides practical guidelines for the postnatal management of antenatally detected hydronephrosis.

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INTRODUCTION

The detection of renal abnormalities during prenatal ultrasonography was first reported by Garrett *et al*[1] in 1970. Since then routine use of ultrasonography for detection of congenital anomalies has become a part of routine care during the antenatal period. Currently it is estimated that genitourinary anomalies comprise nearly 20% of all prenatally detected fetal anomalies[2]. Amongst these hydronephrosis is one of the most commonly detected anomalies seen in approximately 1% to 5% of all pregnancies and it occurs due to various causes[3] (Table 1). Thus we have an increasing number of patients who are presenting to the clinician with a presumptive diagnosis rather than a symptom and that too before they are born[4]. Logic dictates that this early detection should help in improving post natal outcomes and help in better preservation of the renal function. Lee *et al*[5] in their meta-analysis found that 12%-88% of these children will have demonstrable pathology depending on the degree of prenatally detected hydronephrosis. Hence a thorough postnatal evaluation of the upper and lower tracts is mandatory postnatally. But this also means that 88%-12% of these children will have no demonstrable pathology postnatally. This is borne out by various studies showing that the most common cause of antenatally detected hydronephrosis is transient or non obstructive dilatation of the pelvicalyceal system[6,7]. Thus, postnatally, the clinician is faced with dilemma to differentiate the hydronephrosis which will resolve spontaneously from the one which will become clinically significant and would need

Table 1 Differential diagnosis of prenatal hydronephrosis

Etiology	Incidence
Transient/physiologic	50%-70%
PUJ obstruction	10%-30%
Vesicoureteral reflux	10%-40%
Ureterovesical junction obstruction	5%-15%
Multicystic dysplastic kidney	2%-5%
Posterior urethral valves	1%-5%
Ureterocele	1%-5%
Others like ectopic ureter, *etc.*	< 1%

PUJ: Pelviureteric junction.

Table 2 Descriptive definition of hydronephrosis by Antero Posterior Diameter

Classification of hydronephrosis	Second trimester APD in mm	Third trimester APD in mm
Mild	4-7	7-9
Moderate	7-10	9-15
Severe	> 10	> 15

APD: Antero posterior diameter.

Table 3 Society of fetal urology grading of hydronephrosis

Grade 1	Urine barely splits the sinus
Grade 2	Moderate renal pelvis splitting confined to renal border with dilated major calyces
Grade 3	Pelvis distended outside the renal border, major and minor calyces are dilated; the parenchyma is spared
Grade 4	Parenchyma is thinned

surgery. This differentiation needs to be done by utilizing appropriate investigations using the lowest radiation and least invasive techniques so that timely surgical intervention can be done, in those who need it, to prevent renal function deterioration[4]. This article reviews the primary literature and consensus statements pertaining to antenatally detected hydronephrosis and sets forth our own recommendations regarding management of infants with this finding.

DIAGNOSING HYDRONEPHROSIS ANTENATALLY

The diagnosis of fetal pelvis dilatation and its natural history postnatally is best understood if we understand that the definition of hydronephrosis has undergone a sea change. Traditionally hydronephrosis was defined as dilatation of the pelvicalyceal system due to partial or complete obstruction. Now clinicians understand that hydronephrosis is aseptic dilatation of the collecting system and it may or may not be associated with obstruction. Investigators have proposed that the term pyelectasis be used to describe dilatation of renal pelvis whereas pyelocaliectasis and hydronephrosis include dilatation of calyces. However, all these three terms are used interchangeably and are used to describe a dilated pelvicalyceal system regardless of its etiology[8].

The antenatal ultrasound screening is most commonly performed at 18-20 wk of gestation. This is the time when the renal architecture becomes visibly distinct. Normally the renal pelvis and calyces are not seen, if seen then it indicates hydronephrosis. The sonologist should be vigilant in the antenatal period to differentiate a dilated collecting system from the hypoechoic sonolucent pyramids which may mimic hydronephrosis. Once the diagnosis of a dilated collecting system is made, it should be objectively described using one of the various classification systems. The majority of authors use either the Antero posterior diameter (APD) system or the Society of Fetal Urology (SFU) classification. With the sophisticated ultrasound machines with better resolution detecting smaller dilatations of the renal pelvis, the cut off value of the renal pelvis dilatation necessitating cognizance and achieving clinical significance has been a matter of debate. In the early 80's a threshold value of 10 mm indicated the need for further investigations in the post natal period[8]. In 1990, Mandell proposed a classification system based on APD and gestational age that helps to categorize antenatal hydronephrosis in the mild, moderate and severe variety[9]. This was further substantiated by the work of Corteville *et al*[10] and should now be taken as a standard classification of antenatal hydronephrosis based on APD[11,12]. A number of other studies have noted persistent postnatal uropathy when the APD measures > 6 mm at < 20 wk, > 8 mm at 20-30 wk and > 10 mm at > 30 wk gestation[13,14]. Recently cut off of 6 mm at 20 wk and 10 mm at 30 wk have been suggested for pyelectasis and an APD cut off of 10 mm at 20 wk and 12 mm at 30 wk for hydronephrosis[15]. An interesting feature of this study has been the effort to separate pyelectasis from hydronephrosis so that postnatal ultrasound can be avoided in a number of patients. However given the subjective nature of sonography and the factors like maternal hydration affecting the measurements, this issue has not been resolved as of today and hence clinicians should follow a standard classification and grade the dilatation as mild, moderate and severe as suggested in Table 2.

Another classification used to describe hydronephrosis is the Society of Fetal Urology classification which was first described in 1993[16]. This system describes the renal pelvis dilatation along with the dilatation of the calyces and hence its effect on the parenchyma (Table 3). However this grading system is not universally followed and due to the ambiguity in inter and intra observer agreement especially in grade 3 and grade 4 hydronephrosis, modifications have been proposed[17,18]. Even these modifications have not gained universal acceptance.

Given these discrepancies, it is imperative that worldwide a uniform system of classifying and grading hydronephrosis should be followed. In order to overcome these variations and negate the effect of hydration and full bladder, group from Hong Kong has proposed a Hy-

dronephrosis Index and have given nomograms to help clinicians judge the degree of renal pelvic dilatation based on the gestational age[19]. The hydronephrosis index is defined by the APD of the fetal kidney divided by the urinary bladder volume. It is an interesting index but its clinical usefulness and specificity in fetuses with abnormally dilated renal pelvis or gross hydronephrosis is not established.

Till a reliable method is described, which overcomes the variables of maternal hydration, bladder fullness of fetuses and the operator dependency, clinicians should mention the method used to diagnose antenatal hydronephrosis and grade its findings. If the APD is used than the presence or absence of associated calyceal dilatation should also be mentioned.

OTHER FINDINGS

Besides the diagnosis of hydronephrosis, the antenatal ultrasonography should document the amniotic fluid level, degree of urinary bladder distension; it's emptying and wall thickness visualization of ureter, presence of normal or any abnormality in the opposite kidney and the echogenicity of the kidneys. These additional findings often contribute to establishing the postnatal diagnosis[20]. In cases of posterior urethral valves the level of amniotic fluid is a significant predictor of renal function and clinical outcome[21].

TIMING AND FREQUENCY OF ANTENATAL ULTRASOUND

Currently, there is no agreed upon protocol for the antenatal evaluation and its follow up. The first anomaly scan is done usually between 18-20 wk, this should reliably diagnose antenatal hydronephrosis. The subsequent frequency of follow up ultrasound is often based on the severity of findings and the pathology suspected. There is usually no added advantage of doing very frequent ultrasound examinations for it adds very little to the diagnosis and subsequent management and only aggravates parental anxiety. Once the diagnosis is made then the next ultrasonography can be done in the third trimester between 28-32 wk. However more frequent ultrasounds, every 4-6 wk, will be needed in cases having bilateral hydronephrosis, posterior urethral valves, prune belly syndrome and severe hydronephrosis in a solitary kidney. Ultrasound findings in these clinical scenarios have an important bearing on the decision making in deciding the obstetric course of the patient.

ANTENATAL COUNSELLING

Once the diagnosis of ante natal hydronephrosis (ANH) is made, the parents are engulfed by a myriad of emotions. The two important things that the clinician is often asked to answer is-Should antenatal intervention be done and what happens postnatally. Addressing the parental anxiety and concerns is as important as the clinical management of the child.

Prenatal intervention

Studies have shown that urinary obstruction can cause renal dysplasia and relief of that obstruction can prevent dysplasia if performed early enough[22]. The goal of fetal intervention would be to relieve this obstruction and allow for normal renal development. This in turn would maintain the amniotic fluid levels to allow for normal lung development. Currently, fetal intervention is recommended for those with documented lower tract obstruction, the commonest and most widely studied being posterior urethral valves, where intervention would significantly benefit the overall fetal (and its renal function) prognosis. Open fetal surgery, vesico-amniotic shunt, renal pelvis aspiration, vesicocentesis, fetoscopic fulguration of posterior urethral valves etc have been tried. Though this sounds fascinating, its attendant problems and risks cannot be overlooked. Also does it alter the prognosis significantly and does the benefit outweigh the risks should be evaluated diligently.

At present fetal intervention is indicated in cases where the life of the fetus is at risk, typically a second trimester fetus with significant oligohydramnios, suspected good renal function and absence of other life threatening anomalies[21]. However, this is often too late to prevent renal dysplasia. The procedure is associated with significant risk of infection and also significant fetal and maternal morbidity and fetal mortality[23,24]. Thus, except in a select few cases fetal intervention should not be done and even these cases should be done in centres where the necessary expertise and experience is available.

What happens postnatally?

When the diagnosis of ANH is made the parents often have apprehension that the child will need surgery postnatally[25]. They need to be assured and often a session of counseling with the pediatric urologist who will be taking care of the child postnatally goes a long way to allay the apprehensions of the parents. What should be emphasized that though there is no cause for alarm in majority of cases, a proper and rigorous follow up is a norm in majority of the cases.

POST NATAL MANAGEMENT

"A perfection of means but a confusion of conclusion seems to be our problem-Albert Einstein". There is no ambiguity regarding that all antenatally detected hydronephrosis should be evaluated by an ultrasound postnatally[5] (Level I evidence Grade A recommendation). Since infants are relatively dehydrated at birth, the initial postnatal ultrasonography should be performed after 48 h of birth. Day two of life is preferred to enable adequate hydration after delivery but circumstances pertaining to early discharge following delivery may not allow this. Also breast fed neonates may not be adequately

Table 4 Measures to be taken within first 48 h after birth in infants diagnosed with antenatal hydronephrosis

USG	Suspected lower tract obstruction, *e.g.*, Posterior urethral valves, prune belly syndrome Bilateral hydronephrosis with or without hydroureter Solitary kidney with APD > 15 mm or SFU grade 2 or more
Antibiotic prophylaxis	Suspected lower tract obstruction APD > 10 mm or SFU grade 2 or more in the third trimester Solitary kidney with hydronephrosis of any grade Bilateral hydronephrosis
VCUG	Suspected posterior urethral valves antenatally
Catheterization	Suspected lower tract obstruction-posterior urethral valve or prune belly syndrome

VCUG: Voiding cysto urethrogram; APD: Antero Posterior Diameter; SFU: Society of Fetal Urology; USG: Ultrasonography.

hydrated until a steady milk flow is established. Hence the first postnatal ultrasound is preferably done between 5-7 d after birth[12,26,27]. The exceptions to this caveat are: (1) Suspected lower tract obstruction *e.g.*, Posterior urethral valves; (2) Severe bilateral hydronephrosis with or without hydroureter; and (3) Solitary kidney with hydronephrosis especially if the APD is > 15 mm or it is SFU grade 2 or more in the third trimester. Early sonography in these situations has obvious bearing on further management.

SHOULD CHEMOPROPHYLAXIS BE STARTED IMMEDIATE POSTNATALLY?

Whenever there is hydronephrosis the treating clinician is worried about two things-obstruction and infection. The obstruction needs to be established in most cases with ANH. However the clinician is worried about the possibility of infection in a dilated system with stasis of urine. So, should neonates and infants with ANH be put on antibiotic prophylaxis? Till date there are no prospective studies providing level I evidence to support the use of prophylaxis. The available literature is conflicting. Studies have shown that the risk of infection increases with the degree of hydronephrosis[28-31]. Coelho *et al*[28] found the incidence of urinary tract infection (UTI) to be 10% for those with mild hydronephrosis, 20% for those with moderate and 40% for those with severe hydronephrosis. Girls appear to be at greater risk than boys[29]. However these studies are observational in nature and not standardized as regards, the method of urine collection, definition of infection, selection of patients for voiding cystourethrogram and use of prophylactic antibiotics.

More and more data is coming regarding the limited usefulness of prophylactic antibiotics and with the varying practice patterns due to variations in geographic location, clinician experience and above all variable health care practices in developing countries, as of yet, no standardized uniform guidelines have been proposed. However undeniably patients with mild hydronephrosis are at much less risk of infection as compared to those with moderate to severe hydronephrosis[12,32-36]. If prophylaxis is started than the choice of antibiotics are Amoxicillin (15 mg/kg) or Cephalexin (2 mg/kg). Based on the available evidence we propose the following to be done within the first 48 h after birth in neonates born with antenatally diagnosed hydronephrosis (Table 4).

ULTRASOUND AT 5-7 D AFTER BIRTH

All infants detected to have ANH should be evaluated by a postnatal ultrasound, which is usually done at 5-7 d after birth[11,37] (for the reasons described above). The following should be the aim of doing this evaluation using a tool which is easily available, provides good anatomical information, is non invasive and is not associated with any radiation: (1) Confirm the presence of hydronephrosis; (2) Grade the degree of hydronephrosis; (3) Plan further tests and evaluation and management strategies based on the ultrasound findings; and (4) Decide the need for antibiotic prophylaxis.

The ultrasonography should be done with the baby being well fed. It is the practice of one of the authors (Sharma A) to start the examination of these babies by scanning the bladder first. If the bladder is full, usually the baby voids and the degree of bladder emptying is known immediately giving a fair idea regarding the absence of outflow obstruction. Also once the bladder is empty, the effect of a distended bladder on the filling and emptying of the collecting system resulting in fallacious diagnosis of pyelectasis is avoided. The mechanism by which a full bladder causes dilatation of the renal pelvis and the maximal degree of normal dilatation is not known. However it is accepted that when urinary bladder is distended than false positive cases may occur[19]. Hence if a sonologist sees mild degree of hydronephrosis than whether it persists or disappears after bladder emptying should be looked for and mentioned in the report.

Ultrasonography at 5-7 d would show one of the following scenarios: (1) No hydronephrosis-Normal pelvicalyceal system; (2) Unilateral hydronephrosis; (3) Bilateral hydronephrosis; (4) Unilateral Hydronephrosis with hydroureter; and (5) Bilateral hydronephrosis with bilateral hydroureter. Let us see each scenario and discuss its management.

NO HYDRONEPHROSIS-NORMAL KIDNEYS

Postnatal ultrasound will be normal in 41%-88% of cases diagnosed to have hydronephrosis antenatally[10,11,38]. Why this happens is a matter of speculation. Constantinou[39] suggested that a pacemaker in the renal pelvis activates the smooth muscle of the renal pelvis to initiate peristaltic contractions. The direction of the peristalsis is from the renal calyces and pelvis towards the urinary bladder. Any immaturity of the pacemaker in the renal

Table 5 Management recommendations in neonates with antenatal hydronephrosis but Normal Post natal ultrasound

USG	At 1 mo and at 3-6 mo
VCUG	Not recommended if two USG are normal
Antibiotic prophylaxis	Not recommended routinely Would be prudent to be started if the follow up is not reliable For those not getting prophylaxis, parents should be told to get a urine routine if the neonate shows any signs of not being well

VCUG: Voiding cysto urethrogram; USG: Ultrasonography.

pelvis might lead to poor co-ordination of the peristaltic activity[40-42]. Thus there is impediment of the emptying of the renal pelvis resulting in urinary stasis in the renal pelvis. Also, disco-ordination of muscle cell excitation can spread in any direction so that retrograde peristalsis can occur[43]. It has been speculated by Leung *et al*[19] that the pacemaker in the renal pelvis does not mature at an early gestational age. Maturation of this pacemaker and ureteral peristalsis starts around 28 wk of gestation, after which equilibrium is gradually established between pelvicalyceal filling and bladder filling/emptying in the fetus. This probably explains the disappearing fetal hydronephrosis postnatally when the physiological function of the urinary tract becomes more mature.

Even if the first postnatal scan does not show hydronephrosis a repeat scan at 3-6 mo is mandatory. If the scans, on both occasions, do not show hydronephrosis, than a diagnosis of transient hydronephrosis can be safely and surely made. Emphasizing the need for a second scan is of paramount importance as late worsening or recurrent hydronephrosis is seen in nearly 15% of infants[44-46].

These infants have a 25% incidence of associated vesico ureteral reflux (VUR)[47]. Hence some investigators have proposed antibiotic prophylaxis and a Voiding Cysto Urethrogram (VCUG) study in these patients[48-51]. The objections to VCUG being performed in all cases has been based on the feeling that it is not an entirely benign procedure due to its invasive nature, radiation exposure, expense and up to 15% rate of post procedure urinary tract infection[52]. Also majority of the VUR in this category of patients would be low grade with a high chance of resolution of spontaneously. Ismaili *et al*[53] found that if two successive ultrasonography were normal than VCUG was not justified. Two recent studies have also shown that routine VCUG and antibiotic prophylaxis are not to be recommended in these patients who are at low risk of infection[6,54,55]. But as those cases where the follow up is unlikely to be very rigorous and methodical as occurs in the low socio economic group especially in developing countries, advising prophylactic antibiotics would be a natural extension of the logic to make attempts to prevent renal damage.

We propose the following recommendations based on the presently available literature in this category of patients (Table 5).

UNILATERAL HYDRONEPHROSIS BUT NO HYDROURETER

This constitutes the largest category of patients with prenatally detected hydronephrosis. 50%-70% of these would have transient or physiologic hydronephrosis which regresses over a period of time and has no clinical implications; pelviureteric Junction (PUJ) obstruction accounts for the remaining 30%-50% of cases[56,57].

The following questions need to be addressed when these patients are being evaluated: (1) When and how to evaluate them initially? (2) How to do follow up? (3) When to do a functional study? (4) How to differentiate non obstructed from obstructed systems? (5) How long to follow them? and (6) When to Intervene?

When and how to evaluate Initially?

There is no ambiguity regarding the fact the first evaluation should be on the 5th to 7th day after birth and is by ultrasound. However the agreement ends here. How to grade hydronephrosis has been a matter of much debate. The landmark study by Dhillon *et al*[58] in 1998, proposed the measurement of APD of the renal pelvis as a means of judging the severity of hydronephrosis and predicting the need for surgery. While the APD measurement provides an objective means of predicting pathology, many felt that other features are also important in determining the severity of hydronephrosis. Therefore, features such as calyceal dilatation and parenchymal thinning should also be considered in grading the severity of hydronephrosis. These factors were taken into account by the Society of Fetal Urology and a grading system for hydronephrosis was proposed[3]. This is a five point severity stratification system which also helps in predicting the need for surgical intervention[59]. Although SFU is a useful system, two alternative grading systems have been proposed. Sibai *et al*[60] proposed sub classifying SFU grade 4 into Grade 4 A-with segmental cortical thinning and Grade 4 B-diffuse cortical thinning. Onen[61] proposed subcategorizing patients with SFU grade 4 into those kidneys with mild to moderate *vs* severe parenchymal compromise to account for the underestimation of disease severity in patients with intra renal pelvic configuration.

Whatever system is followed, after the first postnatal ultrasound, the clinician should be able to categorize these patients in the mild, moderate and severe hydronephrosis categories so that further management can be decided[12]. The categorization of this category of patients in Mild, Moderate and Severe types, based on APD and SFU grading is given in Table 6. After the Initial Ultrasound at 5-7 d after birth the next follow up ultrasound should be done at 4 wk.

How to follow up?

The important questions to be answered during follow up of these infants are: (1) Do they need prophylactic antibiotics; (2) Do they need VCUG; and (3) When to

Table 6 Categorization of patients with unilateral hydronephrosis with no hydroureter into mild, moderate and severe hydronephrosis based on Antero Posterior Diameter /Society of Fetal Urology Grading

	Mild	Moderate	Severe
APD	< 20 mm	20-30 mm	> 30 mm
SFU	Grade 1 and 2	Grade 3	Grade 4

APD: Antero posterior diameter; SFU: Society of Fetal Urology.

repeat ultrasound?

Do they need prophylactic antibiotics

Regardless of gender prophylactic antibiotics are not recommended for patients with mild degree of hydronephrosis because of the low risk of developing a urinary tract infection or need for subsequent surgery[6,28,35]. But chemoprophylaxis is indicated in those with moderate or severe degree of hydronephrosis till VCUG is done.

Do they need VCUG

Patients with mild degree of hydronephrosis do not need VCUG. Though a small subset will have associated VUR, majority of the times it is a low grade VUR which subsides on its own[47]. However those with moderate to severe hydronephrosis need a VCUG. VUR would be diagnosed in about 20% of these patients[62,63].

The timing of VCUG in this group of patients should be at 4-6 wk. These patients are on prophylactic antibiotics, hence to diagnose or rule out VUR, it would be prudent to wait till the neonate is old enough. Whether it should be a conventional VCUG or a radionuclide cystogram is a matter of personal preference and debate. A conventional VCUG would not only diagnose lower grade of VUR but would also exclude the possibility of posterior urethral valve, which can present indolently[64].

It is recommended that if no reflux is seen then chemoprophylaxis can be stopped unless it is a solitary kidney (to avoid the slightest chance of infection affecting a solitary renal unit). In those with VUR chemoprophylaxis should be continued[12,65].

When to repeat ultrasound?

Irrespective of the grade of hydronephrosis a repeat ultrasound is warranted at 4 wk after birth. It confirms the severity of hydronephrosis and also gives an insight into progression/regression of hydronephrosis. Also the hydronephrosis can be categorized into mild, moderate and severe type again. Changes in the severity of hydronephrosis can occur as kidneys mature and/or signs of obstruction manifest[12].

If there is mild hydronephrosis (APD < 20 mm or SFU Grade 1 or 2) and for moderate hydronephrosis (APD 20-30 mm or SFU Grade 3)-confirmed at 1 mo a repeat ultrasound is indicated at 3 mo and then six monthly till the age of 3 years and then yearly till the age of six years. Whenever the sonography shows resolution of hydronephrosis a repeat ultrasonography at 3-6 mo is warranted to confirm the finding as recurrence is noted in previously resolved hydronephrosis[34].

For severe grade of hydronephrosis (APD > 30 mm or SFU Grade 4)-ultrasound at 1 mo confirms the findings and then further sonography is done based on the need for intervention. If conservative management is opted (in cases with differential function > 40%) then ultrasonography should be done at monthly intervals for 3 mo, then bimonthly till the age of 1 year. Any sign of increasing hydronephrosis would warrant intervention or a further radionuclide study to determine the need for intervention.

When to do a functional study?

A diuretic renogram is indicated in those with severe degree of hydronephrosis at 4 wk after birth. All other patients can be followed with ultrasound as mentioned above, with a radionuclide study done when there are signs of increasing hydronephrosis. The functional evaluation should be by mercapto acetyl triglycine (MAG3) or ethyl cysteine (EC) Renogram using a F-15 or F0 protocol. Due to lack of maturity of the kidneys and a very high background activity resulting in erroneous calculation of differential function a DTPA renogram should be avoided in the first 6 mo of life[66-68].

How long to follow them?

If there is no increasing hydronephrosis on serial ultrasounds then also the child needs to be followed up till the age of 6 years. A stable dilated system at 6 years would not warrant further study except around puberty when it would be worthwhile having a look at the kidneys by ultrasound to rule out any deterioration of hydronephrosis with the spurt in growth that occurs. This area, where the dilatation has stabilized in the early childhood, has hitherto not been investigated thoroughly and further studies are awaited to look into the fate of those kidneys later in life. A radionuclide study at 6 years, before stopping follow up, would be useful to confirm the good functional status of the kidney and establish a baseline value for further comparison in future.

When to intervene?

Patients with unilateral hydronephrosis is the category where the clinician faces the biggest dilemma of differentiating a non obstructed dilated system, where hydronephrosis will regress spontaneously over a period of time (or remain stable) from a dilated but obstructed system. This needs to be diagnosed as early as possible so that intervention can be done before renal damage occurs. The dilemma gains much significance as the clinician has to choose between conservative approach *vs* surgery. Till an answer to this dilemma is obtained, there is much parental anxiety, as the need for surgery hangs like a sword of Damocles on the head of the patient.

Two modalities have been extensively studied to provide an answer to this vexing question-Ultrasonography

and Radionuclide studies. Though a diuretic renogram has been considered the gold standard to diagnose obstruction, numbers of studies have questioned its ability, especially in the way it is done at present, to diagnose obstruction and more importantly diagnose cases which will need surgical intervention. Hafez *et al*[69] in 2002 had shown that drainage curves from the initial renogram are not always predictive of cases which need surgical intervention. For many years the output function (drainage) has been empirically estimated on the basis of the slope of the frusemide curve, a T half > 15-20 min reflecting an obstruction and a short T half excluding obstruction[70]. This method is still largely used many urologists. Of course a good renal emptying practically excludes any significant risk factor related to obstruction; but what should be the conclusion when the T half is high? If the function has been compromised then the diagnosis of obstruction is beyond doubt. But can the diagnosis of obstruction be made based on the curve pattern in the presence of well preserved renal function, the answer is probably not. The major pitfall in this interpretation is what has been called the "reservoir function". When there is a dilated system, the tracer, even under the influence of frusemide has to fill the renal pelvis before leaving the kidney, even if there is no significant restriction to urinary flow. Thus, despite all technical precautions, one can end up the test with no or limited renal pelvis emptying, simply due to this reservoir effect[66-68]. It is therefore not acceptable to conclude that the kidney is obstructed simply because of poor drainage. It has been shown during longitudinal conservative follow up of these children that the drainage might improve considerably and spontaneously, sometimes after several years[71]. Newer parameters like output efficiency and Normalized Residual Activity have been evaluated and are found to be more reliable[66-68]. However they have not gained universal acceptance and also there values have not been standardized. Two new parameters which have shown promise in differentiating an obstructed from a non obstructed system are-Post micturition and post erect images acquired 1 h after tracer injection[66-68] and the cortical transit time[72,73]. The post micturition post erect images taken at 60 min showing retained tracer are more indicative of poor drainage and obstruction then the post frusemide curves. They can be taken easily and even an infant can be held in the arms of the parent to obtain the post erect images. However this also has not been incorporated in routine diuretic renogram all over the world.

Cortical Transit time has shown promise to identify those renal units which are at risk of deterioration of renal function due to obstruction. It is the passage of the tracer from the outer cortex to the inner structures *i.e.*, the medulla and collecting system. In a normal kidney one expects a rapid transit with more or less homogenous kidney filling in about 2 min. A delay in this suggests obstruction. It has also been found that kidneys with delayed cortical transit times are not only at high risk of deterioration of renal function, but also show good post operative improvement in 80% of cases[72,73]. However, 20%-30% of these kidneys would not show good recovery of function. Also a large prospective study is needed to confirm that cortical transit time is the best predictor of which children should be operated upon[67,68]. Till one of these parameters are universally accepted, the current practice of getting a well tempered renogram, lacking sensitivity and specificity, serves only to get the differential renal function and is a poor man's Di Mercapto Succinic Acid Scan[36,74]. The decision to operate is simple when the differential renal function is < 40%. But the dilemma persists in kidneys with function > 40%.

It is imperative to make this differentiation between a dilated but non obstructed system form an obstructed system at an optimum period of time as delay in his can lead to irreversible damage to the obstructed kidneys and intervention later may not lead to partial/complete recovery of the lost function. When the differential function is > 40%, the infant is under observation. Ultrasound is the most universally accepted, non invasive and non ionizing tool used to evaluate these children during this period of observation. Investigators have studied whether ultrasonography can be used to predict the need for surgery. Dhillon[58], from the Great Ormond street group was the first to describe the predictive value of anteroposterior diameter of the renal pelvis for determining the need for pyeloplasty. This landmark study found that amongst those with an APD > 40 mm, 80% needed surgery; while those with an APD between 30-40 mm; 55% needed surgery and no intervention was needed in those with an APD < 12 mm. However, mere APD cannot definitively predict the need for surgery as a good number of patients with an APD between 20-40 mm did not need surgery. Burgu *et al*[75] also found that an APD of, 20 mm correlated with persistence of differential renal function and that stable or decreased APD on serial imaging was also predictive of retained or improved function. Other investigators have used renal parenchymal area, calyx to parenchymal ratio and pelvis cortex ratio and hydronephrosis index to evaluate the hydronephrosis postnatally and predict the need for surgery[76,77]. However these parameters have not found widespread acceptance.

Recently, an interesting observation has been published by Sharma *et al*[78], demonstrating the utility of comparing APD measurements in patients with unilateral hydronephrosis in supine and prone positions. They found that in those cases where the APD decreases in prone position by > 10% as compared to supine position, the hydronephrosis decreases over a period of time or does not increase, resulting in preserved differential function. These cases did not need surgery. In contrast, if the APD does not change in prone position or increases in prone position then these cases needed surgical intervention as their differential function showed a substantial drop[78]. This small study of 39 patients from a single center is based on the simple principle that the pelvicalyceal system drains better in prone position, hence the obstructed systems would not show better drainage and

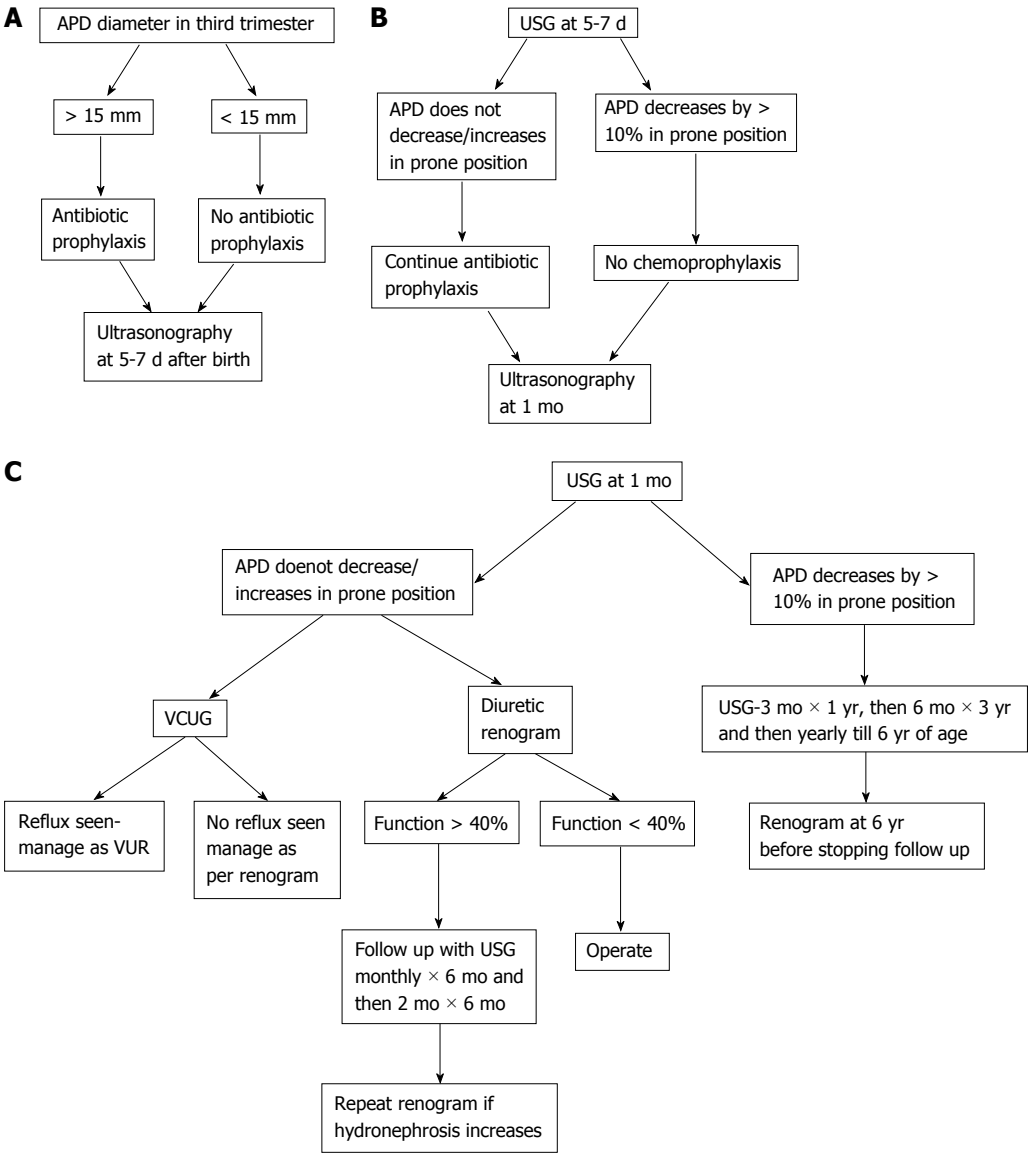


Figure 1 Proposed management plan for patients with Unilateral Hydronephrosis with no hydroureter (A-C). APD: Antero Posterior Diameter; VCUG: Voiding cysto urethrogram; USG: Ultrasonography; VUR: Vesico ureteral reflux.

the APD would remain the same or increase in prone position as the urine from the different calyces pools in the pelvis. If it is a dilated but non obstructed system that in the dependent prone position there would be better drainage and the APD would decrease in prone position as compared to supine position. These measurements of course would have to be done with an empty bladder as a full bladder interferes with the drainage form the pelvicalyceal system. At present, this seems to be the simplest way of differentiating a dilated but non obstructed system from a dilated and obstructed system. Also, variables like the degree of hydration would not affect the conclusion drawn.

The aim of evaluation by noninvasive and inexpensive modality like ultrasonography is to diagnose those patients at risk of deterioration of renal function, differentiate them from those who would do well in the long run and help in judiciously utilizing renogram to intervene at the optimum moment before renal function is affected. We propose the following algorithm to manage these patients with unilateral hydronephrosis (Figure 1).

BILATERAL HYDRONEPHROSIS

Infants with bilateral hydronephrosis are at an increased risk of infection compared to children with unilateral hydronephrosis. The risk of renal function deterioration is high in this group[36]. In this group of patients difference in differential function on renogram is not a reliable way of predicting the need for surgery as both the renal units may have deterioration of function which would not be reflected in the percentage difference in function. Literature is sparse in providing guidelines to manage this group of patients. We propose the following algorithm to manage these patients (Figure 2). This logical proposal is based on the literature available for unilateral hydrone-

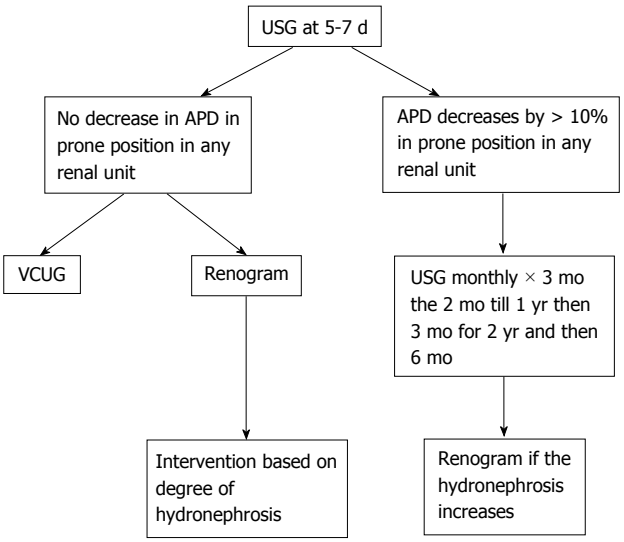


Figure 2 Management of patients with bilateral hydronephrosis with no hydroureter. APD: Antero Posterior Diameter; VCUG: Voiding cysto urethrogram; USG: Ultrasonography.

phrosis and needs to be substantiated by a larger multicenter study.

UNILATERAL HYDRONEPHROSIS WITH UNILATERAL HYDROURETER

This is the group of patients who has a megaureter, the nature of which needs to be ascertained. Literature in this group of patients is quite clear regarding the following points in their management: (1) Definition of megaureter-retrovesical ureteric diameter > 7 mm from 30 wk gestation onwards is taken as megaureter[79]; (2) Antibiotic prophylaxis-is recommended for the first 6-12 mo of life[80,81] as the risk of UTI is higher with uretero vesical junction obstruction than with PUJ obstruction; (3) VCUG-an early VCUG is recommended as 14% of these patients may have an associated posterior urethral valves[82]. VCUG not only would rule out bladder outflow obstruction but also would confirm or rule our reflux and thus define further course of management; (4) Renogram-based on the data from Great Ormond Street a Diuretic renogram is indicated using MAG3 or EC in patients with ureteric dilatation > 10 mm[83]; and (5) Defining obstruction-Interpretation of renogram in the presence of a dilated ureter may be difficult, as delayed transit may be caused by an increased capacity of the dilated ureter and pelvis. Poor drainage is also apparent because the bladder is full and the effect of gravity is incomplete[84]. For these reasons interpretation of the wash out curves should be made in the light of differential renal function and the degree of renal pelvis dilatation. An initial differential renal function of < 40% or a drop in function by > 5% on serial scans is taken as significant. On the other hand delayed transit on diuretic renogram in the presence of stable or improving dilatation and a differential function above 40% in an asymptomatic patient are not strong indicators of obstruction. A close follow up with serial ultrasounds is recommended in this group[85].

BILATERAL HYDRONEPHROSIS WITH BILATERAL HYDROURETER

Most of these cases are associated with Bladder outflow obstruction and/or bilateral reflux. The following recommendations are just an extension of the rationale thinking based on the now standardized protocol in the management of unilateral hydronephrosis with hydroureter: (1) Antibiotic prophylaxis-recommended; (2) VCUG-to be done at the earliest under antibiotic cover to confirm or rule out posterior urethral valves; (3) Renogram-to be done using MAG3 or EC within the first 4 wk of life, in cases of bilateral megaureter(not refluxing and not associated with posterior urethral valves); and (4) Definition of obstruction-Differential renal function should be interpreted in clinical context, since values within normal range will be seen when there is bilateral renal damage and/or in the presence of chronic renal failure.

CONCLUSION

Postnatal management of prenatally detected hydronephrosis is a topic which has evoked widespread interest. The issue which remains ambiguous at present and is the area of much study and research is how to differentiate a dilated but non obstructed system from a dilated and obstructed system. The utilization of sonography and the acceptance of parameters like output efficiency normalized residual activity and cortical transit time on renogram would be able to provide definitive answers to this dilemma in the near future.

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REVIEW

Periodontitis: Tip of the iceberg in chronic kidney disease

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Abstract

The prevalence of chronic kidney disease (CKD) is constantly escalating not only in industrialized countries but throughout the world. It is of major significance because of to its high morbidity and mortality. Strategies to tackle this worldwide health problem include identification of its associated risk factors, comorbidities, and complications as well as proper management to handle all the pertinent issues. Periodontal disease, a treatable infectious state of the dental supporting tissues, is common in CKD patients. Its association with CKD is believed to be in a reciprocal or bidirectional fashion and has been massively studied. This paper, therefore, aims to review the recent evidence pertaining to the association between periodontal disease and a variety of renal illnesses. Most of the current evidence was collected from cross-sectional studies and clinical trials. There is substantial evidence indicating that periodontal disease contributes markedly to the chronic systemic inflammatory burden, leading to cardiovascular and cerebrovascular complications, the principal causes of death among chronic renal disease patients. Furthermore, several studies demonstrated that proper periodontal intervention could help improve systemic inflammation and even nutritional status among CKD patients, resulting in a better quality of life. Suggestions have been made that periodontal disease should be diagnosed early, and managed and controlled to, at least, eradicate a source of inflammation in this population. Awareness of such an important issue should be increased in the relevant medical personnel.

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Key words: Periodontitis; Chronic kidney disease; Dialysis; Kidney transplantation; Inflammation

Core tip: Periodontitis is gaining extensive public recognition due to its devastating impact on systemic diseases. Its association with chronic kidney disease is believed to be in a reciprocal or bidirectional fashion and has been extensively studied. In this review article, careful selection of involved studies was performed. This paper, thus, illustrates both the supporting and conflicting results of current publications pertaining to the association of periodontal disease and a variety of renal illnesses including glomerular diseases, and dialysis and kidney transplant populations.

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INTRODUCTION

Chronic kidney disease (CKD) is recognized as a significant global public health issue[1,2] because it leads to high morbidity and mortality[3,4]. The prevalence of CKD is progressively increasing[5] across the world[6,7]. Globally, CKD affects more than 50 million people[8] and more than 1 million of these undergo renal replacement therapy (RRT)[9]. CKD poses extensive burdens not only on the inflicted individuals particularly in terms of quality of life, but also on society as a whole in terms of medical care and subsequent costs. Many developed nations spend more than 2%-3% of their annual health-care budget to

provide treatment for end-stage renal disease (ESRD)[10]. For instance, a study in the United States revealed that the total management cost of CKD alone was $8000 per patient. In addition, if CKD-related comorbidities existed, the cost of care increased to $14000[11]. In 2007, the United States Medicare expenditures on CKD patients exceeded $60 billion, representing 27% of the total Medicare budget[10]. Likewise, in England, the National Health Service (NHS) disclosed that the annual cost of medical care for kidney disease was about the same as those for American patients. The estimated cost of English CKD patients was about one-third of all NHS expenditure and more than a half of the sum was spent on RRT[12].

As a result, it is important that all modifiable risk factors for CKD be identified and controlled such as blood pressure, blood glucose level, and calcium or phosphorus level[3]. Moreover, there is a significant non-traditional risk factor, chronic systemic inflammation, and its source should be identified and treated.

Periodontal disease (PD), a treatable infection of the oral cavity, is gaining more attention nowadays. PD *per se* serves as a "source of social inequality, reduced quality of life, reduced chewing function, esthetic impairment, tooth loss and disability"[13]. In relation to other diseases, a large number of studies have been carried out to demonstrate its association with numerous systemic diseases such as cardiovascular disease[13], CKD[5,14,15], ESRD[16], glomerulonephritis (GN)[17], diabetes mellitus (DM)[18], rheumatoid arthritis[15,19], chronic obstructive pulmonary disease, cognitive impairment, obesity and cancer[15].

In relation to CKD, studies have shown an increased rate of PD in this population[20], which probably results from the state of inflammation and malnutrition. The observed prevalence of PD in CKD is globally elevated and is noted as the fourth most costly disease to manage in developed countries[21]. Several studies demonstrated that CKD patients suffered more from PD than healthy subjects. For instance, one study revealed a 100% prevalence of mild to moderate gingivitis in CKD subjects compared with 85% in the general population[22]. Similarly, an investigation in the United States by the Third National Health and Nutrition Survey Ⅲ disclosed an increased prevalence of moderate periodontitis (14.6%) among CKD individuals in comparison with the control group (8.7%)[22,23].

A number of retrospective and prospective studies have also shown a marked association between periodontitis and mortality in CKD subjects, even after adjustment for various confounders such as age and smoking. The studies revealed a notable increase in mortality rate of CKD patients with periodontitis of particularly moderate-to-severe degree[20,24]. Because of the above-mentioned significance of PD, this paper aims to review the recent evidences pertaining to the close association between PD and CKD.

Definitions

CKD is defined as a condition where there is persistent kidney damage along with progressive and irreversible renal function loss[25-27] through such mechanisms as "renal hyperfiltration, increased intra-glomerular pressure, arterial hypertension, renin-angiotensin system activation, proteinuria and renal ischemia"[26]. Diagnostic criteria for CKD involve either the presence of reduced glomerular filtration rate (GFR) or markers of renal damage such as albuminuria, abnormal urine sediment, electrolytes or structure and histology for 3 or more months, and a history of kidney transplantation (KT)[28].

The National Kidney Foundation's Kidney Disease Outcome Quality Initiative classified CKD into 5 stages according to the severity of the condition. Thus, microalbuminuria represents the degree of renal damage and the measured GFR signifies the reduced renal function[29]. Simply put, CKD is classified based on Cause, GFR category, and Albuminuria category (referred to as CGA)[28].

PD is a group of infectious diseases affecting the dental supporting tissues[29-31], and is characterized by progressive destruction of the tooth supporting apparatus[13,15,32]. Commonly noted signs of PD comprise "gum tenderness, gum bleeding, gum recession, alveolar bone loss, tooth mobility, and tooth loss"[33].

PDs are derived from the microflora formed in the biofilm or dental plaque, adhering to the teeth. Significantly, periodontal pathogens are capable of invading the dental superficial tissue (gingiva) to the deeper structures (bone and ligaments)[16,30,31]. They contribute to the chronic systemic inflammatory burden by causing both local infection and disseminating into the bloodstream, leading to bacteremia[13,29]. An inflammatory cascade is then activated, as clearly seen by an elevation of inflammatory mediators, particularly C-reactive protein (CRP) and interleukin-6 (IL-6)[5,18,29], which adhere to and proliferate in the coronary endothelial cells, resulting in formation, maturation and exacerbation of atheroma, platelet aggregation and impairment of vascular relaxation[13,14,34]. These mechanisms eventually result in atherosclerotic complications, the primary cause of high morbidity and mortality among the CKD population[5,33].

Classification of PD encompasses plaque-induced gingivitis, and chronic and aggressive periodontitis. Plaque-induced gingivitis involves only tissue of the gingivae, characterized by gingival erythema, edema, hemorrhage and tissue enlargement. It is treatable and reversible, in contrast to the other 2 conditions. The pathogenesis of periodontitis includes reduced salivary production, as well as increased salivary pH and salivary urea concentration, a shift of normal oral flora from Gram-positive to Gram-negative, and secondary hyperparathyroidism, the role of which remains to be elucidated[11].

ASSOCIATION BETWEEN PERIODONTITIS AND KIDNEY DISEASES

CKD and periodontitis

Several studies have established the relationship between

Table 1 Common orofacial problems associated with chronic kidney disease itself or caused by therapy[35]

Organs	Manifestations	Organs	Manifestations
Oral mucosal lesions	Ulceration	Mouth	Poor oral hygiene
	Uremic stomatitis		Uremic odor, bad odor/halitosis
	Mucosal petechia/ecchymosis		Uremic frost
	Metastatic soft tissue calcifications		
	Macules, nodules		
	Papillomas		
	Pyogenic granuloma	Salivary glands	Acute suppurative sialadenitis
	Fibro-epithelial polyps		
	White patch, erythematous patch		
	Angular cheilitis/candidiasis	Tongue	Tongue coating
	Oral hairy leukoplakia		Geographic tongue
	Lichen planus-like disease		Black hairy tongue
	Epstein-Barr virus like lesions		
	Non-Hodgkin's lymphoma		
	Kaposi's sarcoma		
Bone	Decreased thickness of cortical bone	Periodontium	Gingival overgrowth
	Radiolucent lesions		Increased deposits of calculus
	Abnormal bone healing after extraction		Severe periodontal destruction
	Osteolytic areas		Increased tooth mobility
	Premature bone loss in the jaw		
	Decreased trabeculation	Teeth	Premature tooth loss
	Bone demineralisation		Malocclusion
	Brown tumour of the maxilla		Erosion of lingual tooth surface
	Pulp calcification		
	Pulp narrowing		
	Delayed eruption		
	Necrotic teeth		

systemic diseases, including CKD, and oral disease as an association rather than causal relationship[14,16,33,35,36]. Many described that the link between the 2 entities was bidirectional[29,30] or reciprocal[5,27]. That is, CKD and RRT, in any form, can exert an effect on oral tissues as well as dental management among these individuals. Periodontitis, likewise, adds greatly to the overall systemic inflammatory burden and the management of ESRD patients[5].

A large survey of 11200 adults demonstrated the bidirectional relationship between PD and CKD. Multivariable logistic regression models were used to evaluate the direct effect of PD on CKD, while simultaneously controlling for a direct effect of many other factors such as DM, hypertension, and socioeconomic status. Adults with PD and edentulous adults were approximately twice as likely to have CKD (OR = 1.62 and 1.83, respectively) after adjusting for 14 other potential risk factors. Moreover, the PD score was statistically important, such that for every one unit increase in the continuous PD score, the risk of having CKD increased by 1% when adjusting for other factors[34].

Impact of chronic renal disease on periodontal tissues

Numerous studies have agreed on the fact that ESRD patients neglect or pay less attention to dental care, and access and utilize dental resources and procedures less often, hence, are more likely to present with poor oral hygiene. This was attributed largely to the immense physical and psychological burden and time-consuming RRT sessions. Furthermore, confounding factors, for instance, DM, smoking, dialysis period, age, degree of medical management of renal failure complications, and demographic variables, may potentially impact on the seeking of dental care[5]. The impact of CKD and RRT was identified in oral tissues, such as xerostomia, delayed tooth eruption, enamel hypoplasia and altered salivary pH[5,30,33,34]. The tongue and salivary glands are also affected (Table 1)[35].

A frequent oral problem is cyclosporine-induced gingival hyperplasia among RRT patients particularly those with a renal transplant[5,31,34]. In ESRD patients on hemodialysis (HD), there were elevated levels of plaque and calculus, gingival inflammation, and increased rates and severity of PD. The uremic state was considered an important underlying cause, along with immune system alteration and disturbances, and hence, a reduction in the host response[2,5,31]. In the pre-dialysis population, a study in Sweden was performed in 51 subjects presenting with a variety of CKDs, and close to dialysis commencement. These participants were given a comprehensive dental inspection and the results undoubtedly revealed poor oral health in these ESRD individuals[37].

Nevertheless, not all studies reported similar findings with regard to the extent and severity of periodontitis among the CKD population. In recent literature, conflicting study results were reported. Many studies published in some Asian, and North and South American countries (Taiwan, Canada, United States and Brazil) demonstrated a higher prevalence of periodontitis in CKD and a higher frequency of chronic severe periodontitis among HD patients. In contrast, both cross-sectional and clinical trial studies from some European countries such as Spain and

the Netherlands found no statistically significant association between periodontitis and HD patients, compared with healthy control group[35].

In the same way, investigation of the association of CKD and PDs did not show uniform findings even though performed in various ethnic groups. In a cross-sectional study among non-Hispanic blacks and whites, non-Hispanic whites and those with a low income and lower educational level showed the strongest association between PD and CKD after adjustment of such variables as age, race or ethnicity, diabetes and dental care use[14]. In contrast, other studies done in the same ethnic groups reported that such an association in non-Hispanic whites was unremarkable compared with Mexican-American and non-Hispanic black CKD patients[2].

Impact of periodontal diseases on CKD

The appreciable influence of PD on CKD is amplified by the systemic inflammatory burden[5,31,33-35]. Periodontal pathogens are able to not only cause a local inflammatory reaction but also invade into the bloodstream and cause bacteremia[33,34]. The inflammatory process involves induction of several acute-phase mediators, such as elevated CRP, blood sugar and low-density lipoprotein level as well as a reduction in high-density lipoprotein level and peripheral neutrophil counts and function. This then activates the inflammatory cascade. Activation of the complement system, accumulation of pathogens in the vascular endothelium, atheroma formation and impaired vascular relaxation are all involved in the pathogenesis of atherosclerosis[13,14,35]. Subsequently, this leads to the final events of myocardial infarction and a cerebrovascular accident, the primary causes of death in CKD patients[5,33].

It is often mentioned that a greater number of pathogens are present in HD patients. *Porphyromonas gingivalis* (*P. Gingivalis*) is one of those species playing vital roles in bringing about serious outcomes in these populations. A further study regarding major periodontal pathogens and their severity among CKD patients as compared to systemically healthy controls was performed in Brazil. Sixty-six eligible chronic periodontitis patients comprised 19 healthy subjects, 25 pre-dialysis CKD patients, and 22 ESRD patients receiving RRT treatment. These patients underwent periodontal assessment in terms of periodontal pocket depth (PPD), gingival recession, and clinical attachment loss (CAL). Subgingival plaque was collected and then analyzed by polymerase chain reaction. The findings suggested a higher severity of periodontitis in CKD patients compared with their counterparts. *Eikenella corrodens* was the most prevalent periodontal pathogen found in parallel between the control and pre-dialysis groups. Conversely, the RRT group had greater numbers of *P. Gingivalis* and *Candida albicans* which are of importance in the CKD population as they often predispose these patients to opportunistic infections, signifying that administration of an antifungal agent is crucial when chronic periodontitis occurs[26].

Aside from being the source of inflammation and infection, poor oral health may act as a contributor to protein-energy wasting in the CKD population. It presents as anorexia, muscle atrophy, low anabolic hormones, insulin resistance and raised energy expenditure through a number of pathways. A variety of medications taken by CKD patients produce xerostomia which, in turn, causes deglutition problems. A metallic taste is also reported in one-third of advanced CKD individuals, affecting the flavor of food and leading to diminished nutrient intake. Poor oral hygiene, likewise, acts as a contributor to cardiovascular complications in CKD as a byproduct of the inflammatory cascade[35].

Recently, a systematic review evaluating the association between periodontitis and CKD as well as the effect of periodontal treatment on the estimated GFR was published. Four cross-sectional studies, one retrospective and 3 interventional, were included. The correlation between periodontitis and CKD was demonstrated, with an odds ratio of 1.95 (95%CI: 1.35-2.01) by pooled estimates. Interestingly, all interventional studies reported a positive effect of periodontal therapy on estimated GFR[38]. Unfortunately, the reviewer considered that the report was not a well conducted systematic review[39].

Glomerular diseases and periodontitis

In contrast to frequent studies on the association between periodontitis and CKD, limited studies in GN have been mentioned. Ardalan *et al*[17] reported a preliminary study of the association, recruiting 10 subjects with unknown primary GN (7 mesangioproliferative GN, 2 membranoproliferative GN and 1 of unknown origin). The severity of PD was determined by plaque index (PI), gingival index (GI) and PPD. All received appropriate dental treatment after initial examination. After therapy, median urine protein excretion was reduced significantly from 3100 to 900 mg/d ($P = 0.008$), and 40% of the patients were found to have decreased CRP levels. The study reported a high rate of PD through such mechanisms as direct glomerular invasion by periodontal pathogens and an indirect systemic inflammatory burden of CRP. The authors, therefore, concluded that the association between periodontitis and primary GN was plausible[17]. In addition, another comprehensive study analyzed tonsil flora in immunoglobulin A nephropathy (IgAN) patients, the most common primary glomerular disease. Sixty-eight IgAN patients and 28 controls were enrolled. *Treponema* spp. or *Campylobacter rectus*, anaerobic bacteria reported to be causative agents of PDs, played a marked role in the remission of proteinuria (HR = 2.35, $P = 0.019$), by which the IgAN subjects were found to have a higher incidence than those without these organisms[40].

The most common secondary glomerular disease is diabetic nephropathy, a complication of longstanding DM. DM is an unequivocally major risk factor of periodontitis. Commonly, the risk of periodontitis is increased about 3-fold in diabetic compared with non-diabetic populations. The level of glycemic control was determined as a key risk indicator[41]. The poorer the HbA_{1C} level among diabetic patients, the greater the risk of developing periodontitis. This, in turn, may predict the

development and progression of ESRD itself. Diabetic nephropathy patients possessed worse dental health, in terms of more dental caries and deep periodontal pockets, as well as lower salivary secretion. These patients also tended to have higher observed yeast counts, predisposing to oral candidiasis[6].

A study in the Gila River Indian community of type 2 DM patients investigated the effect of periodontitis on development of macroalbuminuria or ESRD. Sixty percent of the subjects had moderate to severe periodontitis and 20% were edentulous at baseline and during follow-up. Thirty-six percent developed macroalbuminuria and about 13% progressed to ESRD. Interestingly, the incidence of macroalbuminuria and ESRD was increased as the degree of periodontitis progressed; thus, it predicted the development of overt diabetic nephropathy and ESRD in a "dose-dependent" manner[18]. A similar study in Pima Indians with type 2 DM was performed. The relationship between number of deaths per 1000 persons and years of follow-up increased with the continuum from none/mild, moderate to severe periodontitis. After adjustment for age, sex, duration of DM, HbA1c, macroalbuminuria, magnetic resonance imaging (BMI), serum cholesterol, hypertension, electrocardiographic abnormalities and current smoking status, patients with severe periodontitis a 3.2-fold risk of cardio-renal mortality (ischemic heart disease and diabetic nephropathy combined) compared with the controls[42].

Dialysis and periodontitis

Major changes in the oral condition usually accompany the initiation of dialysis. Studies have illustrated higher rates of oral pathology in dialysis patients, presenting with one or more oral manifestations. Oral features include uremic odor, dry mouth, taste disturbance, tongue coating, mucosal petechiae and ecchymosis, reduced salivary flow, mucosal inflammation and oral ulceration[5,16,35]. Progression of PD has been reported as severity increases along the continuum from pre-dialysis, peritoneal dialysis to HD. However, studies on the prevalence of these symptoms in peritoneal dialysis and HD patients are still sparse[35].

Peritoneal dialysis

A study in Poland compared the periodontal status between 3 adult CKD groups, those on maintenance HD, continuous ambulatory peritoneal dialysis (CAPD), and pre-dialysis treatment. The study involved 202 dialysis patients (141 on HD and 61 on CAPD), and 160 CKD patients (35 on HD, 33 on CAPD and 38 pre-dialysis). Two control groups were allocated: a group of 26 healthy individuals with advanced periodontitis and 30 individuals from the general population. The severity of PD was clinically measured by the GI, papillary bleeding index, PI, CAL and community periodontal index of treatment needs (CPITN). HD patients showed the most concerning values in all parameters of PD severity[43]. A study in Brazil in 3 similar groups as above revealed that pre-dialysis patients, compared with CAPD and HD subjects, showed greater bleeding on probing, and frequency of generalized chronic periodontitis, which was enhanced by smoking. Pre-dialysis and HD groups paralleled such findings in having higher frequency of severe chronic periodontitis and percentage of sites with CAL > 6 mm. Due to the heterogeneity of the data, PD seems to have a higher prevalence of PD than the general healthy population, but lower than in the HD population. Conversely, CAPD patients and systemically healthy individuals appeared to share similarities in the periodontal conditions[44].

In Turkey, many studies were performed. One study revealed that GI, PI and calculus surface index were significantly higher in PD and HD groups than the healthy controls[45]. In addition, PD patients presented with higher salivary flow rate, salivary pH, salivary buffering capacity, decayed, missing, and filled teeth index and numbers of filled teeth than the HD counterparts[46]. Another study on the relationship between periodontitis and risk of atherosclerosis was published. The authors evaluated periodontal status of 110 eligible PD patients by using PI, GI and PD index (PDI). Atherosclerotic risk and nutritional and inflammatory markers were also assessed. There was an association between poor periodontal status and parameters of malnutrition, inflammation and atherosclerotic risk. Multiple regression analysis demonstrated that age, albumin level and duration of dialysis were independently associated with the severity of periodontitis. This study consequently confirmed the relationship between both periodontitis and non-surgical periodontal treatment and chronic systemic inflammation[47].

Recently, in a study conducted in Thailand, clinical periodontal status was evaluated in 32 stable PD patients by PI and PDI. At baseline, high sensitivity CRP positively correlated with clinical periodontal status (PI; $r = 0.57$, $P < 0.01$ and PDI; $r = 0.56$, $P < 0.01$). After completion of periodontal therapy, clinical periodontal indexes were significantly lower and CRP significantly decreased from 2.93 to 2.21 mg/dL. In addition, increased blood urea nitrogen (BUN) from 47.33 to 51.8 mg/dL, reflected nutritional status improvement. Erythropoietin dosage requirement decreased from 8000 to 6000 units/wk while the hemoglobin level remained stable. The authors, hence, concluded that periodontitis is a potential treatable source of systemic inflammation in PD patients and periodontal treatment can improve systemic inflammation, nutritional status and erythropoietin responsiveness among these patients[48].

Hemodialysis

Among the 3 modalities of RRT, HD, peritoneal dialysis and KT, HD is the most common among ESRD adult patients[49]. ESRD patients, particularly those on maintenance HD, are at great risk of cardiovascular complications, resulting in high morbidity and mortality[5,49]. In the United States, atherosclerotic events were reported as the

leading cause of death, accounting for 44% of all deaths among ESRD individuals, followed by infection.

To support this, a prospective observational study was performed in Taiwan, aiming to investigate the relationship between periodontitis and cardiovascular disease (CVD) mortality in HD patients. The dental health status of 253 included HD patients was indicated by 3 methods of examination, namely, PI, GI and PDI. After a 6-year follow-up period, the subjects with moderate-to-severe periodontitis were found at greater risk of having increased CVD-related mortality (1.83-fold), even after adjustment for various confounders such as age, smoking and educational level[24]. Similarly, with the same objective as above, a retrospective analysis was carried out in the United States One hundred and sixty-eight adults were recruited and given a dental examination to determine PD. After 18 mo of follow-up, the study concluded that HD subjects with moderate-to-severe periodontitis had a 5-fold increase in CVD mortality. Adjustment for other covariables did not significantly affect such a strong association between the 2 conditions[20].

In another study, the 253 Taiwanese HD subjects were included in a study to investigate the adverse effect of periodontitis on inflammation and malnutrition status. Three methods of assessment included PI, GI and PDI. Data on demography such as age and sex, biochemistry such as BUN, Cr, CRP, albumin and ferritin, and hematology were collected for analysis. The results conclusively revealed a significant prevalence of periodontitis among the studied patients. Not only did these individuals face an increased risk of CVD, they were also more likely to have protein-energy malnutrition. An increased degree of inflammation was observed from elevated levels of serum ferritin and CRP. The authors strongly believed that an association between clinical periodontal status and systemic inflammation existed[32]. Accordingly, effective periodontal management could result in CRP reduction and might imply a lower level of risk in developing systemic complications in this population[27], although a reduction in cardiovascular complications remain to be explored[5].

Most studies on the effect of periodontal treatment in the HD population showed a positive effect on inflammation[27,50-53]. In addition, the effect of periodontal therapy in 30 stable HD patients demonstrated a significant improvement in clinical periodontal status (both PI and PDI), nutritional markers (pre-dialysis BUN and serum albumin) and erythropoietin responsiveness after completion of treatment[50]. Nevertheless, some studies failed to show this effect. An example was a randomized controlled trial investigating an effect of intensive periodontal therapy on metabolic and inflammatory markers in 342 HD patients. The study reported an insignificant difference between the treated and control groups for serum albumin or IL-6 level at any time when adjusted for BMI, diabetes and PI. The limitation of the study was the small sample size, only 53 randomly assigned patients, the relatively healthy subjects, and the imbalance in numbers with diabetes[54].

Kidney transplantation and periodontitis

Kidney transplantation (KT) is the most efficient and preferred choice of long-term RRT despite disadvantages such as risk of opportunistic infection, graft-*vs*-host rejection, hypertension, and the tendency for reduced renal function with increased age[5,55].

Gingival hyperplasia is a notable condition observed among renal transplant patients, secondary to post-transplantation immunosuppressive agents such as cyclosporine and prednisolone. Primarily, cyclosporine was said to be the focal cause of gingival hyperplasia in a dose-dependent manner. Additional use of calcium channel blockers such as nifedipine may enhance the incidence and accelerate the severity of gum overgrowth. This can be successfully reduced by substituting cyclosporine with tacrolimus and by surgical intervention[5]. An attempt to evaluate the link between KT and oral lesions was carried out in a study including 33 renal transplant patients, 46 dialyzed patients and 37 controls. All subjects were intra-orally examined and oral disorders were identified and treated. Gingival overgrowth was the most prevalent finding among KT patients, resulting from cyclosporine administration either alone or in combination with calcium channel blockers. Other frequent findings comprised of xerostomia, geographic tongue, and oral candidiasis. A metallic taste was observed more in dialysis patients. In summary, the prevalence of oral lesions was higher in renal disease patients[55].

Investigation of the association between periodontal status and renal allograft function, estimated by GFR, was carried out in the United States. KT patients were categorized into 2 groups: deterioration or stable/improvement of renal function between 2 time points at least 6 mo apart. Chronic periodontitis, defined by $\geqslant 6$ sites with probing depth $\geqslant 5$ mm, or clinical attachment level $\geqslant 4$ mm in at least 6 proximal sites, was significantly more prevalent in patients with reduced renal function ($P = 0.04$). It acted as a statistically significant predictor of improved renal function over time ($P = 0.04$)[56]. Interestingly, the impact of chronic periodontitis on other cardiovascular conditions among these individuals was further studied. A study from Poland illustrated this when it aimed to assess whether periodontitis may contribute to left ventricular hypertrophy (LVH) in KT patients. Ninety-nine patients were classified according to CPITN score into patients with advanced disease (CPITN 3-4) and none/moderate lesions (CPITN 0-2). The advanced lesion group had higher plasma high-sensitivity CRP concentration ($P < 0.05$) and left ventricular mass index (LVMI) ($P < 0.001$), compared with the other group. Besides, LVMI was dependent on CPITN ($P < 0.001$), high-sensitivity CRP ($P < 0.05$), serum cholesterol ($P < 0.05$) and Cr levels ($P < 0.05$). The authors concluded that chronic periodontitis and a concomitant systemic inflammatory reaction in KT patients may be associated with LVH[57].

Finally, inflammation is recognized as playing a pivotal role in transplant rejection. CRP and IL-6, significant

inflammatory markers, are documented as surrogate markers to identify patients who are at greater risk of rejection. In light of this, a hypothesis was proposed that periodontitis may increase the risk of organ rejection by contributing to the systemic inflammatory load in organ transplant patients. A study about the link between periodontitis and serum IL-6 was performed in 47 transplant patients and 18 healthy age-matched controls. The result demonstrated a significantly higher level of serum IL-6 and increased mean probing depth in transplant patients. Multivariable linear regression analysis, after adjustment for sex, diabetes, smoking and immunosuppressant dose, showed that the mean probing depth, number of missing teeth and mean percentage of sites with at least 4 mm of attachment loss were independent predictors for elevated serum IL-6 levels[58]. Besides, a study with a larger group of subjects (90 transplant patients compared with 72 age-matched controls) reported a higher level of IL-6 and CRP in transplant individuals, compared with the control group[59].

CONCLUSION

The current literature strongly supports an association between PD and CKD. They affect each other reciprocally. PD, a treatable infective dental condition, potentially places a devastating chronic systemic inflammatory burden on the CKD population, resulting in significant atherosclerotic complications and death. In the same way, chronic renal disease impacts on oral health in this population with gingival overgrowth as the most prevalent finding due to a cyclosporine-mediated mechanism. Several studies concluded that proper periodontal intervention rendered a promising outcome in the systemic improvement of CKD subjects although its impact on cardiovascular complications remained to be further explored. Thus, PD diagnosis and management deserve better awareness. Further investigation, especially prospective randomized controlled trials, with intervention should be conducted to allow more accurate evaluation. Also, a larger number of participants and ethnic subgroups are required to be recruited in the research to provide more data to make firmer conclusions.

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MINIREVIEWS

Stress urinary incontinence in women and cell therapy: What can we expect from the future?

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Key words: Stem cell; Stress urinary incontinence; Women; Basic science; Animal model

Core tip: This is a review of the current literature regarding the use of stem cell for stress urinary incontinence. It has been focused on cell sources, animal model creation and the possibility of translating this therapy for humans.

Zambon JP, Magalhaes RS, Almeida FG. Stress urinary incontinence in women and cell therapy: What can we expect from the future? *World J Clin Urol* 2014; 3(3): 304-309 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/304.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.304

Abstract

Stress urinary incontinence (SUI) is a common disorder that affects a large number of women and their quality of life. The aim of SUI therapy is to restore the existing urethral function *via* physical therapy, biofeedback, pelvic floor rehabilitation, pharmacological therapy, bulking agents and surgical approaches. Currently, the gold standard for the management of SUI is the tension-free vaginal sling, which provides structural support to the female urethra. However, even minimally invasive surgical procedure such as "slings" carries risks for the patients, lost efficacy over the time and has long-term complications. For this reason, new therapeutic modalities are needed. Cell therapy has been emerged as an alternative to be used on the treatment of different diseases. The use of stem cells as a therapeutic option for SUI is an attractive alternative because, theoretically, injected cells could restore functional muscle cells and aid in sphincter closure in women with sphincter-associated incontinence. This study aims to review the current literature regarding evidences for using stem cell therapy on stress urinary incontinence in women.

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INTRODUCTION

Stress urinary incontinence (SUI) is a common disorder that affects a large number of women and their quality of life. According to the National Center for Health Statistics 16% of adult United States women suffer from moderate to severe urinary incontinence, which has a significant socioeconomic impact with an estimated treatment cost of up to $16 billion annually in the United States[1-4].

The pathophysiology of SUI is multifactorial and poorly understood. It appears to be due to the dysfunction of the sphincteric mechanism of the urethra, changes in connective tissues, poor blood perfusion in the periurethral and submucosal areas, and neuronal dysfunction[5,6].

The aim of SUI therapy is to restore the existing urethral function *via* physical therapy, biofeedback, pelvic floor rehabilitation, pharmacological therapy, bulking agents and surgical approaches. Currently, the gold standard treatment for the management of SUI is the tension free vaginal sling, which provides structural support to the female urethra through a minimally invasive surgical procedure. However, even minimally invasive surgical

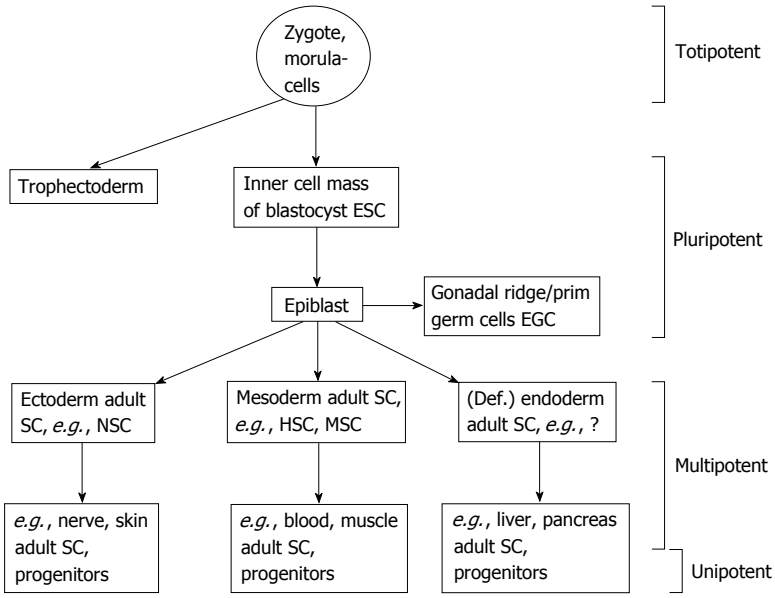


Figure 1 Stem cells and its potential for differentiation. Adapted from Ref. [12]. ESC: Embryonic stem cells; EGC: Embryonic germ cells; SC: Stem cells; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells.

procedure carries risks for the patients, lost efficacy over the time and has long-term complications such as voiding dysfunction and "*de novo* urgency". For this reason, new therapeutic modalities are needed[7-9].

Cell therapy has been emerged as an alternative to be used on the treatment of different diseases. Despite being described for more than 40 years in hematopoietic system regeneration, their therapeutic potential has been started only in 1999, when the cover of Science, one of the most respected scientific journal, announced "Breakthrough of the year: stem cells show their potential"[10].

The use of stem cells as a therapeutic option for SUI is an attractive alternative because, theoretically, injected cells could restore functional muscle cells and improve the urethral sphincter activity in women with sphincter-associated incontinence. Cell types considered for this therapeutic purpose include bone marrow stem cells (BMSC), adult mesenchymal stem cells (MSC), human cord blood stem cells, human amniotic stem cells (HASC), induced pluripotent stem cells (iPSC)[11]. This study aims to review the current literature regarding evidences for using stem cell therapy on stress urinary incontinence in women.

STEM CELLS AND ITS POTENTIAL FOR DIFFERENTIATION

Stem cells are undifferentiated cells that are defined by their abilities of self-renewal and differentiation capacity[12,13]. There is a lack of defined morphologic and molecular characteristics of stem cells. Therefore, they are classified according to their potency. The hierarchic order for stem cells ranges from totipotency to pluri and multipotency to unipotency[14]. Totipotent cells are capable of forming cells of the ectoderm, mesoderm, endoderm, and trophoblast. Pluripotent cells can give rise to cells of the three germ layers, but not extra-embryonic tissues. Multipotent cells can be isolated from the developing germ layers or descended adult organs and are capable of self-renewal and differentiate into multiple cell types. Ultimately, the unipotent cells can differentiate into only one cell type[15-18] (Figure 1).

EXPERIMENTAL ANIMAL MODEL: AN ESSENTIAL STEP TO DEMONSTRATE THE EFFECTIVENESS OF CELL-BASED THERAPY FOR STRESS URINARY INCONTINENCE

The development of a reliable experimental animal model is the first step to demonstrate the effectiveness of cell-based therapy for stress urinary incontinence. However, once the physiopathology of SUI has not been well established a reliable animal model for urinary incontinence remains a big challenge[4,11].

Mice, rats, rabbits, pigs, and more recently monkeys have been used in experimental studies. There are different ways to induce urinary incontinence in animals such as vaginal balloon dilation, urethrolysis, urethrolysis and cardiotoxin injection, electrocauterization of pudendal branches, and bilateral pudendal nerve transection. Despite some studies have shown promising outcomes, the main question that should be addressed is: Any of these current methods reproduce the pathophysiology stress urinary incontinence in women[19-25]?

Badra *et al*[4] published the first study performed in monkeys demonstrating a long-term animal model of stable urethral deficiency. They emphasize that nonhu-

man primates have a natural menarche, a 28-d menstrual cycle, natural menopause as well as age associated health risks that closely mirror those of women.

The objectives of creating a reliable animal model are to avoid methodological bias and outcomes misinterpretation. In the literature, most of the studies, which demonstrate promising outcomes have a short follow up and were performed in small young animals. Therefore, the translation of this therapy for humans needs further and more detailed investigation[9,11].

STEM CELLS AND STRESS URINARY INCONTINENCE

There are two different mechanisms of actions for cell therapy. First, the cells can differentiate, integrate into the site of injury and then, replace the damaged tissue. Second, cells can release cytokines, chemokines, and growth factors, which act in paracrine or endocrine manner. Ideally, autologous cells are always recommended to decrease the risk of immune response and rejection[26].

Embryonic stem cells

Embryonic stem cells (ESC) are pluripotent cells derived from blastocysts. These cells propagate readily and remain undifferentiated when cultured with leukemia inhibitory factor (LIF). When LIF is withdrawn, ESCs form aggregates called embryoid bodies that generate a variety of specialized cell types. However, the extraction of these cells involves the destruction of embryos, therefore their use is associated with controversial ethical dilemmas. Despite the self-renewing potential and the capability of differentiation into tissues derived from the three germ layers, embryonic stem cells are associated with uncontrolled growth and teratoma formation. For this reason, embryonic stem cells have never been tested for stress urinary incontinence[27].

Adult stem cells

The regenerative capacity of a tissue is determined in part by whether it contains endogenous stem cells. These stem cells remain quiescent in the niche for long periods until they are activated by the requirement of new cells to maintain the tissue or because of the tissue damage. Despite the limited potential of differentiation and self-renewal, adult stem cells are an attractive source of cells for urinary sphincter regeneration. Most of the studies regarding cell-based therapy and SUI were performed using adult stem cells derived from adipose tissue, skeletal muscle, bone marrow, human cord blood, and amniotic fluid[27].

Bone marrow-derived stem cells: The bone marrow contains two major populations of stem cells: hematopoietic stem cells (HSCs) and MSC, which provide stromal support for HSCs. It has been demonstrated that bone marrow derived stem cells have *in vitro* myogenic differentiation potential, and when placed in culture they expressed myosin heavy chain and desmin[8,9,12].

There is a lack of studies correlating SUI and BMSC. Kinebuchi *et al*[28] transplanted BMSC into rat's injured urethral sphincter. After 13 wk, cell-treated rats did not have improvements on leak point pressure whether compared to controls. On the other hand, Kim *et al*[29] demonstrated that the periurethral injection of BMSC increased the leak point pressure and urethral closure pressure at one-month follow up.

The outcomes regarding bone marrow-derived stem cells and SUI are controversial. Some studies have shown histological and functional recovery, whereas other studies have shown no improvements. These conflicting results may be related to different methodologies, animal models, route of administration, and amount of injected cells. The current literature does not have strong evidences that support the use of BMSC for SUI.

Adipose derived stem cells: The adipose tissue is derived from mesoderm, is easily accessible and overwhelmingly abundant. It has been demonstrated that under specific conditions the adipose tissue has stem cells, which can differentiate into myogenic, adipogenic, osteogenic, chondrogenic, and neurogenic tissue[30].

Shi *et al*[31] injected a tissue engineering bulking agent composed of adipose derived stem cells (ADSC) and silk fibrin microspheres in 4 different points around the urethra of rats with urinary incontinence. In this study, urinary incontinence was established by bilateral pudendal nerve transection and confirmed by decreasing leak-point pressure (LPP). Injection of silk fibroin microspheres without cells could recover LPP and lumen area at 4 wk but its efficacy disappears at 8 and 12 wk follow up. The injection of microspheres with ADSC brought long-term efficacy at 8 and 12 wk post-injection and improved the urethral sphincter regeneration[31].

Li *et al*[32] developed a model of postpartum urinary incontinence in rats through the placement of vaginal balloon dilator for four hours followed by bilateral ovariectomy. They labeled isolated ADSC's from the peri-ovarian fat with thymidine analog 5-ethynyl-2-deoxyuridine. After four weeks leak-point pressure and bladder capacity were significantly higher in ADSC-treated rats compared to the balloon-injured ovariectomized rats. Histological analysis of ADSCs treated group demonstrated a higher density of peri-urethral blood vessel and the preservation of the urethral fibromuscular structure[32].

Jack *et al*[30] isolated pluripotent adipose derived stem cells from human lipoaspirate. These cells were stained with Vybrant Red and injected around the urethra of athymic rats. Eight weeks later, injected cells remained viable and randomly distributed in the local submucosa and lamina propria[30].

Muscle derived stem cells

There are different cell populations that can be isolated form skeletal muscle biopsies. These cells include myoblasts, satellite cells, muscle progenitor cells, and muscle derived stem cells (MDSC). As well as adipose derived

stem cells, it has been demonstrated that adult stem cells isolated from skeletal muscle also have multilineage differentiation capacity[33].

Xu *et al*[34] isolated MDSCs from the hind gastrocnemius of 4-wk-old Wistar rats and infected them with lentivirus encoding green fluorescent protein. In this study, urinary incontinence was established by bilateral pudendal nerve transection and confirmed by LPP decrease. MDSCs were injected at proximal urethra and the animals were sacrificed at four weeks. MDSC-treated rats had higher leak-point pressure and sphincter muscle thickness than controls[34].

Badra *et al*[4] developed a model of urinary sphincter deficiency in adult premenopausal female cynomolgus monkeys cauterizing and then bilaterally transecting the pudendal nerve. Autologous muscle progenitor cells were injected at four locations (12, 3, 6 and 9 o'clock positions) around the sphincter area. At 12 mo follow up, cell-treated monkeys had an improvement in resting, somatic and adrenergic nerve stimulated maximal urethral closure pressure, and a greater percentage of sphincter area occupied by muscle. The GFP labeled precursors cells were found in the skeletal muscle layer and expressed desmin and connexin-43. According to the authors it seems reasonable to assume that their outcomes have a high translational values[4].

Cord blood stem cells

Cord blood is a rich source of stem cells, including hematopoietic and mesenchymal stem cells, which have been used in the treatment of many diseases, including leukemia, lymphoma and anemia[35].

There are few experimental studies that use stem cells derived from cord blood for SUI therapy[35-37]. Lim *et al*[35] reported the short-term effects of human umbilical cord blood mononuclear transplantation in incontinent rats. Urinary incontinence was created through the electrocauterization of periurethral soft tissue and confirmed by leak point pressure measurement. At four weeks, cell-treated rats had significant higher leak-point pressure than controls; however, the injected cells were not found in the urethral tissue[35].

Human amniotic fluid stem cells

Amniotic fluid, due to its contact with the fetus, has been considered an interesting source for undifferentiated or partially differentiated cells. HASC express surface markers and transcription factors distinctive of embryonic stem cells. These include octamer-binding transcription factor 4 and stage specific embryonic antigen-4. HASCs have high replicative self-renewal potential and multilineage differentiation capacity[38-41].

Chun *et al*[42] injected three different lineages of early differentiation human amniotic fluid-derived cell to restore urinary sphincter function in mice with urinary incontinence. In this study, urinary incontinence was created through bilateral pudendal nerve transection and the follow up was two and four weeks. HASC's were early differentiated into muscle, neuron, and endothelial progenitor cells, and then injected into the urethral sphincter as a single, double, or triple combination. Urodynamic study showed that mice treated with the three cell populations had better outcomes than the other ones. These functional results were confirmed by histological and immunohistochemical analysis, as evidenced by the formation of new striated muscle fibers and neuromuscular junctions at the cell injection site[42].

iPSC

iPSC were first described by Takahashi *et al*[43] in 2006 when they reprogrammed human fibroblasts to become pluripotent stem cells by the addition of four different genes: Oct3/4, Sox2, c-Myc, and Klf4. Despite being a good source of cells, not all adult stem cells can be reprogrammed using the same method, which means that each cell type may have critical factors. Unlike embryonic stem cells, iPSC have no ethical issues and no immune rejection. On the other hand, these cells are reprogrammed through the addition of oncogenes, which increase the risk of uncontrolled growth[44]. These cells have never been tested to treat stress urinary incontinence.

REGENERATIVE MEDICINE OF THE CONNECTIVE TISSUES-PELVIC PROLAPSE AND LIGAMENT REINFORCEMENT

The current surgical approaches for pelvic organ prolapse treatment are associated with high rates of long-term complications such as erosions, infections, pain, and vagina shrinkage. There is a lack of studies in the literature with regards to cell-therapy and pelvic organ prolapse[45].

Boennelycke *et al*[46] used fresh muscle fiber fragments seeded on synthetic biodegradable methoxy polyethylene glycol-polylactide-co-glycolic acid scaffolds implanted subcutaneously on the abdomen of rats. After 8 wk, new striated muscle was created and the scaffolds had disappeared[46]. Petros *et al*[47] implanted mersilene tapes between rectus abdominis and midurerhra in 13 canines for periods of 6-12 wk. They observed a linear deposition of collagen around all implanted tapes and some interstitial macrophages. In this study, the main goal was to reinforce damaged pubourethral ligaments using an autogenic collagenous ligament[47].

Regarding prolapse and regenerative medicine approaches, the current literature does not have sufficient data to support cell-based therapy. Further studies using a reliable animal model, different cell sources and scaffolds should be addressed before clinical use.

STEM CELL THERAPY AND STRESS URINARY INCONTINENCE-CLINICAL OUTCOMES

The translation of basic science to humans is the main

goal of scientists and researchers. When a new therapy reaches acceptable outcomes in animal models and has the potential to be applied in humans, some issues should be considered before moving forward. Despite expectations inherent to new breakthroughs, a carefully analysis of pros and cons regarding the procedure should be considered to avoid potential avoidable mistakes.

Aref-Adib *et al*[11] published a systematic review addressing the use of stem cell therapy for stress urinary incontinence in humans. They assessed different outcomes such as adverse effects, incontinence, quality of life, urodynamic, transurethral ultrasound and urethral EMG. Out of 89 studies, only eight met the inclusion criteria, which was a quality score tool designed for use in systematic reviews. According to the authors, the outcomes from the studies published to date are promising. Stem cell therapy may improve patients' quality of life as well as objective measures of urinary incontinence. Furthermore, the procedure is safe with minimal adverse effects. However, despite promising results, there is no long-term follow up data and many patients lost to follow up without any explanation and withdrew from the studies[11].

Shirvan *et al*[48] described the safety assessment of urethra injections of autologous nucleated cells harvested from peripheral blood along with platelets in 9 women with stress urinary incontinence. At 6 mo follow up all the patients considered themselves completely cures with 8 women completely continent and one marked improvement. The idea is that peripheral blood has multipotential cell, which can improve the tissue regeneration[48].

FUTURE PERSPECTIVES AND DIRECTIONS

The stress urinary incontinence is a benign condition that has other effective, simple, cheap and safe treatment alternatives. I believe that stem cell therapy may be an alternative to treat those patients who has urethral sphincter deficiency and did not respond to well-established procedures. Historically, any new therapeutic modality has an initial enthusiasm; however, as well demonstrated in this paper, there are still few evidences, which support cell-based therapy for SUI. The enthusiasm surrounding the new therapeutic possibility should be replaced by moderation, reflection, and careful analysis of the available data associated with consistent experimental studies. The indiscriminate use without sufficient basic evidence could lead to discredit and retrocession.

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MINIREVIEWS

Innovative microsurgical treatment of male external genitals lymphedema

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Abstract

Secondary lymphedema of male external genital organs, characterized by increase in genital organs volume, tissue fibrosis, erysipelas, and objective difficulties in the normal use of lower limbs and the penis, is a very common and impairing consequence of invasive surgery, radical lymphadenectomy and radiotherapy of the pelvic-inguinal area. Standard surgical approach to lymphedema are either very invasive and/or at high risk of lymphedema recurrence and do not guarantee an efficient long-term treatment. Alternatively, we developed a microsurgical technique to perform direct anastomoses between the lymphatic collectors of the spermatic funiculum afferent to the external iliac chains and the vessels tributary to the spermatic vein. This innovative approach, although surgically demanding, provided a long term successful treatment of external genitals with no clinical complications, low invasivity, rapid post-surgical recovery, minor tissue demolition and satisfactory post-surgical functional and esthetic results. In addition, lympho-venous microsurgery seems to trigger the local development of new lymphatic vessels that not only canalize along new collecting channels, but also form complex meshes in proximity to the anastomosis area, thus improving lymphedema also in adjacent tissues like lower limbs, supplied by lymphatics emptying into common developed lymphatic shunt.

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Key words: Microsurgery; Lymphatic meshes; Secondary lymphedema; Lympho-venous anastomosis; Lymphangiogenesis

Core tip: Treatment of secondary lymphedema of male external genital organs through invasive standard surgical techniques may be complicated by impairing consequences for the patient and often do not guarantee an efficient long-term outcome. Alternatively, microsurgical suture of lympho-venous anastomoses between lymphatic collectors of the spermatic funiculum efferent to the external iliac chains and the pampiniform plexus tributary to the spermatic vein provides a successful long term treatment of genitals lymphedema and triggers the development of new lymphatic meshes in proximity to the anastomosis area, thus improving lymphedema also in tissues, like lower limbs, supplied by lymphatics emptying into common developed shunts.

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INTRODUCTION

The lymphatic system is a closed vascular network, whose smallest vessels (initial lymphatics) originate as

a dead-end tubes directly from the extracellular tissue space[1] to subsequently confluence in progressive larger collecting lymphatics that paralleling main artery and veins, propel the lymph unidirectionally. At variance with what observed in initial lymphatics, the wall of collecting lymphatics is supplied with one or more layers of smooth muscle cells arranged in the media externally to the endothelial cells layer and interspersed with collagen and elastic fibres. Fluid flux along the lymphatic conduits is maintained unidirectional by the presence of primary valves in the wall of initial lymphatics[2,3] and secondary or intraluminar valves all along the lymphatic network. Intraluminar valves are formed by two endothelial leaflet attached at opposite sites to the lymph channel wall, connected with zonulae and sustained by a basement membrane composed of collagen, fibrillar material and scanty fibroblasts[4]. Two consecutive valves, arranged at a distance ranging between approximately 1 μm and approximately 15-20 μm delimit the "lymphangion"[1] a sort of primitive heart often considered the "functional unit" of the lymphatic vascular system.

In its flow along the main lymphatic ducts, the lymph is conveyed through secondary lymphoid organs, the lymph nodes. The lymph enters the node through afferent lymphatic vessels, percolates through the node's inner structure dense of lymphocytes and emerge through efferent vessels to be eventually returned into the venous blood stream.

The upper and lower extremities are drained by a superficial and a deep lymphatic network, both equipped with intraluminar valves and smooth muscle cells. Lymph from the skin and superficial connective tissues are carried by superficial ducts, while the muscles and the periosteum drain into the deeper network which accompanies the main vascular fascia. The superficial and deep networks connect only at the level of the popliteal nodes, the adductor canal of the lower leg and the supratrocheal node of the arm. Collecting ducts from the upper limbs drain into the axillary lymph nodes while those of the lower extremities drain into inguinal lymph nodes. The latter also receive lymph from the gluteal area, the uterus, the anterior abdominal wall, the external genital organs and the lower anal canal.

Lymphatic drainage of the external male genitals occurs through two main pathways. A superficial diffuse lymphatic mesh which supplies the scrotal tissues, empties into collecting ducts running along the scrotal raphe and reaches the inguinal lymph nodal chains. A deeper lymphatic network which supplies the gonads is basically organized in two plexuses: (1) one from the testis parenchyma which run proximally along the testicular artery and discharges into the para- and pre-aortic lymph nodal stations; and (2) the other, from the vaginal tunica, which runs in the spermatic funiculum and empties into the external iliac lymphnodes.

The lymphatic vessels efferent from the external, internal iliac and obturator nodes merge into the common iliac lymphatic duct and travel through the corresponding lymph nodes which also receive lymph from the perineum, the buttock, thigh and the pelvic viscera. Efferent lymphatics from iliac nodes drain into para-aortic lymph nodes plexus; the latter, which also receive from all abdominal organs, discharge into the cysterna chyli, a dilated saccular structure located along the abdominal aorta. The larger and main lymphatic collector, the thoracic duct, departs from the cysterna chyli, runs dorsally to the aorta and eventually empties into the venous stream at the junction between the left internal jugular and subclavian veins. The right lymphatic duct collects instead the lymph from the right arm, the right and most of the left lung, the heart, the right side of head, neck and the right anterior chest to empty into the right brachiocephalic vein at the confluence of the right internal jugular and subclavian veins.

The connection between the large collecting ducts and the venous system is usually controlled by unidirectional valves that prevent backflow of blood into the lymphatic vessel. Additional communications between smaller collecting lymphatic and veins may be found along the iliac, renal, subclavian, azygos, and portal veins. However, functional study have revealed that these lymphaticovenous communications are not recruited under normal conditions and may become functional only in pathological conditions[5,6]. No direct lympho-venous anastomosis have instead been detected in lymph nodes.

Because of its peculiar anatomical arrangement the lymphatic system fulfils several important functions in the body: (1) by removing fluid from the interstitial tissue and returning it to the blood stream, it maintains the normal tissue hydration and plasma volume; (2) it returns proteins and high molecular weight soluble molecules from the interstitial tissue to the blood; (3) it returns leukocytes, cell debris and tumoral cells to the blood stream; and (4) the dissemination of lymph nodes along the lymphatic network provides an important contribution to host immune defence by presenting antigens and antigen-presenting cells to the B and T lymphocytes in the lymph node hylum.

SECONDARY LYMPHEDEMA: A COMPLEX IMPAIRMENT OF TISSUE FLUID HOMEOSTASIS

Transport of fluid and solute from the interstitial space to the lymphatic lumen occurs in initial lymphatics by bulk flow through the highly permeable interendothelial junctions[7] that allow free passage of fluid, interstitial hydrophilic molecules of any size, cells, viruses and bacteria. Since the initial lymphatic endothelium offers no effective sieving to large macromolecules, the protein concentration and the colloid osmotic pressure of lymph in initial lymphatics equals that of the surrounding interstitium.

Therefore, the requirement necessary for the formation of the lymph is that the hydraulic pressure in the

interstitium be higher than that existing in the initial lymphatics, while propulsion of the lymph along the lymphatic conduits is sustained by intraluminar pressure gradients developing between adjacent lymphangions. The maintenance of the pressure gradients which sustain lymph formation and propulsion is supported by both local tissue movements such as cardiogenic oscillations, heart activity[8] and respiratory muscle contraction[9], as well as by the action of synchronous contraction of the smooth muscle wall of the collecting lymphatic vessels[1]. Through these mechanisms, lymph flow may increase up to 20-30 folds its physiological value, to counteract an increased interstitial fluid volume depending, for example, upon an increased fluid filtration across the blood microvasculature wall. Such a modulation allows an efficient control of tissue fluid volume only until attainment of a saturation threshold, beyond which the filtration rate through the wall of blood capillaries overwhelms the maximal flow capability of the normally performing lymphatic network, leading to a progressive development of tissue edema. Expansion of tissue fluid is instead defined lymphedema when it depends not only from an increased capillary filtration rate, but rather to an absent or greatly impaired lymphatic drainage. Depending on the underlying cause lymphedema can be distinguished in: (1) primary lymphedema, caused by absence or deficient development of the lymphatic vasculature and/or lymph nodes. In its more severe forms, the primary lymphedema appears at birth (congenital primary lymphedema), and is accompanied by functional impairment of most organs, peritoneal and pleural effusions, pulmonary edema, *etc*. Congenital lymphedema is likely due to genetic faults or abnormalities in the formation of lymph nodes and/or lymphatic vessels due to a defect of the lymphangiogenic vascular endothelial growth factor receptor (*VEGFR-3*)-gene or of the *FOXC-2* (fork head transcription factor for the formation of collecting vessels) gene, both involved in the development and control of lymphangiogenesis. The primary idiopathic lymphedema occurs instead in adolescence or within the 35th year of age and typically affects the upper and lower limbs and/or the external genitalia[10]. The causes of this pathology are still unclear although phenomena like abnormalities of lymph nodes and/or lymphatic vessels, hypoplasia of the lymphatic structures or extracellular matrix degradation might all interplay to determine the primary lymphatic drainage impairment. Due to its complexity, congenital and idiopatic lymphedema represents a challenging, often unsolvable clinical and, more so, surgical problem which, at the moment, goes beyond the focus of the present Review.

Instead, we will focus on a recent application of microsurgery in the treatment of secondary lymphedema, a more localized lymphatic insufficiency caused by excision, lesion or radiation of the lymphatic vasculature and of the lymph nodes secondary, as an example, to major surgery and/or oncological therapies. Impairment of the lymphatic fluid and solute drainage, such as that observed in secondary lymphedema, causes uncontrolled increase of tissue fluid volume, as well changes in tissue matrix composition, thus deeply compromising tissue function.

Secondary lymphedema of the external genital organs is a very common consequence of invasive surgery of the pelvic[11] and inguinal area[12] with associated radical lymphadenectomy and radiotherapy[13]. In particular, lymphedema of the male genital organs and of the hypogastric area is a very impairing condition characterized by an important increase in the volume of the external genital organs, tissue fibrosis, erysipelas, and objective difficulties in the normal use of lower limbs and the penis. Hence, although pelvic lymph node dissection (PLND) is an essential staging approach to cancer patients, the secondary lymphedema that can develop as a complication of this procedure is very impairing to the patient's normal quality of life, even from a psychological standpoint.

TREATMENT OF THE SECONDARY LYMPHEDEMA OF THE MALE EXTERNAL GENITALS: STATE OF THE ART

In the upper and lower limbs, the first treatment of secondary lymphedema consists in local physical therapy, massage, bandage and external compression, which improves the lymphatic drainage by slowly reducing tissue fluid imbibition, increasing local tissue pressure and/or muscular tone and, eventually, shunts development. However, when the lymphatic drainage pathways have been excised and/or interrupted by fibrous scars developed after surgery and/or radiotherapy, medical treatment of lymphedema very seldom provides satisfactory results[14]. This is particularly true in patients affected by secondary lymphedema of the external male and female genital organs in which effective physical therapy cannot be completely performed and that can therefore be treated only by surgery. Various surgical approaches has been utilized: (1) lymphangiectomy associated to adipectomy, the excision of involved subcutaneous tissue, remains the most common approach to the treatment of chronic male external genital organs lymphedema[15]; and (2) more recently, lymphangenctomy/adipectomy has been improved by a plastic surgery procedure consisting in resection of scrotal and penile lymphedematous tissue, reconstruction with a posterior fascio-cutaneous flap from the scrotum itself and penile reconstruction with a skin graft[16,17].

These classical surgical approaches are usually very invasive and imply important demolition of a large part of the scrotal and penile tissues with high risk of lymphedema recurrence. Therefore, in the last decade, with the development of more sophisticated microsurgical techniques and aiming at favoring new lymphangiogenesis in the lymphedematous tissues, the homologous lymph nodes contained in vascularized tissue flap excised from the inguinal, crural or axillary districts of the same patient have been transplanted in lymphedematous external male genitals or limbs[18]. An alternative microsurgical de-

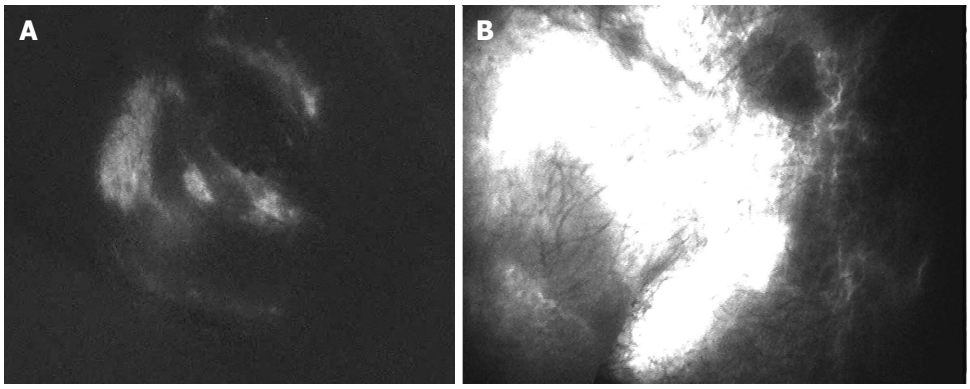


Figure 1 Photodynamic eye lymphography. A: Photodynamic eye (PDE) lymphography of normal testis. The injected indocyanine is canalized into the local lymphatic network and is rapidly drained out of the subcutaneous scrotal tissue; B: PDE lymphography of lymphedematous testis. Indocyanine diffusively accumulates in the scrotal subcutaneous tissue as a consequence of the impaired lymphatic drainage.

rivative procedure utilizes only the subcutaneous scrotal lymphatic vessels, ignoring the alternative draining pathways[19]. In spite of the great technical improvement offered by these pioneering microsurgical approaches, they do not seem yet to offer enough guarantees to ensure an efficient long-term treatment of the extended, severe, secondary lymphedema of the external genitals organs.

A NEW MICROSURGICAL APPROACH IN THE TREATMENT OF MALE EXTERNAL GENITALS SECONDARY LYMPHEDEMA: BILATERAL LYMPHO-VENOUS SHUNT OF THE SPERMATIC CORD

The development of microsurgical techniques has allowed a minimally invasive approach to the treatment of secondary lymphedema of lower or upper extremities and more rarely of other body districts. The present Review describes the application of a new microsurgical lymphovenous derivation technique for the treatment of male external genital organs secondary lymphedema. Indeed, following a careful autoptic observation of the lymph node-chain tributaries of the collecting lymphatic ducts draining the testis during previous external iliac-chain lymphadenectomy, we developed a technique that allowed us to perform direct anastomosis between the lymphatic collectors running within the spermatic cord afferent to the external iliac chains and the vessels tributary to the spermatic veins[14,20]. The idea of developing new drainage pathways of testicular lymphatic collectors in spermatic cord originated from the consideration that excision of lymph node-chain tributaries of lymphatic ducts draining the testis could determine lymphedema of external genitals as a consequence of the insufficient drainage through the superficial external genitals lymphatics.

On the other hand, locoregional PLND with excision of the pelvic fibrous-fatty tissue using as margins the external iliac vein and the posterolateral aspect of obturator fossa, is required in prostate cancer to attain an accurate diagnosis of occult micrometastases and to stratify patients who might benefit from adjuvant therapeutic measures.

From January 2006 to November 2013, 18 patients suffering from lymphedema of male external genitals or male genital and/ or lower limbs lymphedema were screened for lymphedema treatment. Out of these patients, 10 were candidate to microsurgery according to the following inclusion criteria: (1) patients from 25 to 75 years old; (2) at least 3rd degree lymphedema, with no satisfactory and/or durable results after physical and/or pharmacological therapy; (3) free of tumor for at least two years; (4) at least one year since the last post-surgical adjuvant oncological treatment; and (5) less than 15 years since the primary oncological surgery lymphedema development. Patients with co-morbidity as cardiopathy, respiratory diseases, renal failure or severe bilateral lower lymphedema were oriented towards medical and physical therapy.

The pre-operative screening included: (1) lower abdomen and genitals echography, lymphoscintigraphy of lower limbs and inguinal area. Indeed, since there are currently no studies documenting the use of lymphoscintigraphy for the identification of genital organs pathologies, echography was the only diagnostic instrument available for this purpose. Subsequently, the green indocyanine lymphography, Photodynamic eye (PDE, Hamamatsu Photonic K.K., Tokyo, Japan) imaging[21] was utilized to investigate the patency of the genitalia lymphatic network (Figure 1). Briefly, 0.2 to 1 mL green indocyanine were injected bilaterally at the base of the scrotum through an insulin needle. After few minutes of massage, the fluorescent dye in subcutaneous lymphatic vessels at a maximum depth of 2-3 cm were visualized through a portable near-infrared camera system. Images were conveyed on a PC monitor and stored. The Echo-color doppler of lower limbs was also performed to exclude vascular disease such as deep veins thrombosis.

In the first two candidate patients, lymphedema affected both the external genitals and the lower limb: therefore, a preliminary lympho-venous shunt of lower limb was performed followed, after six months, by the

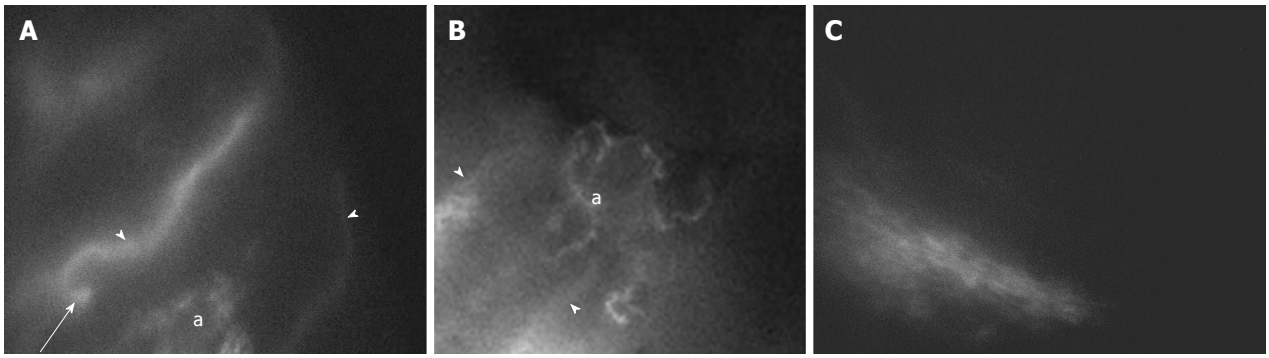


Figure 2 Photodynamic eye lymphography of lymphatic meshes. A and B: Photodynamic eye (PDE) lymphographies of the complex mesh of small lymphatic vessels (a) developed between lymphatic collectors (arrowheads) at 3 mo from surgery in responder patients. Arrow: lympho-venous anastomosis; C: PDE lymphography showing, in a non responder patient, the complete absence of lymphatic network at 3 mo from surgery.

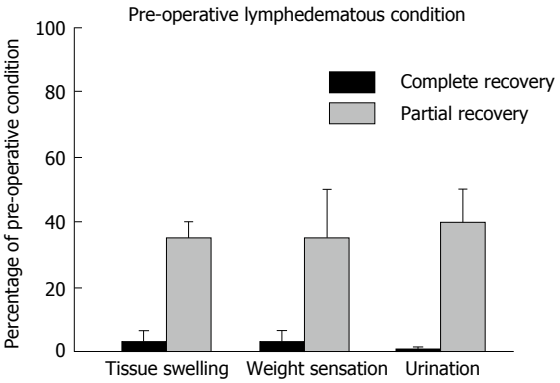


Figure 3 Post surgical self-evaluation of tissue swelling, weight sensation and urination expressed as percentage of the pre-operative lymphedematous condition in patients showing a complete or only partial recovery at three months after surgery. Based on patients evaluation, even partial recovery represented a significant improvement with respect to pre-operative condition. In responding patients the objective clinical outcome was accompanied by a subjective evaluation of almost complete recovery of the normal tissue swelling, weight sensation and urination. Zero percentage on the Y axis corresponds to the normal condition. Histograms presents mean values ± SD of the mean.

planned lympho-venous shunt of spermatic cord. Under general anaesthesia, bilateral funiculum lymphatic collectors (diameter approximately 500 μm) were visualized under stereomicroscopic magnification (40 ×) after cutaneous inguino-scrotal, cremaster and vaginal tunica incision. Identified lymphatic collectors were used to perform (with interrupted stitches, Vicryl 10-0 or 9-0) the lympho-venous termino-lateral or termino-terminal anastomosis, depending upon the proximity and the pathway followed by either the veins of the pampiniforms plexus or the spermatic vein previously prepared through adventixectomy. The number of anastomosis (up to four) depended upon the number of lymphatic collectors found, the higher the anastomosis number the higher the chances of a more effective drainage.

The microsurgical procedure lasted between five and seven hours, depending upon the extent of tissue fibrosis and surgery complexity: indeed, the microsurgical approach is highly demanding in terms of intra-operative technical skill, surgical and laboratory training and on-site imaging equipment. On the other hand, the described microsurgical effort is worth, as it offers several major advantages with respect to the standard approach, such as: (1) absence of clinical complications, (2) low invasivity and more rapid post-surgical recovery; and (3) minor tissue demolition and more satisfactory post-surgical functional and esthetic results.

Since low-weight molecular heparin has been used during the surgery, incidence of thrombosis into the lymphatic channels or in the anastomosis was minimized. Antibiotic was maintained for seven days, while heparin therapy continued for at least 30 d. Neither warfarin nor thrombin inhibitors were necessary.

LONG TERM CLINICAL OUTCOME OF THE MICROSURGERY

At three months after surgery, the patency of lympho-venous anastomosis was assessed by PDE: of the 10 patients who underwent microsurgery of spermatic cord, 8 showed early improvement, while an almost complete (95%-100% of scrotal swelling) remission was observed at six months after surgery. The clearest observation was the correspondence between the clinical outcome, the restored motility of lymphatic collectors observable during PDE lymphography[22], and the anastomosis patency. Indeed in responding patients, the distribution and canalization of the injected fluorescent dye improved over time with clearly visible patent anastomosis (Figure 2A and B). Clear identification of patent scrotal subcutaneous and spermatic lymphatic vessels in responding patients is accompanied by a significant reduction of tissue fluid volume and of the hypogastric abdominal wall thickness. Viceversa, only two patients (not-responders) showed much slower improvement, with only partial recovery (approximately 60%) at six months. In these non-responding patients, PDE images obtained at three months after microsurgery (Figure 2C) highlight the disperse distribution of fluorescent dye in the subcutaneous with difficult localization of the upstream lymphatic collectors and anastomosis.

The post-surgical patient self-evaluation demonstrated (Figure 3): (1) improvement of scrotal tissue softness

and scrotal skin normochromic aspect; (2) pain absence, (3) disappearance of the edematous volume with evident reduction of scrotal and penile dimensions with normal palpability of the testis and decreased weight sensation, (4) decreased urination and (5) the disappearance of episodic erysipelas with respect to the pre-operative condition. The postsurgical improvement assessed at three months after surgery by PDE and self evaluation was maintained over several years in all responding patients. The restoration of the preoperative scrotal size may sometime require an additional plastic surgery to remove the excessive scrotal skin.

COMBINED SWELLING OF EXTERNAL GENITALS AND LOWER LIMBS

The lower limb is supplied by: (1) a superficial lymphatic network, merging into lymphatic collectors directed to the inguinal lymph nodes from which the lymph is carried through the iliac collectors into the cisterna chili and thereafter to the thoracic duct and, eventually, into the subclavian veins; and (2) a deeper lymphatic network, whose collectors follow the sub-fascial limb neuro-vascular plexus to reach the peri-aortic lymph node chains and the thoracic duct. As a consequence, patients undergoing to inguinal and/or pelvic lymphadenectomy may develop a secondary lymphedema of the lower limb. We chose to micro-surgically treat the secondary lymphedema of the lower limb by performing a lympho-venous termino-terminal anastomosis between inguinal pre-nodal lymphatic collectors of the limb and collateral branches of the saphena or, when collaterals were not available, a termino-lateral anastomosis between inguinal pre-nodal lymphatic collectors and the saphena itself[14,20]. In the lower limb, the lympho-venous anastomosis is preferable compared to the lymphatic grafting technique usually performed in the treatment of upper and/or lower limb lymphedema[23]: (1) to more efficiently exploit the strong muscular contraction of the thigh musculature; and (2) to avoid the risk of inducing lymphedema in the previously healthy controlateral leg.

A common clinical observation is that lymphedema of the external genitals is often combined with either unilateral or bilateral chronic swelling of the lower limbs. In the first two cases coming to our observation, a two-stage treatment was performed: first, the microsurgical lympho-venous shunt of the lower limb and then, after six months, the derivative lympho-venous anastomosis of genital organs. The results of this approach clearly indicated that surgical treatment lymphatic collectors of the thigh draining lower limb edema may result in an improvement of the genitalia edema (Figure 4); in turn, shunting of the spermatic cord in treating of genital organ edema often results in recovery from lower limb edema so that lower limb surgery was deemed unnecessary. This could be explained by the fact that, after excision of inguinal lymph nodes, lymph from lower limbs is shunted through the scrotal and testicular pathways. Similarly, when testicular drainage is restored, edema of the scrotum and penis is also significantly improved, possibly because of lymphatic connections between the testicular and scrotal lymphatics networks. These results point to the existence of complex shunt network connecting the local lymphatic drainage pathways. It is worth noting that the target of physical therapy is to improve lymph-edematous tissue drainage bypassing the obstruction by recruiting shunted pre-existing lymphatic vessels usually supplying adjacent tissue districts.

POST-SURGICAL RESPONSE OF THE LYMPHATIC VASCULATURE

In addition to recruitment of patent superficial and spermatic cord lymphatic vessels, the development of peri-anastomotic lymphatic meshes in different tissues such as the spermatic cord, the inguino-crural and brachial regions was observed and documented for the first time in several patients who positively responded to microsurgery. Meshes were instead never encountered by PDE in non-responding patients or in normal tissues. Lymphatic meshes (Figure 2) consist of several lymphatic vessels sprouting from the anastomosis and/or between afferent lymphatic collectors and merging into well-canalized and complex networks. Subcutaneous meshes could be detected as early as three months after surgery and their complexity and extension seemed to improve over time, as shown by PDE images at 6 mo.

Lymphatic meshes were observed not only in patients who underwent spermatic funicular microsurgery, but also in patients who positively responded to the treatment of the lower limb swelling (Figure 5). On the contrary, no canalized lymphatics were evidenced at the same post-surgical follow-up in non-responding patients, who showed no significant improvement when compared to the pre-surgical PDE.

The development of this network might well explain the successful outcome of the microsurgical procedure in responding *vs* non-responding patients with little or no improvement. Indeed, it seems difficult to attribute the positive results of the surgical treatment to improvement of only one single lymphatic draining district. It is at present not known whether lymphatic meshes of the type observed in this study after microsurgical procedure may develop also in conservative physical therapy.

The post-surgical development of peri-anastomotic lymphatic meshes might serve as: (1) capacitance reservoirs to improve the draining capability of the existing lymphatics and maximize the drainage capacity of the postsurgically-canalized lymphatic vessels; and (2) intermediate compartments placed in series between the smallest initial lymphatics taking place from the edematous tissue and the larger collecting vessels transporting the lymph out of the tissue. At present, we are unable to precisely establish whether these lymphatic meshes reflect recruitment of previously existing but obliterated networks or, rather, new lymphangiogenesis. The fact that

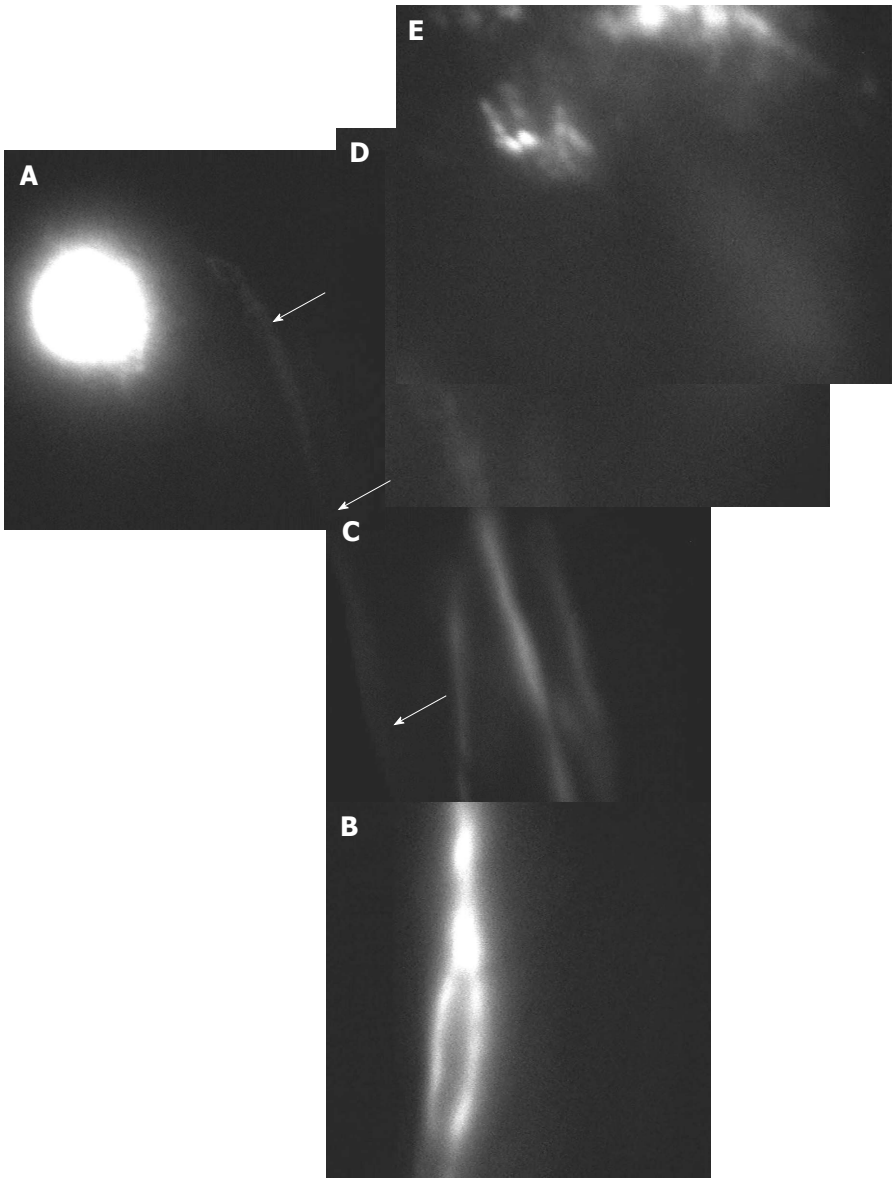


Figure 4 Photodynamic eye lymphography performed at three months after lympho-venous shunt of the the spermatic cord in a patient affected by lymph-edema of the lower limb and of the external genitals secondary to radical prostatectomy and lymphadenectomy of the pelvic-inguinal lymph-nodes chain. After injection (bright spot, A) in the scrotum, fluorescent indocyanine travels along a lymphatic vessel (arrows) in the thigh to connect with the lymphatic network of the lower limb (B). After injection in the foot, indocyanine flows along the lower limb lymphatics vessels (B and C) to be shunted directly into the external genitals lymphatic plexus (D and E) bypassing the excised pelvic-inguinal lymph-nodes chain.

meshes were never observed in PDE lymphography of normal healthy tissues, but only during the post-surgical follow up in previously oedematous tissues of patients which positively respond to microsurgery might support the hypothesis of local lymphangiogenesis triggered by the minimally invasive microsurgical procedure within a process of extracellular matrix and lymphatic repair. In particular, the visual observation that the sprouting was particular intense in proximity of the patent anastomosis, is in line with the experimental observation[24] that, in the rat tail in presence of interstitial fluid flow, such as that induced by the new patent lympho-venous anastomosis, lymphatics may bypass a tissue lesion, reconstructing the original lymphatic pathway.

Although the lymphatic system still represents the least known compartment, particularly in humans, of the cardiovascular-extracellular tissue system, a growing amount of information is available on lymphatic cell biology and on the mechanical and molecular factors promoting functional lymphangiogenesis[25-32]. During embryogenesis the endothelial cells of the cardinal vein express the lymphatic endothelium-specific hyaluronan receptor (LYVE-1) and the VEGFR-3, which serves as a signalling receptor for the lymphatic-specific growth factors C (VEGF-C) and VEGF-D. Activation of VEGFR-3 signal is sufficient to induce lymphangiogenesis[30]. Later in adult life both LYVE-1 and VEGFR-3 are expressed by the lymphatic, but not by the blood endothelial cells. Subsequently, a still undefined mesenchymal factor induces, in specific endothelial cells of the cardinal

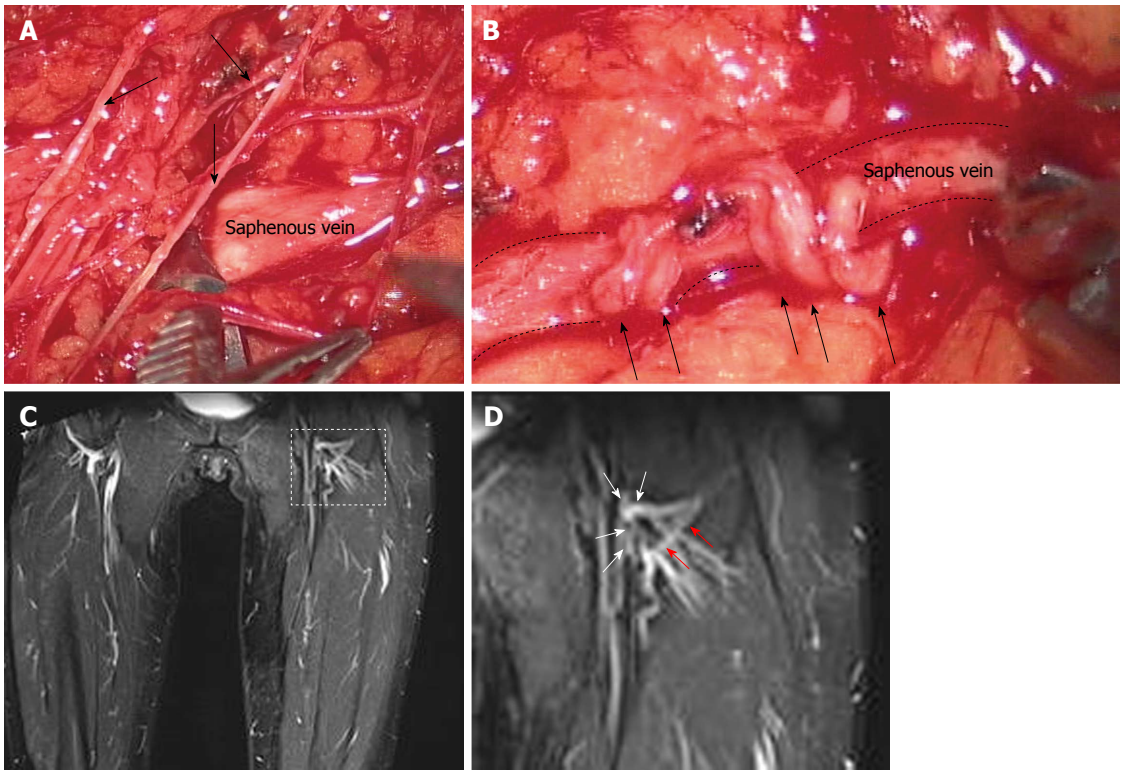


Figure 5 Lymphatic meshes were observed not only in patients who underwent spermatic funicular microsurgery, but also in patients who positively responded to the treatment of the lower limb swelling. A: Microsurgical identification of the saphenous vein and of lymphatic vessels (arrows) in the crural area; B: Latero-terminal lympho-venous anastomosis, confectioned with interrupted stitches, between lymphatic vessels (arrows) and the saphenous vein (dashed line) in proximity of the femoral-saphenous cross; C: Magnetic resonance image of the lower limb obtained in the same patient of A, B at 8 years after microsurgery. Details of the left limb crural area (delimited by the dashed line) are presented in D; D: White arrows identify the sites were latero-terminal lympho-venous anastomosis were confectioned, while red arrows focus on a new developed lymphatic collector.

veins the expression of the protein Prox1[31,32] which in the adult, is expressed by the lymphatic endothelium and by several non endothelial cells like lens, retina, central nervous system, heart, liver and pancreas[33-35]. Prox1-positive cells emerge from the original vessels to form new lymphatic sprouts. Prox1 is necessary to the development and differentiation of lymphatic endothelial phenotype[31,32], a phenomenon which implies induction of lymphatic specific genes such as VEGFR-3, podoplanin and desmoplakin-1[25,28] and simultaneous repression of blood vascular specific genes like collagens, laminin, VEGF-C, E-selectin, neuropilin-1 and interleukin-8. The transmembrane mucin-type glycoprotein podoplanin, expressed during embryogenesis by all cardinal vein and Prox1-sensitive cells seems to play an important role in lymphatic endothelial cells migration, adhesion and vessel formation[29].

At present, no information are available concerning the expression of pro-lymphangiogenetic endothelial and tissue molecules in human lymphedematous compared to normal tissue in murine models of primary lymphedema[33], dermal tissue swelling, interstitial fluid and solute transport critically depend on collagen and lipid dermal composition, suggesting that lymphatic function may be modulated by extracellular structure and macromolecular content. However, the behavior of normal lymphatic vessels exposed to either radiation or trauma, as in secondary lymphedema patients, may be quite different from those induced by primary genetic mutation.

CONCLUSION

The choice of the microsurgical lympho-venous derivation in the spermatic cord is highly recommended in the treatment of secondary severe chronic lymphedema of the external male genitals. Indeed, in addition to its direct significant advantages with respect to the standard approaches, such as: (1) minor tissue demolition and more satisfactory post-surgical functional and esthetic results; (2) absence of clinical complications; and (3) low invasivity and more rapid post-surgical recovery, the microsurgical lympho-venous derivation offers the unique possibility of improving lymphedema in adjacent tissues which are supplied by lymphatic collectors emptying into common lymphatic shunt.

Last but not least, lympho-venous microsurgery triggers the development of a complex network of new lymphatic vessels in proximity to the anastomosis area. This phenomenon, that we documented for the first time in human patients, seems to play a crucial favorable role in guaranteeing the long term recovery from chronic lymphedema after microsurgical treatment.

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MINIREVIEWS

Is periprostatic adipose tissue associated with aggressive tumor biology in prostate cancer?

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Abstract

The prevalence of overweight and obesity and their health-related problems have been increasing. Obesity is increasingly recognized as a risk factor in different types of cancer in humans. The mechanisms supporting the link between obesity and cancer development have not been fully understood. Leptin, a circulating cytokine produced by adipocytes, may influence prostate cancer (PCa) progression in different ways. Body mass index seems to be an unreliable predictor for the development of PCa, but its influence on progression and poor oncological outcomes seems to be clear. Given the fact that abdominal fat is the most metabolically active fat, with different metabolic and paracrine effects, related anthropometric measurements may lead to a better estimation of PCa risk. Metabolically active periprostatic abdominal fat may also play an important role in releasing cytokines and growth factors that may promote tumor cell proliferation or even create a favorable environment for aggressive tumor biology. Different imaging measurements, *e.g.*, periprostatic adipose tissue (PPAT) thickness, may be significant predictors of PCa. Several genes in the PPAT of obese men have been identified to contribute to chronic immuno-inflammatory responses which eventually lead to cell cycle alteration with oncological potential. *In vitro* studies showed the importance of PCa and its interaction with its microenvironment particularly in patients with aggressive PCa. Different types of cytokines, such as interleukin-6, may promote a tumorigenic microenvironment. This article endeavors to review the current literature on the association of PPAT with aggressive tumor biology in PCa.

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Key words: Prostate cancer; Obesity; Periprostatic fat

Core tip: Globally, prostate cancer (PCa) is highly prevalent. Although the prevalence of PCa is similar across different populations, major differences in PCa incidence and mortality are seen worldwide. A contribution of environmental factors, such as obesity, may play an important role. Most studies used body mass index as a factor of obesity. However, only visceral fat is metabolically active. In a study by van Roermund *et al*, periprostatic fat measured on a computed tomography scan correlated with tumor aggressiveness. In this review, we aim to give more insight into the relationship between periprostatic fat and Pca aggressiveness by reviewing the recent literature.

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INTRODUCTION

Over the past few decades, the prevalence of overweight and obesity have been increasing in developed countries[1]. Obesity is currently seen as a serious health problem in particularly well-developed populations. More

recently, it has been hypothesized that obesity could potentially influence the risk of developing cancer. Several studies already showed a correlation between obesity and different types of cancer[2-4]. However, the relationship between obesity and the risk of developing prostate cancer (PCa) is still unclear, as studies are inconsistent in their outcome[5,6]. The incidence of clinically significant PCa is highest in well-developed countries, and in Europe, PCa is the second most common cause of cancer-related death in men[7].

Although many risk factors for PCa are well known, including age, ethnic background, and family history, the mechanisms of the interactions between lipids and carcinogenesis are not fully understood. Multiple studies suggest an important role for metabolic active periprostatic adipose tissue (PPAT) in the risk of developing PCa[8-12]. This review specifically considers the relationship between PPAT and the risk of developing aggressive PCa, and discusses the underlying mechanisms and clinical consequences.

RESEARCH

For this review a literature search in PubMed was performed using the MeSHsearch terms; "periprostatic fat", "obesity" linked with "prostate cancer". Studies relating to periprostatic adipose tissue and PCa were selected, and limited to articles in the English language. Studies identified in references and considered relevant were also included.

OBESITY AND PROSTATE CANCER

Obesity is considered as one of several factors potentially related to the development of clinically significant PCa. Earlier postmortem research showed a high prevalence of PCa, occurring in approximately 80% of men > 70 years of age who died from other causes[13,14]. The high prevalence of PCa in elderly males supports a hypothesis that progression of latent PCa to clinically significant PCa might be associated with environmental and lifestyle factors. In particular, abdominal obesity may be an important factor influencing progression of latent PCa to clinically significant PCa, rather than development of latent disease.

Evidence that body mass index (BMI) is related to a higher incidence of PCa is still inconsistent. A prospective cohort study in 900000 adults showed a significant positive linear trend in death rates with increasing BMI for all cancers, including PCa[3]. In that study, men with a BMI of at least 35.0 appeared to have a significantly elevated relative risk of cancer-related death compared with normal weight males. In relation to these data, a systematic review of MacInnes evaluated the association between anthropometric measurements and the risk of PCa[15]. In that meta-analysis a weak positive correlation between the risk of PCa and BMI and waist-hip ratio (WHR) was found. Moreover, subgroup analysis showed a positive correlation between the risk of advanced PCa and BMI and WHR, supporting the hypothesis that obesity might influence PCa progression or tumor biology. In contrast, no direct correlation between PCa and weight was found in the study.

A large European prospective cohort study in 150000 men suggested that higher waist circumference and WHR may be associated with increased risk of advanced PCa and high-grade PCa amongst individuals with lower BMI. No association was seen in males with higher BMI levels, suggesting that abdominal adiposity mainly played an important role in influencing PCa[16]. An explanation for the inconsistency in the results can be found in the fact that BMI, or even WHR, are inadequate measures of obesity as they include adipose and non-adipose body components. Given the fact that abdominal fat is the most metabolically active fat tissue, we should explore other methods for estimation of adipose tissue, such as fat distribution, which may eventually link obesity to PCa.

Several studies have correlated obesity with higher PCa grade and poorer oncologic outcomes[17-20]. Capitanio *et al*[17] showed that BMI was independently associated with PCa volume at radical prostatectomy. These results were confirmed by Freedland *et al*[18] in over 2200 patients treated with radical prostatectomy, and showed that obese males were more likely to have high-grade tumors. In addition, more adverse pathological features and a higher risk of biochemical recurrence were seen in obese patients. Significantly higher rates of PCa recurrence over time were observed in obese patients (BMI $\geqslant$ 30) compared with their non-obese counterparts in two other studies[19,20].

Possible mechanisms linking obesity and cancer involve endocrine disturbances such as increased serum estrogen, insulin, insulin-like growth factor and leptin, and decreased free testosterone[21-25]. However, the mechanisms by which obesity leads to an increased risk of PCa are not known, and may occur at multiple levels. Recently, different *in vitro* studies on leptin have been performed. Leptin is a cytokine produced by adipocytes. Like insulin, leptin controls body weight homeostasis through food intake and energy balance[26]. Circulating serum leptin levels correlate with body fat in humans. Hoda *et al*[21] showed that a chronic systemic increase in leptin might enhance the growth of prostate or other cancer cells by activation of the mitogen-activated protein kinase pathway. Moreover, leptin stimulated growth of breast, esophagus, and prostate cancer cell lines in different studies[24,27,28]. In addition, leptin may accelerate PCa progression by promoting androgen-independent cell proliferation[25].

PERIPROSTATIC ADIPOSE TISSUE

PPAT surrounds the prostate capsule[29]. In an earlier study of Hong *et al*[30], 100 prostatectomy specimens were analyzed, and showed that adipose tissue covering the prostatic surface was present in 48% of prostates, with the least adipose tissue found posteriorly. By analyz-

ing PPAT, valuable new insights could be attributed to the role of adipose tissue in PCa pathophysiology. This particular adipose tissue is not only used for storage of triglycerides, but also plays a role in releasing cytokines and growth factors that may promote tumor cell proliferation or play a role in creating a favorable environment for aggressive tumor biology[10,11,31,32]. Finley *et al*[10] analyzed PPAT of patients who underwent a radical prostatectomy. They suggested that once cancer cells extend beyond the prostate capsule, these PPAT-secreted factors, may influence the phenotypic behavior of malignant cells *via* extracellular matrix components or direct cell-to-cell contact. This cellular mechanism and PPAT-secreted factors have been proposed in a limited number of different publications which we will discuss below.

IMAGING

Only three studies used *in vivo* imaging techniques in investigating the relationship between PPAT and PCa. van Roermund *et al*[8] retrospectively analyzed whether periprostatic fat and subcutaneous fat measured by computed tomography correlated with advanced PCa stage in a group of patients who underwent external radiotherapy or brachytherapy for localized PCa with different risk classifications according to Ash *et al*[33]. A significantly larger total periprostatic fat area and higher periprostatic fat density was seen in patients with high risk PCa, compared with low and intermediate risk patients[8]. An association was found between more periprostatic fat and higher periprostatic fat density, and prostate-specific antigen (PSA) $\geqslant$ 10 ng/mL, a T3 tumor, and a Gleason score $\geqslant$ 8. A different study, analyzing PPAT as a marker for PCa aggressiveness in patients only treated with brachytherapy, showed no correlation between PPAT and advanced PCa stage[34]. A confounding factor might be that brachytherapy is mainly used in low-risk PCa patients, which might explain the absence of a correlation in this particular study.

Bhindi *et al*[9] evaluated whether PPAT thickness on transrectal ultrasound could be a predictive factor for the detection of PCa in men with no prior diagnosis of PCa. Besides known predictors of PCa, such as older age, African ethnicity, family history of PCa, abnormal digital rectal examination (DRE) and elevated PSA level, PPAT thickness was also a significant predictor for detection of PCa or high-grade PCa. They suggested that for each millimeter increase in PPAT thickness, the odds of detecting any PCa or high-grade PCa increased by 12% and 20%, respectively. So beside other clinical parameters, including age, abnormal DRE and PSA level, PPAT may have a clinically significant role in diagnostic algorithms for PCa in the future[9].

GENETICS

The individual genome represents the starting point for transformation of prostate epithelial cells and its microenvironment. Knowledge of the PPAT genetic profile may uncover more about the relationship with PCa tumor biology. Until now, only one study reported on the gene expression of PPAT in relation to PCa. Ribeiro *et al*[35] aimed to determine the spectrum of genes expressed in PPAT to evaluate the influence of obesity on PCa and vice versa. PPAT was obtained from 18 patients, with an equal distribution of obese and lean men. Patients underwent a retropubic radical prostatectomy for PCa (organ-confined and extra-prostatic) or open prostatectomy in the case of benign prostatic hyperplasia. Results showed that several genes in the PPAT of obese men contributed to chronic immuno-inflammatory responses, *i.e.*, anti-lipolytic, lipogenic, proliferative and anti-apoptotic activities. These immuno-inflammatory responses lead to white adipose tissue overgrowth and alteration of the cell cycle to promote oncogenesis[35,36]. These findings support the importance of PPAT and the presence of a complex relationship between obesity and PCa.

MICROENVIRONMENT

Tumor cell progression depends on the tumor characteristics as well as on the surrounding microenvironment. Moreover, after highlighting the importance of the genetic profile, we should not underestimate the influence of interactions between tumor cells and their microenvironment[10,31,37-39]. The microenvironment is able to influence the proliferation, migration and metastatic behavior of tumor cells by modulating the extracellular matrix and growth factor production[40]. In breast cancer, a paracrine effect of adipose tissue on the microenvironment of the tumor is already associated with poorer oncologic outcomes[41]. Tokuda showed in an *in vitro* study that PCa cells grow differently in an adipocyte-rich environment, compared with control cultures[39]. In particular, higher cytokine expression in PCa cells was seen when cultured in an adipocyte-rich environment.

A pilot study by Venkatasubramanian *et al*[42] on PPAT from obese PCa patients aimed to explore whether PPAT from obese patients differed from that of lean patients, with specific attention to differences in the microenvironment. *Ex vivo* magnetic resonance imaging and histology were used on the collected tissue of patients who underwent a radical prostatectomy for primarily low-grade PCa. PPAT from obese patients showed significantly increased proliferation of PCa, compared with PPAT from lean patients.

Visceral adipose tissue differs from peripheral adipose and secretes different types of cytokine-like adipokines, leptin and adiponectin, numerous inflammatory mediators, interleukins, chemokines and growth factors. Finley *et al*[10] explored the association of cytokines and growth factors secreted by PPAT in patients with aggressive PCa. In this study, significantly higher levels of interleukin-6 (IL-6) were shown in PPAT of patients with higher grade tumors. Higher IL-6 levels were associated with downstream activation of signal transducer and activa-

tor of transcription 3 phosphorylation, which promotes cell cycle progression, tumor invasion and host immune system evasion. This suggests that PPAT modulates PCa aggressiveness by serving as a source of IL-6. This modulating role of PPAT is confirmed by Ribeiro *et al*[11], who showed locally increased activity of matrix metalloproteinase (MMP), a proteolytic enzyme facilitating and promoting PCa cell survival and migration. Interestingly, higher levels of MMP9 were seen in obese men with esophageal adenocarcinoma, and is associated with poor tumor differentiation in these patients[43]. In PCa, MMP activity in PPAT is significantly increased compared with that in the PPAT of patients with benign prostatic hyperplasia[31].

CONCLUSION

Though limited, studies evaluating the association between PPAT and PCa describe evidence for an interaction between PCa and its surrounding tissues. Data suggest that local adipose tissue can alter the behavior of cancer cells in different ways. Microenvironment-related release of factors such as cytokines and interleukins, play an important role in promoting survival and migration of PCa cells, and thus PCa progression. Different genes in PPAT of obese patients also contribute to a favorable environment for tumor growth. Given that PPAT thickness can be related to PCa aggressiveness, this may represent a diagnostic tool with important prognostic value, since BMI is unreliable as a prognostic factor. The findings of this review of the literature should promote future studies to further investigate the value of PPAT in PCa etiology and its relationship to the development of aggressive PCa. New therapeutic strategies could be developed, and more studies of the diagnostic value of PPAT should be pursued.

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MINIREVIEWS

Morphological and functional evaluation of chronic kidney disease using magnetic resonance imaging

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Abstract

X-ray computed tomography (CT), ultrasonography (US) and radionuclide scanning are important clinical methods for evaluating morphology of the kidney. These modalities are also applicable for estimating kidney function with time lapse analysis using proper contrast-media as may be necessary. In the case of US, it can estimate kidney function based on the measurement of blood flow using the Doppler effect. Formerly, magnetic resonance imaging (MRI) was an inappropriate diagnostic imaging technique for abdominal organs because of their respiratory displacements. However, MRI is now actively used for kidney as well as liver or other parenchymal organs, in tandem with the technological advances. Unlike unenhanced X-ray CT, "conventional" MRI can distinguish the border between cortex and medulla in T1 or T2 weighted images. It was known that the border blurred with decreasing kidney function. Moreover, several other particular imaging methods were introduced in recent years, and these could be called "functional" MRI. In this review, the following are discussed: functional MRI for chronic kidney disease, which include blood oxygenation level-dependent MRI for evaluation of hypoxia, diffusion-weighted imaging for evaluation of fibrosis, diffusion tensor imaging for evaluation of microstructure, and arterial spin labeling to evaluate the amount of organ perfusion, accompanied with several related articles. The ultimate goal of functional MRI is to provide useful *in vivo* information repeatedly for daily medical treatment non-invasively.

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Key words: Magnetic resonance imaging; Chronic kidney disease; Blood oxygenation level-dependent effect; Diffusion-weighted imaging; Diffusion tensor imaging; Arterial spin labeling; Fibrosis; Hypoxia; Functional magnetic resonance imaging

Core tip: Recent advances in diagnostic imaging technology allow us to evaluate the function of parenchymal organs such as kidney and liver. For example in the kidney, magnetic resonance imaging technique can visualize changes in the ratio of oxy-/deoxy-hemoglobin, the accumulation of extra cellular matrix, the alteration of microstructure of tubules and blood capillaries in the interstitium, and the amount of organ perfusion without using any contrast medium. Thus, with imaging technique, the nephrologist can evaluate a kidney from multiple points of view. This is an emerging modality, and it's hoped-for mission is providing significant information for daily clinical decisions. Accumulation of further knowledge is keenly anticipated.

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INTRODUCTION

Magnetic resonance imaging (MRI) until now has had an

important role as a diagnostic imaging method for urology and nephrology. Gadolinium contrast medium was used frequently for examinations in the past. However, since nephrogenic systemic fibrosis was identified, the frequency in use of the contrast media in renal disease seems to have decreased. However, significance of the role of MRI is increasing. MRI, which is superior in contrast resolution of soft tissues to X-ray computed tomography (CT) and ultrasonography, provides important information for internal architecture of the kidney and adrenal gland, or qualitative diagnosis of tumor lesions (*e.g.*, presence of fat composition) particularly in cases of kidney dysfunction for which it is difficult to use iodine-based contrast medium. Furthermore, magnetic resonance angiography is useful for evaluation of renal artery lesions similar to ultrasonographic examinations. Even for similar tomography, MRI, unlike X-ray computed tomography, enables the evaluation of the inner structure such as the corticomedullary border in the kidney without using contrast media. Changes related to renal functions have been known to occur, such as the disappearance of the corticomedullary border as renal dysfunction worsens. Moreover, what is frequently used for clinical MRI examinations presently is nuclear magnetic resonance of the hydrogen (proton) atomic nuclei abundantly contained in human bodies as moisture, which enables various evaluations by devising imaging methods as one of the features of MRI.

ORIGIN OF FUNCTIONAL MRI

Development of origin of functional MRI (fMRI) originally started as a noninvasive imaging method for brain functions. The phenomenon that is frequently targeted in fMRI is blood oxygenation level-dependent (BOLD) effects, indicating that difference in susceptibility of oxidized hemoglobin and reduced hemoglobin exerts a particular influence on the T2* relaxation time. In an activating site of the brain, the blood flow that surpasses it increases with an increase of the oxygen consumption, the reduced hemoglobin which is paramagnetic material decreases, and prolongation of the T2* relaxation time occurs. In other words, it is a method to grasp an activating site of a brain not by electrical activity of cerebral nerve cells but by changes in accompanying hemodynamics[1]. Brain MRI is captured continually and an activating site is mapped in comparison with the resting state, while having the subject repeat specific tasks such as visual/auditory stimulation and finger motion.

fMRI IN THE KIDNEY

Hypoxia and fibrosis are important factors determining progression of chronic kidney disease

Renal functions are evaluated in tests related to the renal blood flow such as the glomerular filtration rate and renal plasma flow (RPF), which is still a gold standard. However, in imaging studies, in addition to X-ray CT with contrast medium and MRI, quantitative blood flow evaluation is realized by using carbon dioxide contrast medium and ultrasonography. It has already been shown that the renal blood flow measured by these imaging studies correlate well with estimated glomerular filtration rate (eGFR) with serum creatinine, and it can be regarded as functional image evaluation of kidney. The decrease of renal blood flow in chronic kidney disease (CKD) occurs not only in large or medium vascular diseases such as renal-artery stenosis but also accompanies degeneration of the kidney as in glomerulosclerosis and tubular atrophy/interstitial fibrosis. A number of past studies have revealed that a decrease in the renal blood flow causes hypoxia, which causes further inflammation and fibrosis and lowers the renal blood flow, with ensuing CKD[2]. From the above, it was decided that MRI for evaluating the clinical condition of CKD including blood flow and partial pressure of oxygen, as well as fibrosis of the kidney is called fMRI for kidney. It is distinguished from the conventional MRI, which focuses on forms/morphology. The renal fMRI, which is expected to be applied to future clinical practice, is described below (Figure 1).

BOLD MRI: Evaluation of hypoxia

The first report was from Prasad *et al*[3] in 1996. His group evaluated kidneys based on T2* weighted images and revealed that when energy consumption of the renal tubule is reduced with administration of furosemide and a large quantity of drinking water, T2* relaxation rate: R2* (= 1/T2*) in the medulla decreases. They concluded that the change was reflected by a decrease in reduced hemoglobin, capturing improved oxygenation in the site. Further, partial pressure of oxygen was higher in the cortex than in the medulla even before the drug administration and drinking water load and its change was still poor thereafter. Based on the past results, it was expected with more blood flow per unit weight in the cortex than in the medulla and the high partial pressure of oxygen in the tissues that much energy is consumed by the medulla for urine concentration. Then the difference in the partial pressure of oxygen in renal parenchyma and the physiological function of each tubular segment was reconfirmed by fMRI, which is a totally different method. In 2005, the relationship between the microelectrode method, which is a direct oxygen partial pressure evaluation method, and R2* values of BOLD MRI was studied using porcine kidneys. Changing inspired oxygen pressure using a respirator continually, the authors measured pO_2 by the microelectrode method and R2* values of the MRI for both medulla and cortex of kidney and a significant correlation between them was found[4]. As described above, the utility of MRI has been recognized since it is an experimental method that can examine an acute and transient oxygen pressure change in a noninvasive manner before and after medication in place of the microelectrode method. In 2006, Thoeny *et al*[5] studied 15 cases of renal-transplant recipients and healthy subjects after drinking sufficient water. They reported a significant correlation

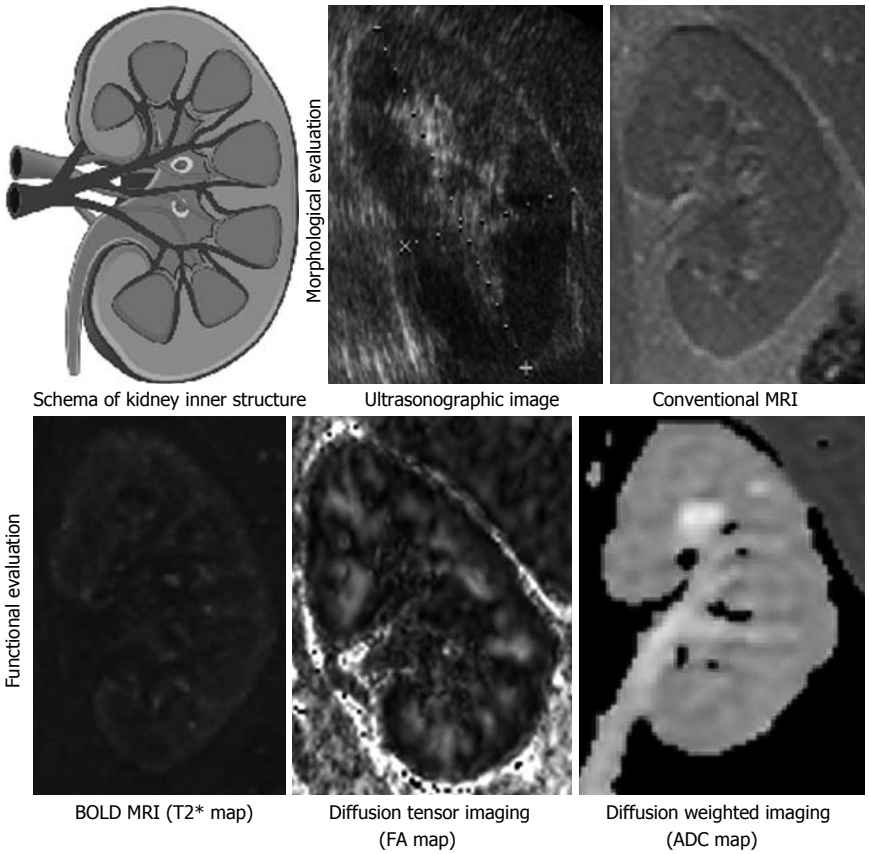


Figure 1 Various modalities for kidney. It is noteworthy that the cortex and medulla are distinguishable on the basis of function or microstructure in functional MRI. For example, in T2* map of BOLD MRI, the medulla was seen darker than the cortex because of the difference in amount of deoxy-hemoglobin; in FA map of diffusion tensor imaging, FA of medulla was higher than the cortex probably because straight microstructure of medulla including distal tubules and surrounding blood capillaries restrict irregularity of Brownian movement of protons. Contrast and angle are adjusted respectively. Each of the kidney images is of a different patient. BOLD: Blood oxygenation level-dependent; MRI: Magnetic resonance imaging; FA: Fractional anisotropy; ADC: Apparent diffusion coefficient.

of the serum creatinine level and R2* value of MRI, with the apparent diffusion coefficient (ADC) of diffusion weighted images described below[5]. It was reported for the first time that R2* values enable evaluation not only of the relative change in transient oxygenation but also oxygenation (or hypoxia) when renal function and hemodynamics are stable. It was also an important report in indicating the possibility of MRI as an evaluation method for chronic hypoxia in CKD.

Diffusion-weighted imaging: Evaluation of fibrosis

The diffusion weighted image (DWI) is one that visualizes the difference in random movements of water molecules in tissues (Brownian motion). It is thought that perfusion in capillaries and diffusion in the target tissues are more purely evaluated for small *b* values (s/mm^2) and great *b* values, respectively. Further, ADC values calculated with plural diffusion weighted images from different *b* values can be quantitatively evaluated without being affected by T2-weighted images. Namimoto *et al*[6] measured ADC values of cortex and medulla of 16 kidneys from 8 CKD cases in which renal function had deteriorated, and 8 kidneys from 16 cases of subjects with normal renal function. They showed ADC was significantly decreased in both cortex and medulla in CKD[6]. For *b* values, Thoeny *et al*[5] studied ADC for $b = 0.50, 100$ s/mm^2 (low); that for $b = 500, 750, 1000$ s/mm^2 (high); and separately for the average (avg) ADC. They found all ADC (low), ADC (high) and ADC (avg) decreased in both cortex and medulla of CKD patients as compared with those of normal kidneys from healthy volunteers although there were only 18 healthy volunteer cases and 15 CKD cases[7]. In healthy kidneys, both ADC (high) and ADC (avg) were always lower in the medulla than in the cortex whereas a difference was not seen in ADC (low) between cortex and medulla. Possibly this is because the regularly-structured renal tubules and blood capillaries, which are a characteristic of medulla, are generated by the diffusion limit of water molecules. In 2007, for 110 kidneys from 55 cases, the relation of ADC values ($b = 0$ and 500 s/mm^2) was studied with 99mTc-diethylenetriaminepentaacetic acid renal dynamic scintigrams and their positive correlations were found[8]. It has been known for a long time that the remaining renal function of CKD correlates well with degrees of tubular degeneration and fibrosis of interstitial tissue. It has been inferred that ADC values are an index of fibrosis from studies on imaging principle and cirrhosis; in 2010, Togao *et al*[9] reported that ADC values decreased renal parenchyma on the ligation side and it is the change corresponding to fibrosis that was

histologically demonstrated, using the unilateral ureteric ligation model, which is a model of postrenal failure[9].

Diffusion tensor imaging

Originally, diffusion of water molecules is random three-dimensionally, and does not have specific directionality though diffusion that has "directionality" when there is structure disturbing the diffusion occurs. This is called "anisotropic diffusion" and diffusion tensor imaging (DTI) is the method to image this anisotropic diffusion as well as evaluating it quantitatively. The myelin sheath of nerve fibers is an obstacle to the diffusion of water molecules in the brain and spinal cord and therefore courses of the nerve fibers can be visualized in DTI. Fractional anisotropy (FA) is an index that reflects diffusive anisotropy. This is an index that shows how the diffusion deviates from isotropic diffusion (fully random diffusion without diffusion restrictions), and it gets close to 0 in the case of isotropic diffusion and to 1 when the anisotropic diffusion is large. Moreover, in tractography the direction of anisotropy is tracked linearly and displayed three-dimensionally, and in particular an image of a brain seems to imitate the course of nerve fibers[10]. Since DWI has begun to be applied to kidneys, anisotropic diffusion is likely to occur in the renal structure, which has regularity in renal tubules and vascular courses. As a result of studying FA values of kidneys for 10 healthy subject cases, FA values of medullas that had regular radial structures were higher than those of cortex, indicating that DTI enables evaluation of renal microstructure. In a study on kidney transplantation, a significant positive correlation was recognized in FA values and eGFR, and it is presumed that change in the inner structure accompanied by a decrease of the renal function was captured[11]. The past studies reported a significant correlation between the renal function and FA values of medulla, indicating mainly that they reflect the degrees of disorder of the tubular structure[12].

Study results of fMRI for CKD and transplanted kidney

The preceding material described methods of renal fMRI that have been most widely reported. Hypoxia and fibrosis, two major factors that are related to the development of CKD, can be evaluated. If it can be applied to clinical practice as in a periodic examination, clinical conditions of CKD at the time of the primary diagnosis can be understood and can capture the changes in the kidneys before alterations are detected in the serum creatinine levels and proteinuria. In addition, it is useful for measurement of effect after treatment intervention, and can contribute to efficient innovative drug development according to the authors. Indeed, reports on its usability as a laboratory study have been increasing. The author measured T2* values of the BOLD MRI and ADC values of DWI for CKD of 43 diabetic nephropathy cases and 76 nondiabetic nephropathy cases, (approximately 120 cases in total) and examined their correlations with eGFR and renal major axes[13]. The results revealed that T2* values and ADC values had significant correlation with eGFR and renal major axes in nondiabetic nephropathy and that major axes of kidneys with decreasing GFR were shortened (= atrophy), and fibrosis developed in such kidneys and were hypoxic. Since renal biopsy was performed for 37 cases, deposition of collagen fibers was measured by Masson trichrome stain as evaluation of interstitial fibrosis. In the kidneys in which interstitial fibrosis had developed, the ADC value significantly decreased, which was the result expected by imaging principle and animal experiment. The degrees of atrophy of the kidneys and ADC values were correlated even for diabetic nephropathy and "fibrosis kidney = kidney atrophic" though hypoxic degrees were independent from fibrosis and eGFR. The cases indicating high hypoxia without noteworthy fibrosis nor decrease of eGFR were remarkable, because future aggravation of renal function is expected. In addition, the hypoxia is likely occurring by a reversible cause and therefore it is amenable for stricter intervention by nephrologists. Recently, results of tracking recipients from 9 renal transplant cases for 3 years have been reported[14]. ADC values and the R2* values of the transplanted kidney by cortex and medulla were compared soon after the transplantation (7 ± 3 mo) and approximately three years later (32 ± 2 mo). In the eight cases in which renal function was stable for three years, other measured values except the R2* values of cortex were almost similar and no difference was noted. On the other hand, in the one case in which renal function decreased, increase in R2* values and decrease in ADC values were recognized, suggesting aggravation of hypoxia and development of fibrosis. These results showed reproducibility that can be evaluated longitudinally for at least DWI and BOLD MRI. In addition, the interesting point is that change in measured values accompanied by the degenerating renal function was captured in the same case. Further, even when renal function is stable on the blood test, significant change in the R2* values suggest progress of hypoxia in cortex is recognized, and it is particularly worth noting that it reminds us of its relation with the immunosuppressive drug used frequently after transplantation, particularly calcineurin inhibitor.

Other imagings

There is an imaging method called arterial spin labeling (ASL), which is a method to evaluate brain perfusion. This is a method by which the spin in blood flowing into the target organ is labeled by magnetizing with radio frequency to evaluate the amount of organ perfusion with the blood itself as an endogenous tracer. A perfusion image is obtained from the difference of the images before and after the labeling. If however, the signal noise ratio (S/N) is low, it is necessary to image repeatedly and integrate them and therefore the imaging time is increased. Instruments having a magnetostatic field of 3 teslas have been introduced for clinical use. The improvement of images and shortening of imaging time are being attempted and therefore their clinical application is approaching feasibility. There still are a few reports that examined the

amount of kidney perfusion by similar methods. The relationship of kidney perfusion with clearance of para-aminohippuric acid that is RPF was studied in 2010, and a significant correlation was shown with amounts of perfusion per unit weight measured by ASL[15]. In addition, reports on kidney transplantation and reports that clarified a decrease in amount of perfusion in a CKD case in comparison with healthy subjects have come to be recognized[16]. MRI images not only protons but also other atoms. In evaluation of kidneys by Sodium-23 (^{23}Na) MRI, the behavior of Na concentration increasing progressively toward the depths of the medulla from a cortex surface layer can be obtained as an image. MRI also observed that a Na concentration gradient gradually disappears when renal ischemia is caused experimentally. It is probably the change that reflects tubular impairment due to ischemia[17].

CONCLUSION

Most of the research reports have been published within the past ten years and renal fMRI is now in the dawn of its emergence. Correlation with eGFR and RPF, which are classic indices of the renal function, is discussed in most papers. However, in kidneys for which positional information is not as important as in the brain (though there is difference between right and left), it is meaningless to introduce a large-scale device and re-evaluate the indices that are not needed because serum creatinine is sufficient for evaluation. The ultimate goal of the fMRI study was to provide useful information for daily medical treatment such as, for example, a renal biopsy diagnosis by evaluating kidneys non-invasively over time. Accumulation of further knowledge is eagerly anticipated.

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MINIREVIEWS

Metabolic syndrome and lower urinary tract symptoms

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Abstract

Recently, clinical and epidemiologic data indicating the involvement of metabolic syndrome (MetS) in the pathogenesis and progression of lower urinary tract symptom (LUTS)/benign prostatic hyperplasia (BPH) are reported. This review evaluates the reports on the influence of MetS in the development and progression of LUTS/BPH, and discusses possible clinical implications for the management and treatment of this disease. Recent studies on the epidemiological relationship between MetS and LUTS hypothesize that MetS may be associated with an overactivity of the autonomic nervous system for which hyperinsulinemia, a key element of the MetS, might be responsible. An alternative explanation is that LUTS are associated with chronic ischemia of pelvis resulting from atherosclerotic changes in blood vessels, which leads the production of reactive oxygen species, which can damage the bladder detrusor. Control of autonomic nervous system overactivity and control of chronic bladder ischemia have potential as new targets for LUTS treatment. Studies suggest an association of MetS with LUTS/BPH, although further research is needed to understand how MetS influences LUTS/BPH. MetS should be considered a new domain in basic and clinical research in patients with LUTS/BPH and as a target for treatment.

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Key words: Metabolic syndrome; Lower urinary tract symptoms; Sympathetic overactivity; Chronic bladder ischemia

Core tip: Associations between metabolic syndrome (MetS) and lower urinary tract symptom (LUTS) are noteworthy. MetS treatment may have the potential to prevent LUTS from worsening as well as to prevent the occurrence of new LUTS. Medication for LUTS patients with MetS should not only target the lower urinary tract, but should also be recognized as systemic treatment. Medications which are essentially thought to be unrelated to LUTS treatment may have potential for development as a specific medical treatment for LUTS with MetS.

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INTRODUCTION

Lower urinary tract symptoms (LUTS) are a symptom complex which decreases quality of life in both the males and females. It is known that the number of LUTS patients increases in an age-dependent manner. It has been reported that bladder outlet obstruction (BOO), which occurs in patients with benign prostatic hyperplasia (BPH), is the main factor that triggers LUTS and efforts have focused on BOO. However, recent reports have shown that various etiologies are also associated with LUTS. Metabolic syndrome (MetS) is a cluster of several metabolic abnormalities or risk factors, including visceral obesity, dyslipidemia, hypertension, insulin resistance, and glucose intolerance[1]. Recently, it has been suggested that MetS may play an integral role in LUTS/BPH etiologies. In order to improve the quality of LUTS treatment, it will be useful to understand how MetS influences LUTS.

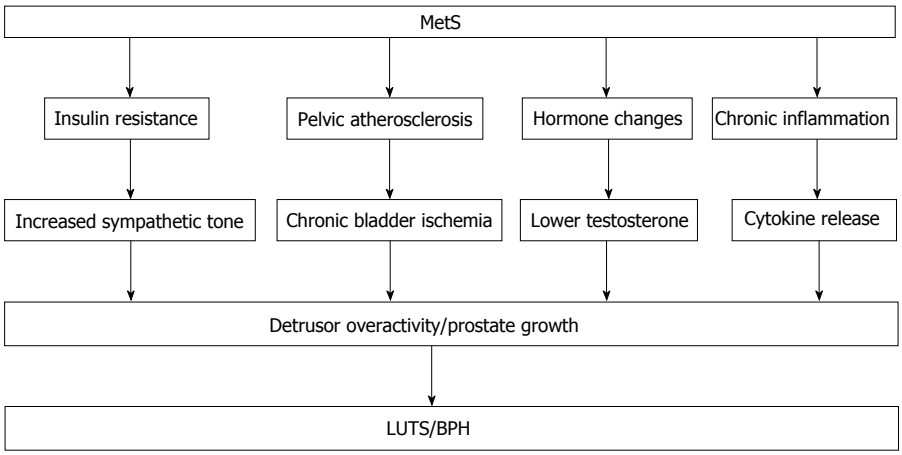


Figure 1 Potential role of metabolic syndrome on the lower urinary tract symptom. MetS: Metabolic syndrome; LUTS: Lower urinary tract symptom; BPH: Benign prostatic hyperplasia.

The present review highlights current knowledge about the influence of MetS on LUTS/BPH and about the expected effect of MetS treatment on LUTS improvement. The application of this knowledge in clinical use should contribute to improvements in the treatment quality of LUTS patients with MetS.

METS AND LUTS/BPH

Associations between LUTS/BPH and various components of MetS (obesity, hypertension, and fasting blood glucose) have been previously reported[2-4]. Moreover, cases of LUTS/BPH have also been positively associated with the number of MetS components[5].

It is known that hyperinsulinemia caused by insulin resistance, the core pathophysiology of MetS, may increase prostate smooth muscle tone[6], resulting in the increased prostate volume[7,8]. The insulinlike growth factor (IGF) pathway may contribute to the relation between insulin resistance and prostate enlargement. Insulin presents a structural similarity to IGF-1 and can bind its receptor, result in activating of a complex pathway influencing proliferation of prostatic cells. Another relevant study has demonstrated that the chronic inflammation induced by MetS may lead to the prostate enlargement[9,10]. It has been proven that insulin resistance and inflammation increase as the number of MetS components increases[11]. T-cell activity in prostate with inflammation infiltrates may result in increased stromal and epithelial cell proliferation that is sustained by an autoimmune mechanism. Repetitive tissue damage and the subsequent wound healing induced by inflammation may result in prostate enlargement[7].

Alternative hypotheses include overactivity of the sympathetic nervous system by hyperinsulinemia, which may lead to more sever LUTS independent of prostate enlargement, and pelvic atherosclerosis causes chronic ischemia of the bladder, which may lead to functional impairments clinically manifested as LUTS (Figure 1).

SYMPATHETIC OVERACTIVITY AND LUTS

Insulin resistance is thought to be the central underlying mechanism of MetS and is known to cause sympathetic overactivity[12]. Recent studies trying to show the epidemiological link between MetS and LUTS hypothesize that MetS is related with increased autonomic nervous system activity for which hyperinsulinemia, a key element of MetS, might be responsible[13,14]. Briefly, hyperinsulinemia that induces increased autonomic nervous system activity results in prostate enlargement and detrusor overactivity. McVary *et al*[14] revealed that LUTS are related with changes in blood pressure. The important role of α1-adrenoceptor (α1-AR) in the sympathetic nerve terminals of the prostate has been indicated. It has been also indicated that dynamic obstruction is mediated by stimulation of α1-AR. Previous studies indicate a relationship between overactivity of autonomic nervous system and LUTS. Spontaneously hypertensive rats (SHRs) have been reported to void more often than normotensive control rats[15], and have also been reported to have an overabundance of sympathetic fibers innervating the bladder. Urodynamic studies on SHRs have indicated spontaneous bladder contractions at low volumes, an effect that is largely ameliorated by α1-AR blocker and appears to be mediated by increased nerve factor. Thus, sympathetic overactivity has been suggested to have relation with bladder storage function. Autonomous nervous system overactivity because of MetS might contribute to detrusor overactivity. Hammarsten *et al*[16] represented an association between a more rapid enlargement of prostate and diseases related with sympathetic activation, including obesity, non-insulin-dependent diabetes mellitus and treated hypertension (HT). Furthermore, it has been proven that the over input of autonomic neural stimulation to the prostate may lead to the prostatic enlargement[17].

CHRONIC BLADDER ISCHEMIA AND LUTS

An association among smoking, vascular risk factors and LUTS has been reported[18]. Furthermore, previous study has shown decreased bladder compliance, reduced contractile force in the bladders and increased micturition frequency in pelvic ischemic model of rabbits[19]. Various researchers have shown the influence of chronic ischemia of the bladder detrusor resulting from atherosclerotic changes in blood vessels on LUTS; specifically atherosclerosis narrows the bladder vessels. Bladder filling in atherosclerotic models results in a significant decrease in blood flow of the bladder. Repeated ischemia and reperfusion produces reactive oxygen species, which damages the tissues[20,21]. Shimizu *et al*[22] found that an angiotensin receptor blocker (ARB), olmesartan, protects bladder function in a chronic bladder ischemic model by recovering blood flow and decreasing oxidative stress. Protective effects of coenzyme Q10[23] and melatonin[24] on bladder hyperactivity in a chronic bladder ischemia model due to their action as antioxidants have been indicated. Treatment targeting the bladder blood flow and protection from oxidative stress caused by chronic ischemia can be a new strategy for LUTS treatment.

MEDICINAL TREATMENT FOR LUTS

Medical treatment of voiding and/or storage symptoms is now the initial choice of therapy and α1-AR blockers remain the most widely used pharmacological agents aimed at the dynamic component of prostatic obstruction[25]. α1-ARs predominate in prostatic stroma at the mRNA and protein levels, and it is reasonable to think that they may play a role in combatting the dynamic component of obstruction[26]. Increased activity of the autonomic nervous system may result in an acceleration of the sympathetic tone of the lower urinary tract, lead to C-fibers stimulation, which in turn causes storage symptoms. By bringing these findings together, it is suggested that α1-AR-blocker exerts an suppressive effect on C-fiber urethral afferent nerves by inhibiting the muscle tone of prostate activated by HT, which may lead to improve storage symptoms. We previously showed that the effect of tamsulosin on storage symptoms was more prominent in patients with HT than in patients without it[27]. An α1-ARs subtype analysis showed that the expression levels of α1-AR mRNAs in the detrusor were equally at low densities[26], indicating that α1-AR blockers have little effect on contractility of detrusor, which in turn may have little effect on voiding symptoms. Although α1-AR-blockers improve functional obstruction in BPH, detrusor contractility may not be improved in elderly patients.

There is no special treatment for LUTS patients with MetS at the present time. Strict control of MetS may be needed.

EFFECT OF METS TREATMENT ON LUTS

Increased physical activity have been associated with a decreased risk of LUTS[28], although the mechanism of this effect is not completely understood. MetS patients may receive medications for HT, hyperlipidemia and diabetes. These medications may possibly improve LUTS as well as control MetS. As the populations of LUTS and HT both increase with aging, a great number of LUTS patients receive medications for HT. Ang-Ⅱ is a potent vasoconstrictor and plays an important role in the systemic renin-angiotensin system (RAS), which controls blood pressure by regulation of vascular tone and sodium homeostasis. Medications which inhibit the RAS, such as ARBs, are mainstays in HT treatment. In addition to its systemic effects, Ang-Ⅱ may affect cellular proliferation and function in several organ systems, including bladder and prostate, through local RAS. It has been well established that Ang-Ⅱ receptors exists in the bladder wall and Ang-Ⅱ on the detrusor muscle has contractile effects[29,30]. Because Ang-Ⅱ is the principal mediator of the RAS and strongly stimulates cell growth[31], researchers have also reported its possible role as a mediator in collagen production and smooth muscle growth in BOO[32,33]. Previous studies report that Ang-Ⅱ induces bladder muscle contractions[29,34]. Ang-Ⅱ in the bladder is known to regulate smooth muscle growth and connective tissue production[35], and has been partly associated with the pathogenesis of urinary dysfunction with subsequent outlet obstruction[36] and stress urinary incontinence in rats[37].

It has been proven that morphological changes are remarkable in the bladder smooth muscle cells, connective tissues, and innervation in the detrusor muscles in patients with chronic BOO[38]. It has been shown that repeated captopril administration, the angiotensin-converting enzyme inhibitor, in neonatal BOO rabbits significantly reduced the expression level in total DNA and decreased the amount of total collagen in the bladder[39]. It may hypothesized that these pathological variations in the BOO bladder may be caused by the activation of Ang-Ⅱ-mediated changes. It has been indicated that urethral resistance in a rat model of stress incontinence may be reduced by the blockade of Ang-Ⅱ receptors[37]. Taken together, it can be proposed that the development of BOO results in a significant change of Ang-Ⅱ receptors in the lower urinary tract. It has also been indicated that the Ang-Ⅱ antagonist telmisartan suppresses both the suppression of Ang-Ⅱ receptors in the bladder and the bladder hypertrophy in BOO rats[36]. Consequently, telmisartan is effective in attenuating the increase in bladder weight in BOO. We previously showed that ARB administration in hypertensive patients improves LUTS[40]. One possible mechanism indicating the improvement of storage symptoms by ARB administration may be the effect of stabilization of the smooth muscle activity by down regulation of the local RAS in the bladder. It is also

discussed that Ang-Ⅱ may contribute in the prostate. It has been reported that RAS has been overactivated in BPH[41]. The elevated local levels of Ang-Ⅱ in the prostate in BPH patients has also been reported[42]. Ang-II may have effect on cell proliferation and smooth muscle tone in the prostate *via* Ang-Ⅱ receptors. Nevertheless, the functional role of elevated Ang-Ⅱ in the prostate is not clearly understood. It is expected that ARB may improve LUTS by down regulating noradrenaline release in the prostate. Recent reports indicated that the effect of apoptosis induced by ARB in prostatic tissue of SHR[43]. ARB may improve voiding symptoms by minimizing the prostatic microenvironment, result in lowering urethral resistance. As ARB can also improve blood flow, as mentioned above, the possibility exists that ARB may improve LUTS.

OBJECTION TO THE ASSOCIATION OF METS AND LUTS

The role of MetS in the occurrence of LUTS remains controversial. Some reports, in particular in Asian populations, found no association between MetS and LUTS[44-46]. Moreover, a report from Korea showed favorable effects of MetS on LUTS[47], that is, the opposite result. The differences in these results could stem from several factors. First, the ages of the study participants are different among studies. Second, the criteria used to define the presence of MetS or LUTS vary among studies. And third, the prevalence of MetS and BPH are lower in Asian populations.

It is important to emphasize that most studies indicating the possible association between MetS and LUTS are derived from observational studies, which are often limited to a specific area or population. Future clinical trials are expected.

CONCLUSION

Associations between MetS and LUTS are noteworthy. MetS treatment may have the potential to prevent LUTS from worsening as well as to prevent the occurrence of new LUTS. Medication for LUTS patients with MetS should not only target the lower urinary tract, but should also be recognized as systemic treatment. Statin and ARB, which are essentially thought to be unrelated to LUTS treatment, may have potential for development as a specific medical treatment for LUTS with MetS.

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MINIREVIEWS

Percutaneous nephrolithotomy *vs* laparoscopic ureterolithotomy for large upper ureteral stone: A review article

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Key words: Upper ureteral stones; Percutaneous nephrolithotomy; Laparoscopic ureterolithotomy; Laparoscopy; Ureteral calculi

Core tip: It's an honor to write a prestigious editing board; there is a review article study to compare between percutaneous nephrolithotomy and laparoscopic ureterolithotomy for treatment of large upper ureteral stones. These two procedures are different in many aspects and it's not clearly defined that which one is preferred option.

Shadpour P, Modaresi SS, Maghsoudi R, Roohinezhad R. Percutaneous nephrolithotomy *vs* laparoscopic ureterolithotomy for large upper ureteral stone: A review article. *World J Clin Urol* 2014; 3(3): 336-339 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/336.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.336

Abstract

To investigate the best treatment option for large upper ureteral stone, percutaneous nephrolithotomy or laparoscopic ureterolithotomy. We searched three key word of upper ureteral stone, laparoscopic ureterolithotomy, percutaneous nephrolithotomy in PubMed, Scopus and Ebsco. We found approximately twenty suitable articles about this subject since January 1980 until January 2014. All articles studies and reviewed meticulously and brief review of these articles was written and some Ideas of experts was added. In many studies, it is suggested that success rate and complications of laparoscopic ureterolithotomy and percutaneous nephrolithotomy are the same, but percutaneous nephrolithotomy has less hospital stay time, duration of surgery and it is more cost effective. Overall it seems that percutaneous nephrolithotomy for treatment of upper ureteral stones is preferable rather than laparoscopic ureterolitothomy

INTRODUCTION

Large impacted upper ureteral calculus defined as a stone that is located above the rim of pelvic Inlet bone. Upper ureteral stones may be single or multiple and may be associated with kidney stones.

The best treatment modalities for large proximal ureteral stones are not well defined[1] and include extracorporeal shock wave lithotripsy, ureterolithotripsy, percutaneous nephrolithotripsy, laparoscopic ureterolithotomy and open surgery. For large upper ureteral stones, both percutaneous and Laparoscopic ureterolithotomy are possible less invasive modalities, but how we can choose one of these two procedures, is not clearly defined.

The aim of this review article study is to compare between two main approaches to this stones that are

percutaneous nephrolithotomy (PNL) and Laparoscopic nephrolithotomy.

RESEARCH

This review was conducted on a request from Baishideng Group. For this study we searched all full text papers indexed in three major resources of medical literature (PubMed, Scopus and Ebsco) using the following key words: Upper ureteral stone, laparoscopic ureterolithotomy and percutaneous nephrolithotomy. We defined large upper ureteral stones as a stone larger than 1 cm.

All papers fulfilling these inclusion criteria were included: (1) Involving treatment of large proximal ureteral stones; (2) Published between Jan 1980 and Jan 2014; and (3) If several studies were reported by the same center and authors, the most recent publication was included in writing of this review. Commentaries and case reports were excluded. After a rapid scan of the literature, ultimately 21 articles were found to be relevant to these criteria and used for compiling this review article.

DISCUSSION

Upper ureteral stones are a prevalent problem. There are many different treatment modalities that include medical expulsive treatment, shockwave lithotripsy, ureteroscopic stone extraction; PNL and laparoscopic ureterolithotomy. In large upper ureteral stones, PNL and laparoscopic ureterolithotomy are the two main approaches reported in the literature. Indications of these two procedures are nearly same and include large upper ureteral stone that is resistant to shock wave lithotripsy and stones that are not suitable for other treatment modalities. Contraindications are also not specific and are the same general contraindications for laparoscopy and PNL. These two procedures may be compared in many different aspects such as invasiveness, cost, learning curve, complications, success rate, duration of surgery and hospital stay. Therefore the choice between two modalities for each given patient would only be prudent once these differences have been taken into account.

For the ureteral stone accompanied by complex kidney stones that need surgery, PNL is the modality of choice. In upper ureteral stones located at a reasonable distance from the uretero pelvic junction [*i.e.*, close to the ureteropelvic junction (UPJ)] and accessible from the renal pelvis too, PNL is preferred[2].

But if the stone is located far from the UPJ, the surgeon must first displace the stone from its position at the upper ureter into the pelvis, and then perform routine PNL. This approach, commonly termed push-back PNL is the alternative to laparoscopic ureterolithotomy.

In one study, Li *et al*[3] evaluated stone location based on vertebral level. They concluded that if a ureteral stone is located higher than the upper border of the fourth lumbar vertebra, it is inappropriate for ureteroscopic lithotripsy and should be treated by another treatment modality such as PNL or laparoscopy. In their study, stone free rate for PNL was 96.4%. Mean operation time and post operative hospital stay were 108.78 min and 2.49 d respectively[3].

For the upper ureteral stone accompanied by kidney stones, Xiong *et al*[4] conclude PNL to be the reasonable choice for stone treatment, In their study, they performed PNL on 108 patients with kidney and upper ureteral stones. Stone clearance rate was 99.1%, hemoglobin decrease was 4.8 ± 2.7 g/L and no blood transfusion was needed[4].

PNL is a trans-parenchymal procedure, but in laparoscopic ureterolithotomy, the kidney remains intact. This difference between the two approaches has been underlined by some authors, stating that they may theoretically expect bleeding and kidney injury to dominate in PNL compared to laparoscopic ureterolithotomy. In reality, in uncomplicated PNL, bleeding is not significant. Lang[5] reported their post PNL transfusion rate to be 0.43%.

Through the unavoidable step of tract dilation, PNL inherently causes some renal parenchymal invasion. Renal dimercapto succinic acid scans performed to assess the state of cortical function at varying intervals post op, shall reveal focal cortical defects at the point of access, but at the same time usually confirm that as result of the removal of obstructive stones, overall renal function will be preserved or even improve in the post operative scan after PNL[6].

In laparoscopic ureterolithotomy it is clear that the procedure does not involve direct parenchymal trauma. In one study Yasui *et al*[7] evaluated the impact of laparoscopic ureterolithotomy on renal function by performing renal scintigraphy using 99mTc-mercaptoacetyltriglycine (99mTc-MAG3) before and 3 mo after surgery. Ultimately they conclude that pre-operative and post-operative affected renal function images shown no significant change in MAG3 clearance[7].

One of the most important positive aspects of choosing laparoscopy for the treatment of large upper ureteral stones is its high success in achieving stone free state. This has been confirmed by several studies in which success rates were reported to stand between 96% to 100%[8-10].

Some factors may hamper the success of laparoscopic ureterolithotomy including severe fibrosis and tissue adherence surrounding the ureteral stone site. This can make dissection very difficult and occasionally impossible, and these factors are limitations of laparoscopic ureterolithotomy especially in retroperitoneal route[11].

Another complication of laparoscopic ureterolithotomy that directly impacts its success is upward displacement of the ureteral stone into the renal pelvis. Upward migration may preclude laparoscopic access to the stone. This situation can occur when a non-impacted stone is located close to the UPJ and the adjacent proximal ureter is very dilated. In such a circumstance, it's better to switch the treatment plan to push-back PNL. Several studies have reported the success rate for push-back PNL between 82.8% and 99.1%[4,12,13].

Table 1 Summary of some characteristics of two treatment modality for large upper ureteral stones: Laparoscopic ureterolithotomy, push back percutaneous nephrolithotomy

	Laparoscopic ureterolithotomy	Pushback PNL
Stone free rate range (%)	96.5-100	82-99
Operation time range (min)	45-190	30-160
Hospitalization time (h)	24-144	12-96
Transfusion rate average (%)	Rare	0.43

PNL: Percutaneous nephrolithotomy.

Regarding the approach to the kidney for percutaneous treatment of upper ureteral stones, there is essentially two ways to reach the stone. One is by creating access through a tract from superior calyx. Upper ureteral stones are readily accessible *via* this approach, provided the ureteral segment proximal to the stone is dilated enough to permit navigation by the nephroscope[13].

The second approach is push-back PNL. For this purpose the stone must first be displaced from the upper ureter into the renal pelvis by retrograde ureteroscopy, followed by obtaining a routine subcostal inferior calyx access for PNL. But at times the stone is tightly impacted. This renders the push-back strategy difficult and even perilous. Precisely such impacted ureteral stones should better be approached electively by laparoscopic ureterolithotomy, as push-back PNL is apt to fail.

In comparing the two surgical procedures, duration of anesthesia and surgery have also been evaluated in several studies. All allude to the slightly lengthier approach being laparoscopic ureterolithotomy which takes anywhere between 45 and 190 min on average[14-18]. Laparoscopic ureterolithotomy is fairly time consuming, while push-back PNL is relatively quicker. Upper ureteral stones chosen for push-back PNL were usually solitary. This might have contributed to the observation that in many small studies, push-back PNL takes between 30 to 160 min[3,4,12,19].

Duration of hospital stay is an important consideration. Most health systems favor limiting hospital stay as a strategic objective to help lower the financial burden of treatment. In PNL, the duration of hospital admission has been reported between 12 and 96 h[12,20]. For laparoscopic ureterolithotomy this period spans between 1 and 6 d[10,14,16] (Table 1).

Another aspect of any less invasive surgery is the direct cost of such procedures which generally require advanced equipment and disposable devices, in addition to trained surgeons and operating room staff. This factor has not been covered in any of the papers contributing to this article.

CONCLUSION

Both laparoscopic ureterolithotomy and push-back PNL can be used for treating large upper ureteral stones.

These two modalities are similar in many aspects such as success rate and complication rate; but differ in such areas as duration of anesthesia and hospital stay, indirectly leading to treatment cost. As result it appears that push-back PNL is slightly favorable over laparoscopic ureterolithotomy for treating large upper ureteral stones.

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MINIREVIEWS

Varicocele and infertility: Role of pressure flow dynamics

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Abstract

Varicocele is prevalent in infertile individuals as well as in normal adolescents and adults. It has an increasing trend with growing age. Infertile individuals with varicocele, develop varying degrees of sperm abnormalities that range from mild to severe semen abnormalities, even azoospermia may develop. The main proposed features of these abnormalities are incompetence of one-way valves of the draining veins of testes, that allow backflow of blood into testes. This backflow produces abnormally high intra-testicular pressure and temperature, that has been confirmed by thermography and pressure estimation in various studies. Microsurgical varicocelectomy may reverse the pathologic effects on spermatogenesis in most patients, which points towards the cause and effect relationship of varicocele with testicular damage. We propose that the prolonged effect of gravity might or may not be the initiating factor for varicocele, as in our experience, around 1/4th of hypogonadotropic hypogonadism patients who had no varicocele before treatment, developed varicocele within 3 to 6 mo of treatment with gonadotropins. Occasionally varicocele is produced by "Nutcracker phenomenon", which is compression of left renal vein between the abdominal aorta and superior mesenteric artery. The deleterious effects of varicocele may develop slowly, causing delayed secondary infertility or rapidly, leading to azoospermia or individual may be spared of damage due to unknown factors that need further research.

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Key words: Varicocele; Scrotal Doppler ultrasonography; Testicular blood flow; Pressure flow dynamics; Testes; Infertility; Oligospermia; Aesthenospermia

Core tip: Varicocele is prevalent in infertile individuals as well as in normal adolescents and adults. It has an increasing trend with growing age. Infertile individuals with varicocele, develop varying degrees of sperm abnormalities. Microsurgical varicocelectomy may reverse the pathologic effects in most patients, which points towards the cause and effect relationship of varicocele with testicular damage. The question as to how some individuals are spared of the deleterious effects of varicocele, needs further research.

ur Rehman K, Zaman MQ, ur Rehman H, Zaneb H. Varicocele and infertility: Role of pressure flow dynamics. *World J Clin Urol* 2014; 3(3): 340-343 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/340.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.340

INTRODUCTION

Varicocele is a disorder of the draining veins of testes. Normally these veins have competent valves, which are required to prevent backflow of blood into testes. In individuals with varicocele, these valves become incompetent, leading to reflux/backflow of venous blood into testes[1-3]. This backflow increases intra-testicular pressure and heat, while decreasing oxygen concentration in testes. These

factors cause testicular injury through inflammatory mediators and production of free radicals[4]. As a result, testicular function, especially spermatogenesis, is affected which may cause male infertility. The consequences of varicocele are variable in different individuals, some may suffer from infertility whereas others may not[3,5].

The pathophysiology of varicocele is yet not clear although its association with male infertility is known since 1950[2]. It is suggested that not a single factor, but various factors are involved in the pathological effect of varicocele. Patient's life style and genetic factors may be among them[2]. The effects of varicocele are more prominent on the left side, due to higher hydrostatic pressure in the left internal spermatic vein. Also the blood column on the left side, after incompetence of one-way venous valves is longer (40 cm) on the left side. It has been suggested that varicocele is a bilateral disease. In a series of venographies, varicocele was found to be bilateral in 84% cases. Amongst them, collateral venous channels and retroperitoneal venous bypasses were observed in 70% cases on left side and 75% on right side[6-8].

RESEARCH

A thorough literature search was conducted at the Medline database. The controlled vocabulary of the medical subject headings included varicocele and pressure, varicocele and blood flow, varicocele and hemodynamics, varicocele and Doppler ultrasonography and varicocele and scrotal Doppler ultrasonography (CDUS). The references retrieved were reviewed and relevant references were chosen according to their relevance for the subject. The references list from each reference was further checked to identify relevant references. Only English language articles were selected.

PREVALENCE OF VARICOCELE

World Health Organization has reported that among men with normal sperm quality, 11.7% have varicocele whereas 25.4% individuals with infertility have varicocele[9]. The prevalence of varicocele in middle school boys has been found to be 16.5%. Its prevalence increases with age, around 10% per decade and reaches 75% at the eighth decade of life[10,11].

When individuals with varicocele were investigated by CDUS, it was found that 94% had grade 2 and 3 reflux on Doppler study. In individuals without clinical varicocele, 40% were found to have reflux of grade 1 and 2 in their testicular veins[12].

DIAGNOSTIC IMAGING FOR VARICOCELE

Scrotal CDUS has been used for determining the flow of varicocele veins including their backflow on valsalva maneuver[12]. If blood flow in a varicocele vein reverses its direction by raised intra-abdominal pressure, it may be considered significant for the diagnosis of varicocele[13]. In the same study, similar kind of blood flow pattern was also detected in 54% individuals without varicocele, which invites ambiguity. The backflow pattern has been graded during valsalva maneuver, from Grade 1 (reflux < 2.0 s) to Grade 3 (reflux > 2.0 s)[14]. The CDUS findings have been proposed to be clinically useful because the maximal reflux velocity and total cross-sectional area of the affected testicular veins can predict the number of internal spermatic veins requiring ligation during microsurgical subinguinal varicocelectomy[15].

PRESSURE FLOW DYNAMICS OF VARICOCELE

In normal population, testicular veins, like other veins, have low-pressure dynamics with no backflow. It has been proposed that if venous valves are incompetent, high intravenous pressure in the internal spermatic veins may be transmitted to the testes. This happens due to the intra-abdominal destination of these veins. High intra-abdominal pressure, in the absence of proper functioning venous valves, delivers backpressure into the testicular veins[16]. The reflux of blood in the internal spermatic veins of testes has been found to be similar at all levels of veins irrespective of its branching pattern. However, the velocity of blood in these veins decreases as the number of branches increase closer to the testes[15]. It has been found that varicocele veins have high hydrostatic pressure that exceeds the pressure in testicular arterial microcirculation, leading to ischemic damage[6]. It has been reported that animal studies have shown conflicting evidence of both increased as well as decreased testicular blood flow after experimentally induced varicocele[17]. This is probably due to the fact that animal models do not mimic the human disease process well, at least in its diversity of pathophysiological phenomenon.

In clinical situations, patients, with upper motor neuron lesion and spastic paralysis of the abdominal muscles, develop varicoceles whereas lower motor neuron lesion patients, with flaccid paralysis rarely, develop varicocele[18]. This can be explained by the phenomenon that spastic paralysis produces high pressure in the abdomen whereas flaccid pressure does not. This study favors the concept of high abdominal pressure as a major cause of varicocele related changes. Hydrostatic pressure in varicocele veins depends on the height of blood column rather than vein diameter[6]. The taller individuals have higher prevalence and severity of left-sided varicocele and it is probably related to the length of left internal spermatic veins and the consequent increased hydrostatic pressure in them[19]. During sclerotherapy of internal spermatic veins of varicocele patients, it was observed that most men had absent valves of the internal spermatic veins[20]. It is the destruction of one-way valves of the veins that allows transfer of pathologic hydrostatic pressure towards testicular venous microcirculatory system. In varicocele, this pressure is around five times higher than normal and exceeds arteriolar pressure, leading to hypoxia. This is also true for right-sided varicocele[16].

It has been suggested that varicocele is either of "pressure-type" or "shunt-type". The "pressure type" varicocele is due to valvular incompetence of internal spermatic vein (testicular vein) whereas "shunt type" is caused by the cremasteric vein and/or deferential vein incompetence[21]. The authors were also of the opinion that spontaneous reflux causes the shunt type varicocele, *i.e.*, the dilatation of medium-sized and large varicocele veins, whereas Valsalva-induced reflux results in "pressure type" or the stop type mechanism, associated with subclinical varicocele[22,23]. This needs further investigation for better understanding.

"Nutcracker syndrome" is another proposed, though rare explanation for varicocele in many studies. According to this view, varicocele results from compression of left renal vein between the abdominal aorta and superior mesenteric artery that may result in varicocele and left flank pain. This phenomenon has mainly been reported in the pediatric population[24-26]. Adolescent patients with the nutcracker phenomenon and left varicocele may present with symptoms of hematuria, proteinuria, scrotal discomfort, and flank pain. In 12 such patients, a shunt anastomosis of the proximal part of the spermatic vein and inferior epigastric vein was done and varicocele was treated by ligation of the left spermatic vein. The symptoms disappeared in all the patients. The diameters and peak velocities of left renal vein significantly decreased and left testicular volume significantly increased after surgery[27].

Experimental anatomic modeling suggests that in varicocele, both testicular as well as epididymal venous outflow is deranged resulting in pathological compensatory hemodynamic changes. Intra-testicular pressure increases, intra-organ vascular integrity is impaired leading to extravasation and testicular venous infarction. There is local arterial hypertension and inter-arterial shunting of the arterial blood. This triggers the mechanism of secondary arterial ischemia of testis and epididymis. The circulatory disorder is pronounced in case of testicular venous outflow blockade which is severe in combined testicular-cremasteric venous block which causes hemodynamic collapse[28].

Varicocele veins have a higher intravascular pressure compared to other veins and it leads to deterioration in blood flow of testes[29,30]. It was reported that normal individuals have 59.9 mm. Hg venous pressure on the left spermatic veins which in varicocele patients was higher by 19.7 mm. Hg at rest and 22 mm. Hg during Valsalva' s maneuver in varicocele patients[31]. It has been observed that after transplant nephrectomy varicocele develops in 23.3% male patients[32].

It has been observed that in normal individuals on valsalva test, femoral vein caliber increases by 22.6% ± 5.7%, whereas in patients with varicocele, it rises to 61.1% ± 10.0%. It has been suggested that the formation of tension chamber in femoral vein may be the cause of ilieo-spermatic reflux of varicocele[33].

In our recent publication, we have reported that hypogonadotropic hypogonadism patients, on presentation, have small size testes and barely appreciable blood flow with no evidence of varicocele. After treatment with gonadotropins, 23% patients developed varicocele within 3-6 mo of treatment[34]. We propose that as this change appeared in a short period of time, which is not enough for the gravity effect to take place, it is most likely that the varicocele veins along with incompetent valves were already there but remained undetectable due to poor arterial blood flow, which was insufficient to fill up the veins. This finding of course needs further investigation; however it favors the hypothesis that the prolonged effect of gravity may not be, at least, the initiating factor for the development of varicocele. Similarly, the effect of constipation on varicocele was studied and found that chronic constipation alone, does not cause varicocele, but it can facilitate its effects[35].

A body of evidences demonstrated that varicocele causes serious damage to testes, which can be successfully improved by microsurgical varicocelectomy, thus proving the cause-effect relationship[36]. Even in non-obstructed azoospermic patients suffering from varicocele, sperms appeared in ejaculate in 34.6% patients after microsurgical varicocelectomy[37]. Based on aforementioned evidences, it seems appropriate that varicocele produces harmful effects but what we still don't know is that how some individuals are spared of these effects.

In conclusion, varicocele is a disease that may cause deleterious effects quickly, leading to azoospermia or slowly, causing infertility due to semen abnormalities or the individual may be spared due to various unknown factors that need further research.

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MINIREVIEWS

Vitamin C supplementation in patients on maintenance dialysis

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Abstract

As one of the most important water-soluble non-enzymatic antioxidants, vitamin C consists of ascorbic acid and its oxidized form, dehydroascorbic acid. Maintenance hemodialysis (MHD) patients have a generally lower plasma vitamin C level compared with general population. Moreover, dialysis patients also exhibit a low plasma vitamin C level, which is largely related with increased inflammation, refractory anemia and oxidative stress. In this review, we described, in great detail, the vitamin C deficiency in MHD patients and its effects on anti-oxidation, anti-inflammation, pro-oxidation and secondary hyperparathyroidism. In addition, we described the possible potential value of vitamin C in anemia, and the side effects of over-doses of vitamin C supplementation in this particular population. In summary, MHD patients may benefit from vitamin C administration. However, further research should be carried out to confirm its potential beneficial effects, optimal dosage and side effects from vitamin C supplementation.

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Key words: Vitamin C; Supplementation; Maintenance dialysis; Anti-oxidation; Anti-inflammation; Anemia; Oxalosis

Core tip: In this review, we described the vitamin C deficiency in maintenance hemodialysis patients and its effects on anti-oxidation, anti-inflammation, pro-oxidation and secondary hyperparathyroidism. In addition, we described the possible potential value of vitamin C in anemia, and the side effects of over-doses of vitamin C supplementation in this particular population.

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INTRODUCTION

As one of the most important water-soluble non-enzymatic antioxidants, vitamin C consists of ascorbic acid and its oxidized form, dehydroascorbic acid. The former is easily to be oxidized into the unstable dehydroascorbic acid while exposed under chronic or acute oxidant conditions, such as in smokers and in diabetes. Humans can not synthesize ascorbate due to the lack of the gene encoding the enzyme gulonolactone oxidase, which is involved in the last step in biosynthesis of L-ascorbic acid[1]. Therefore, vitamin C can be obtained only from fresh fruits and vegetables, such as strawberry, kiwi, orange juice and broccoli.

In normal population, the plasma vitamin C level ranges from 30 to 60 μmol/L[2]. Plasma level of vitamin C in maintenance hemodialysis (MHD) patients is generally lower[3,4] compared with general population. Moreover, dialysis patients exhibit a low plasma vitamin C level, which is largely related with increased inflammation, refractory anemia, oxidative stress, and secondary hyperparathyroidism (SHPT)[5-8].

Previous investigations demonstrated that vitamin C

supplementation possesses promising effects in MHD patients, such as better improvement of oxidative stress[9,10], inflammation[11] and anemia[12,13]. Therefore, vitamin C adjuvant therapy has been highly recommended for this particular group of patients.

In this review, we described the possible contents of physiological functions of vitamin C, deficiency of vitamin C in MHD patients as well as effects of vitamin C on anti-oxidation, anti-inflammation, pro-oxidation, anemia and SHPT in MHD patients. Moreover, dosages of vitamin C supplementation and side effects of vitamin C due to its metabolite, oxalosis accumulation, were also discussed.

PHYSIOLOGICAL FUNCTIONS OF VITAMIN C

Vitamin C can act as both antioxidant and as prooxidant[9,10,14-16]. It is generally regarded as a protective antioxidant due to its direct oxyradical scavenging properties[17,18]. Recent research revealed that vitamin C plays many prominent roles, and it functions in the biosynthesis collagen, norepinephrine and carnitine as a cofactor for several enzymes[19-21]. Moreover, vitamin C also plays a key role in peptide amidation and tyrosine metabolism. In addition, vitamin C has a potential to enhance the non-heme iron absorption[22] and alter the metabolism of the iron (Fe) from inert tissue stores[12,23,24]. At the gastrointestinal tract with an alkaline pH, vitamin C provides auxiliary aids in maintaining iron in a more soluble state, which is more readily absorbed across the intestinal mucos[22,25].

Scurvy is a disease resulting from deficiency of vitamin C in diet[26]. It is preceded by certain symptoms, including increased bone resorption[27], gingival problems[28], weakness, fatigue, irritability, vague myalgias, joint pains, connective tissue disorders, mood changes and poor wound healing.

DEFICIENCY OF VITAMIN C IN MHD PATIENTS

It is well known that MHD patients have a generally lower plasma vitamin C level compared with normal population[3,4]. MHD patients exhibit remarkably low vitamin C levels in plasma, frequently < 10 μmol/L or even < 2 μmol/L[3,29]. In our previous study, a plasma vitamin C level of < 4 μg/mL (22.8 μmol/L) is presented in 64.4% dialysis patients[5]. The observed low level of plasma vitamin C might be attributed to the dietary restrictions on fruit and vegetable intake, and impaired metabolism in uremia during dialysis procedure[4,30-32], as well as increased inflammation[5], oxidative stress[6] and SHPT[8]. Previous investigations demonstrated that dialysis treatment induces a decrease in plasma vitamin C level, which is approximately reduced by 33%-50% from the baseline values[3,33] and equal to a removal of 100-300 mg vitamin C during a dialysis session[4,34]. Therefore, vitamin C deficiency might be more frequently detected in these individuals. Conventional hemodialysis (HD), on-line hemodiafiltration and high-flux hemodialysis eliminate plasma vitamin C in a similar way[5,33]. These findings could be associated with its small molecule (176.1 Da), low protein-bound and high soluble characteristic in water.

EFFECTS OF VITAMIN C ON ANTI-OXIDATION AND ANTI-INFLAMMATION IN MHD PATIENTS

Widely observed in long-term dialysis patients, oxidative stress is associated with chronic inflammation and increased mortality risk. Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage in uremic patients[35,36]. The relationship between oxidative stress and inflammation is certainly bidirectional since oxidative stress affects inflammatory status and inflammation exerts influence on the state of oxidative stress. Inflammation in uremic patients can be triggered by oxidative products during dialysis procedure, such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2)[37]. On the other hand, inflammation may further aggravate oxidative stress through potentiating respiratory burst activation in monocytes and neutrophils[38].

As an important anti-oxidant, vitamin C possesses beneficial effects on reducing ROS and improving inflammatory status. Acute administration of vitamin C reduces oxidant stress levels and improves NO-mediated resistance vessel dilatation in renal failure[39]. Moreover, Tarng *et al*[10] reported that in MHD patients vitamin C supplementation for 8 wk reduces the 8-OHdG level of cellular DNA, an index of oxidative DNA damage in ROS-mediated diseases[40]. Abdollahzad *et al*[9] found that malondialdehyde levels are decreased and lipid profiles are improved in MHD patients orally supplemented with 250 mg vitamin C every other day for 12 wk. In our recent crossover study, we found that the level of hypersensitive C-reactive protein (hs-CRP) in MHD patients is lowered by oral vitamin C supplementation of 200 mg/d for 3 mo, and the hs-CRP level is increased again after the vitamin C supplementation is withdrawn[11]. Considered as a co-antioxidant, vitamin C can regenerates a-tocopherol (vitamin E) from the a-tocopheroxyl radical, produced *via* scavenging of lipid-soluble radicals[41]. High doses of vitamin C administration in a low infusion rate during HD session can prevent an increase in lipid peroxidation, which might be probably associated with the enhanced rate of endogenous vitamin E regeneration[42].

However, some other studies did not show this beneficial effect in MHD patients[43-45]. For example, in Fumeron's study[45], 250 mg vitamin C is orally given to 33 MHD patients thrice weekly after each dialysis session for 2 mo, and no improved situation of oxidative/anti-oxidative stress and inflammation has been observed. Chan *et al*[44] also reported that there is no effect on markers of

oxidative stress in MHD patients after 250 mg vitamin C supplementation thrice weekly for 8 wk, either intravenously or orally. Kamgar *et al*[43] recently reported that the CRP level exhibits a decrease trend in MHD patients after an oral vitamin C supplementation of 250 mg/d for 2 mo. Similar findings have been observed by Ramos *et al*[46].

These conflicting data may be partially explained by the following reasons: (1) the inflammatory status of patients in some previous studies is altered due to daily oral vitamin C supplementation; (2) certain factors are different in the study populations, such as age, dialysis vintage, smoking status and proportion of diabetes; (3) difference in doses, duration and route of vitamin C administration; and (4) different markers of oxidative stress have been used in the above-mentioned studies.

EFFECTS OF VITAMIN C ON PRO-OXIDATION IN MHD PATIENTS

Vitamin C also acts as a pro-oxidant due to promoting Fenton chemistry, which converts the Fe^{3+} into Fe^{2+} and catalyzes the formation of ROS[47-49].

Eiselt *et al*[50] documented a pro-oxidative effect of vitamin C during intravenous iron sucrose and vitamin C administration in MHD patients. In this study, they found that intravenous administration of Fe together with vitamin C supplementation results in a greater increase in plasma thiobarbituric acid reacting substances compared with single intravenous administration of Fe. Ferretti *et al*[15] indicated that intravenous administration of vitamin C increases lipid hydroperoxides and advanced glycation end product levels and decreases paraoxonase activity in hemodialysis patients. In addition, De Vriese *et al*[16] also found similar results by oral vitamin C supplementation in MHD patients.

POTENTIAL VALUE OF VITAMIN C IN ANEMIA IN MHD PATIENTS

Although anemia management has been improved in patients with chronic kidney disease (CKD), anemia is still prevalent in these patients[51]. Approximately 35% of patients with end stage renal disease have refractory anemia[52]. This persistent anemia might be explained by relative resistance to erythropoietin (EPO) due to functional iron deficiency, which is a situation characterized by low transferrin saturation[52].

Latest study showed that the level of plasma vitamin C has a positive correlation with the hemoglobin level[53] as well as a negative correlation with the EPO resistance index[54-56], and ascorbic acid has also been used to improve response to erythropoiesis-stimulating agents. Vitamin C plays an important role in the utilization of iron from storage sites. As an electron donor, vitamin C can reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in order to mobilize storage iron, including the portion of tissue iron as hemosiderin[57], resulting in an activated iron bioavailability and enhanced production of red blood cells.

Previous work showed that ascorbic acid administration ameliorates the hemoglobin level in MHD patients with iron-overloading[12,13]. These findings have been subsequently confirmed in anemic MHD patients receiving iron administration accompanied by vitamin C[7,54,58]. However, in patients with normal iron status, the situation of EPO needs and transferrin saturation is not improved with vitamin C supplementation[12]. A recent meta-analysis[52] of the available studies indicated that vitamin C supplementation is closely related to a decreased rHuEPO dose and an increased transferring saturation, but no apparent effect on ferritin levels has been observed.

Patients on dialysis have a shortened red blood cell half-life compared with the normal level of 120 d[59,60]. A series of factors have been demonstrated to contribute to this reduced lifespan of red blood cells, including increased oxidative stress and decreased antioxidant products[60,61], the breakdown of phospholipid asymmetry in red blood cell membranes[61,62], increased level of parathyroid hormone[63,64] as well as deficiencies of carnitine[65] and zinc[66]. In addition, although the exact mechanism remains unclear, the half-life of circulating red cells might be extended by vitamin C supplementation[67].

VITAMIN C DEFICIENCY AND SHPT

SHPT is common in chronic hemodialysis patients[68], and it develops because of the increased parathyroid hormone (PTH) synthesis, secretion and the impaired renal clearance[69]. About 50% MHD patients have increased PTH levels[68]. High level of plasma PTH has been linked to uraemic toxin[70], leading to an increased cardiovascular mortality in MHD patients[71].

The effect of vitamin C on SHPT remains unknown in MHD patients. Richter *et al*[8] showed that higher level of plasma vitamin C is correlated with lower level of bio-intact PTH in MHD patients. Vitamin C has an effect on post-receptor events in the calcium-sensing receptors on parathyroid cells, which might partially explain the inverse interaction between the vitamin C level and PTH[72]. Moreover, the cyclic adenosine monophosphate response to PTH can be also enhanced by vitamin C supplementation[73]. Sanadgol *et al*[74] reported that the mean level of serum PTH is decreased in the first 2 mo compared with baseline after a 3-mo intravenous administration of vitamin C (200 mg, thrice weekly). However, this effect is gradually diminished at month 3, which might be explained by the reduced sensitivity of calcium-sensing receptors on parathyroid gland cells with the passage of time. Interestingly, Biniaz *et al*[75] did not find the beneficial effect of vitamin C on SHPT in a double blinded, placebo-controlled study in MHD patients.

DOSAGES OF VITAMIN C SUPPLEMENTATION

In healthy subjects, a daily administration of 90 mg or

75 mg vitamin C is enough for men or women, respectively[76]. For MHD patients, the recommended dosages are more controversial. Generally speaking, vitamin C supplementation is recommended for MHD patients due to the restrictions on their dietary intake as well as losses during dialysis procedure. Many clinicians only recommend vitamin C to a conservative range of 60-100 mg/d with the consideration of oxalate accumulation in the tissues of renal failure patients. These recommendation may not be optimal, because the loss of vitamin C during a single dialysis treatment may reach several hundred milligrams of vitamin C[4,34], resulting in vitamin C deficiency common in MHD patients.

Many literatures documented that the oral doses of vitamin C in the range of 100-200 mg/d[11,77-80] are considered as the sufficient and safe dosages[79], or intravenous administration of 300 to 500 mg vitamin C thrice weekly at the end of dialysis is regarded as "guidelines for intravenous ascorbic acid adjuvant therapy"[56,81,82]. However, some other studies hold a contrary opinion[83]. This conservative recommendation is mainly due to the side effects of oxalosis following the vitamin C administration.

OXALOSIS

In the past several decades, researchers have also investigated side effects of vitamin C supplementation. Since oxalate is one of the products of vitamin C metabolism, vitamin C supplementation in MHD patients may enhance oxalate plasma levels in patients with uremia. Significant plasma oxalate levels induced by vitamin C supplementation (500-1000 mg/d for 3 or more than 3 wk) have been observed[7,84-86]. Canavese *et al*[83] reported that plasma oxalate levels are progressively increased even the dosage of vitamin C is set at 500 mg/wk. The peak level is observed after 1 year of treatment, and then it is leveled off thereafter. Therefore, the safety of this protocol in terms of oxalate metabolism should be carefully considered.

However, some studies showed that this potential hazard may be prevented by taking certain measures, such as avoiding vitamin B_6 deficiency[87] and improving dialysis technology[88,89]. Tomson *et al*[88] showed that tissue oxalate accumulation is completely absent in well-dialyzed CKD patients.

CONCLUSION

Taken together, vitamin C deficiency is common in MHD patients. Therefore, vitamin C supplementation is crucial for MHD patients. Patients can potentially benefit from vitamin C supplementation by its effects on anti-oxidative stress, anti-inflammation, SHPT and improved anemia. However, vitamin C overdose should be avoided due to its secondary oxalosis.

To date, recommendations of vitamin C therapy in MHD patients can not reach consensus because of the wide use of variety of dosages, route of administration, durations and the severe side effects of oxalosis. Oral doses of vitamin C in the range of 100-200 mg/d, or intravenous administration of 300 to 500 mg vitamin C thrice weekly at the end of dialysis are commonly regarded as adequate to prevent ascorbate deficiency in MHD patients.

In summary, MHD patients may benefit from vitamin C administration. However, further large-scale randomized-controlled clinical trials should be carried out to confirm its beneficial effects, optimal dosage and side effects from vitamin C supplementation.

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ORIGINAL ARTICLE

Programmed cell death protein 4 expression in renal cell carcinoma, penile carcinoma and testicular germ cell cancer

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Abstract

AIM: To investigate the expression of programmed cell death 4 (Pdcd4) tumor suppressor gene in tissue specimen of renal cell carcinoma (RCC), testicular germ cell cancer and penile cancer.

METHODS: Pdcd4 expression was studied using immunohistochemistry in 188 cases of RCC and 28 controls (including 9 oncocytoma); in 74 cases of penile carcinoma (including 17 metastatic tissue samples) and 26 controls; in 11 cases of seminoma, in 14 cases of non-seminoma and 5 controls.

RESULTS: Control tissues exhibited strong core and cytoplasmatic Pdcd4 staining. In contrast, core and cytoplasmatic Pdcd4 levels were significantly decreased in cancer tissues.

CONCLUSION: Our data support a role for Pdcd4 (down-) regulation in urologic tumors. Interestingly, Pdcd4 expression seem to be a potential diagnostic marker for renal or penile tumors.

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Key words: Programmed cell death 4; Seminoma; Non-seminoma; Testicular cancer; Renal cell carcinoma; Penile carcinoma; Expression; Apoptosis; Immunohistochemistry

Core tip: Programmed cell death 4 has increasingly become the focus of investigative tumor research in the last years. It has shown to be involved in many tumorous entities, some of which we present for the first time in this paper. Its involvment in apoptosis, invasion and metastasis has been proved in numerous works and showed to be a target for diagnostic and therapeutic measures. We investigate its role and cellular expression patterns in urologic tumors, especially some, that haven't been investigated to this extent or at all to this date.

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INTRODUCTION

Programmed cell death 4 (Pdcd4) expression is known

Table 1 Clinicopathological parameters for patients with seminomatous testicular germ cell cancer *n* (%)

Variables	Carcinoma (*n* = 11)	Control (*n* = 5)
Age in years mean (range)	40.58 (33-53)	30.75 (24-42)
Pathological stage		
pT1	7 (63.6)	NA
pT2	2 (18.2)	NA
pT3	2 (18.2)	NA
Lymphnode metastasis		
cNx	1 (9.1)	NA
cN0	7 (63.6)	NA
cN1	1 (9.1)	NA
cN2	2 (18.2)	NA
M-stage		
cMx	1 (9.1)	NA
cM0	10 (90.9)	NA
Tumor marker		
HCG increased	5 (45.5)	NA
LDH increased	3 (45.5)	NA
AFP increased	0 (0)	NA
Clinical tumor stage		
CS I A	5 (45.5)	NA
CS I S	3 (27.3)	NA
CS II B	1 (9.1)	NA
CS II C	2 (18.2)	NA

NA: Not applicable; HCG: Human choriogonadotropin; LDH: Lactatdehydrogenase; AFP: Alpha-fetoprotein; CS I A: Clinical stage 1A.

to be suppressed in many tumors, for example urothelial- or colorectal cancer[1,2]. As shown in a previous study, Pdcd4 expression levels may have a tumor specific expression pattern and a potential as a diagnostic marker[1]. It inhibits RNA binding of the initiations factors eIF4A and eIF4G[3,4]. The *Pdcd4* gene is located on chromosome 10q24 and encodes a 469 aminoacids long protein. A major regulator of Pdcd4 expression, microRNA 21 (miR-21) is induced by the transforming growth factor-β pathway. High miR-21 expression leads to a suppression of Pdcd4 expression and an induction of metastasis, invasion and intravasation in cell culture[5]. In Pdcd4 overexpressing cells, the subsequent carbonic anhydrase Ⅱ down-regulation shows its influence on the translational level[6]. At the transcriptional level Pdcd4 influences the *uPAR* gene promoter through phosphorylation of the Sp transcription factors in colorectal cells[7]. Interestingly, this pathway is not confirmed for breast cancer: the lack of suppression of uPAR transcription by Pdcd4 overexpression therefore shows a possible tissue specific role of Pdcd4 and its involvement in carcinogenesis[8].

Renal cell carcinoma

Renal cell carcinoma (RCC) stands for 2%-3% of all human cancers with an incidence of 5.8 and a mortality of 1.4 per 100000. Mostly men in the age of 60 to 70 are affected. Important risk factors are smoking, obesity and hypertension as well as having a first-degree relative with RCC. The different subtypes of RCC are clear cell RCC (ccRCC), papillary RCC (pRCC), chromophobe RCC (chRCC) and sarcomatoid RCC (sRCC).

Testicular cancer

This entity represents between 1%-1.5% of male cancers and about 5% of all urological tumors, with an incidence of 3-10 per 100000 males/year Peak incidence is in the third decade of life for non-seminoma and in the fourth decade for seminoma. Risk factors are cryptorchidism, undescended testes, Klinefelter's syndrome, positive familial history, a contralateral tumour or a precancerous lesion and infertility. The two subtypes include seminoma and non-seminomatous germ cell tumor (NSGCT).

Penile cancer

Penile cancer has an incidence of < 1/100000 males in western countries. It is related to race and ethnicity, most frequently affecting white hispanics (1/100000). Risk factors are social, cultural, hygienic and/or religious practices.

The role of Pdcd4 in penile- and testicular carcinoma has not been reported or investigated to this date. Recent studies to RCC stipulate a role for Pdcd4 in tumor progress and survival[9]. We thus investigated its expression pattern in human renal-, testicular-and penile tissue in the largest cohorts published to this date.

MATERIALS AND METHODS

Patients

The expression of Pdcd4 in tumorous and normal tissue was studied using a tissue microarray as described in a previous study[10]. The microarray included 188 RCC, 74 penile squamous cell carcinoma and 25 testicular cancer (11 seminoma, 14 NSGCT) samples with their complementary controls; see Supplementary tables for clinicopathological parameters. The study was approved by our institutional ethics committee (ethic vote 199/10). The clinical pathological parameters are listed in Tables 1-4.

Immunohistochemistry

The construction of the tissue microarray was reported earlier[1]. Immunohistochemical staining was performed automatically (DAKO TechMate™ 500, Danmark) for Pdcd4 [1:400, Anti-Pdcd4 (rabbit) antibody, United States] following the manufacturer´s instructions as described in a previous work[1]. Negative and positive controls were run using rabbit *ig*G-isotype in a concurrent manner. Inflammatory, stromal and normal cells expressed Pdcd4 and therefore served as internal positive control. The stained microarrays were archived with a pathology scanner (Pannoramic MIDI Scanner, 3D-HISTECH, Hungary) for subsequent analyses (Figures 1 and 2).

Pdcd4 expression was scored by one pathologist who was unaware of the patients' clinical history. According to Mudduluru *et al*[11], we evaluated the core and cytoplasmatic immunostainings. Core Pdcd4 staining was classified in four groups according to the amount (in percent) of stain-positive nuclei (core quantity: score): none: 0; ⩽ 30%: 1; 30%-70%: 2 and ⩾ 70%: 3. Cytoplasmic and core stainings were matched by the intensity of the staining results (none, weak, intermediate or strong). The sum

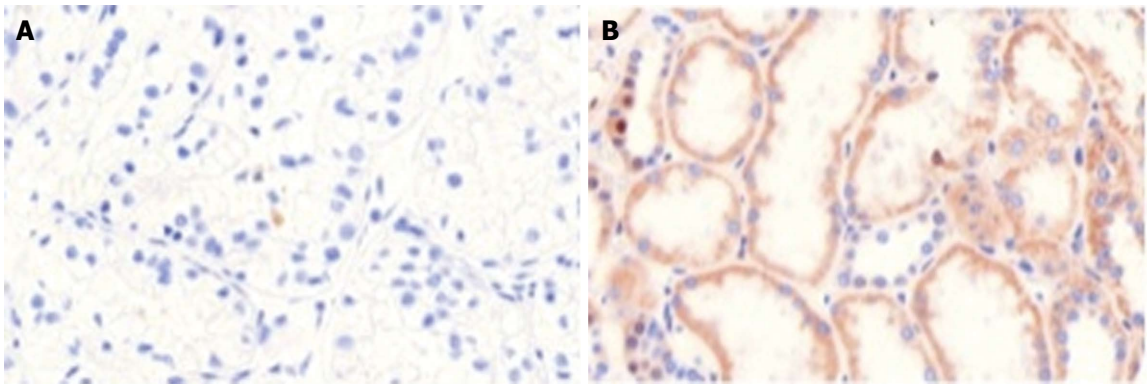


Figure 1 Histopathological staining results of malignant (pT2) renal tissue (A) and of a control sample (B) (haematoxylin and eosin staining, magnification factor: 40 ×).

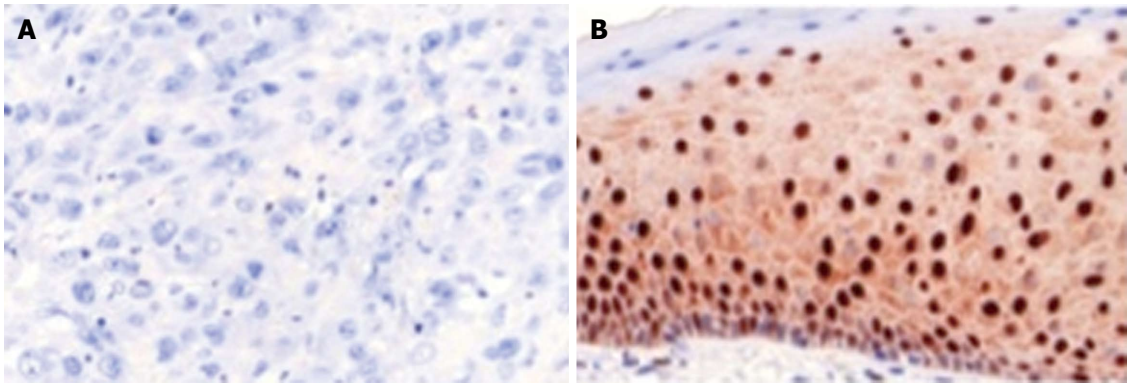


Figure 2 Histopathological staining results of malignant (pT3) penile tissue (A) and of a control sample (B) (haematoxylin and eosin staining, magnification factor: 40 ×).

Table 2 Clinicopathological parameters for patients with non-seminomatous testicular germ cell cancer *n* (%)

Variables	Carcinoma (n = 14)	Control (n = 5)
Age in years mean (range)	32.58 (16)	30.75 (18)
Pathological stage		
pT1	5 (26.3)	NA
pT2	5 (26.3)	NA
pT3	3 (15.8)	NA
pTx	1 (18.2)	NA
Lymphnode metastasis		
Nx	7 (36.8)	NA
N0	4 (21.1)	NA
N3	3 (15.8)	NA
M-stage		
Mx	4 (21.1)	NA
M0	9 (47.7)	NA
M1	1 (5.3)	NA
Tumor markers		
HCG increased	13 (68.8)	NA
LDH increased	7 (36.8)	NA
AFP increased	12 (85.7)	NA
Clinical stage		
I S	8 (42.1)	NA
II A	2 (10.5)	NA
II C	3 (15.8)	NA
NA	1 (5.3)	NA

NA: Not applicable; HCG: Human choriogonadotropin; LDH: Lactatdehydrogenase; AFP: Alpha-fetoprotein.

of these scores was assesed as well.

Statistical analysis

Clinicopathological parameters were correlated with Pdcd4 expression using the χ^2-test. Statistical analyses were performed with SPSS, version 20 (IBM Corporation, United States). Statistical significance was concluded at $P < 0.05$.

RESULTS

RCC

Cytoplasmic and core Pdcd4 levels were increased in normal renal tissue compared to RCC ($P < 0.001$). Pdcd4 expression was reduced in patients with locally advanced RCC. Furthermore, statistical analysis showed high significance levels in staining patterns between the renal tumor types (sRCC, pRCC, ccRCC and chRCC). Especially cytoplasmatic staining showed the strongest correlation levels ($P < 0.0001$). Further significant results showed in T-stage dependent stainings ($P = 0.001$) and tumor size ($P = 0.011$). Figure 3 presents tumor type dependent staining results.

Penile cancer

Both, core ($P < 0.001$) and cytoplasmic ($P = 0.047$)

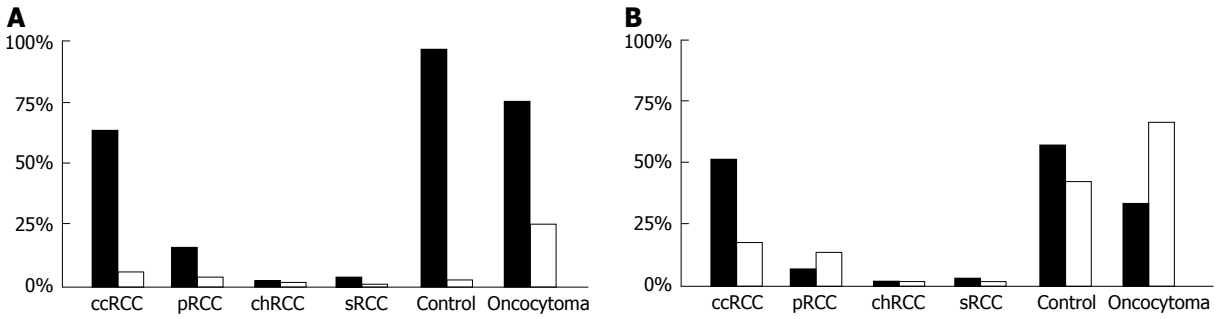


Figure 3 Percentual distribution of core intensity (A), cytoplasmatic-(B) and programmed cell death 4 staining results in correlation to renal cell carcinoma subtype and controls. Left weak/negative staining, right strong/positive staining result. RCC: Renal cell carcinoma; ccRCC: Clear cell RCC; pRCC: Papillary RCC; chRCC: Chromophobe RCC; sRCC: Sarcomatoid RCC.

Table 3 Clinicopathological parameters for patients with renal tumors *n* (%)

Variables	RCC (*n* = 188)	Normal/oncocytoma (*n* = 28)
Age in years mean (range)	60.4 (0-85)	56.86 (26-75)
Sex		
Male	128 (68.1)	12 (42.9)
Female	60 (31.9)	16 (57.1)
Pathological stage		
pT1	81 (37.5)	NA
pT2	40 (18.5)	NA
pT3	57 (26.4)	NA
pT4	3 (1.4)	NA
Missing	7 (16.2)	NA
Lymphnode metastasis		
pN0	96 (44.4)	NA
pN1	6 (2.8)	NA
pN2	9 (4.2)	NA
pNx	72 (33.3)	NA
Missing	5 (15.3)	NA
Metastasis		
M0	114 (52.8)	NA
M1	24 (11.1)	NA
M2	1 (0.5)	NA
Mx	42 (19.4)	NA
Missing	7 (16.2)	NA
Grade		
G1	56 (25.9)	NA
G2	111 (51.4)	NA
G3	12 (5.6)	NA
G4	2 (0.9)	NA
Missing	7 (16.2)	NA

NA: Not applicable; RCC: Renal cell carcinoma.

Pdcd4 levels were decreased in penile carcinoma tissue when compared to non-malignant penile skin (Figure 4). Furthermore, we observed lower Pdcd4 levels in locally advanced and less-differentiated penile carcinomas. A strong correlation in differentiating tumorous from healthy tissue was found. Especially core stainings showed the strongest significance ($P < 0.0001$) level in this group. T-Stage dependent stainings also correlate with core and cytoplasmatic Pdcd4 expression, insinuating a stage dependent expression and regulation of Pdcd4 (Figure 5). Other significant results were calculated when immunohistochemistry (IHC) results were compared to grading and tumor recurrence. There is no correlation with nodal status.

Testicular cancer

In seminoma and NSGCT, only weak correlation levels to clinicopathological parameters were calculated. Thus, Pdcd4 staining results correlate in differentiating healthy from malignant tissue in both entities ($P = 0.007$). Another notable significance is cytoplasmatic stainings to alpha-fetoprotein (AFP)-levels ($P = 0.032$) (Figure 6). This insinuates a potential for Pdcd4 as a diagnostic marker in this tumorous entity.

DISCUSSION

Our results show a decreasing Pdcd4 expression in urologic tumors (*i.e.*, RCC and penile carcinoma). As shown in a previous study, Pdcd4 expression levels may have a tumor specific expression pattern[1].

Shiota *et al*[12] showed that Pdcd4 interacts with the DNA binding domain of Twist1, through inhibition of the DNA binding ability and Y-box binding protein-1 (YB-1) expression, reducing cell growth. The immunohistochemical stainings showed an inverse correlation between core Pdcd4 and YB-1 expression.

Shi *et al*[13] could show an effect of miR-21-expression on docetaxel resistance, insinuating a more complex role of Pdcd4 in tumor aggressivity and chemoresistance. Pdcd4 overexpression in the study of Shiota *et al*[12] showed a high sensitivity to chemotherapeutics (cisplatin and paclitaxel). Thus, the data displayed there (clinicopathological parameters and follow-up) and the data of our study is not extensive enough to correlate Pdcd4 expression levels and tumor chemosensitivity. A COX regression analysis could not confirm this hypothesis of Pdcd4 as a prognostic marker in survival or progress. The weak correlation levels are presumably due to the small cohort restraining the arithmetic power of this analysis. Because of these results and the cohort size, a multivariate analysis was redundant. Another possible explanation for these results are the weak responses of metastatic disease to further therapy in advanced tumor stages.

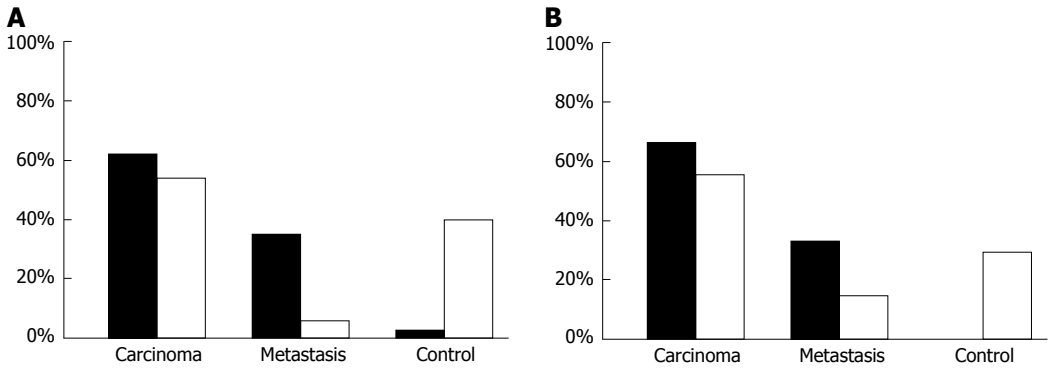


Figure 4 Percentual distribution of core (A) and cytoplasmic programmed cell death 4 (B) levels in penile carcinoma tissue compared to metastasies and controls. Left weak/negative staining, right strong/positive staining result.

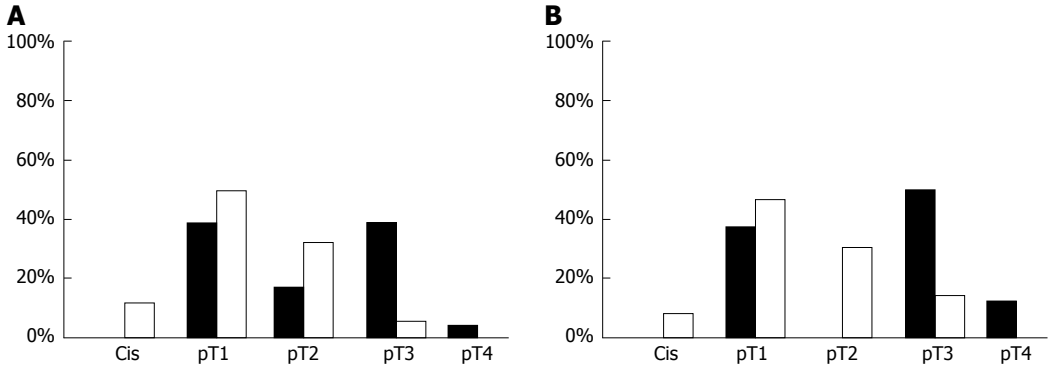


Figure 5 Percentual distribution of core (A) and cytoplasmatic (B) programmed cell death 4 staining results in correlation to T-stage of penile carcinoma and controls. Left weak/negative staining, right strong/positive staining result.

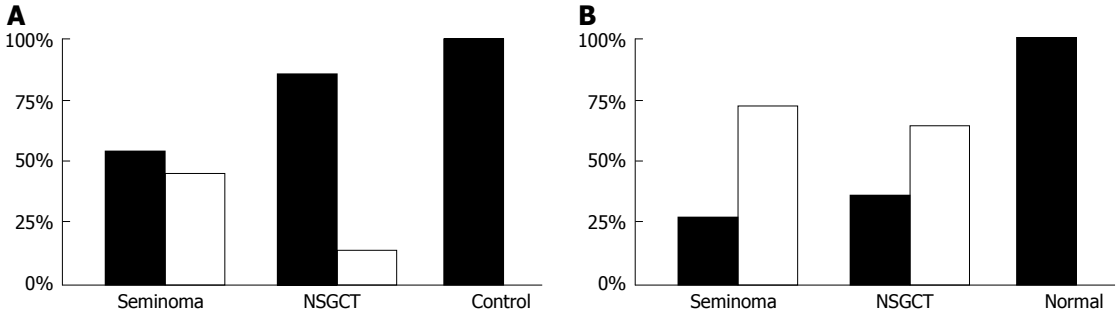


Figure 6 Percentual distribution of core (A) and cytoplasmatic (B) programmed cell death 4 staining results in correlation to Seminoma-, non-seminomatous germ cell tumor and controls. Left weak/negative staining, right strong/positive staining result. NSGCT: Non-seminomatous germ cell tumor.

A recent study by Li *et al*[9] showed a T-stage dependent expression of Pdcd4. Thus, the significant correlation between Pdcd4 expression and survival could not be confirmed in our study. A possible explanation for this is our differentiation of RCC subtypes in our study design. Our analysis shows a clear difference in Pdcd4 expression patterns within the subgroups, this being probably due to their distinct tumor biology. IHC Pdcd4 expression was positive in the core and cytoplasmatic compartment in RCC samples: The levels changed according to tumor progression (T-stage dependent). This supports the hypothesis of tumor-specific expression patterns of Pdcd4 and a possible shuttle function between the cellular compartments. Thus, Li *et al*[9] described a solely core expression; the use of a different antibody for IHC could explain these diverging results. However, this differentiation is important regarding the good discriminative potential of our IHC for differing malignant RCC tissue from the benign renal tissue and oncocytoma. Prognostic relevance such as described by Li *et al*[9] could not be verified in our study.

A high sensitivity and specificity could be confirmed for penile carcinoma as well. These data are similar to results in bladder carcinoma samples obtained in a previous study and suggerate a similar regulation in these tumorous entities[1]. To assess these similarities and to evaluate

Table 4 Clinicopathological parameters for patients with penile carcinoma *n* (%)

Variables	Carcinoma (*n* = 57)	Metastasis (*n* = 17)	Control (*n* = 26)
Age in years mean (range)	63.32 (35-93)	62.96 (11-87)	64.15 (37-97)
Pathological stage			
Cis	4 (7)	NA	
pT1	26 (45.6)	NA	
pT2	15 (26.3)	NA	
pT3	11 (19.3)	NA	
pT4	1 (1.8)	NA	
Lymph node metastasis			
cN0	50 (87.7)	NA	
pN+	7 (12.3)	NA	
M-stage			
cM0	57 (100)	NA	
Grading			
G1	9 (17)	NA	
G2	36 (67.9)	NA	
G3	8 (15.1)	NA	
Missing	4 (7)	NA	
Surgical margins			
R0	55 (79.7)	10 (58.8)	
R1	2 (2.9)	0 (0)	
R2	0 (0)	2 (11.8)	

NA: Not applicable.

the role of a shuttle function of the gene, we calculated different IHC-expression variables (core and cytoplasmatic intensity and quantity). A statistical evaluation (χ^2-test) of these IHC expression patterns with established variables (T-stage, resection status and tumor grade) support the similarities between these epithelial tumors. Thus, a validating COX regression analysis could again not confirm this hypothesis of Pdcd4 as a prognostic marker in survival or progress.

In testicular samples, expression levels correlated significantly in the malignant/healthy group, in T-stage-dependent staining results and to AFP-levels. This insinuates a potential for Pdcd4 as a diagnostic marker. However, our cohort did not have a sufficient size to evaluate clinical outcome. Accordingly, a ROC analysis revealed only weak sensitivity and specificity and a survival analysis (COX) was not possible. Another problem in elucidating the role of Pdcd4 in NSGCT is the cellular inhomogeneity of the tumor cells within each probe (chorion-, embryonal-, cystic-carcinoma and yolk sack tumor).

Our study supports the important role for Pdcd4 downregulation in renal-, testicular- and penile carcinoma. Interestingly, Pdcd4 expression seem to be a potential diagnostic marker for renal or penile tumors.

COMMENTS

Background

Programmed cell death 4 (Pdcd4) is involved in the process of apoptosis. It was first described in 1995 by Shibahara *et al*. Its upregulation has an effect on transcription, translation and many signal transduction pathways. In many tumorous entities its expression levels are suppressed, so that it was stipulated that it has a tumor-suppressive function through the initiation of cellular apoptosis. Especially translation and transcription pathways seem to be affected by the active gene. This could not be verified in all tumorous entities, so that the authors assume a cancer-specific expression pattern. Wei *et al* (2009), Matsuhashi *et al* (2007) and Mudduluru *et al* (2009) compared expression levels in healthy and tumorous tissues of ovarian-, squamous- and colorectal-carcinoma. In these probes there was a "shift" from the nuclear-in healthy probes to the cytoplasmatic compartment in malignant tissue. These results could not be verified in all tumorous entities. Studies with urologic tumors have shown diverging results concerning the expression levels or were not published at all. In a previous work, the authors could show a very significant correlation between the tumor stage and expression patterns in transitional cell carcinoma.

Research frontiers

Shi *et al* could show an effect of microRNA 21 (miR-21)-expression on docetaxel resistance, insinuating a more complex role of Pdcd4 in tumor aggressivity and chemoresistance. In this study, there was a negative correlation between low miR-21 expression levels with high docetaxel chemosensitivity and high Pdcd4 expression. The expression levels of Pdcd4 could be used to elaborate an individual therapeutic strategy and a prognostic evidence for each patient.

Innovations and breakthroughs

The studies of Woodard *et al* (2008) and Jansen *et al* (2004) demonstrated high Pdcd4 expression in clear cell renal cell carcinoma cell lines after having treated these with Fluvostatin and a higher geldamycin sensitivity in cells with high Pdcd4 expression levels. Fluvostatin being an inhibitory agent for pAkt and S6K1, inhibitors of Pdcd4. A recent study by Li *et al* (2012) showed a T-stage dependent expression of Pdcd4. This analysis shows a clear difference in Pdcd4 expression patterns within the subgroups, this being probably due to their distinct tumor biology. Immunohistochemistry (IHC) Pdcd4 expression was positive in the core and cytoplasmatic compartment in RCC samples: The levels changed according to tumor progression (T-stage dependent). This supports the hypothesis of tumor-specific expression patterns of Pdcd4 and a possible shuttle function between the cellular compartments.

Applications

This study supports the important role for Pdcd4 downregulation in renal-, testicular- and penile-carcinoma. Therapeutic and prognostic possibilities are discussed for other tumourous entities (*i.e.*, squamous carcinoma, Dou *et al* 2014). Especially chemosensitivity and therapeutic options can be evaluated for patients showing high IHC Pdcd4 expression levels.

Terminology

Pdcd4: Programmed cell death 4; NSGCT: Non-seminomatous germ cell tumor.

Peer review

The manuscript by Fisher *et al* is to investigate the Pdcd4 expression in renal cell carcinoma, penile carcinoma, and testicular germ cell cancer. The authors found that the Pdcd4 is strongly expressed in core and cytoplasm, but core and

cytoplasmic Pdcd4 levels is significantly decreased in the cancer tissues. These findings are important and may reveal a novel biomarker for renal and prostate cancers.

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RETROSPECTIVE STUDY

Single-site laparoscopic partial nephrectomy: Where are we going?

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Abstract

AIM: To review an evolution of laparoscopic surgery, there has been a growing interest in laparoendoscopic single-site surgery (LESS).

METHODS: A comprehensive electronic literature search was conducted using PubMed database to identify all publications relating to LESS-partial nephrectomy (PN). The research includes articles published from April 2008 to January 2014. We focused our attention only on articles in which were cited the single-site surgical technique (laparoscopic and robotic), tumour stage and grade, mean tumour size, intraoperative variables, blood loss and transfusion rate, length of post-operative stay and complication rates, Clavien classification, positive of surgical margins, pain assessment at discharge.

RESULTS: A total of 9 studies were collected with 221 patients included. The mean patients age was 62 years. The mean tumor size was 2.35 cm with a mean operative time of 181 min (range 111-270 min) and 58.3% were done by robot. The mean ischemia time was 23.6 min. The 25.8% of patients underwent an unclamp LESS-PN. Mean estimated blood loss was 296 mL and median length of hospital stay was 4 d. The rate of severe post-operative complications (≥ Clavien grade Ⅲ) was 5.4%. Not all surgical series of LESS-PN or Robotic-LESS-PN shows conversion in Multiport Laparoscopic or Open Surgery. Regarding oncologic outcomes, surgical margins were positive 4% of patients (9/221), no distant or port-site metastases were recorded.

CONCLUSION: LESS-PN and RLESS-PN are feasible and associated with reduced postoperative pain, shorter median hospital stay, shorter recovery time, and better cosmetic satisfaction without compromising surgical and oncological safety.

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Key words: Nephron sparing surgery; Partial nephrectomy; Laparoendoscopic single-site surgery; Single-port access surgery; Single-incision laparoscopic surgery; Robotic single-port partial nephrectomy

Core tip: In recent years, there has been a growing interest in laparoendoscopic single-site surgery (LESS). Some authors has used da Vinci surgical system for LESS surgery. Although almost every laparoscopic procedure in urology has been duplicated by using a LESS approach, only a few studies have reported problems and challenges encountered during LESS partial nephrectomy. The aim of our study is to evaluate the current literature in order to assess the efficiency, safety, and potential advantages of LESS-partial nephrectomy and Robotic-LESS PN.

Castellucci R, Cindolo L, Alvaréz-Maestro M, Giusti G, Be-

rardinelli F, Pellegrini F, Schips L. Single-site laparoscopic partial nephrectomy: Where are we going? *World J Clin Urol* 2014; 3(3): 358-363 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/358.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.358

INTRODUCTION

Renal cell carcinoma represents 2%-3% of all cancers with an age-standardised rate incidence and mortality of 5.8 and 1.4 per 100000, respectively, in more developed areas[1].

The widespread use of modern imaging methods has resulted in a marked increase in the number of renal tumors detected incidentally in patients with non-urological symptoms[2]. These tumors are often of lower grade and stage, and nephron-sparing surgery (NSS) is a good treatment option for small (≤ 4 cm) renal lesions[2,3].

Currently available evidence suggests that localized kidney cancer is best managed by NSS whenever technically feasible[4].

Although open partial nephrectomy (PN) is considered the "gold standard" in the surgical therapy of T1 renal tumors, advances in laparoscopic surgery have led to increasing use of laparoscopic PN (LPN) for NSS[2].

LPN has also gained popularity, but is currently performed mainly in high-volume reference centres, and its diffusion has been limited by the steep learning curve[5].

Laparoscopic NSS combining the preservation of renal function and the minimal invasiveness of laparoscopy represents a robust alternative to open surgery especially because the incidence of benign lesions on final histopathology is high (nearly 30%) in small incidentally discovered renal masses[2,6].

In recent years, there has been a growing interest in laparoendoscopic single-site surgery (LESS), an evolution of laparoscopic surgery. "Single-site surgery" is the term given to various laparoscopic techniques that use a single skin incision to gain access to the abdominal cavity[7]. It has been proved to be applicable in the clinical field, being safe in hands of experienced laparoscopic surgeons in well-selected patients[8,9].

LESS tries to overcome even the rare port-related complications of laparoscopy and seems to achieve a fast and painless postoperative recovery with excellent cosmetic results[10,11].

Cindolo *et al*[12,13] were pioneers in the field of LESS-PN, and described the unclamped technique in 2009, achieving favourable early outcomes. Although almost every laparoscopic procedure in urology has been duplicated by using a LESS approach, only a few studies have reported problems and challenges encountered during LESS partial nephrectomy[14,15]. In spite the development of new specific equipment for LESS, the surgical instruments have limited range of motion, and clashing of instruments is a major disadvantage[14]. For this reason, some authors have used da Vinci surgical system for LESS surgery[16,17]. The introduction of robotic technology has provided some attractive features such as magnified 3D vision, articulating instruments, scaling of movement, tremor filtration, fourth robotic arm assistance, and a live intraoperative ultra-sound platform. Consequently, these features have reduced the crowding of instruments, enabled better precision with tumor resection and renal reconstruction. Nevertheless, there is still paucity of relevant studies of Robotic LESS-PN (R-LESS-PN) on its intermediate term clinical outcomes as it is a relatively new approach[16].

The aim of our study is to evaluate the current literature in order to assess the efficiency, safety, and potential advantages of LESS-PN and R-LESS PN.

MATERIALS AND METHODS

Bibliographic research

A comprehensive electronic literature search was conducted using PubMed database to identify all publications relating to LESS-PN. The research includes articles published from April 2008 to January 2014; it was conducted using a free-text protocol that included the following terms: nephron sparing surgery, partial nephrectomy, laparoendoscopic single-site surgery, single-port access surgery, single-incision laparoscopic surgery, robotic single-port partial nephrectomy.

The inclusion criteria for LESS were single, exophytic, cortical, small (4.0 cm) renal masses suitable for standard laparoscopic PN without ischemia. Patients with renal tumors up to stage T2 in the absence of nodal and systemic metastases were considered for the procedure, while those ones with significant cardiovascular and respiratory comorbidities or uncorrected coagulopathy were excluded. Even though patients with body mass index (BMI) < 30 were selected for this procedure during the early part of our learning curves, at present, we do not consider obesity as exclusion criteria.

Among all the articles found, we selected only those that were single-centre studies (not multicentre studies); we analysed only articles single-site surgical technique series (laparoscopic and robotic) that included information about tumour stage, tumour grade, mean tumour size, mean operative time, blood loss, transfusion rate, length of post-operative stay and complications. In addition the following data were collected: age, gender, BMI, intraoperative variables (number of additional ports), post-operative complications (Clavien classification), positive surgical margins, pain assessment at discharge [visual analogue scale (VAS) scale]; In the study incision length and patient subjective scar satisfaction were also evaluated[18]. In all the studies, all patients underwent renal ultrasonography and computed tomography scan before surgery, to give detailed information on tumour size, location, extent of parenchymal infiltration and proximity to the pelvicalyceal system.

Table 1 The surgical relevant data of all studies

Ref.	*n*	Robotic	Age (yr)	BMI	Mean tumor size (cm)	Mean operative time (min)	Mean EBL (mL)	Length of stay (d)	Mean ischemia time (min)	Transfusion rate (%)	Complication > Ⅲ grade clavien	Additional trocars % (diameter)	Positive margins	VAS	Conversion in MLS	Conversion in OS
Desai *et al*[20]	6	0	62	25	3	270	475	7.2	20	0	2	100% (6 mm × 2 mm; 1 mm × 5 mm)	0	-	-	-
White *et al*[21]	15	4	-	-	3.01	196	422	4.5	NA	26	0	13% (NA)	1	6	2	1
[2]Kaouk *et al*[16]	7	2	63.5	27.5	2.1	165	260	3.3	0 in 6 pts 16 in 1 pts	14	0	14% (NA)	1	1.35	-	-
Choi *et al*[15]	59	56	-	-	2.6	212	171	4.5	27.5	13	1	Most of the cases (12 mm)	2	-	2	2
Bazzi *et al*[22]	17	0	60.6	26.8	1.8	176.6	170.6	3.4	28.6	0	3	0	0	1.58	1	1
[2]Rais-Bahrami *et al*[23]	15 (14 pts)	0	57.9	29.3	2.3	167.3	293.3	2.7	24.6 (9 pts) 0 (6 pts)	0	2	0 (1 case converted LPN)	0	2.1	1	0
[1]Tiu *et al*[24]	67	67	52.4	23.2	2.4 (47)	178	271	4	24	10.64	0	Cases right 5	2	-	0	0
			51.9	24.7	5.4 (20)	197	408	5.3	31	10	0	mm-trocar	1	-	0	1
Schips *et al*[19]	21	0	58.4	25	1.8	111	196	4.4	0	0	2	50% (2 mm × 3 mm; 5 mm × 5 mm)	2	2.1	3	0
Springer *et al*[18]	14	0	52.5	26.2	2	120	165	4	0	0	0	76% (11 mm × 3 mm)	0	1	0	0

[1]Tiu uniforms procedures depending on the size of the neo-formations, in the first row showns formations < 4 cm in the second those > 4 cm; [2]In these cases, authors specify the number of procedures that executed without ischemia from those in which the removal executed with ischemia. BMI: Body mass index; EBL: Estimate blood loss; OS: Conversion in open surgery; LPN: Laparoscopic partial nephrectomy; NA: Not attempted; VAS: Visual analogue scale; MLS: Multiport laparoscopic surgery.

We selected studies that described both LESS-PN and R-LESS PN (with or without ischemia).

RESULTS

A PubMed search revealed 9 surgical series of LESS NSS describing 221 cases overall including the authors' updated experience[15,16,18-24]. No randomised or comparative clinical trials were found. The surgical relevant data are described in Table 1. The mean average age was 62 years with a mean BMI of 25. Overall, the mean tumour size was 2.35 cm, the mean operative time was 181 min (range 111-270). Fifty-eight point three percent of the cases were R-LESS-PN. The mean ischemia time was 23.6 min (range 31-16 min), 25.8% of the cases were done without ischemia.

Mean estimated blood loss was 296 mL (range 165-475 mL) and median hospital stay was 4 d (range 2-7.2 d).

Severe (≥ Clavien grade Ⅲ) postoperative complications occurred in 5.4% of patients (12/221).

In some surgical series of LESS-PN or R-LESS-PN conversion to multiport laparoscopic or Open Surgery (OS) was necessary. However, only 14/208 cases needed conversion.

Reviewing the literature the use of an additional trocar to either multiport laparoscopic surgery (10 cases) or OS (4 cases) is valid option during the LESS procedure. The majority of cases only one additional trocar was used.

Rha used an additional 8-mm trocar to lift the liver in procedures of the right kidney[25]. In another paper an additional 3-mm trocar placed in the majority of patients was used to better control haemostasis during the removal of the tumour[18]. Some authors however, like Bazzi *et al*[22] did not used it at all.

Regarding oncologic outcomes, surgical margins were positive in 4% (9/221). Out of these 7/9 had renal cancer cells at the level of the inked parenchymal excision surface (although in four cases the intraoperative frozen section was negative); the remaining 2/9 were benign lesions (one angiomyolipoma and one recurrent oncocytoma). In most of the articles the patients were discharged with minimal discomfort, as demonstrated by their pain assessment scores (median VAS = 1)[18,19,21-23].

DISCUSSION

During the last decade the use of high-tech devices has become more and more common in urological procedures; efforts are done in the development of minimally invasive surgery. The conventional laparoscopic surgery is now paving way to the new technologies including LESS[26].

In fact, robotic and laparoscopic LESS partial nephrectomy represents an attractive and minimally invasive treatment option for patients with small renal tumours. Although the use of an additional 5-mm subxiphoid liver retraction port for right-sided renal tumours was a deviation of the strict philosophy of LESS surgery; however, it has become an accepted practice[25].

Several studies have been performed to evaluate the efficacy and surgical feasibility of LESS in current clinical practice[24,25,27]. In the oncological arena Bensalah confirmed that LESS is safe, but revealed that the indication and tumor location, rather than margin status, were significant predictors of local recurrence with a mean follow-up of 37 mo[28].

The oncological and functional outcomes of R-LESS-PN has been published by Tiu *et al*[26]. In their work they evaluated 39 patients who underwent R-LESS-PN with a minimum of 2 years follow-up. They showed comparable results with other minimal invasive surgical options for the management of renal tumours. They concluded that were still to address the current challenge of R-LESS surgery before this technique might be considered as the standard of care[26].

The main limitations of LESS-PN are: lack of availability of dedicated tools, impossibility of triangulation of the instruments using conventional laparoscopic instruments, and limits in the range of movement, that causes inter-instrument interference both inside and outside the operative field[29].

For these reasons Stolzenburg *et al*[30] tried to improve efficiency moving on from articulating instruments to the curved (pre-bent) laparoscopic instruments. These procedures were performed using different ports and instruments proving the feasibility of the laparoscopic single-site surgery instruments with variable equipment, at least in the hands of experienced laparoscopic surgeons[30].

The introduction of robot-assisted techniques minimize some of these problems despite the current da Vinci system (Intuitive Surgical Inc, Sunnyvale, CA, United States), is not designed to be used in this way. The more common problems are instrument conflicts (internal or external), significant gas leak, and the insufficient tissue retraction due to the absence of the fourth robotic arm[31].

Nevertheless, the development of new devices for LESS (*e.g.*, prebent instruments, streamlined and flexible optics, and magnetic anchors) will surely reduce the technical difficulties that were reported when this technique was just beginning[32].

Song have shown that the recovery of renal function after partial nephrectomy is impacted by patient age, comorbidities, and baseline renal function, along with the amount and depth of parenchyma excised. Interestingly, these investigators have reported warm ischemia times to have no impact on renal function[33].

The goal of LESS should be the Trifecta achievement defined as the combination of warm ischemia time < 20 min, negative surgical margins, and no surgical complications[11].

In this respect, Buffi *et al*[34] observed a significant longer operative time and mean ischemia time in R-LESS-PN cases than with the multiport robotic partial nephrectomy procedure. They also observed that patients with increased tumour size, moderate and high Preoperative Aspects and Dimension Used for an Anatomic Classification and Radius/Exophytic/Nearness to collecting system/Anterior/Location scores, infiltration of the collecting system, and renal sinus involvement had an increased probability for trifecta failure[34].

Cost-efficacy is also an important issue that should be taken into account. Pini *et al*[35] have compared the real costs of the various procedures (PN, LPN, laparoscopic nephrectomy, N-LESS and LESS-PN). They analysed the intra and post-operative costs, concluding that the minimally invasive surgery is the most expensive in the intraoperative phase (use of materials, operating time, *etc.*), but less wasteful post-operatively (days of hospitalization, post-surgical complications). Unfortunately the German government (as Italian government) uses a prospective payment system based on diagnosis-related groups. This system calculates the cost encoding peri- and postoperative complications and hospitalization time, without any distinction of surgical approach, blood transfusions, need of perioperative Double J stenting and intraoperative frozen section. For this reason, the total cost remained almost unmodified among all groups that they analysed. In conclusion, they stated that local health systems should consider a subclassification with different reimbursement, which should incentive NSS and minimal invasiv surgery approaches[35].

The evidence available in the literature indicate that LESS-PN and RLESS-PN are feasible and associated with favourable reduced postoperative recovery. However, the prolonged learning curve with LESS is a major drawback. Continued innovation may allow single-port surgery to become more easily incorporated into standard practice.

COMMENTS

Background

During the last years it was developed a new minivasive tecHnique, laparoendosocopic single site (LESS) surgery, that is applied to small renal tumours. The purpose of this method is to be less invasive than usual one, because of its only one access point. Therefore this innovative approach should lead to a minor morbidity for patients and a more suitable cosmetic result, especially in young patients.

Innovations and breakthroughs

In this paper the authors assembled various articles, published all over the world, trying to offer a topic overview.

Applications

This technique could be used for all the small tumors. However, the prolonged learning curve with LESS is the major drawback.

Peer review

This is a reasonably well written review of LESS partial nephrectomy.

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SYSTEMATIC REVIEWS

Use of intralesional collagenase in the treatment of peyronie's disease: A review

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Abstract

AIM: To review the relevant literature in an effort to examine the body of evidence available to date.

METHODS: Ovid MEDLINE search database was queried using MeSH terms "penile induration", "peyronie's disease", "Collagenases" and "Collagenase" using various permutations. No temporal parameters were employed.

RESULTS: In all, 5 relevant clinical trials were isolated from 34 results. These trials were analyzed using the Oxford Centre for Evidence-Based Medicine criteria. They were further examined based on study design and methods; the primary and secondary outcomes were reviewed for treatment efficacy and collagenase-related side effects.

CONCLUSION: Intralesional collagenase appears to be safe and effective in the non-surgical treatment of Peyronie's disease. However, the data remains limited and further inquiries into the safety of collagenase, treatment standardization and standardized outcomes reporting remain necessary. Furthermore, studies comparing intralesional collagenase to alternative medical and surgical therapy will be important in guiding the future treatment decision process.

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Key words: Peyronie's disease; Collagenase; Reconstructive urology; Plaque; Intralesional injection

Core tip: In December of 2013, the United States Food and Drug Administration approved the use of collagenase clostridium histolyticum (CCH) for the treatment of Peyronie's disease (PD). In all, 5 relevant clinical trials were isolated from 34 results. With limited data on medical PD treatments, the studies to date appear to support CCH as a reasonably safe and well-tolerated non-surgical intervention. However, because no studies compared CCH to other medical interventions and no trials have been conducted to assess the ultimate need for surgical intervention, further comparative investigations are necessary to determine the ultimate role that the intralesional collagenase may play in the treatment of PD.

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INTRODUCTION

In December of 2013, the United States Food and Drug Administration (FDA) approved the use of collagenase clostridium histolyticum (CCH) for the treatment of Peyronie's disease (PD). This approval was based on several clinical trials, most notably the recently completed

concurrently run Investigation for Maximal Peyronie's Reduction Efficacy and Safety Studies (IMPRESS) I and Ⅱ trials. The goal of this paper is to describe the body of evidence leading to the FDA's approval of this drug in the treatment of PD.

The prevalence of PD is strikingly high with epidemiological estimates ranging from 3.2% in Europe[1] to 8.9% in the United States[2]. Yet despite its high prevalence, PD remains both underreported and difficult to treat[3,4]. Many men do not seek treatment, oftentimes due to embarrassment or lack of access to accurate information. The presentation of PD is varied and includes penile pain with erection, angulation of the penis, erectile dysfunction, and the presence of a palpable plaque, typically located on the dorsum of the penis[5-10]. Furthermore, men with PD are at a higher likelihood to have an inability to perform intercourse as curvature of 60 degrees of higher has been associated with a threefold increase in the odds of sexual disability[11]. These factors and the complicated nature of PD make it psychologically and physically detrimental to not only the patient but also his sexual partner[12,13].

Although 250 years have passed since Francois Gigot de la Peyronie described the first case series of PD, little understanding of its etiology has emerged[14,15]. The disease is believed to occur secondary to abnormal collagen deposition and scar formation from buckling trauma to the tunica albuginea of the penis. The current pathophysiological model of PD reflects the body's response to microtrauma to the tunica albuginea where a disordered healing process allows scarring to occur. This scarring restricts symmetrical expansion of the tunica albuginea during an erection, thus resulting in angulation of the penis. As with all scar formation, the major step is collagen synthesis. It is theorized that microtrauma combined with an underlying genetic predisposition allows for abnormal collagen deposition and scar formation[16].

The formation of scar is a balance between collagen formation and its degradation through collagenase-a naturally occurring enzyme that functions to break down collagen. This enzyme, produced by humans, is also formed by bacteria. Clostridium histolyticum, a gram-positive anaerobic bacterium, is well known to cause destructive, rapidly spreading infections through soft tissue planes which occurs mainly through the function of collagenase. This bacterial enzyme was first isolated in 1953[17]. It has since been purified and is now commercially available. CCH (Xiaflex, Auxillium Pharmaceuticals) is a purified mixture of two collagenases, AUX-1 and AUX-2. The first *in vitro* biochemical study of CCH, published in 1962, demonstrated collagenolytic activity on animal tissues[18]. Gelbard *et al*[19] subsequently examined the *in vitro* effects of CCH on the tunica albuginea of patients with and without PD. Their research demonstrated a significant collagenolytic effect of CCH on both normal tissue and tissue with Peyronie's plaques. Importantly, vascular smooth muscle was not digested and preservation of all vessels except small venules was observed. This led to the notion that collagenase would be a safe treatment for PD and other similar afflictions, including Dupuytren's disease (DD).

In February 2010, CCH was approved by the FDA for treatment of DD. The approval came as a result of a large double blind randomized, placebo controlled trial (DBRCT)[20] demonstrating that localized injection of CCH, in conjunction with passive joint manipulation, reduced the contracture to full extension within 30 d of last injection, compared to placebo (from 43.9 to 80.7 degrees *vs* from 45.3 to 49.5 degrees, $P < 0.001$). This observation was achieved with minimal adverse events (AEs). The pathological and epidemiological similarity between DD and PD and the promising of DD trials lead to the initiation of two DBRCTs to examine the effects of CCH on PD[21].

MATERIALS AND METHODS

Relevant articles for this review were obtained through the Ovid MEDLINE search database. Search strategy included MeSH terms "penile induration", "peyronie's disease", "Collagenases" and "Collagenase". The search was carried out as follows: ("penile induration" OR "Peyronie's disease") AND ("Collagenases" OR "collagenase"). This produced in 34 results, which were further narrowed down by clinical trials focusing on collagenase and PD yielding a total of five relevant trials. Due to the limited availability of clinical trials on this subject, no year limits were used in this search and publication dates ranged from 1985 through 2013.

Evaluation of these clinical trials was based on the Oxford Centre for Evidence-Based Medicine criteria.

RESULTS

A total of 5 clinical trials have examined the use of CCH in the treatment of PD (Tables 1-4).

The use of collagenase in the treatment of PD

The first clinical trial examined the safety of intralesional collagenase to treat PD[22]. It included 31 men, each with a mean curvature of 42 degrees, 10 of which had undergone prior non-CCH treatments for their condition. Collagenase was injected daily for three days at a dose of 470 to 620 μg/mL for the first 15 patients and 910 μg/mL for the rest. Total dose ranged between 470 to 2730 μg/mL per patient, with a mean of 2330 μg/mL. This low dosing regimen was chosen to assess both safety and efficacy during this phase 1 trial. Objective improvement was observed in 20 patients; 4 had resolution of their plaques, and 16 had penile curvature reduction by 20%-100%. These results were seen in the majority of patients within 2 wk of treatment. Pain with erection was eliminated in 13 of the 14 patients who entered this study with erectile pain. Three out of 4 men who were unable to have intercourse regained the ability after treatment. The one who failed had a pre-treatment 180-degree penile bend. AEs

Table 1 Study designs

Ref.	No. of patients	Study design	Length of study	Control group	Length of follow up
Gelbard *et al*[22]	31	Prospective	Mean of 22 mo per patient	None	9.8 mo (mean)
Gelbard *et al*[23]	49	Double blind, RCT	3 mo	Placebo	3 mo
Jordan[24]	25	Prospective	9 mo	None	None
Gelbard *et al*[25]	147	Double blind, RCT	Up to 18 wk	Placebo	None
Gelbard *et al*[26]	832	Double blind, RCT	Up to 52 wk	Placebo	None

RCT: Randomised controlled clinical trial.

Table 2 Study treatments

Ref.	Intralesional injection therapy dose and schedule
Gelbard *et al*[22]	One daily dose of 470620 μg/mL (15 patients) or 910 μg/mL (16 patients) for three consecutive days
Gelbard *et al*[23]	CCH single injection (6000-14000 Units)
Jordan[24]	Three 10000 Units injections administered over 7-10 d, repeated at 3 mo
Gelbard *et al*[25]	CCH 0.58 mg (10000 Units), 2 injections 24-72 h apart, repeated every 6 wk for up to 3 cycles
Gelbard *et al*[26]	CCH 0.58 mg (10000 Units), 2 injections 24-72 h apart, repeated every 6 wk for up to 4 cycles

CCH: Collagenase clostridium histolyticum.

Table 3 Adverse events

Ref.	Adverse effects (*n*)
Gelbard *et al*[22]	Ecchymosis (21), Pain (2), Albuginea rupture (1)
Gelbard *et al*[23]	Tenderness at injection site (Majority), tunica albuginea rupture (1)
Jordan[24]	Edema/pain/or ecchymosis (20), No serious AEs
Gelbard *et al*[25]	Bruising (96), Edema (50), Pain (58), No serious AEs
Gelbard *et al*[26]	Ecchymosis (441), Edema (30), Pain (250), Corporeal rupture (3), Penile hematoma (3)

AEs: Adverse events.

Table 4 Study outcomes

Ref.	Outcome	Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence
Gelbard *et al*[22]	Improvement seen in 20 patients (4 had plaque disappearance, 16 had curvature decrease by 20%-100%)	2
Gelbard *et al*[23]	Overall, 36% responsed to treatment. Improvement greatest in patients with lesser degree of pre-treatment curvature	2
Jordan[24]	Significant mean changes from baseline angular deviation and plaque width at 3, 6, and 9 mo	2
Gelbard *et al*[25]	Improved penile curvature (29.7% *vs* 11.0%, $P < 0.05$) and patient reported bother scores for treatment group *vs* placebo	1
Gelbard *et al*[26]	Improved penile curvature (34% *vs* 18%, $P < 0.0001$) and patient reported symtom bother score for treatment group *vs* placebo	1

were minimal and included ecchymosis (21 patients) and pain (2 patients). One patient had an albuginea rupture two weeks after treatment. This was a 23 years old who experienced pain with a popping sensation during intercourse. The penis was bandaged and he was instructed to avoid intercourse for 3 wk. After healing, the degree of penile curvature was straighter than before treatment. Mean follow up for this study was 9.8 mo.

Collagenase vs placebo in the treatment of PD: A double-blind study

Eight years later, a trial of 49 patients was conducted stratifying patients by degree of curvature. The patients were divided into three groups: group 1; 30 degrees or less and/or palpable plaque less than 2 cm, group 2; 30 to 60 degrees and/or plaque 2 to 4 cm, group 3; over 60 degrees and/or plaque greater than 4 cm[23]. Pre and post intervention deformity was measured with vacuum induced erection photography. The patients in each group were randomized to receive either treatment or placebo (saline injections). The three treatment groups received a total of 6000 units, 10000 units, 14000 units, respectively. All injections were administered into the Peyronie's plaque, and patients were instructed to avoid intercourse for 2

wk after treatment. Patients were evaluated at 1 week, 1 mo, and 3 mo after treatment. Overall, 36 percent (8 of 22 patients) of the treatment group responded to CCH, compared to 4 percent (1 of 27 patients) for placebo, $P < 0.007$. In general, patients from group 1 had a higher response rate to CCH compared to group 2 and 3 (100%, 36%, 13%, respectively). Response differences for treatment *vs* placebo in groups 1 and 3 were not statistically significant. One category 3 patient experienced a tear of the tunica albuginea during intercourse 3 wk after treatment. The tear was treated conservatively and resolved. The majority of patients experienced tenderness at the injection site, yet this AE was observed as frequently in both the treatment and placebo groups.

The use of intralesional clostridial collagenase injection therapy for PD: A prospective, single-center, non-placebo-controlled study

A 2008 prospective trial including 25 patients with PD was conducted with a more uniform treatment protocol: three injections of intralesional CCH at 10000 units/0.25 cm^3 per dose, administered over 7-10 d[24]. This process was repeated at 3 mo after first treatment. Plaque size and angle deformity was assessed at 3, 6, and 9 mo. Eighteen of the 25 patients completed the treatment and follow-up. Primary outcome was a > 25% reduction from pretreatment angular deviation, considered a successful response. A secondary end point was a patient questionnaire. This questionnaire included a subjective patient evaluation of both visual outcomes and restoration of sexual function. Results demonstrated significant decreases in mean deviation angle observed at months 3 and 6 (*P*-values at 9 mo were not reported). Positive treatment response for the primary outcome (> 25% angular reduction) peaked at month 3 yet declined by month 9, likely due to drop-out of successful patients. However, patients who experienced treatment success in month 3 continued to experience success at month 9. Additionally, significant decreases were observed in plaque width at 3, 6, and 9 mo and plaque length at months 3 and 6. The patient questionnaire revealed overall global evaluations of the patient's disease condition improved. More than 50% were considered "much improved" or "very much improved". One third responded "minimal improvement" or "no change". AEs occurred in eighty percent of patients and included edema, penile pain, or ecchymosis. No serious AEs were observed.

As stated previously, by February, CCH had been FDA approved to treat DD at a dose of 10000 Units per injection. Studies have shown that intralesional injection followed by finger extension and manipulation, intended to further break down the cord, had shown the greatest improvement in plaque breakdown[20]. These protocols were thus applied to studies regarding CCH and PD.

Phase 2b study of the clinical efficacy and safety of CCH in patients with Peyronie's disease

A phase 2b DBRCT study including 147 subjects examined the effect of CCH and "modeling," or bending of the flaccid penis in order to break up a PD plaque[25]. Subjects were divided into 4 groups to receive CCH or placebo (saline injections) (3:1 randomization) with or without penile plaque modeling (1:1 randomization). Penile plaque modeling consisted of stretching the penis for 30 s, followed by 30 s in the nonmodeled state, repeated for 3 cycles at a time. Subjects in the CCH group received 2 injections at 10000 units/0.25 cm^3 per dose, given 24 to 72 h apart, repeated for up to 3 cycles at 6-wk intervals. Outcomes included change in penile curvature based on goniometer measurement, patient reported outcomes *via* questionnaire (PD-PRO and IIEF), and AEs. There were significant differences in penile curvature in the CCH *vs* placebo groups when modeling was applied (32.5% *vs* 2.5%, $P < 0.001$). Minimal difference was observed between treatment and placebo groups in the absence of modeling. Patient questionnaires revealed that CCH treated patients has a significantly better PD symptom bother score than those on placebo ($P = 0.05$). However there was no significant difference in the other questionnaire domains (intercourse discomfort, constraint, penile pain). Common AEs in CCH groups included bruising (86.5%), edema (45%), and pain (52.3%). No serious AEs related to treatment were observed. No systemic immunologic events were reported.

Clinical efficacy, safety and tolerability of CCH for the treatment of peyronie's disease in 2 large double-blind, randomized, placebo controlled phase 3 studies

Most recently, the Investigation for Maximal Peyronie's Reduction Efficacy and Safety Studies (IMPRESS) I and Ⅱ trial, a phase 3 DBRCT with the largest cohort of 832 subjects, examined CCH *vs* placebo in patients with PD[26]. Modeling was not performed. Dosing and scheduling was identical to the prior phase 2b study, although cycles could be repeated up to 4 times. Subjects treated with CCH had a mean 34% improvement in curvature, compared with 18% in the placebo group ($P < 0.0001$). PD symptom bother score was also significantly improved in the treatment *vs* placebo groups ($P = 0.0037$). AEs in the CCH group were similar to those observed in the phase 2b trial, including ecchymosis (80%), edema (55%), and pain (45.4%). However, six serious AEs were observed. Three men experienced corporeal rupture, all of which were successfully repaired surgically. The other three experienced penile hematoma, one resolving without intervention, one resolved with aspiration, and one successfully repaired surgically. No systemic or immunologic events were observed.

DISCUSSION

The recent FDA approval of CCH for the treatment of PD marks an important step in the treatment of this widespread and often debilitating disease. CCH is the first non-surgical therapy to become FDA approved for this purpose. Though limited clinical data currently ex-

ists on intralesional collagenase-in all, 5 clinical trials were isolated-the results appear promising. Though side effects throughout the trials were relatively common, they were mild and transient. Serious AEs were rare, and included corporeal rupture and penile hematoma. These serious AEs were reported both in the trials from 1985, 1993, and the most recent IMPRESS trial.

Improvement in penile curvature, both objective and subjective, was reported in all five trials; the highest level of objectivity was reached in the IMPRESS trial through the use of goniometer. Most importantly, two thirds of the sexually active men in the IMPRESS trial cohort received a validated PD symptom questionnaire taking into account the psychological burden that PD has on the patient.

There are limitations to this area of research. Although results support CCH as safe and effective, none of these trials compared CCH to other treatment modalities, or to placebo. Furthermore, the same team of researchers authored 4 out of 5 publications, which could contribute to bias. Yet given the excellent safety results, coupled with its efficacy displayed in DD trials, the authors of this paper still recommend CCH as a safe and effective treatment option for PD.

To date, no studies comparing CCH to other medical interventions have been conducted and no assessment of the ultimate need for surgical intervention has been performed. However, given the paucity of data on medical PD treatments, the studies to date appear to support CCH as a reasonably safe and well-tolerated non-surgical intervention for PD.

COMMENTS

Background

Trials on the use of intralesional collagenase for the non-surgical treatment of Peyronie's disease (PD) first appeared in 1985. However, in the three decades leading up to the United States Food and Drug Administration's (FDA) approval of this therapy, limited studies have been conducted to measure its efficacy and safety. Furthermore, few, if any review articles are available regarding this therapy.

Research frontiers

Collagenase clostridium histolyticum (CCH) was recently FDA approved for the treatment of PD. There have been a handful of trials focusing on the use of CCH to treat PD, yet few examined the safety and efficacy of CCH. To the authors' knowledge, there have been few, if any, review articles discussing these trials in detail, including CCH background, safety, efficacy, and future directions of research.

Innovations and breakthroughs

CCH is a promising, non-surgical alternative to treat PD. This review article discusses the CCH background, summarizes clinical trial results, and comments on future research directions. The authors believe this is a novel article and one that can help provide useful information to both clinicians and researchers who aim to treat this disease.

Applications

This article provides summaries of the efficacy, safety, and future directions of intralesional collagenase. This information can be applied to clinical practice, as well as future research.

Terminology

There are no terms in this article which the authors feel cannot be understood by the majority of readers.

Peer review

This article summarize the five clinical trials published from 1985 to 2013 about safety and advantage in the use of CCH in the treatment of PD. The studies support CCH as a reasonably safe and well-tolerated non-surgical intervention for PD: rare serious adverse events included corporeal rupture and penile hematoma.

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SYSTEMATIC REVIEWS

Best surgical treatment for very large benign prostatic obstruction

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Abstract

AIM: To investigate the best surgical treatment for very large benign prostatic obstruction (BPO).

METHODS: A revision of literature was conducted in PubMed database with 167 search results. Key words for the search were benign prostatic hyperplasia, surgical treatment, large, and volume. Inclusion criteria for this study were surgical treatment of benign prostatic obstruction for prostates equal to or larger than 80 cc. Among article search results, 9 completed inclusion criterion and were revised. Each surgical technique included in those articles was compared to each other. The results were observed, and conclusions derived from this are presented. There is no statistical analysis.

RESULTS: Of the 5 techniques presented in the revised articles [open transvesical enucleation, holmium laser enucleation of the prostate (HoLEP), photoselective vaporization of the prostate using potassium titanyl phospate laser, transurethral resection with bipolar energy, and transurethral enucleation with bipolar energy], open transvesical enucleation best permits the resolution of obstructive symptoms. It presents excellent maximum flow rates, high resected tissue volume and maintenance of results over time. These characteristics explain why it has been the gold standard treatment for prostates greater than 80 cc. However, it is at the expense of greater blood loss, urethral catheter and hospital stay times. Since its initial application in 1996, the transurethral enucleation of the prostate by means of a holmium laser has become a procedure that has similar surgical outcomes with fewer complications when compared to open surgery making it an interesting alternative for very large BPO. Nonetheless, no procedure has removed open surgery as the gold standard for very large BPO.

CONCLUSION: Open surgery has proved to be the gold standard for very large BPO. HoLEP appears as a minimally invasive alternative with same benefits but less morbidity.

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Key words: Benign prostatic obstruction; Surgical treatment; Prostatectomy; Holmium laser enucleation of the prostate

Core tip: Though the gold standard for surgical treatment of very large benign prostatic obstruction has been open prostatectomy, in the last three decades there has been a notorious absence of publications showing the outcomes of this surgery. The only procedure with similar results and fewer complications seems to be the holmium laser enucleation of the prostate making it an interesting alternative when confronted with large sized prostates. New methods of treating

large prostates have an interesting challenge since both open surgery and holmium laser enucleation of the prostate present favorable results.

Sáez ID, de la Llera JF, Horn CD, López JF, Chacón RA, Figueroa PA, Vivaldi BI, Coz F. Best surgical treatment for very large benign prostatic obstruction. *World J Clin Urol* 2014; 3(3): 370-375 Available from: URL: http://www.wjgnet.com/2219-2816/full/v3/i3/370.htm DOI: http://dx.doi.org/10.5410/wjcu.v3.i3.370

INTRODUCTION

Open prostatectomy has been one of the oldest procedures practiced in urology to treat large benign prostatic obstruction (BPO) and it still remains valid today. Historical accounts divide open prostatectomies according to different techniques: perineal, suprapubic and retropubic approach[1,2].

The first open prostatectomy described was through the perineal approach by Covillard. In 1639, he became the first to remove a prostatic middle lobe through perineal approach. However, it was Goodfellow that became the first to perform open perineal prostatectomy routinely. Numerous physicians perfected the technique employed afterwards but it was Young at Johns Hopkins University in 1905 who published his operative technique[3].

Suprapubic prostatectomy techniques followed perineal approaches. In 1894, Eugene Fuller is regarded as the first man to perform a complete suprapubic removal of a prostatic adenoma. However, it was Peter Freyer in 1900 that gained most fame performing a transvesical prostatectomy. His publication of cases in the British Medical Journal popularized this procedure[4].

In terms of retropubic prostatectomy, Van Stockum is regarded as the first person to complete an enucleation of prostatic adenoma using this surgical approach in 1908. It was Millin, in 1945, who popularized this approach publishing his operative technique and results[5]. His technique has been employed then since worldwide for over 65 years with minor modifications.

The introduction of instruments such as the Stern-McCarthy resectoscope in 1926 opened a new era in urology and BPO surgery. It became one of the leading discoveries in the field of urology in the 20th century. This resectoscope was utilized worldwide as one of the first endourological approaches to BPO. It became the first minimally invasive procedure for endoscopic treatment of pathologies such as bladder neoplasms and BPO. Perhaps the most important advance made to this instrument was provided by Iglesias in 1975 who modified it to allow both irrigation and suction[6]. With the development of new lenses this enhanced vision of the operatory field and shortened operatory times.

Numerous endoscopic techniques have been described and published since Iglesias published his results with his modified resectoscope. The most important breakthrough since then has been the development of transurethral resection of the prostate (TURP). Initially developed with monopolar and posteriorly with bipolar energy, TURP became the treatment of choice of BPO, especially with prostate sizes between 30 and 80 cc[7]. Utilized to this day worldwide, TURP has fulfilled a crucial role in the treatment of prostatic obstructions symptoms with small and middle sized prostates. Nonetheless, there is little evidence that discusses the role of TURP in prostates sized over 80 cc and especially over 120 cc.

Numerous articles have been published showing that novel techniques may be the new state of the art treatment for such condition. Procedures such as transurethral incision of the prostate, transurethral needle ablation of the protate, transurethral ultrasound guided laser induced prostatectomy, Prostatron, Thermex and visual laser ablation of the prostate have been employed to treat small and midlle sized prostates. With initially positive results, most of these techniques have failed in their attempt to become the new gold standard treatment for such a disease. Most have never been an option for the treatment of very large BPO. They have been employed only in the setting of small and middle sized prostates. These instruments have quickly fallen from being strongly advertised solutions to BPO to adorning the basements of hospitals around the world.

Various other techniques have been developed to defy open prostatectomy as the treatment of choice for very large BPO. The most accepted of these has been that of the holmium laser enucleation of the prostate (HoLEP). Initially described in 1996 in New Zealand by Gilling *et al*[8], this procedure has seen to have benefits over other endoscopic procedures such as TURP for the treatment of large prostates.

MATERIALS AND METHODS

To evaluate the best surgical treatment for very large BPO we conducted a meticulous search and revision of available literature.

A systematic search was conducted at PubMed database with the following keywords: benign prostatic hyperplasia, surgical treatment, large, volume. A total of 167 articles were found (including randomized controlled trials, prospective and retrospective series). All publications were reviewed and those that fulfilled inclusion criterion were considered in our study. These criteria were the following: prostatic volume greater than 80 mL sized by transrectal ultrasound (TRUS), measurement of international prostate symptoms score symptoms, maximum urinary flow, prostatic specific antigen (PSA) values pre and post surgery and registry of surgical complications with a follow up of at least 12 mo. Studies in which authors employed resective techniques had to include measurement of enucleated or resected tissue. Those who used vaporization techniques had to include exclusively measurement of prostatic volume by TRUS before and

Table 1 Overview of the published studies

Ref.	Follow up (mo)	Patients (*n*)	Change in PSA		Change in symptoms (IPSS)		Change in Q max		Amount of tissue removed		Time urethral catheter (h)	Length of stay (h)
			ng/dL	%	Absolute	%	mL/seg	%	mL	%		
Transvesical												
Alivizatos *et al*[9], 2008	12	60	-4.3	-68.2	-13	-62	7.1	88.7	86	89.5	120	144
Ou *et al*[10], 2013	12	49	-4.4	-78.5	-19.3	-76.8	11.8	231.3	109.8	77.8	182	223
Rao *et al*[11], 2013	12	40	-3.91	-86.5	-21	-85.7	19.7	333.8	75.2	68.3	148	223
HoLEP												
Elzayat *et al*[12], 2006	24	225	-8.1	-90	-14.3	-79.4	20	250	86	68.2	30	29
Matlaga *et al*[13], 2006	12	86	-8.86	-90.2	-14.5	-73.9	15.8	173.6	140	82.3	15.1	26
Kuntz *et al*[14], 2008	60	42			-19.2	-86.4	20.5	539.4				
PVP with KTP												
Rajbabu *et al*[15], 2007	24	54	-4.6	-41.8	-16.3	-74	11	137	59	43.7	23	11
Alivizatos *et al*[9], 2008	12	65	-3.8	-61.2	-11	-55	7.4	86	38	40.8	24	48
Bipolar TURP												
Zhu *et al*[16], 2009	36	132	-4.17	-57.3	-18.5	-86.4	15.7	237.8	58.1	72.9	69	117
Enucleacion bipolar												
Rao *et al*[11], 2013	12	43	-4.22	-88.4	-21.4	-86.2	20.8	358.6	65.9	56.8	79.2	129
Ou *et al*[10], 2013	12	45	-4.3	-72.8	-17.6	-75.8	9.6	162.7	98.7	74.7	103	139

PSA: Prostatic specific antigen; IPSS: International prostate symptoms score; HoLEP: Holmium laser enucleation of the prostate; TURP: Transurethral resection of the prostate; KTP: Potassium titanyl phosphate; PVP: Photo vaporization of the prostate.

after the intervention as well.

Of the 167 articles selected for revision, only 9 fulfilled criteria for inclusion (Table 1). These investigations analyze 5 therapeutic alternatives for benign prostatic hyperplasia surgery: transvesical or transcapsular enucleation of the prostate, endoscopic resection with bipolar energy, vaporization with potassium titanyl phospate (KTP) laser, transurethral enucleation with Holmium laser and transurethral enucleation-resection with bipolar energy (mushroom technique). A detailed analysis of these articles is presented, grouped by operatory technique.

RESULTS

Simple open prostatectomy (transvesical)

Developed by Freyer in 1900 and still a valid procedure today, three contemporary articles describe this technique. Alivizatos *et al*[9], Ou *et al*[10] and Rao *et al*[11]. Together they compile 149 patients with a mean follow-up of 12 mo. With an average prostate volume of 115 cc, they achieve a mean enucleation of 78 cc of prostatic tissue. This corresponds to the greatest resected volume of all techniques analyzed. PSA values dropped 77% (from an average of 5.3 to 1.2 ng/dL). This technique owns the highest recatheterization rate with an average of 4.7% of patients (Rao *et al*[11] has a 7.5% recatheterization rate). This series also has the greatest number of reinterventions with 5.3% of patients undergoing a new surgical intervention.

This technique has the longest need of urethral catheterization and hospital stay with 150 and 196 h, respectively. This procedure also has the highest transfusion rate, with an average of 9.8% of patients transfused.

Holmium laser enucleation of the prostate

Published by Gilling *et al*[8] in 1996, this technique uses the Holmium YAG laser, allowing to transurethrally enucleate prostatic lobes in a similar fashion as when done with fin-

Table 2 Complication rates of each technique

	Reoperation (%)	Urethral stenosis (%)	Transfusion (%)
Technique			
Transvesical	5.3	7.9	9.8 (6.1-13.3)
HoLEP	1.33	0.66	0.84 (0-7.14)
PVP (KTP)	2.52 (0-3)	1.68 (0-2)	0
Bipolar TURP	4.5	4.5	0
Bipolar enucleation	2.5 (0-2.2)	2.4 (2.2-2.5)	7.5 (0-6.6)

HoLEP: Holmium laser enucleation of prostate; PVP: Photo-selective vaporization of prostate; KTP: Potassium titanyl phosphate; TURP: Transurethral resection of prostate.

ger enucleation in open techniques.

Three study groups employ this technique with a total of 353 patients and a 32 mo average follow-up (Elzayat *et al*[12], Matlaga *et al*[13], Kuntz *et al*[14]).

It is the technique that resects the second greatest amount of prostatic tissue with an average 75.2% reduction of prostate volume. It also has the greatest PSA drop with a 90.1% reduction rate (from 9.41 to 0.93 ng/dL). It provides the greatest improvement of maximum urinary flow with an average increase of 17.9 mL/seg with respect to baseline values.

Nonetheless, this technique is known to be the slowest procedure. In effect, it is the slowest of all with 121 min mean intervention time.

Only 1 patient required recatheterization and 1 required reintervention due to persistent hematuria. Transfusion rate was 0.34% (3 of 353 patients). Patients needed a total of 22.5 h of urinary catheterization, while hospital stay was 27.5 h, second shortest of techniques compared.

Photoselective vaporization of the prostate using KTP laser

This is the only non resective technique reviewed. It does not extract tissue, hence, there are no biopsy results or accurate total tissue removal volumes (% prostatic size reduction is obtained with the difference between pre and post operative transrectal echography). This does not permit adequate comparison with other techniques.

This technique is presented in 2 series (Rajbabu *et al*[15], Alivizatos *et al*[9]), with a total of 119 patients and 18 mo mean follow-up.

Reduction of prostatic volume is 42%, constituting the technique with the lowest volume drop. PSA drop and maximum urinary flow are also the lowest with an average decrease of 51.5% (from 8.6 to 4.4 ng/dL) and 9.2 mL/s improvement, respectively.

It has no transfusion rate and has a short catheter and hospital stay time (23.5 h and 29.5 h, respectively).

Bipolar energy

Two techniques utilize this method and were included for revision.

Transurethral resection with bipolar energy

Basically, it is similar to classical TURP but with the use of bipolar energy. This allows irrigation with a saline solution, consequently permitting to extend operatory times with less risk of post TURP syndrome.

There is one article that uses this procedure (Zhu *et al*[16]), with a total 132 patients and a 36 mo follow-up.

This series presents a reduction of prostatic volume of 72.9%, behind transvesical adenectomy and HoLEP. Drop of PSA was 57.3% (7.27 to 3.1 ng/dL), while improvement of flow was 15.7 mL over baseline.

This study group published a 4.5% reoperation rate (second after open surgery). Average usage of urinary catheter and hospital stay was that of 69 and 117 h, respectively.

Transurethral enucleation with bipolar energy (mushroom technique)

Two articles employed this procedure (Rao *et al*[11], Ou *et al*[10]). Both compared this intervention with transvesical open surgery (results previously mentioned).

These studies comprise a total of 88 patients and have a follow up of 12 mo. Preoperative mean volume was 124 cc with a reduction of tissue of 65.7%. This places it in fourth place, only overcoming vaporization with KTP laser. However, it has the second greatest PSA fall, behind HoLEP, with a reduction of 80.6% (5.33 to 1.07 ng/dL). Requirements of catheterization and total hospital stay were just behind open transvesical surgery, with average times of 91.1 and 134 h.

DISCUSSION

Within the spectrum of techniques employed to treat prostatic obstructive hyperplasia, TURP is considered the gold standard for small and medium sized prostates (80 mL or smaller). When confronted with larger sized prostates, studies do not compare TURP to other techniques. Also, no significant sized series have been published to show results in this large volume subgroup.

Of the 5 techniques presented, there are no doubts that open transvesical enucleation permits the resolution of obstructive symptoms. It presents excellent maximum flow rates, high resected tissue volume and maintenance of results over time. In fact, it is the treatment of choice for very large BPO according to american urological association (AUA) and European guidelines. These characteristics explain why it has been the gold standard treatment for prostates greater than 80 cc[12] but at the expense of greater recatheterization, blood loss, urethral catheter and hospital stay times (Table 2). Favoring open surgery is the fact that this procedure can be completed in basically equipped surgical wards and should be mastered by all urologists worldwide.

In the last few years HoLEP has had an important role in the discussion of the best treatment of large benign prostates. This technique has similar results when compared to open prostatectomy both in resected vol-

ume and long term studied parameters. In particular, Kuntz *et al*[14] has a 5 years follow up in which the score symptoms of the AUA and maximum flow rate have maintained inalterable over time in both the open adenectomy and HoLEP groups. This has not been reported for other techniques, converting HoLEP in the sole option that equals benefits to open surgery but with lesser transfusion and shorter catheter and hospital stay rates.

Some benefits reported for KTP laser fulguration are similar to those obtained with HoLEP. These include lower catheter requirements, hospital stay and transfusion rates. However, HoLEP reports better flow rate and symptom results in the long term.

Most urologists state that the HoLEP learning curve is slow and is estimated at a mean of 30 cases. It also requires highly sophisticated equipment and trained personnel to implement this procedure efficaciously.

Other interesting results are those achieved by the fall of PSA by enucleation with the bipolar loop (mushroom), situating itself immediately behind HoLEP, even better than open surgery. This may be due to both the enucleation of tissue as well as the fulguration of the prostatic capsule, which reduces the number of glandular cells of the prostate. It would be interesting to have a close follow up and comparison of these techniques on prostatic cancer incidence in the very long term.

In light of this review, it seems valid to state that the ideal technique for treating prostates larger than 80 cc is enucleation. While this is performed transvesically or minimally invasively, it is this condition that differences results from resective or fulgurative techniques.

In attempts to maintain this condition of enucleation, alternative techniques have appeared that combine the benefit of being less invasive and the advantages of open surgery. Small series of laparoscopic enucleation, utilizing the Millin technique or through a unique transvesical trocar were first described by Mariano *et al*[17] in 2002. Recent publications such as García-Segui *et al*[18] in 2012 compared 17 laparoscopic extraperitoneal to 18 open Millin technique patients. The laparoscopic group had a lower hemorrhage rate and lower irrigation, catheterization and hospital stay time. There are no reports in the medium or long term. The largest series employing this technique is McCullough *et al*[19], who publishes in 2009 a series of 280 cases, 96 of them laparoscopically approached, with results discretely superior to open surgery. There are no prospective randomized trials with adequate follow up that permit to conclude the potential benefits of these techniques. Hence, we do not recommend it over those analyzed in this revision.

It would be of great use to incorporate user satisfaction and hospital cost surveys to treated patients to permit a more valid conclusion on whether to incline the balance toward open surgery or HoLEP.

Open prostatectomy has been the gold standard of treatment of very large BPO for the last 65 years. It is a procedure that is practiced routinely worldwide and solves obstructive symptoms efficaciously. In the past years, new technologies have been developed to treat very large BPO. This review shows that of these procedures, HoLEP is the only one that has come to compete with open surgery side by side. However, it is still an incognito if HoLEP will resist the trial of time and become the standard of care surgery for this illness in 65 more years.

COMMENTS

Background

Management of very large benign prostatic obstruction (BPO) (80 cc or greater) remains a challenge in urological practice. For the past 65 years, open prostatectomy has been the gold standard procedure for these cases. Nowadays, in the minimally invasive era, the search for an alternative with less morbidity, shorter hospitalization stay and recovery time force the authors to evaluate and become familiar with other techniques recently developed but that remain unknown or are unavailable for the majority of urologists worldwide.

Research frontiers

Of the minimally invasive alternatives available to manage very large BPO, there is little evidence published to sustain Holmium laser enucleation of the prostate (HoLEP) as the best surgical approach. More research is needed to compare HoLEP with other minimally invasive procedures and open surgery to sustain it is a new gold standard for this morbid condition.

Innovations and breakthroughs

Open surgery remains the sole alternative for very large BPO when associated with certain comorbid conditions. Such is the case when very large BPO coexists with significant bladder stone burden and/or large bladder diverticula. No other procedure simultaneously resolves these comorbid conditions as effectively as open surgery. It is also important to note that HoLEP was described over 18 years ago. Since its introduction it has proved great effectiveness, but with limited diffusion. Most articles portraying the benefits of this technique have only been published in the last few years. A possible explanation of this phenomenon is the country of origin where it was initially described. If HoLEP had been developed in Europe or in the United States of America, would it have taken over 15 years to become a worldwide recognized technique for BPO treatment?

Applications

According to the authors' research, they consider it necessary to be familiar with minimally invasive approaches to treat BPO. Of these techniques, perhaps urologists should be specifically acquainted with HoLEP, as it has proven to be a minimally invasive and less morbid alternative for these patients.

Terminology

HoLEP: Holmium laser enucleation of the prostate. It is an endoscopic approach that allows enucleating hyperplastic tissue in a similar manner as open surgery but using a resectoscope tip as a fingertip. Laser energy fulgurates the perforating vessels in the enucleation plane. At the end of the procedure, prostate lobes resected placed in the bladder lumen are morcelated and aspirated by an endoscopic morcellator introduced through the resectoscope; PVP: Photoselective vaporization of the prostate. It consists in the ablation of prostatic tissue of the transition zone as potassium titanyl phospate laser energy is applied using a side fire fiber.

Peer review

The paper is a thoroughly insight in the surgical treatment of large (> 80 mL) and very large (> 120 mL) prostates.

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CASE REPORT

Pre-fabricated radial forearm phalloplasty with cadaveric bone graft

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Abstract

Phalloplasty is a complex set of procedures used in efforts to improve the anatomical, physiological, and aesthetic deficiencies caused by loss or absence of the penis. Methods have evolved significantly, and the use of free tissue transfer has become common amongst reconstructive surgeons. The inclusion of bone autograft, usually radius or fibula, within the neophallus has caused significant morbidity, and efforts continue to find the optimal solution. We present a novel approach using a pre-fabricated, radial forearm fasciocutaneous free flap containing cadaveric bone graft for phalloplasty following traumatic penis amputation.

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Key words: Phalloplasty; Pre-fabrication; Cadaver allograft; Radial forearm; Penile reconstruction

Core tip: While there are many options for penile reconstruction, many techniques have associated morbidity when attempting to recreate rigidity in the neophallus by using bone autograft mostly from either the radius or fibula. In our case report, we use a novel approach to addressing rigidity in the neophallus without causing associated morbidity by using a pre-fabricated, radial forearm fasciocutaneous free flap containing cadaveric bone graft for reconstructive phalloplasty.

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INTRODUCTION

Phalloplasty is a complex set of procedures that can pose significant anatomical, physiological, and aesthetic challenges, and the surgical techniques implemented have evolved significantly over the past sixty years. In the 1930s, Borgoras used bipedicled abdominal tube flaps to reconstruct the penis[1]. This method was later replaced by the use of fasciocutaneous and extended pedicle island flaps with continued suboptimal results[2,3]. In the 1980s, microsurgical advancements made the use of free flaps a better option, and the use of sensate, radial forearm fasciocutaneous or osteocutaneous flaps and fibular osteocutaneous flaps improved sensation and rigidity of the neophallus, giving a more desirable and functional outcome[4-7]. This method comes with significant donor site morbidity including extensive donor site scarring, bony fracture, and wrist and ankle instability[2,7]. Implant placement in the radial forearm free flap also has risks, including extrusion, costs, infection, and delayed placement (usually 1 year). We report a novel approach using cadaver bone graft to provide penile rigidity without sacrificing donor site morbidity in a patient undergoing phalloplasty following traumatic penis amputation.



Figure 1 Preoperative view demonstrating perineal urethrostomy.

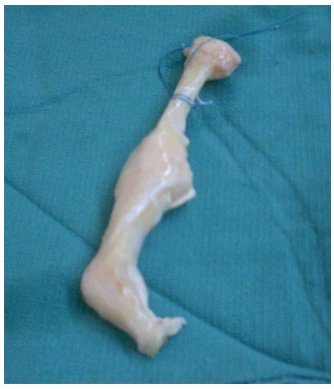


Figure 2 Cadaveric bone allograft construct, using metacarpal and proximal phalanx.

CASE REPORT

The patient is a 17-year-old male who sustained a gunshot wound to the lower abdomen and perineum at the age of nine. All penile substance and his left testicle were lost. He underwent emergent surgery with creation of a perineal urethrostomy. He has developed normally without other medical problems. The perineal urethrostomy allows him to urinate without incontinence. He has a viable right testicle within his scrotum (Figure 1). Through the work of a charitable organization, he was transferred to our institution for penile reconstruction. This is a retrospective case report and received an exempt status from our institutional review board.

The patient underwent general endotracheal anesthesia and was placed in low lithotomy position. His non-dominant left arm was chosen as the donor site for construction of the neophallus. An Allen's test was performed on the left wrist which demonstrated adequate flow to the hand *via* the ulnar artery. Two operative teams were utilized in the operation.

Dissection in the abdomen proceeded through the previous abdominal scar with an extended right Pfannenstiel incision for harvest of the inferior epigastric artery and vein recipient vessels under loupe magnification. These vessels were traced to their origin, and the ilioinguinal nerve was dissected for microscopic nerve anastomosis. The remnant scrotal tissue was elevated to isolate the perineal urethra, and the pudendal nerve and genitofemoral nerve were identified for later nerve anastomosis.

A template was used to design the neophallus on the volar surface of the forearm creating a 5.5 inch long neophallus. Using a tourniquet, the radial forearm flap was elevated in a suprafascial fashion, extending radially to the flexor carpi radialis and ulnarly to the brachioradialis, with identification of the radial artery and its venae comitantes and their inclusion within the flap. The vascular pedicle was dissected to its origin at the brachial artery. The lateral and medial antebrachial cutaneous nerves were also included within the flap.

The neophallus was created by first establishing the urethral conduit along the ulnar side of the flap. This area was de-epithelialized for a width of 0.5 cm, giving a 26 mm diameter neourethra. The ulnar aspect of the flap was rolled over an 18-French Foley catheter and secured to the radial aspect of the flap with an inner layer of interrupted 3-0 absorbable suture and an outer layer of running, locking, 3-0 absorbable suture, creating a water-tight closure of the urethra. The remainder of the radial forearm flap was rolled to create a cylindrical penile shape. To give added infrastructure to the neophallus, cadaveric phalangeal and metacarpal bones were placed in its dorsal aspect. These bones were provided by the University of Miami Tissue Bank (Miami, Florida). The cadaveric bones were stripped of periosteum and soft tissue, trimmed into a more narrow shape, and drill holes were created within the substance of the bones to allow for vascular penetration (Figure 2). The bones were anchored to each other with suture, allowing for minimal movement between them. The bone construct was then placed into the flap, dorsal to the neourethra. The metacarpal bone was placed proximal to the proximal phalanx.

The neophallus was then transferred to the perineal area. The neourethra was sutured to the urethral remnant in the perineum in two layers with absorbable suture. The bone construct within the neophallus was secured to the pubic symphysis. Microvascular anastomosis was performed between the radial artery and right inferior epigastric artery, as well as between the radial artery venae comitantes and the inferior epigastric venae comitantes. Adequate flow was established through the vasculature, but due to the small size of the veins, the patient was given a Heparin bolus and started on a drip. Microscopic neurorrhaphy was performed with anastomosis between the lateral antebrachial cutaneous nerve to the ilioinguinal nerve, and between the medial antebrachial cutaneous nerve to the branch of the genitofemoral nerve and pudendal nerve.

Drains were placed within the abdominal wall dissection and perineum. The perineum was closed with a scrotoplasty to ensure complete soft tissue coverage of the right testicle. A split-thickness skin graft was placed over the donor site on the left forearm. The glans penis was sculptured on the neophallus, using the Norfolk technique to increase its prominence. A skin graft was also



Figure 3 Immediate post-operative view.

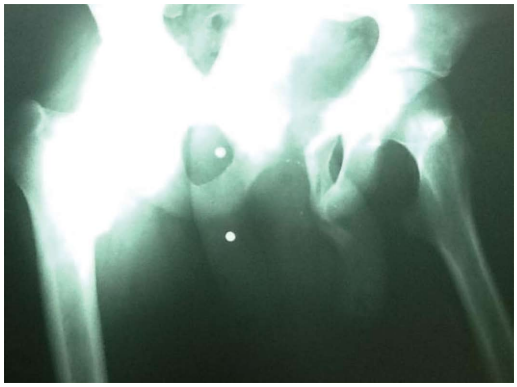


Figure 4 X-ray views demonstrating bone allograft construct within the substance of the neophallus.

placed to further create a transition zone from the shaft to the glans (Figure 3).

The patient was extubated and transferred to the Intensive Care Unit. On post-operative day 2, the patient was taken back to operative room for evacuation of a hematoma that had caused arterial insufficiency to the flap. The microvascular anastomosis was intact, with slight compromise of arterial inflow and venous outflow; however, upon evacuation of the hematoma, there was excellent vascular supply to the flap. The abdominal wall was loosely closed, with a portion of the skin left open due to tension. This area was covered with bovine collagen-dermal bilayer matrix.

The patient was discharged from the hospital and to his native country on post-operative day 28, and he was satisfied with his overall result. The Foley catheter was removed at 6 wk. The patient developed a urethral-cutaneous fistula three months post-operatively at the penoscrotal junction, but he remained continent of urine. On radiograph, the bone allograft construct is viable without evidence of osteomyelitis (Figure 4). At 6 mo, the patient has a rigid and viable neophallus (Figure 5).

DISCUSSION

As the technical innovation of phalloplasty continues to evolve, from a fasciocutaneous flap to a radial forearm fasciocutaneous or osteocutaneous flap or fibula osteocutaneous flap to a sensate, prelaminated fibula flap[8], we propose a new approach using cadaveric bone graft reinforced by free radial forearm fasciocutaneous flap for penile reconstruction. We recognize that the radial forearm osteocutaneous flap has morbidity, including donor site functional and aesthetic deformities requiring skin grafting, risk of radius fracture, and aesthetic deformity from autologous bone harvesting[2]. Additionally, the radial osteocutaneous phallus has been reported to develop a "softened phallus" from cortical degeneration. Kim described 12 softened penises out of 58 radial forearm osteocutaneous phalloplasties[9]. Cavadas described a second stage reconstruction using a free fibular osseous flap for rigidity after a radial forearm fasciocutaneous flap[10].



Figure 5 Six months follow-up demonstrating viable neophallus.

Employing the development and application of over five million musculoskeletal allografts and considering the significant morbidity associated with harvesting of the radius bone autograft, we elected to use a metacarpal and phalangeal allograft from the University of Miami Tissue Bank. Allografts are chemically processed with low dose radiation to prevent the transmission of infection while maintaining biological integrity. Although there is a theoretical risk of disease transmission, it is rare. By using a metacarpal and phalangeal allograft, this eliminates the need for a second operation to provide rigidity with an implant and minimizes donor site morbidity. We were able to provide adequate rigidity and support to the neophallus, as well as place a spacer for a second stage procedure in the event the implant was unsuccessful. This was particularly useful in this humanitarian case, as commonly surgeons only have one opportunity to impact the patient's care.

Penile prosthesis implantation has also been used in reconstructive phalloplasty, usually as a second stage to the procedure. This form of reconstruction has the disadvantage of significant cost and has not always been covered by insurances in the United States. There are also the added risks of extrusion, infection, and revision surgery[11]. This form of reconstruction was not available for this humanitarian case, as the patient was unable to make multiple trips to our institution for staged operations.

In our case, the patient has good overall success. He has regained his self-esteem as well as the physiological use of his neophallus, although he has not reported sexual intercourse. His complication of urethral fistula is consistent with the reported incidence of 63.6%[2]. This has been managed conservatively without further sequelae. The drawback of this report is that long term rigidity has yet to be determined, and long term photographs have not been able to be obtained due to patient limitations. In spite of this, the use of an allograft bone implant has many advantages, and further study of this technique is warranted to determine its inclusion in the ongoing evolution of phalloplasty.

COMMENTS

Case characteristics

A 17 year-old male with a traumatic penis amputation.

Clinical diagnosis

Absence of penis with the presence of a perineal urethrostomy.

Differential diagnosis

Penile amputation.

Imaging diagnosis

Absence of penile substance.

Treatment

The patient was treated with a pre-fabricated radial forearm phalloplasty with cadaveric bone graft.

Related reports

Phalloplasty consists of a complex set of procedures, each with its own morbidity, and the issue of creating and maintaining rigidity in penile reconstruction is challenging.

Term explanation

Phalloplasty is the reconstruction or creation of the penis by surgical methods. Pre-fabrication refers to the method of placing a tissue or implant into another vascularized tissue bed and then transferring this construct into a body of tissue that is desired to be reconstructed.

Experiences and lessons

This case report represents a unique method of penile reconstruction in which the authors create rigidity through the use of a cadaveric bone graft rather than utilizing a bone autograft that causes morbidity at the donor site.

Peer review

This article demonstrates a novel surgical technique of a pre-fabricated radial forearm phalloplasty with cadaveric bone graft in reconstruction of the penis following a traumatic amputation. This type of operation is intended to limit the morbidity of using bone autograft, from either the radius or fibula, as well as complete the operation in one stage, as compared to the multiple stages used with placement of a penile prosthesis implantation.

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