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REVIEW

Choice of dialysis modality prior to kidney transplantation: Does it matter?

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Abstract

The population of patients with end stage renal disease (ESRD) is increasing, lengthening waiting lists for kidney transplantation. Majority of the patients are not able to receive a kidney transplant in timely manner even though it is well established that patient survival and quality of life after kidney transplantation is far better when compared to being on dialysis. A large number of patients who desire a kidney transplant ultimately end up needing some form of dialysis therapy. Most of incident ESRD patients choose hemodialysis (HD) over peritoneal dialysis (PD) as the modality of choice in the United States, even though studies have favored PD as a better choice of pre-transplant dialysis modality than HD. PD is largely underutilized in the United States due to variety of reasons. As a part of the decision making process, patients are often educated how the choice regarding modality of dialysis would fit into their life but it is not clear and not usually discussed, how it can affect eventual kidney transplantation in the future. In this article we would like to discuss ESRD demographics and outcomes, modality of dialysis and kidney transplant related events. We have summarized the data comparing PD and HD as the modality of dialysis and its impact on allograft and recipient outcomes after kidney transplantation.

Key words: Dialysis; Kidney transplant; Outcomes; Peritoneal dialysis; Health literacy

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Core tip: Patients with end stage renal failure need some form of dialysis therapy as a bridge while they wait for kidney transplantation. In this paper we discuss if dialysis modality pre transplantation has any impact on transplant related outcomes.

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INTRODUCTION

Kidney transplantation is the ideal form of renal replacement therapy (RRT) in patients with end stage renal disease (ESRD). Preemptive kidney transplantation is ideal for many, as it is associated with lower rates of acute rejection, increased allograft and patient survival[1]. However, a preemptive kidney transplant (17% overall) is not always possible for many reasons which were explored by Jay *et al*[2], which included disparities in health insurance, race/ethnicity, patient education level, socioeconomic status, access to healthcare, diabetes status and regional variations. It is also well established that patient survival and quality of life after kidney transplantation is far better when compared to being on dialysis[3].

According to statistics, close to 10% of the population are diagnosed with chronic kidney disease around the world. Also only appropriate 10% of this patient population receives some treatment in the form of dialysis or transplant to stay alive. There were 30869 adults patients newly added to the waiting list and 33291 patients were removed from the list according to annual report from SRTR registry released in 2016. Unfortunately, a quarter of those patients were removed due to death or decline in medical condition[4]. Patients waiting for kidney transplant are also gradually getting older (median wait for a newly listed 2010 candidates was 3.9 years[5]), thereby the burden of kidney disease is rising in the elderly population. There has been some improvement in the dialysis related mortality overall but the organ shortage and continued increasing list of patients waiting for a transplant is still haunting the nephrology community. The average time on the waitlist for a deceased donor can be quite variable depending on age, blood group, panel reacting antibodies, history of prior transplantation, race/ethnicity and regional factors[4]. Hence, patients end up needing some form of RRT while they wait for transplantation.

Peritoneal dialysis (PD) leads to minimal disruption of the patient's life, thereby allowing the patient to continue to work or school or other usual activities, along with encouraging patient empowerment in self-management. Hence, for the patients who plan on receiving a transplant after starting dialysis, it can be a better bridge therapy to kidney transplantation, especially, when a lot of patients initiating hemodialysis (HD) *via* catheters are associated with adverse outcomes[6]. As a part of the decision making process, the education generally includes how the choice of therapy would fit into the patient's life however it is not clear and hence not discussed, how a dialysis modality may affect eventual kidney transplantation in the future. A number of studies have addressed the outcome of kidney transplantation after PD versus in-center HD, reporting mixed results. A meta-analysis by Tang *et al*[7] in 2016 concluded that PD was a better choice of pre-transplant dialysis modality than HD. Another study by Jones *et al*[7] in 2018 found PD as a viable bridge therapy for patients waiting for simultaneous liver-kidney transplantation. In another Cohort of 92884 patients, HD as a choice of RRT was associated with an increased risk for graft failure and recipient death[9]. On the other hand, study by Resende *et al*[10] and Dipalma *et al*[11] did not find any impact of dialysis modality on graft function or patient's survival after transplantation.

Our goal of this discussion is to review the current evidence in regards to choice of RRT and impact on kidney transplantation outcomes. We have organized the review into two categories: short-term outcomes, including delayed graft function (DGF), and allograft thrombosis; and long-term outcomes, including mortality. At first, we would like to review the demographics and outcomes of ESRD in the United States, as this crucial decision regarding modality choice can have large impact on choices of significant number of ESRD patients.

ESRD DEMOGRAPHICS

As per the United Network for Organ Sharing, in 2017, there were 94897 patients on the waiting list for kidney transplantation. Among those, majority were aged 50+ years (43% of patients were between 50-64 years of age and 23% of patients were 65+

years of age). Only, 19849 patients (40% of patients were age 50-64 years and 18% of patients were 65+ years of age) received kidney transplantation alone in the United States of America (USA) in the year of 2017[12].

Unites States Renal Data System (USRDS) is the most robust national database in the USA on all patients with ESRD covered by Medicare and Medicaid. At the end of 2015, there were 207810 patients living with a functioning kidney transplant and 83978 dialysis patients (17% of all prevalent dialysis patient population) were on waiting list for kidney transplantation[5]. In the USA, there were 124114 incident ESRD patients in the year 2015 with an unadjusted incident rate of 378 per million population, which is increasing steadily since 2012[13]. Unfortunately, approximately one third (36%) of those patients did not receive significant pre-ESRD care and 80% of patients initiated HD with a catheter as opposed to preferred arteriovenous access[6,13]. Majority of incident ESRD patients chose HD (87.8%) over PD (9.6%) as the modality of choice in the USA[13]. As per the latest data, there were 703243 prevalent ESRD patients in the USA (on December 2015) with an unadjusted prevalence rate of 2128 per million populations, which is also steadily increasing by adding about 20000 patients each year[13]. Among all prevalent ESRD patients, 63.2% of patients were on HD, 29.6% had a functioning kidney transplant and only 7% of patients were utilizing PD. In-center HD accounts for almost all of HD (98%) modality and only a very small percentage of patients perform home HD (2%)[13].

It is in stark contrast to countries like Hong Kong (70%), the Jalisco region of Mexico (51%), New Zealand (30%), Thailand (29%), Qatar (27%), Colombia (27%), Australia (20%) and Canada (20%), where much higher proportion of patients utilize PD as compared to the patients in the USA[14]. PD is an acceptable and could be a preferred form of RRT owing to flexibility, autonomy, care satisfaction[15], better preservation of residual renal function[16], better hypertension control[17], lower intra-dialytic hypotension episodes[18], lower risk of dementia, slower cognitive decline[19,20], better anemia management with lower doses of erythropoietin stimulating agents (ESA) and lower proportions of patients needing ESAs[21]. It is largely underutilized in the USA due to variety of reasons which have been explored by many researchers and found causes to be multifactorial which were physician specific (lack of experience, inadequate training, comfort with HD); patient specific (lack of adequate PD education, health literacy, burden of therapy, age, comorbidities); modality specific (concerns for mortality, solute clearance, peritonitis, treatment failure, regulatory issues on PD fluid, easy availability of HD); and financial incentives for HD units[22-24].

ESRD OUTCOMES

In recent times, success of PD technique has improved and risk of peritonitis had dwindled[22,23]. Review of the data also suggests that as per the USRDS[25], in 2015, adjusted mortality rate for patients on HD was slightly higher than patients on PD (169 per 1000 patients years *vs* 159 per 1000 patients years; respectively) and much higher than patients who received kidney transplantation (29 per 1000 patients years). A very interesting trend of mortality with age and time on dialysis has been noted.

Among those patients who started RRT with HD in 2015, mortality rates in patients < 65 years of age decreased from 200 deaths per 1000 patient-years in month 2 to 134 deaths per 1000 patient-years in month 12. Mortality rates in patients aged ≥ 65 years were much higher as compared to patients with < 65 years but also noted to decrease similarly (615 deaths per 1000 patient-years in month 2 to 278 deaths per 1000 patient-years in month 12).

In contrast, among patients who started RRT with PD[25], mortality increased in both patients < 65 years of age (28 deaths/1000 patient-years in month 1 to 64 deaths/1000 patient-years in month 12) and ≥ 65 years of age (124 deaths per 1000 patient-years in month 1 to 223 deaths per 1000 patient-years in month 12). This study showed two important findings, mortality rates for PD patients were much lower as compared to HD and secondly elderly patients tend to do better on PD versus HD. However, one concern from this mortality data arises that whether it is PD or HD, elderly patients age ≥ 65 years suffer from far more increased risk of mortality as compared to patients < 65 years of age. As the ESRD patient population is aging and dying waiting for a transplant, it will be imperative to increase utilization of kidney transplantation at the earliest and offer a better RRT modality.

In-fact, overall adjusted survival probability of incident patients on PD is much better at the end of 3 years than patients on HD (68% *vs* 57%). Expenditure of PD is also better than HD (75140 $ per patient per year *vs* 88750 $ per patient per year) but much higher than cost for transplant patients (34084 $ per patient per year)[26]. HD and PD patients have similar hospitalizations rate (1.7 per patient year) but almost double

of patients with kidney transplantation (0.8 per patient year). Patients on HD gradually has lower hospitalization rates as time goes on but patients on PD tends to have slightly higher hospitalization rates with time (1.4 PPY in 2013 but increased to 1.6 PPY at end of 3rd year) but still remained lower than HD cohort (1.7 PPY)[27]. This data suggests that PD is a more cost effective modality with somewhat lower risk of mortality as compared to HD in pre-transplant period.

While on the waitlist for a kidney transplant, mortality for PD and in-center HD patients was found to be similar by Inrig *et al*[28]. This prospective observational study used a cohort of patients placed on the transplant list who initiated dialysis (n = 12568) between May 1, 1995 and October 31, 1998. Two-year mortality was 6.6% among PD patients and 6.9% among HD patients, with no significant differences [hazard ratio (HR) 1.01; 95% confidence interval (CI) 0.82 to 1.23] when controlled for baseline characteristics, comorbidities, and laboratory variables. This study used the modality the patient was on at 90 d of dialysis as the treatment group, and excluded those who died in the first 90 d. Of note, in this study 24% of the patients were on PD, indicating that PD patients are much more likely to be listed for a kidney transplant early since the percentage of PD utilization nationally is much lower.

Delayed graft function for kidney transplant

DGF defined as need of dialysis within seven days of kidney transplantation, occurred in 21.3% of patients transplanted in 2008 in the USA[29].

Numerous studies as mentioned in Table 1 have investigated DGF rates and have found mostly similar to lower rates of DGF in PD versus HD patients[29-39]. Some of the earlier studies were performed in an era when different immunosuppressive regimens were used[31-34]. A large study by Snyder *et al*[38] investigated this question in 2002 using USRDS data with over 22000 patients; also found a lower incidence of DGF among PD patients (RR = 0.74, 95%CI: 0.67-0.81, P < 0.0001) after adjustment of multiple clinical covariates. They also noted that PD patients were 1.39 times more likely to get transplanted as compared to HD patients (95%CI: 1.35-1.43, P < 0.0001). In a more recent study by Molnar *et al*[39] of 14508 dialysis patients who underwent kidney transplantation for the first time, the case-mix-adjusted risk of DGF was 34% lower for patients on PD *vs* HD (HR = 0.66 with 95%CI of 0.55-0.79, P < 0.001). However, once adjusted for malnutrition inflammation complex syndrome and donor characteristics, PD was no longer an independent predictor for decreased DGF (HR = 0.82 with 95%CI of 0.60-1.13, P = 0.23)[31]. But, PD was found to be protective against DGF in a subgroup of patients with hemoglobin between 12 and 13 gram/dL. A meta-analysis by Tang *et al*[7] found significantly lower risk of DGF in PD patients as compared to HD patients (OR 0.67, 95%CI: 0.62-0.72, P = 0.024). Lin *et al*[41] also postulated higher risk of DGF in HD patients based upon the observation that there more dialysis events were noted in HD group (1.59 in HD *vs* 0.71 in PD, P < 0.05).

In a retrospective observation study of patients with DGF requiring HD or PD, Thomson *et al*[42] found an increased risk of wound infection/leakage (PD 5/14 *vs* HD 6/63, P = 0.024), shorter length of hospitalization (PD 13.7 d *vs* HD 18.7 d, P = 0.009) and lesser time requiring dialysis post-operatively (PD 6.5 d *vs* HD 11.0 d, P = 0.043) with use of PD however no differences in readmission to hospital within 6 mo, graft loss or acute rejection episodes at one year. GFR also did not differ between the PD and HD groups at one month, six months or at one year[42].

Reasons for better outcome in terms of DGF in PD patients are not entirely clear. PD patients have better preservation of residual renal function[37,38]. There may be lead time bias as well because, generally PD patients may be more motivated and hence may have increased transplant access. Few other reasons like difference in immune function, cytokine production, and different response to ischemic kidneys among PD *vs* HD patients have been proposed as well[37]. In fact, maintenance dialysis prior to transplantation is noted to be a major contributor to DGF[29]. Since, PD is performed daily and patients are less likely to be hyperkalemic, hence are less likely to require additional treatments just prior to kidney transplantation. PD patients are not likely to be volume depleted either; this will also ensure adequate perfusion of the allograft. HD prior to transplant may be associated with volume removal, which in turn may result in eventual decreased perfusion of the transplanted organ and some tubular necrosis[43]. In addition, intra-op aggressive hydration has been proved to be effective in reducing DGF[29,43], which may have been countered against by pre-transplant HD.

Thrombosis of the allograft: Comparing prior HD to PD

In contrast to DGF, thrombosis of the graft may be surprisingly higher in the PD patients (Table 2) as compared to their HD counterparts[38,44-46].

In Snyder *et al*'s[38] subgroup analysis of allografts surviving < 3 mo, patients on PD prior to the transplant had higher adjusted risk for both allograft failure (RR 1.23, 95%CI: 1.09-1.39, P < 0.001) and death-censored allograft failure (RR 1.33, 95%CI: 1.16-

Table 1 Pre-transplant dialysis modality and delayed graft function

Study Period	Authors	Study Design	Study Participants	DGF Incidence	Favors
1983-2006	Caliskan *et al*[30]	Retrospective observational	44 PD and 44 HD patients	No difference in DGF incidence	None
1983-1989	Cacciarelli *et al*[31]	Retrospective observational	cohort of 662 patients	26% of PD and 36% of HD patients	PD
1984-1988	Triolo *et al*[32]	Retrospective observational	18 PD and 18 HD patients	27% patients on PD and 27% patients on HD	None
1988-1995	Fontan *et al*[33]	Retrospective observational	92 PD and 587 HD patients	22.5% in PD and 39.5% of HD patients	PD
1989	Cardella *et al*[34]	Retrospective observational	31 PD and 37 HD patients	35% in PD and 35% in HD patients	None
1990s	Vanholder *et al*[35]	Case-control	117 PD and 117 HD patients	23.1% in PD and 50.4% in HD	PD
1993-2014	Song *et al*[36]	Retrospective observational	97 PD and 178 HD patients	19.6% in PD and 32% in HD	PD
1994- 1995	Bleyer *et al*[37]	Retrospective observational	Cohort of 9291 patients	20% of PD and 28.6% of HD patients	PD
1995-1998	Snyder *et al*[38]	Retrospective observational	5621 PD and 17155 HD patients	12% in PD and 16% in HD	PD
2001-2006	Molnar *et al*[39]	Retrospective observational	2092 PD and 12,416 HD patients	15% in PD and 21% in HD	PD
2002-2011	Prasad *et al*[40]	Retrospective observational	45 PD and 45 HD patients	8.8% in PD and 11.1% in HD	None

DGF: Delayed graft function; PD: Peritoneal dialysis; HD: Hemodialysis.

1.53, $P < 0.0001$) than HD patients[38]. Forty one percent of those on prior PD, who had allograft failure in the first 3 mo, had thrombosis *vs* 30% of those on prior HD (OR 1.59, 95%CI: 1.08-2.36, $P = 0.02$). All other early causes of allograft loss were similar between the two groups. In another study of 84513 renal transplant recipients between 1990-1996, Ojo *et al*[48] found much higher odds of renal vein thrombosis (RVT) in PD patients as compared to HD patients (OR = 1.87, $P = 0.001$). Change in pre-transplant dialysis modality was also predictive of RVT among patients who switched from HD to PD (OR = 3.59, $P < 0.001$) as compared to HD patients who never switched and among patients who switched from PD to HD as compared to HD patients who never switched (OR = 1.62, $P = 0.047$)[48]. In another study of 119 HD and 39 PD patients who underwent simultaneous kidney-pancreas transplantation, renal allograft loss due to thrombosis was much more common in PD patients as compared to HD patients (5.1% *vs* 0%, $P = 0.058$)[50].

Since most patients on PD do not have an arteriovenous access, underlying thrombotic tendencies may be masked, and only uncovered at the time of transplantation. In addition, some PD patients may have been driven to switch after repeated thrombosis of the HD access. Moreover, PD patients are noted to have increased pro-coagulant factors such as apolipoprotein A, factors II, VII, VIII, IX, X, XI and factor XII, and hemo-concentration as compared to HD patients which can predispose them at higher risk of allograft thrombosis[46,48]. The reasons behind increase in such factors are likely due to moderate non-specific inflammatory cell harvesting when the peritoneal membrane gets exposed to dialysis solutions. This leads to macrophage activation and increased presence of thromboplastin and plasminogen activator in the peritoneal cavity.

On the contrary, a study by Pérez Fontán *et al*[47] on 827 patients (127 PD and 700 HD patients), who received deceased donor kidney transplantation between 1988 and 1997, there were similar incidence of primary allograft thrombosis between PD and HD patients (4.7% *vs* 6.1%, P = NS). Arterial and venous thrombosis was also similar in both groups[47]. Studies by Lin *et al*[41] and Escuin *et al*[49] also reported similar results whereby they found no difference in incidence of graft thrombosis among PD versus HD patients.

Risk of infection and diabetes mellitus after transplantation

Patients receive multiple immunosuppressive medications in post-transplant period which increases the risk of infections. Infectious complications related with PD catheter after transplantation remain a concern[42,50]. In a study by Rizzi *et al*[51] on 313 PD patients who underwent transplantation between 2000 to 2015, authors found that 8.9% patients had post-transplant peritonitis especially among those who had DGF requiring dialysis. In addition, PD catheter was associated with an increased risk of exit-site infection and peritonitis even if it's not used[52]. There is also a report of increased conversion from PD to HD after transplant due to leakage of dialysate fluid from surgical incision[52]. Hence, authors had suggested low threshold for PD catheter removal at time of transplantation in patients with low risk of DGF. In patients with an increased risk of DGF, PD catheter could be left in place but to be removed at the

Table 2 Pre-transplant dialysis modality and allograft thrombosis

Study Period	Authors	Study Design	Study Participants	Thrombosis Incidence	Odds Ratio (OR)
1980s-1990s	Van der Vliet *et al*[44]	Retrospective observational	303 PD and 612 HD patients	7.3% in PD and 3.6% in HD patients	$P < 0.02$
1988-1997	Pérez Fontán *et al*[47]	Retrospective observational	127 PD and 700 HD patients	4.7% in PD and 6.1% in HD patients	P = NS[b]
1989-1992	Murphy *et al*[45]	Retrospective observational	202 renal transplant procedures	9 PD versus 0 HD patients	Chi-squared = 9.63; $P < 0.01$
1990-1996	Ojo *et al*[48]	Retrospective Case-control match	63 PD and 161 HD patients	30.7% in PD and 18.9% in HD	OR = 1.87, 95%CI[c] 1.28-2.72, $P < 0.001$
1990-1994	Escuin *et al*[49]	Retrospective observational	138 PD and 892 HD patients	2.17% in PD and 3.47% in HD	P = NS
1992-1996	Vats *et al*[46]	Retrospective observational	1090 PD and 780 HD children	20% in PD and 10% in HD[a]	$P = 0.04$
1995-1998	Snyder *et al*[38]	Retrospective observational	156 PD and 349 HD patients	41% in PD and 30% in HD	OR 1.59, 95%CI 1.08-2.36, $P = 0.02$
1998-2011	Lin *et al*[41]	Retrospective cohort	603 PD and 1209 HD patients	Not available	P = NS

[a]:vascular thrombosis as cause of graft failure;
[b]:non-significant;
[c]:Confidence Interval. PD: Peritoneal dialysis; HD: Hemodialysis.

earliest once no longer needed. Also, incidence of post-operative infections after transplantation was found to be increased in PD patients as compared to HD patients (67.5% *vs* 25.9%, $P < 0.00001$) with an increased median length of hospital stay[53]. Lin *et al*[41] also found higher risks of peritonitis and urinary tract infection in PD patients after transplantation. But, authors reported higher risk of new onset tuberculosis and chronic hepatitis C in patients after 90 d of kidney transplantation treated with prior HD[41].

Risk factors for post-transplant diabetes mellitus (PTDM) was evaluated by Courivaud *et al*[54] among 137 patients and did not find any impact of dialysis modality on development of PTDM. On the contrary, in a cohort of 72 patients, Madziarska *et al*[55] found that PD was associated with an increased risk of PTDM ($P = 0.007$) in the multivariate analysis. In another study of 121 non-diabetic patients by Seifi *et al*[56], authors found when used as pre-transplant modality, PD was associated with an increased risk for PTDM in univariate analysis, but not in multivariate analysis. The factors associated with new onset of diabetes after transplantation are multiple and variable, but not limited to presence of pre diabetes, immunosuppressive medication regimen, improved appetite and weight gain post-transplant among other.

Long-term outcome: Comparing those on prior HD vs PD

Preemptive kidney transplant without dialysis was associated with excellent patient survival compared to HD prior to transplant (HR 0.81 with 95%CI of 0.73-0.89, $P < 0.001$)[9]. Data on long-term graft survival after PD and HD is mixed from most studies. Goldfarb *et al*[9] analyzed 92844 patients who underwent kidney or kidney-pancreas transplants in 1990-1999. They reported better graft outcomes in patients previously treated predominantly with PD as compared to HD patients (HR 0.97 with 95%CI of 0.94-1.0, $P < 0.05$), after controlling for multiple variables. Lin *et al*[41] also reported higher risk of death censored graft failure in a multivariate analysis in HD patients as compared to PD patients after 10 years of follow up (HR 1.31, 95%CI 1.03-1.84, $P = 0.031$). Although, Tang *et al*[7] did not found 5 years graft survival rate to be different with pre-transplant PD as compared to HD technique in their meta-analysis (HR 0.92, 95%CI: 0.84-1.01, $P = 0.08$). Ten year graft survival was reported to be similar between a cohort of 80 HD and 80 PD patients[11]. In another study of 11664 PD and 45561 HD patient, a similar death-censored graft survival was reported ($P = 0.39$)[57]. Discrepancies in these results were evaluated by Kramer *et al*[58] in a cohort of 29088 patients who received kidney transplantation between 1999 and 2008 and found that statistically significant association of PD with better allograft and patient survival in a multivariable cox regression analysis disappeared when used instrumental variable method that used the case-mix adjusted center percentage of PD as predictor variable.

Patient survival may also be better after kidney transplantation in those on preceding PD as compared to HD. The Goldfarb *et al*[9] study also revealed that predominate PD prior to transplant was independently associated with better recipient survival compared to patients on preceding HD (HR 0.96 with 95%CI of 0.92-0.99, $P < 0.05$). Authors also looked at various RRT combinations and outcomes. They found that patient survival was significantly better in those on prior PD only when compared to those whose prior treatment consisted of solely HD (HR 0.90 with CI of 0.86 to 0.94, $P < 0.001$)[9]. In another study by López-Oliva *et al*[59], authors looked at a cohort of 236 patients and reported that long term patient survival was higher for the PD group than the HD group ($P = 0.04$). Interestingly the combination of prior PD and HD had a worse survival than those on HD alone (HR 1.10, with 95%CI of 1.06 to 1.15, $P < 0.001$).

Similarly, a European center in 2006 reported that prior-PD patients fare better and have lower post-transplant mortality than those on preceding HD. The same authors had postulated that exposure to the HD dialyzer membrane could be immunogenic and lead to an increased risk of graft loss. They found that despite using the biocompatible membranes, patient survival on pre-transplant PD was still superior to the HD counterparts[60].

Mortality benefits in PD patients were again seen in the results reported by Molnar *et al*[39] from 2012. They reported that patients who had been on PD before receiving a kidney transplant have an adjusted 43% lower death risk compared to those on prior HD (HR 0.57 with CI of 0.38-0.87). Using propensity matching, those with a high likelihood of being on PD ($n = 4836$) when adjusted for many variables including transplant donor variables had a HR of 0.56 (0.31-0.99, $P = 0.04$) of all-cause death in comparison to previous HD[39]. Cardiovascular mortality in recipients who were on prior PD was lower compared to those on prior HD, controlling for many variables (HR 0.94)[39]. In an another study, superior survival of PD patients after transplantation was reported to be due to lower risk of cardiovascular death in a cohort of 60008 patients[57]. Still, there are many studies reported whereby authors didn't found survival benefit of PD over HD after transplantation[10,11,58]. Reasons for these mixed results is that even though most of the studies looked at standard variables like time and duration of dialysis, comorbidity index, it still does not take into account many other factors which may determine the long term survival benefits post transplantation. The choice of dialysis modality for any patient also leads to selection bias which may confound the end results like patient or graft survival post transplantation.

Mehrotra *et al*[61] looked at the USRDS database to compare the impact of dialysis modality on survival. They reported no significant difference in the risk of death for PD and HD patients during the 5-year follow-up period. Earlier studies from other countries reported to have shown a marked early survival advantage for PD compared to in-center HD[62-64]. The reasons for this are, may be due to better planning before starting PD, as opposed to HD. PD patients are better prepared and more motivated which might to increased access to transplantation care both pre and post. In addition, this could be explained by the better preservation of residual kidney function on PD, which has been repeatedly shown to enhance survival[65,66].

CONCLUSIONS

Incidence and prevalence of ESRDs in the USA is rising; adding to already a large number of patients on dialysis despite the knowledge that kidney transplantation is ideal and associated with far superior clinical outcomes for patients with ESRD than being on dialysis. Majority of patients in the USA choose HD over PD and initiate dialysis with catheters as opposed to preferred arteriovenous access. Current evidence favors PD over HD as modality of choice as it is associated with lower risk of hospitalizations, healthcare expenditures and mortality. Although, conflicting data exists on mortality benefit of PD versus HD; as mortality for PD and in-center HD patients was found to be similar while on the waitlist[28]. In regards to kidney transplantation outcomes, PD was associated with lower risk of DGF and cardiovascular mortality as compared to HD but with higher risk of infectious complications. Reports on allograft thrombosis, 5 years and 10 years graft survival and patient survival showed mixed results.

Overall, we believe that the choice of dialysis modality prior to kidney transplantation matters. While it is difficult to do a large numbered randomized controlled trial in an attempt to answer this extremely question, education regarding pre-transplant dialysis modality choices needs to be multi-faceted and should include all considerations including impact on kidney transplantation; its short term and long

term outcomes along with the impact on lifestyle[67-69]. This education should not biased on health literacy levels, and no matter what modality patients choose, the education and training must be patient centered, using universal approach. PD is an underutilized modality in the USA and can be a therapy of choice with a potential to be associated with improved outcome for transplantation. Further research and attention from nephrologist and transplantation community is needed in this regard.

REFERENCES

1 **Kasiske BL**, Snyder JJ, Matas AJ, Ellison MD, Gill JS, Kausz AT. Preemptive kidney transplantation: the advantage and the advantaged. *J Am Soc Nephrol* 2002; **13**: 1358-1364 [PMID: 11961024]

2 **Jay CL**, Dean PG, Helmick RA, Stegall MD. Reassessing Preemptive Kidney Transplantation in the United States: Are We Making Progress? *Transplantation* 2016; **100**: 1120-1127 [PMID: 26479285 DOI: 10.1097/TP.0000000000000944]

3 **Tonelli M**, Wiebe N, Knoll G, Bello A, Browne S, Jadhav D, Klarenbach S, Gill J. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. *Am J Transplant* 2011; **11**: 2093-2109 [PMID: 21883901 DOI: 10.1111/j.1600-6143.2011.03686.x]

4 **Hart A**, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Robinson A, Wainright JL, Haynes CR, Snyder JJ, Kasiske BL, Israni AK. OPTN/SRTR 2016 Annual Data Report: Kidney. *Am J Transplant* 2018; **18** Suppl 1: 18-113 [PMID: 29292608 DOI: 10.1111/ajt.14557]

5 **United Network for Organ Sharing**. Transplant trends. 2018; Available from: URL: https://unos.org/data/transplant-trends/

6 **Gillespie BW**, Morgenstern H, Hedgeman E, Tilea A, Scholz N, Shearon T, Burrows NR, Shahinian VB, Yee J, Plantinga L, Powe NR, McClellan W, Robinson B, Williams DE, Saran R. Nephrology care prior to end-stage renal disease and outcomes among new ESRD patients in the USA. *Clin Kidney J* 2015; **8**: 772-780 [PMID: 26613038 DOI: 10.1093/ckj/sfv103]

7 **Tang M**, Li T, Liu H. A Comparison of Transplant Outcomes in Peritoneal and Hemodialysis Patients: A Meta-Analysis. *Blood Purif* 2016; **42**: 170-176 [PMID: 27296631 DOI: 10.1159/000446272]

8 **Jones RE**, Liang Y, MacConmara M, Hwang C, Saxena R. Peritoneal Dialysis Is Feasible as a Bridge to Combined Liver-Kidney Transplant. *Perit Dial Int* 2018; **38**: 63-65 [PMID: 29311195 DOI: 10.3747/pdi.2017.00124]

9 **Goldfarb-Rumyantzev AS**, Hurdle JF, Scandling JD, Baird BC, Cheung AK. The role of pretransplantation renal replacement therapy modality in kidney allograft and recipient survival. *Am J Kidney Dis* 2005; **46**: 537-549 [PMID: 16129217 DOI: 10.1053/j.ajkd.2005.05.013]

10 **Resende L**, Guerra J, Santana A, Mil-Homens C, Abreu F, da Costa AG. Influence of dialysis duration and modality on kidney transplant outcomes. *Transplant Proc* 2009; **41**: 837-839 [PMID: 19376365 DOI: 10.1016/j.transproceed.2009.01.063]

11 **Dipalma T**, Fernández-Ruiz M, Praga M, Polanco N, González E, Gutiérrez-Solis E, Gutiérrez E, Andrés A. Pre-transplant dialysis modality does not influence short- or long-term outcome in kidney transplant recipients: analysis of paired kidneys from the same deceased donor. *Clin Transplant* 2016; **30**: 1097-1107 [PMID: 27334715 DOI: 10.1111/ctr.12793]

12 **United States Renal Data System**. Chapter 6: Transplantation. Available from: URL: https://www.usrds.org/2017/download/v2_c06_Transplant_17.pdf

13 **United States Renal Data System**. Chapter 1: Incidence, Prevalence, Patient Characteristics, and Treatment Modalities. Available from: URL: https://www.usrds.org/2017/download/v2_c01_IncPrev_17.pdf

14 **United States Renal Data System**. Chapter 11: International Comparisons. Available from: URL: https://www.usrds.org/2017/download/v2_c11_IntComp_17.pdf

15 **Griva K**, Kang AW, Yu ZL, Mooppil NK, Foo M, Chan CM, Newman SP. Quality of life and emotional distress between patients on peritoneal dialysis versus community-based hemodialysis. *Qual Life Res* 2014; **23**: 57-66 [PMID: 23689932 DOI: 10.1007/s11136-013-0431-8]

16 **Tam P**. Peritoneal dialysis and preservation of residual renal function. *Perit Dial Int* 2009; **29** Suppl 2: S108-S110 [PMID: 19270196]

17 **Agarwal R**, Flynn J, Pogue V, Rahman M, Reisin E, Weir MR. Assessment and management of hypertension in patients on dialysis. *J Am Soc Nephrol* 2014; **25**: 1630-1646 [PMID: 24700870 DOI: 10.1681/ASN.2013060601]

18 **Wanic-Kossowska M**, Czekalski S. Hypotension in renal failure patients undergoing dialysis therapy. *Pol Arch Med Wewn* 2007; 178-173

19 **Wolfgram DF**, Szabo A, Murray AM, Whittle J. Risk of dementia in peritoneal dialysis patients compared with hemodialysis patients. *Perit Dial Int* 2015; **35**: 189-198 [PMID: 25742686 DOI: 10.3747/pdi.2014.00213]

20 **Neumann D**, Mau W, Wienke A, Girndt M. Peritoneal dialysis is associated with better cognitive function than hemodialysis over a one-year course. *Kidney Int* 2018; **93**: 430-438 [PMID: 29042081 DOI: 10.1016/j.kint.2017.07.022]

21 **Richardson D**, Hodsman A, van Schalkwyk D, Tomson C, Warwick G. Management of anaemia in haemodialysis and peritoneal dialysis patients (chapter 8). *Nephrol Dial Transplant* 2007; **22** Suppl 7: vii78-vi104 [PMID: 17724054 DOI: 10.1093/ndt/gfm332]

22 **Burkart J**. The future of peritoneal dialysis in the United States: optimizing its use. *Clin J Am Soc Nephrol* 2009; **4** Suppl 1: S125-S131 [PMID: 19995996 DOI: 10.2215/CJN.04760709]

23 **Mehrotra R**, Kermah D, Fried L, Kalantar-Zadeh K, Khawar O, Norris K, Nissenson A. Chronic peritoneal dialysis in the United States: declining utilization despite improving outcomes. *J Am Soc Nephrol* 2007; **18**: 2781-2788 [PMID: 17804675 DOI: 10.1681/ASN.2006101130]

24 **Chaudhary K**, Sangha H, Khanna R. Peritoneal dialysis first: rationale. *Clin J Am Soc Nephrol* 2011; **6**: 447-456 [PMID: 21115629 DOI: 10.2215/CJN.07920910]

25 **Unites States Renal Data System**. Chapter 5: Mortality. Available from: URL: https://www.usrds.org/2017/download/v2_c05_Mortality_17.pdf

26 **Unites States Renal Data System**. Chapter 9: Healthcare Expenditures for Persons with ESRD. 2017; Available from: URL: https://www.usrds.org/2017/download/v2_c09_MedExp_17.pdf
27 **Unites States Renal Data System**. Chapter 4: Hospitalization. 2017; Available from: URL: https://www.usrds.org/2017/download/v2_c04_Hospitalization_17.pdf
28 **Inrig JK**, Sun JL, Yang Q, Briley LP, Szczech LA. Mortality by dialysis modality among patients who have end-stage renal disease and are awaiting renal transplantation. *Clin J Am Soc Nephrol* 2006; **1**: 774-779 [PMID: 17699286 DOI: 10.2215/CJN.00580705]
29 **Siedlecki A**, Irish W, Brennan DC. Delayed graft function in the kidney transplant. *Am J Transplant* 2011; **11**: 2279-2296 [PMID: 21929642 DOI: 10.1111/j.1600-6143.2011.03754.x]
30 **Caliskan Y**, Yazici H, Gorgulu N, Yelken B, Emre T, Turkmen A, Yildiz A, Aysuna N, Bozfakioglu S, Sever MS. Effect of pre-transplant dialysis modality on kidney transplantation outcome. *Perit Dial Int* 2009; **29** Suppl 2: S117-S122 [PMID: 19270199]
31 **Cacciarelli TV**, Sumrani NB, DiBenedetto A, Hong JH, Sommer BG. The influence of mode of dialysis pretransplantation on long-term renal allograft outcome. *Ren Fail* 1993; **15**: 545-550 [PMID: 8210569]
32 **Triolo G**, Segoloni GP, Salomone M, Piccoli GB, Messina M, Massara C, Bertinet DB, Vercellone A. Comparison between two dialytic populations undergoing renal transplantation. *Adv Perit Dial* 1990; **6**: 72-75 [PMID: 1982844]
33 **Pérez Fontán M**, Rodríguez-Carmona A, Bouza P, García Falcón T, Adeva M, Valdés F, Oliver J. Delayed graft function after renal transplantation in patients undergoing peritoneal dialysis and hemodialysis. *Adv Perit Dial* 1996; **12**: 101-104 [PMID: 8865882]
34 **Cardella CJ**, Harding ME, Abraham G, Robinson C, Oreopoulos D, Uldall PR, Jordan M, Cook G, Struthers N, Honey R. Renal transplantation in older patients on peritoneal dialysis. *Transplant Proc* 1989; **21**: 2022-2023 [PMID: 2652660]
35 **Vanholder R**, Heering P, Loo AV, Biesen WV, Lambert MC, Hesse U, Vennet MV, Grabensee B, Lameire N. Reduced incidence of acute renal graft failure in patients treated with peritoneal dialysis compared with hemodialysis. *Am J Kidney Dis* 1999; **33**: 934-940 [PMID: 10213652]
36 **Song SH**, Lee JG, Lee J, Huh KH, Kim MS, Kim SI, Kim YS. Outcomes of Kidney Recipients According to Mode of Pretransplantation Renal Replacement Therapy. *Transplant Proc* 2016; **48**: 2461-2463 [PMID: 27742322 DOI: 10.1016/j.transproceed.2016.02.096]
37 **Bleyer AJ**, Burkart JM, Russell GB, Adams PL. Dialysis modality and delayed graft function after cadaveric renal transplantation. *J Am Soc Nephrol* 1999; **10**: 154-159 [PMID: 9890321]
38 **Snyder JJ**, Kasiske BL, Gilbertson DT, Collins AJ. A comparison of transplant outcomes in peritoneal and hemodialysis patients. *Kidney Int* 2002; **62**: 1423-1430 [PMID: 12234315 DOI: 10.1111/j.1523-1755.2002.kid563.x]
39 **Molnar MZ**, Mehrotra R, Duong U, Bunnapradist S, Lukowsky LR, Krishnan M, Kovesdy CP, Kalantar-Zadeh K. Dialysis modality and outcomes in kidney transplant recipients. *Clin J Am Soc Nephrol* 2012; **7**: 332-341 [PMID: 22156753 DOI: 10.2215/CJN.07110711]
40 **Prasad N**, Vardhan H, Baburaj VP, Bhadauria D, Gupta A, Sharma RK, Kaul A. Do the outcomes of living donor renal allograft recipients differ with peritoneal dialysis and hemodialysis as a bridge renal replacement therapy? *Saudi J Kidney Dis Transpl* 2014; **25**: 1202-1209 [PMID: 25394436]
41 **Lin HT**, Liu FC, Lin JR, Pang ST, Yu HP. Impact of the pretransplant dialysis modality on kidney transplantation outcomes: a nationwide cohort study. *BMJ Open* 2018; **8**: e020558 [PMID: 29866727 DOI: 10.1136/bmjopen-2017-020558]
42 **Thomson BK**, Moser MA, Marek C, Bloch M, Weernink C, Shoker A, Luke PP. Peritoneal dialysis versus hemodialysis in patients with delayed graft function. *Clin Transplant* 2013; **27**: E709-E714 [PMID: 24138529 DOI: 10.1111/ctr.12266]
43 **Dawidson IJ**, Sandor ZF, Coorpender L, Palmer B, Peters P, Lu C, Sagalowsky A, Risser R, Willms C. Intraoperative albumin administration affects the outcome of cadaver renal transplantation. *Transplantation* 1992; **53**: 774-782 [PMID: 1566343]
44 **van der Vliet JA**, Barendregt WB, Hoitsma AJ, Buskens FG. Increased incidence of renal allograft thrombosis after continuous ambulatory peritoneal dialysis. *Clin Transplant* 1996; **10**: 51-54 [PMID: 8652898]
45 **Murphy BG**, Hill CM, Middleton D, Doherty CC, Brown JH, Nelson WE, Kernohan RM, Keane PK, Douglas JF, McNamee PT. Increased renal allograft thrombosis in CAPD patients. *Nephrol Dial Transplant* 1994; **9**: 1166-1169 [PMID: 7800219]
46 **Vats AN**, Donaldson L, Fine RN, Chavers BM. Pretransplant dialysis status and outcome of renal transplantation in North American children: a NAPRTCS Study. North American Pediatric Renal Transplant Cooperative Study. *Transplantation* 2000; **69**: 1414-1419 [PMID: 10798764]
47 **Pérez Fontán M**, Rodríguez-Carmona A, García Falcón T, Tresancos C, Bouza P, Valdés F. Peritoneal dialysis is not a risk factor for primary vascular graft thrombosis after renal transplantation. *Perit Dial Int* 1998; **18**: 311-316 [PMID: 9663896]
48 **Ojo AO**, Hanson JA, Wolfe RA, Agodoa LY, Leavey SF, Leichtman A, Young EW, Port FK. Dialysis modality and the risk of allograft thrombosis in adult renal transplant recipients. *Kidney Int* 1999; **55**: 1952-1960 [PMID: 10231459 DOI: 10.1046/j.1523-1755.1999.00435.x]
49 **Escuin F**, Del Peso G, Pérez Fontán M, Rodriguez-Carmona A, Martínez A, Lanuza M, Hortal L, Fernández AM, Albero MD, Pérez Contreras J, Selgas R. A comparative survey on the incidence of kidney graft primary vascular thrombosis among CAPD and haemodialysis patients. *Nephrol Dial Transplant* 1996; **11**: 1896-1897 [PMID: 8918657]
50 **Martins LS**, Malheiro J, Pedroso S, Almeida M, Dias L, Henriques AC, Silva D, Davide J, Cabrita A, Noronha IL, Rodrigues A. Pancreas-Kidney transplantation: Impact of dialysis modality on the outcome. *Transpl Int* 2015; **28**: 972-979 [PMID: 25790131 DOI: 10.1111/tri.12565]
51 **Rizzi AM**, Riutta SD, Peterson JM, Gagin G, Fritze DM, Barrett M, Sung RS, Woodside KJ, Lu Y. Risk of peritoneal dialysis catheter-associated peritonitis following kidney transplant. *Clin Transplant* 2018; **32**: e13189 [PMID: 29292535 DOI: 10.1111/ctr.13189]
52 **Issa N**, Kukla A. Peritoneal dialysis immediately after kidney transplantation. *Adv Perit Dial* 2014; **30**: 83-86 [PMID: 25338426]
53 **Passalacqua JA**, Wiland AM, Fink JC, Bartlett ST, Evans DA, Keay S. Increased incidence of postoperative infections associated with peritoneal dialysis in renal transplant recipients. *Transplantation* 1999; **68**: 535-540 [PMID: 10480413]

54 **Courivaud C**, Ladrière M, Toupance O, Caillard S, Hurault de Ligny B, Ryckelynck JP, Moulin B, Rieu P, Frimat L, Chalopin JM, Chauvé S, Kazory A, Ducloux D. Impact of pre-transplant dialysis modality on post-transplant diabetes mellitus after kidney transplantation. *Clin Transplant* 2011; **25**: 794-799 [PMID: 21158919 DOI: 10.1111/j.1399-0012.2010.01367.x]

55 **Madziarska K**, Weyde W, Krajewska M, Patrzalek D, Janczak D, Kusztal M, Augustyniak-Bartosik H, Szyber P, Kozyra C, Klinger M. The increased risk of post-transplant diabetes mellitus in peritoneal dialysis-treated kidney allograft recipients. *Nephrol Dial Transplant* 2011; **26**: 1396-1401 [PMID: 20852070 DOI: 10.1093/ndt/gfq568]

56 **Seifi S**, Rahbar M, Lessan-Pezeshki M, Khatami MR, Abbasi MR, Mahdavi-Mazdeh M, Ahmadi F, Maziar S. Posttransplant diabetes mellitus: incidence and risk factors. *Transplant Proc* 2009; **41**: 2811-2813 [PMID: 19765442 DOI: 10.1016/j.transproceed.2009.07.043]

57 **Schwenger V**, Döhler B, Morath C, Zeier M, Opelz G. The role of pretransplant dialysis modality on renal allograft outcome. *Nephrol Dial Transplant* 2011; **26**: 3761-3766 [PMID: 21427080 DOI: 10.1093/ndt/gfr132]

58 **Kramer A**, Jager KJ, Fogarty DG, Ravani P, Finne P, Pérez-Panadés J, Prütz KG, Arias M, Heaf JG, Wanner C, Stel VS. Association between pre-transplant dialysis modality and patient and graft survival after kidney transplantation. *Nephrol Dial Transplant* 2012; **27**: 4473-4480 [PMID: 23235955 DOI: 10.1093/ndt/gfs450]

59 **López-Oliva MO**, Rivas B, Pérez-Fernández E, Ossorio M, Ros S, Chica C, Aguilar A, Bajo MA, Escuin F, Hidalgo L, Selgas R, Jiménez C. Pretransplant peritoneal dialysis relative to hemodialysis improves long-term survival of kidney transplant patients: a single-center observational study. *Int Urol Nephrol* 2014; **46**: 825-832 [PMID: 24014131 DOI: 10.1007/s11255-013-0521-0]

60 **Van Biesen W**, Veys N, Vanholder R, Lameire N. The impact of the pre-transplant renal replacement modality on outcome after cadaveric kidney transplantation: the ghent experience. *Contrib Nephrol* 2006; **150**: 254-258 [PMID: 16721018 DOI: 10.1159/000093613]

61 **Mehrotra R**, Chiu YW, Kalantar-Zadeh K, Bargman J, Vonesh E. Similar outcomes with hemodialysis and peritoneal dialysis in patients with end-stage renal disease. *Arch Intern Med* 2011; **171**: 110-118 [PMID: 20876398 DOI: 10.1001/archinternmed.2010.352]

62 **Fenton SS**, Schaubel DE, Desmeules M, Morrison HI, Mao Y, Copleston P, Jeffery JR, Kjellstrand CM. Hemodialysis versus peritoneal dialysis: a comparison of adjusted mortality rates. *Am J Kidney Dis* 1997; **30**: 334-342 [PMID: 9292560]

63 **Schaubel DE**, Morrison HI, Fenton SS. Comparing mortality rates on CAPD/CCPD and hemodialysis. The Canadian experience: fact or fiction? *Perit Dial Int* 1998; **18**: 478-484 [PMID: 9848625]

64 **Heaf JG**, Løkkegaard H, Madsen M. Initial survival advantage of peritoneal dialysis relative to haemodialysis. *Nephrol Dial Transplant* 2002; **17**: 112-117 [PMID: 11773473]

65 **Paniagua R**, Amato D, Vonesh E, Correa-Rotter R, Ramos A, Moran J, Mujais S; Mexican Nephrology Collaborative Study Group. Effects of increased peritoneal clearances on mortality rates in peritoneal dialysis: ADEMEX, a prospective, randomized, controlled trial. *J Am Soc Nephrol* 2002; **13**: 1307-1320 [PMID: 11961019]

66 **Lo WK**, Lui SL, Chan TM, Li FK, Lam MF, Tse KC, Tang SC, Choy CB, Lai KN. Minimal and optimal peritoneal Kt/V targets: results of an anuric peritoneal dialysis patient's survival analysis. *Kidney Int* 2005; **67**: 2032-2038 [PMID: 15840054 DOI: 10.1111/j.1523-1755.2005.00305.x]

67 **Jain D**, Green JA. Health literacy in kidney disease: Review of the literature and implications for clinical practice. *World J Nephrol* 2016; **5**: 147-151 [PMID: 26981438 DOI: 10.5527/wjn.v5.i2.147]

68 **Jain D**, Sheth H, Green JA, Bender FH, Weisbord SD. Health literacy in patients on maintenance peritoneal dialysis: prevalence and outcomes. *Perit Dial Int* 2015; **35**: 96-98 [PMID: 25700462 DOI: 10.3747/pdi.2013.00211]

69 **Green JA**, Mor MK, Shields AM, Sevick MA, Palevsky PM, Fine MJ, Arnold RM, Weisbord SD. Prevalence and demographic and clinical associations of health literacy in patients on maintenance hemodialysis. *Clin J Am Soc Nephrol* 2011; **6**: 1354-1360 [PMID: 21551025 DOI: 10.2215/CJN.09761110]

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REVIEW

Insulin receptors in the kidneys in health and disease

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Abstract

Insulin is an important hormone that affects various metabolic processes, including kidney function. Impairment in insulin's action leads to insulin resistance in the target tissue. Besides defects in post-receptor insulin signaling, impairment at the receptor level could significantly affect insulin sensitivity of the target tissue. The kidney is a known target of insulin; however, whether the kidney develops "insulin resistance" is debatable. Regulation of the insulin receptor (IR) expression and its function is very well studied in major metabolic tissues like liver, skeletal muscles, and adipose tissue. The physiological relevance of IRs in the kidney has recently begun to be clarified. The credit goes to studies that showed a wide distribution of IR throughout the nephron segments and their reduced expression in the insulin resistance state. Moreover, altered renal and systemic metabolism observed in mice with targeted deletion of the IR from various epithelial cells of the kidney has strengthened this proposition. In this review, we recapitulate the crucial findings from literature that have expanded our knowledge regarding the significance of the renal IR in normal- and insulin-resistance states.

Key words: Insulin receptor; Insulin resistance; Kidney disease; Renal sodium reabsorption; Gluconeogenesis; Proteinuria; Systemic blood pressure

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Core tip: Dysregulation of the renal insulin receptor (IR) not only affects local renal metabolism, but also disturbs the systemic glucose homeostasis and blood pressure, leading to metabolic abnormalities. The objective of this review is to highlight the pathophysiological stature of renal IRs in the kidney function, as well as, overall metabolism.

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INTRODUCTION

The incidence of insulin resistance is increasing worldwide in parallel with the rate of obesity. Insulin resistance, *per se*, is often subclinical, and defined by inefficient insulin receptor (IR) signaling in major metabolic tissues, including liver, muscle, and adipose, resulting in impaired cellular glucose uptake. The function and role of reduced IR signaling has been extensively studied in these metabolic tissues. In addition to downstream signaling, alterations in the expression, binding, and phosphorylation of the IR itself may affect target cell sensitivity to insulin[1-3]. The kidney expresses IRs[4,5]; however, it is still debatable whether kidney develops classic "resistance" in the same manner as the liver, muscle, and adipose tissues. Reduced expression of IR and its phosphorylated form, the first step in IR signaling, have been demonstrated in renal epithelial cells of diabetic and insulin-resistant rat models[6,7]. Nevertheless, presence of these receptors throughout the nephron segments suggests an important role in renal metabolism. Insulin could undoubtedly regulate several vital kidney functions through its receptors. However, it has been a mere decade since the role of renal epithelial IR in kidney physiology and pathology began to be illuminated. In this review, we bring together the findings from published literatures that have contributed to our understanding in the area. For easy reading we will use the phrase "renal IR" in place of "IR in renal epithelial cells".

INSULIN RECEPTORS AND INSULIN SIGNALING

Insulin, secreted by pancreatic β-cells, is a peptide hormone with pleiotropic actions and plays an indispensable role in human metabolism. Biological effects of insulin are exerted by binding to IRs. IRs belong to the receptor tyrosine kinases and the IR subfamily, which consists of the IR, the insulin-like growth factor (IGF-I/-II) receptors, and the IR-related receptor[8]. The IR is a transmembrane protein that is composed of two α- and two β-subunits forming a heterotetramer α2β2 (Figure 1), with disulfide bonds between the α-subunits and between the α- and β-subunits[9]. The human IR cDNA was isolated and cloned in the 1980s[10,11]. These studies demonstrated that the α- and β-subunits are derived from proteolytic cleavage of a common precursor. Later, Seino *et al*[12] reported that the IR gene (*INSR*) is encoded by 22 exons and 21 introns. Alternative splicing of exon 11 results in two isoforms, A and B with differential insulin affinity, with isoform B having higher affinity.

Insulin binding to extracellular α-subunits confers conformational changes within the molecule, leading to autophosphorylation of specific tyrosine residues in intracellular domains[13]. Upon activation, various adaptors and signaling proteins (IRS, SHC, GRB, *etc*.) are recruited to the receptor to initiate the intracellular signaling cascade and regulate different biological functions[8,13] (Figure 2).

LOCALIZATION OF THE IR IN THE KIDNEY

The attempts to examine the expression pattern of IRs in the kidney had started about four decades ago; however, their physiological role in the kidney has recently come to light[4,14-16]. Renal localization of IRs was first studied by ^{125}I-labeled insulin binding in microdissected rat glomeruli and tubules in 1988. The results showed high affinity binding sites in the proximal and distal convoluted tubules (PCT and DCT), and to a lesser extent in the cortical and outer medullary collecting duct (CD) and thick ascending limb (TAL)[15]. Later, Sechi *et al*[4] exploited an *in situ* autoradiographic technique to observe insulin binding in glomeruli, renal cortex, outer and inner renal medulla. Findings from their studies revealed the highest IR density in the inner portion of the medulla, which also exhibits the maximal insulin activity in the renal tubule. The localization of IR in the proximal tubule (PT), TAL, DCT, and CD have also been shown by immunofluorescence using polyclonal antibodies against the α- and β-subunits of IR[16]. This approach illustrated an exclusive localization pattern of IR as these antibodies did not overlap with IGF-1 receptor and the IR-related receptor in kidney[17,18]. The significance of IR expression in different segments of the nephron

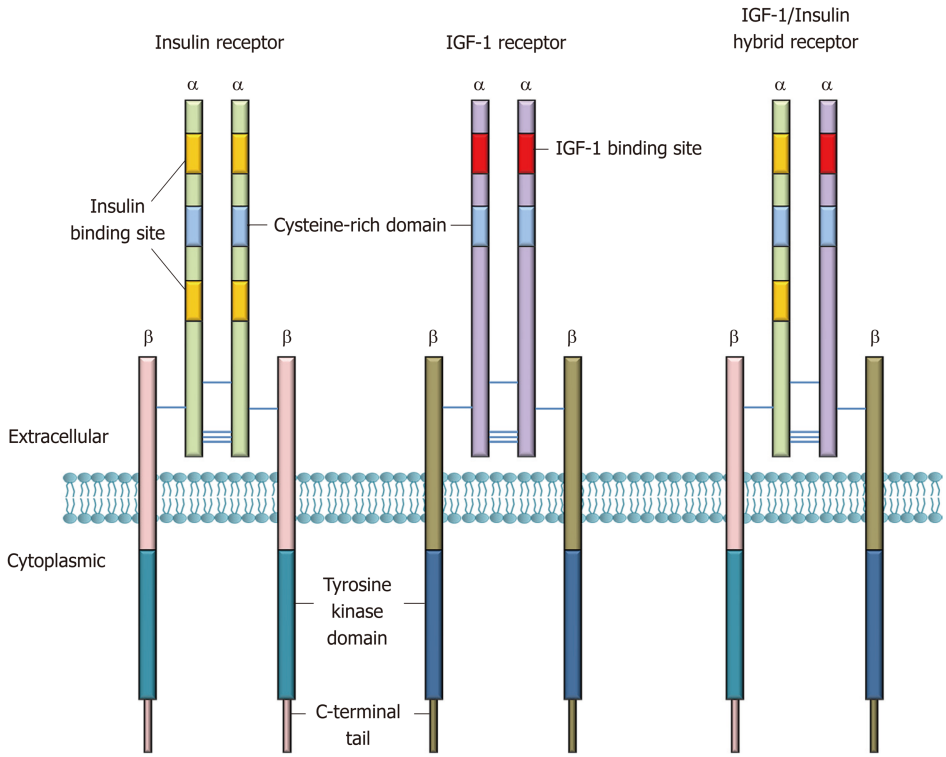


Figure 1 Architecture of insulin and insulin-like growth factor-1 receptors. Insulin and IGF-1 receptors consist of two extracellular α-chains and two transmembrane β-chains. The α-subunits have binding sites for insulin and IGF-1, whereas the cytoplasmic kinase domain comprises major sites for tyrosine autophosphorylation that are crucial for receptor activation. The α- and β-subunits are connected together *via* disulfide linkages (Figure is adapted from reference[9]). IGF: Insulin-like growth factor.

was later confirmed by targeted deletion of IR from these segments[19,20].

RENAL IR IN CARDIOVASCULAR PHYSIOLOGY

Renal regulation of sodium reabsorption is crucial for maintaining homeostasis, fluid balance, and systemic blood pressure. Excessive intake of dietary sodium and/or impaired salt excretion augments the incidences of hypertension[21]. There is substantial evidence suggesting restriction of dietary sodium could decrease cardiovascular risk and reduce blood pressure in normotensive and hypertensive individuals[22,23]. In kidney, sodium reabsorption occurs throughout the tubular segments of nephron including the PT, TAL, DT, and CD[24-26].

Insulin is reported to have antinatriuretic properties and has been shown to increase sodium absorption by regulating the activities of different renal sodium channels including the Na^+/H^+ exchanger type 3, the sodium-bicarbonate cotransporter, and the Na-K-ATPase in PT, the sodium-potassium-chloride cotransporter type 2 and the Na-K-ATPase in TAL, and the sodium-chloride cotransporter and the epithelial sodium channel in DCT and CD[27].

To elucidate the sodium-insulin interaction in the kidney, Sechi *et al*[28], examined renal IR binding and mRNA levels of IRs in rats fed on different salt concentration. They reported an inverse relationship between dietary salt (NaCl) intake and renal IR density. In concordance with this study, Catena *et al*[29] also reported a decrement in IR number and mRNA levels in control rats fed on a high-salt diet. However, IR densities were reported comparable in fructose-fed rats maintained on high- or low-salt diet. Further, a reduced antinatriuretic effect of insulin in high-salt-fed control rats was not observed in fructose-fed rats, implying that the fructose-fed animals lacked the feedback mechanism that limits insulin-induced sodium retention during high salt intake, which may contribute to fructose-induced hypertension[29].

Nevertheless, the expression pattern of IR in the PT, TAL, and CD implies the involvement of IRs in insulin-mediated renal sodium retention[15,30-32]. Therefore, investigating the correlation between IRs and renal sodium reabsorption has been a major focus of researchers to understand the connection between insulin resistance and hypertension. Hypertension is one of the most common cardiovascular complications worldwide. High blood pressure and associated complications lay a

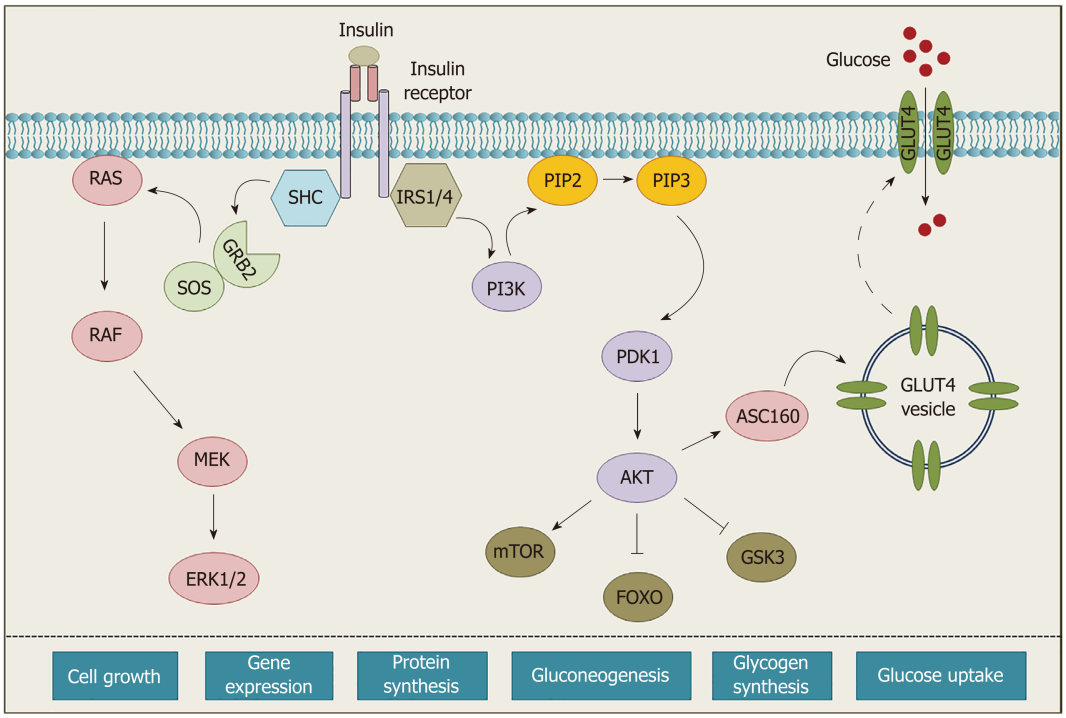


Figure 2 Schematics of the insulin receptor signaling. Binding of insulin to its receptor causes autophosphorylation of specific tyrosine residues. Upon activation IR recruits different adaptor proteins and initiates a cascade of phosphorylation events. These signaling events ultimately lead to activation or repression of an array of proteins, which regulate various biological functions (Figure is adapted from reference[8]).

grave burden on patients. Among various determinants of hypertension, insulin resistance is considered to be a major determinant. Although the precise role of insulin resistance is debatable in the development of hypertension, activation of the sympathetic nervous system, insulin-regulated sodium retention, and activation of the renin-angiotensin system (RAS) are considered as plausible mechanisms[33-35]. The interrelation between insulin resistance and hypertension could either be a non-causal association (two independent processes) or a cause-and-effect relationship, where insulin resistance acts as a cause of hypertension[36].

Interestingly, we observed that specific knockout of renal epithelial cell IR caused elevated systolic blood pressure in mice. Our study has shown that targeted deletion of IRs from renal epithelial cells significantly increased systolic blood pressure and impaired sodium excretion in response to saline load as compared to wild-type (WT). Moreover, intraperitoneal administration of insulin caused a significant drop in blood pressure in WT, but not in IR-knockout (KO) mice. Urinary excretion of nitrates and nitrites (UNOx) was also reduced in KO mice relative to WT mice (Figure 3). These observations suggested that renal IRs could play a key part in the maintenance of normal blood pressure and volume-expansion-associated natriuresis[19]. A study from Bhalla's lab also has shown that renal tubule-specific knockout of IR decreased NCC-mediated sodium reabsorption in high fat-fed mice[37]. However, further investigation is required to comprehensively understand the IR-dependent regulation of sodium retention and associated hypertension during insulin resistance.

Insulin has a complex role in the maintenance of blood pressure. On one hand, insulin-induced sodium retention and increased sympathetic activity is a root cause of hypertension, at the same time, insulin itself has a vasodilatory effect, which is associated with nitric oxide (NO) production[38]. In kidney, hyperinsulinemia affects renal blood flow in a NO-dependent manner[39] and insulin resistance impedes this effect[40,41]. Moreover, experimental diabetes in rats has resulted in reduced renal NO production[42]. Local renal production of NO production has also been implicated in impaired renal blood flow during congestive heart failure[43]. Apart from its vasodilatory effects, NO is also reported to reduce sodium reabsorption in renal tubules[44,45]. Specific deletion of IR from renal epithelial cells has been reported to impair sodium and NO excretion and elevate systemic blood pressure in mice, suggesting a possible role of impaired renal NO production in blood pressure regulation[19]. Moreover, reduced renal expression of the IR in TAL has been linked to salt sensitivity of blood pressure *via* blunted production of NO[46]. These IR-knockout mice also exhibited low protein levels of nitric oxide synthase isoform, NOS1, which is expressed in macula densa cells, TAL, and in CD[46]. Together, these studies support a

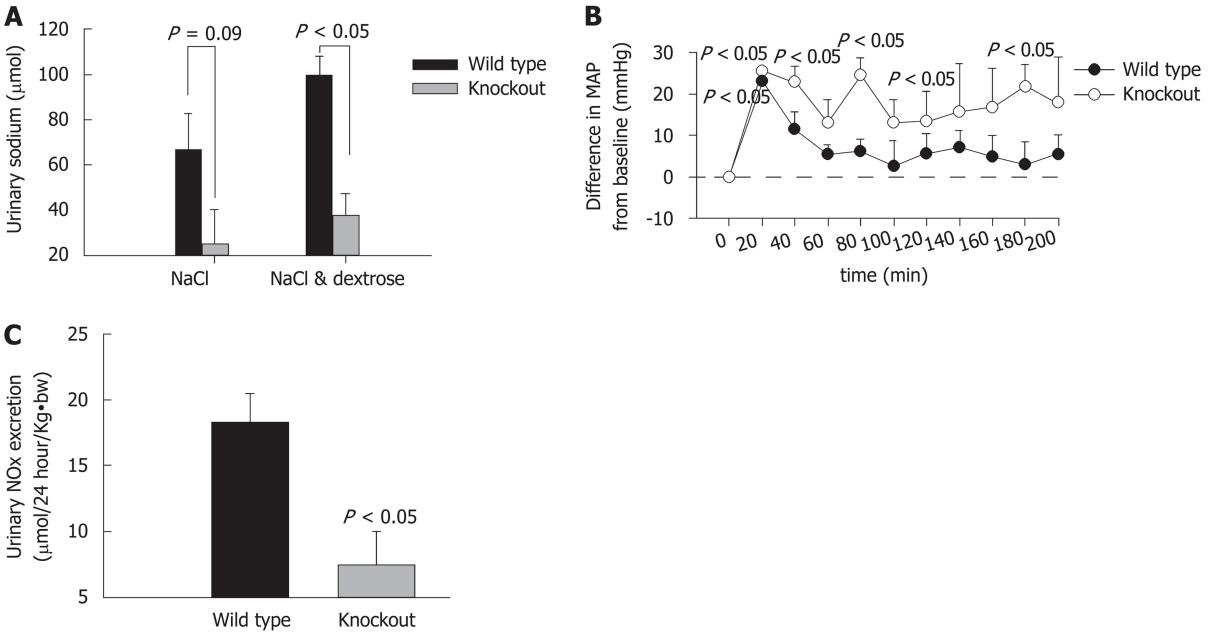


Figure 3 Altered natriuresis and impaired nitric oxide metabolism in insulin receptor-knockout mice. A: Urinary sodium excretion after oral administration of saline with and without dextrose in 4 h; B: Mean arterial blood pressure (ΔMAP) after NaCl and dextrose administration in mice; C: Urinary nitrate and nitrite excretion in wild-type and insulin receptor-knockout mice after 24 h. (Figure is a modification of figures published in reference[19] and taken with permission).

crucial role of renal NO in blood pressure regulation by its autocrine and paracrine actions, particularly in the medullary TAL and CD[47-49].

A fairly recent report from our group showed for the first time that insulin induces eNOS activation and NO generation in the renal inner medullary collecting duct (IMCD) cells[50]. We observed a time- and dose-dependent increase in NO and its metabolite NOx in insulin-stimulated mouse IMCD cells. Moreover, chronic insulin infusion in C57BL/6J mice led to increased expression of endothelial NOS (eNOS) and elevated NO levels in the inner medulla. However, treatment of cells with wortmannin (PI3K inhibitor) and IR-knockdown abolished these effects of insulin *in vitro*, implying the involvement of the IR/PI3K pathway in insulin-stimulated NO generation. Further, targeted deletion of IR from renal tubule epithelial cells resulted in significant downregulation of eNOS in inner medulla with concomitant rise in blood pressure in KO mice. These observations implied that IR signaling in the IMCD could contribute to hypertension in the insulin-resistant state.

The renal RAS is another imperative pathway that regulates systemic blood pressure and maintains water and electrolyte homeostasis. Typically, angiotensin II (Ang II) produced in the RAS pathway interacts with angiotensin type 1 receptors (AT_1R) to exert its biological effects in various tissues including the kidney, the heart, adipocytes, adrenal tissues[51], *etc*. The classical RAS pathway induces sodium reabsorption, vasoconstriction, and blood pressure. Moreover, Ang II has been established to inhibit insulin-mediated PI3K activation and is involved in the pathogenesis of insulin resistance[52]. An interrelation between insulin resistance and the RAS pathway has been reported in hypertensive patients. Although precise mechanism of insulin resistance and RAS is not well established, these two pathways interact at multiple levels to regulate cellular metabolism[53]. Previously, it has been demonstrated that Ang II induces phosphorylation of IRS1 (a key substrate of IR) at Ser616 and Ser312, which is responsible for its inactivation and inhibition of insulin/PI3K signaling cascade[54]. A direct link between renal IRs, RAS, and cardiovascular complications has not been reported and warrants further investigation.

RENAL IR IN SYSTEMIC GLUCOSE HOMEOSTASIS

IR signaling has been reported to maintain blood glucose levels in the liver and other metabolic tissues[55-57], however, there is limited knowledge regarding this action in the kidney. Recent studies have highlighted that renal IR signaling is an equally important contributor and regulator of systemic glucose levels[19,58-60]. The first evidence on kidney's involvement in glucose metabolism came in 1938, where

Bergman *et al* observed that removal of kidneys in hepatectomized rabbits doubled the sugar utilization rate as compared to the hepatectomized animals only[61]. Following which a number of studies substantiated the glucose production activity of the kidney[62,63] and provided evidence that kidney, in addition to acidosis or prolonged starvation, also releases considerable amounts of glucose in normal post-absorptive conditions[64]. Moreover, accumulating evidence predicts that the kidneys impart a critical role in regulating overall glucose homeostasis by various mechanisms, such as reabsorption of glucose from the glomerular ultrafiltrate specifically in the renal epithelial cells, glucose uptake and utilization for meeting the body's energy demands, and gluconeogenesis, *i.e.*, endogenous glucose production from non-carbohydrate sources[55,56].

Similar to liver tissue, renal gluconeogenesis and metabolism were found to be dysregulated in diabetes and the insulin-resistant state[6,7]. There are studies which suggest that renal epithelial cells double their glucose uptake in response to insulin stimulation *via* translocation of GLUT (GLUT1 and GLUT4) to the plasma membrane, which accentuates the effect of insulin on renal gluconeogenesis and on systemic blood glucose levels[65]. Moreover, hyperinsulinemia is reported to inhibit glucose production and stimulate glucose uptake by the renal epithelial cells[60,66]. Both experimental and clinical studies have documented the insulin-mediated regulation of uptake and release of glucose. A hyperinsulinemic clamp study in humans showed a 61% decrease in renal glucose output and approximately a 72% decrease in renal glutamine gluconeogenesis [much higher than liver (25%)] in subjects treated with insulin[67]. Insulin has also been reported to affect the transport of gluconeogenic substrates in the kidney[68]. These studies highlighted the significance and magnitude of renal glucose production, and also revealed higher sensitivity of renal glucose release towards insulin as compared to liver. Moreover, enhanced renal gluconeogenesis in the post-absorptive conditions has been suggested to contribute towards hyperglycemia in Type 2 diabetes in the insulin-resistant state. This is supported by the increased intrinsic gluconeogenesis with simultaneous decrease in IR levels reported in the kidney cortex of Zucker diabetic fatty rats[69]. In addition, a marked decrease in IR expression has been observed in the renal cortex of high-fat diet-fed rats as well as in Type 2 diabetic patients[6,7,69,70].

The role of insulin/IR signaling in regulation of gluconeogenesis transcriptional modulation of gluconeogenic genes, *i.e.*, PCK1 and G6PC is well known in liver[71-73]. However, studies on the role of the IR in renal gluconeogenesis regulation are limited. In 2012, a study from the DeFronzo lab elucidated that insulin negatively regulates gluconeogenesis *via* downregulating the expression of key gluconeogenic genes in the kidney[74]. Around the same time, our group demonstrated that targeted deletion of the IR from the PT resulted in hyperglycemia despite normal whole body insulin sensitivity[20]. More so, an increased activity and elevated mRNA expression of glucose-6-phosphatase (G6Pase, a rate-limiting enzyme in gluconeogenesis) was observed in the PT-specific IRKO mice, signifying the involvement of the IR in regulating the expression of key gluconeogenic genes. Further, reduced IR expression and early IR signaling along with a significant increase in phosphoenolpyruvate carboxykinase (PEPCK) levels were found in kidney cortex of high-fat diet-fed mice[75], providing a clue to the possible mechanism of insulin involving transcriptional regulation of PEPCK, also a rate-limiting gene in gluconeogenesis. In liver, insulin has been shown to suppress the expression of gluconeogenic genes, G6Pase and PEPCK[76]. *In vitro* studies performed in primary PT cells from human kidney (hPT) showed an inhibitory role of insulin on cAMP/DEXA-induced gluconeogenesis, and silencing of IR attenuated this inhibitory effect of insulin on PT-gluconeogenesis in hPT[77]. All these findings clearly state that reduced IR expression/signaling might have a causal function in gluconeogenic gene upregulation and gluconeogenesis.

In vitro studies from our group has demonstrated that loss of IR in human proximal tubule cells attenuated the inhibitory effect of insulin on PEPCK expression in hPT cells[77]. These studies, suggest that impaired insulin sensitivity of PT may affect whole body glucose homeostasis by elevating gluconeogenesis *via* transcriptional induction of gluconeogenic enzymes in the kidney. However, the mechanism by which IR signaling targets gluconeogenic genes in PT needs to be further elucidated. A recent study from Yáñez lab demonstrated downregulation of IR levels, which was accompanied by increased expression and activity of PEPCK in the kidney of both Type 2 diabetic patients (Figure 4) and in a Type 1 diabetic rat model. Moreover, they also observed an apical redistribution of gluconeogenic genes in both the models, implying that insulin signaling may regulate gluconeogenesis through luminal substrate uptake[6]. Recently, Horita *et al*[78], put forward a concept of "selective insulin resistance" in kidney. The state of selective insulin resistance has been recognized in the case of liver, where inhibition of gluconeogenesis by the insulin receptor substrate (IRS) 2 is hindered, whereas IRS1-regulated lipogenesis is not altered. On the

contrary, in kidney, IRS1-dependent inhibition of gluconeogenesis is impaired in the proximal tubule leading to hyperglycemia, while IRS2-dependent signaling is preserved[78-81]. Sasaki *et al*[82] have also reported the role of insulin signaling in maintaining systemic glucose homeostasis in IRS1/IRS2 double KO mice. This study emphasized dual regulation of gluconeogenesis by insulin signaling and glucose reabsorption. This is in consonance with previous studies suggesting impaired glucose levels in diabetic human PT because of enhanced glucose reabsorption and insulin-dependent inhibition of gluconeogenesis[74,83,84], ultimately leading to more glucose release by the kidney as compared to the liver[58]. In the light of these findings, regulation of renal gluconeogenesis is still a matter of debate because both suppression and elevation of gluconeogenic gene expression has been reported in experimental rodent models of diabetes[6,85]. These observations open a whole new avenue for investigating the role of IRs in renal glucose homeostasis.

Together, it can be implied that impaired renal insulin signaling (especially IR signaling) may increase gluconeogenesis, and in the setting of insulin resistance, these impairments can further contribute to other deleterious effects. Therefore, more conclusive studies are warranted to understand the pathophysiological association of renal insulin signaling and glucose metabolism.

RENAL IR IN PROTEINURIA

The presence of proteins especially albumin in urine, aka proteinuria is an important hallmark of renal disease, including diabetic nephropathy. Although glomerular dysfunction is an established cause of proteinuria[86,87], impaired tubular function also contributes to albuminuria in diabetic nephropathy[88,89]. Normally, albumin is reabsorbed by the PT cells through receptor-mediated and fluid phase endocytosis[90]. In the proximal tubules, receptor-mediated reabsorption of albumin is executed by endocytic receptors, megalin and cubilin that are highly expressed in the apical membrane of the PT cells[91]. Existing evidences suggest that besides other factors, insulin could have a potential role in albumin uptake by the PT cells in diabetic and non-diabetic conditions. Retrieval of albumin from ultrafiltrate by the PT cells is crucial for kidney homeostasis. A cohort study on non-diabetic individuals (Relationship between Insulin Sensitivity and Cardiovascular Disease; RISC) proposed a causal relationship between insulin resistance and albuminuria[92]. The RISC study demonstrated that reduced insulin sensitivity, measured by a hyperinsulinemic-euglycemic clamp, is linked to increased risk of albuminuria in a healthy cohort.

Intriguingly, various studies have reported that insulin could also have a potential role in albumin uptake by the PT cells in diabetes. In the STZ-induced diabetic mice model, downregulation of pSer473-Akt expression in the proximal tubule epithelial cells was accompanied by decreased expression of megalin and cubilin establishing the link between insulin signaling and albumin uptake[93]. Recently, Zeng *et al*[94], showed that the ORAI (calcium release-activated calcium channels) are also accountable for the internalization of albumin in proximal tubular epithelial cells *via* clathrin-mediated endocytosis and expression of these channels is insulin-dependent. In concordance with previous studies, Mottl *et al*[95] showed that the urinary ACR was positively associated with insulin-resistant young Type 2 diabetic subjects. Moreover, insulin treatment under high-glucose conditions increased megalin expression and albumin internalization in OK cells[96]. Insulin treatment has attenuated urine albumin excretion in Akita mice also[97]. These reports establish a causal role of PT-specific insulin resistance in the pathogenesis of albuminuria; however, exact mechanism of insulin-dependent albumin uptake needs to be elucidated.

Recent data from our laboratory showed that targeted deletion of IRs from the proximal tubule impairs tubular albumin uptake and results in albuminuria in mice (unpublished data). We have also established circulating insulin levels as a determinant of tubular albumin uptake. Moreover, down-regulation of IR and early IR signaling in the kidney has been reported in Type 2 diabetes and models of insulin resistance[7] , which can contribute to elevated albuminuria. These recent findings support a direct physiological role of PT-specific insulin action on albumin uptake and albuminuria.

CONCLUSION

The physiological relevance of IRs in renal epithelial cells has gained more attention in recent years. Studies based on targeted deletion of IR have now provided sufficient

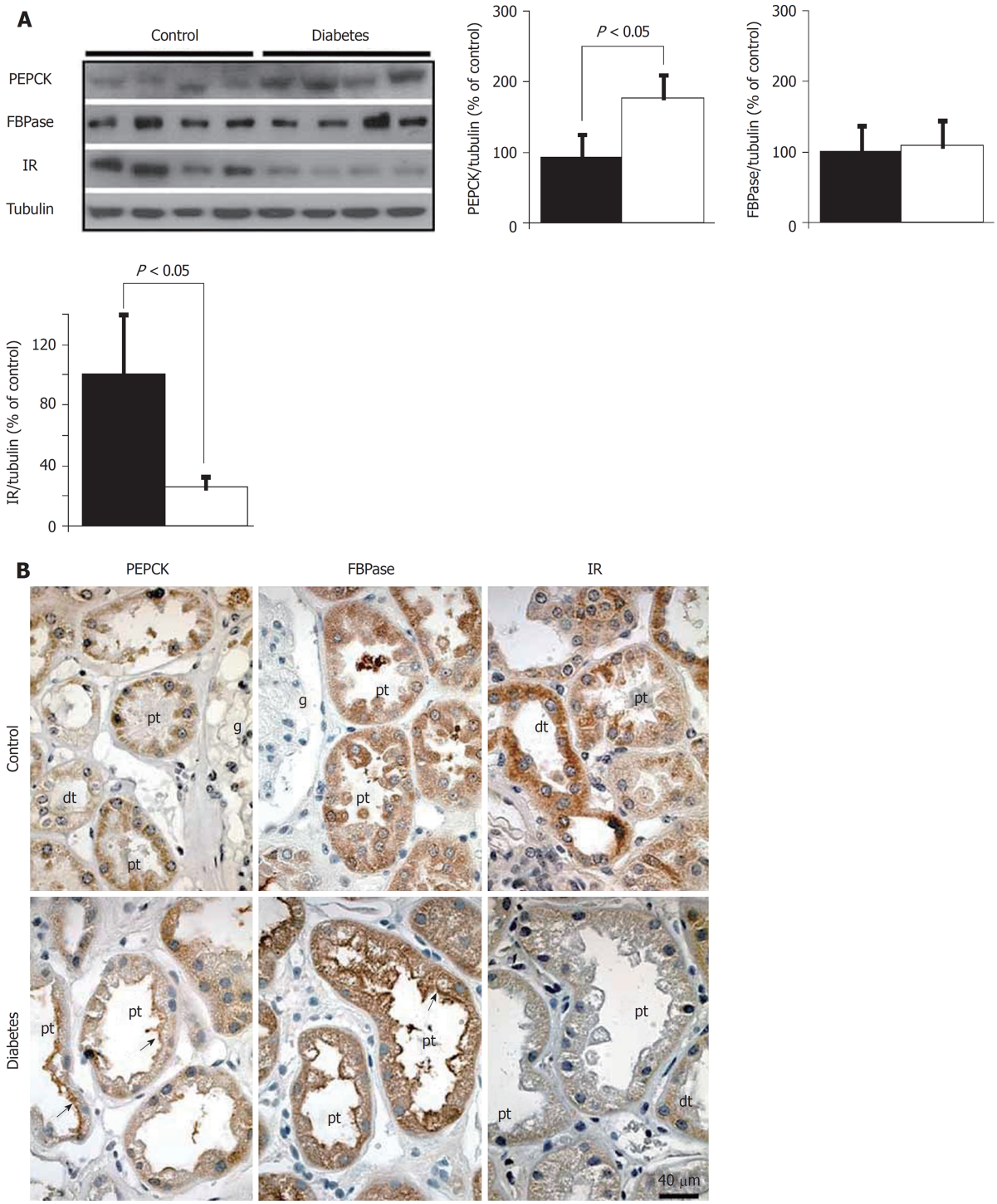


Figure 4 Expression patterns of insulin receptor and gluconeogenic enzymes in normal and diabetic human kidney. A: Expression of FBPase, PEPCK, IR, and tubulin in renal cortex biopsies of control and Type 2 diabetic individuals analyzed by western blotting; B: Immunohistochemical analysis of FBPase, PEPCK, and IR in renal cortex biopsies of control and Type 2 diabetic individuals (Figure is taken from reference number[6] with permission). PEPCK: Phosphoenolpyruvate carboxykinase; IR: Insulin receptor.

evidence to suggest the significance of the renal IR in kidney physiology and pathology. In addition, these studies have enhanced our understanding surrounding the contribution of reduced renal IR observed in the insulin-resistance state. Overall, it can be suggested that modulation of insulin signaling at the receptor level could significantly affect kidney function, which thereby may result in systemic effects. However, more mechanistic studies are warranted to understand the causal role of reduced renal IR in the regulation of blood pressure, systemic glucose levels, and proteinuria.

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REFERENCES

1 **Gavin JR**, Roth J, Neville DM Jr, de Meyts P, Buell DN. Insulin-dependent regulation of insulin receptor concentrations: a direct demonstration in cell culture. *Proc Natl Acad Sci USA* 1974; **71**: 84-88 [PMID: 4359334 DOI: 10.1073/pnas.71.1.84]
2 **Giorgino F**, Almahfouz A, Goodyear LJ, Smith RJ. Glucocorticoid regulation of insulin receptor and substrate IRS-1 tyrosine phosphorylation in rat skeletal muscle in vivo. *J Clin Invest* 1993; **91**: 2020-2030 [PMID: 7683695 DOI: 10.1172/JCI116424]
3 **Grote CW**, Groover AL, Ryals JM, Geiger PC, Feldman EL, Wright DE. Peripheral nervous system insulin resistance in ob/ob mice. *Acta Neuropathol Commun* 2013; **1**: 15 [PMID: 24252636 DOI: 10.1186/2051-5960-1-15]
4 **Sechi LA**, De Carli S, Bartoli E. In situ characterization of renal insulin receptors in the rat. *J Recept Res* 1994; **14**: 347-356 [PMID: 7877134 DOI: 10.3109/10799899409101509]
5 **Nakamura R**, Emmanouel DS, Katz AI. Insulin binding sites in various segments of the rabbit nephron. *J Clin Invest* 1983; **72**: 388-392 [PMID: 6348088 DOI: 10.1172/JCI110979]
6 **Gatica R**, Bertinat R, Silva P, Carpio D, Ramírez MJ, Slebe JC, San Martín R, Nualart F, Campistol JM, Caelles C, Yáñez AJ. Altered expression and localization of insulin receptor in proximal tubule cells from human and rat diabetic kidney. *J Cell Biochem* 2013; **114**: 639-649 [PMID: 23059533 DOI: 10.1002/jcb.24406]
7 **Tiwari S**, Halagappa VK, Riazi S, Hu X, Ecelbarger CA. Reduced expression of insulin receptors in the kidneys of insulin-resistant rats. *J Am Soc Nephrol* 2007; **18**: 2661-2671 [PMID: 17855644 DOI: 10.1681/ASN.2006121410]
8 **De Meyts P**. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. SourceEndotext [Internet]. . South Dartmouth (MA): MDText.com, Inc.; 2000-2016 Apr 27; [PMID: 27512793]
9 **Hale LJ**, Coward RJ. Insulin signalling to the kidney in health and disease. *Clin Sci (Lond)* 2013; **124**: 351-370 [PMID: 23190266 DOI: 10.1042/CS20120378]
10 **Ullrich A**, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 1985; **313**: 756-761 [PMID: 2983222 DOI: 10.1038/313756a0]
11 **Ebina Y**, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou JH, Masiarz F, Kan YW, Goldfine ID. The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 1985; **40**: 747-758 [PMID: 2859121 DOI: 10.1016/0092-8674(85)90334-4]
12 **Seino S**, Seino M, Nishi S, Bell GI. Structure of the human insulin receptor gene and characterization of its promoter. *Proc Natl Acad Sci USA* 1989; **86**: 114-118 [PMID: 2911561 DOI: 10.1073/pnas.86.1.114]
13 **Hubbard SR**. Structure and Mechanism of the Insulin Receptor Tyrosine Kinase. . Handbook of Cell Signaling (Second Edition): Elsevier; 2009; 307-313
14 **Bisbis S**, Derouet M, Simon J. Characterization of insulin receptors in chicken kidneys: effect of nutritional status. *Gen Comp Endocrinol* 1994; **96**: 37-49 [PMID: 7843566 DOI: 10.1006/gcen.1994.1157]
15 **Butlen D**, Vadrot S, Roseau S, Morel F. Insulin receptors along the rat nephron: [125I] insulin binding in microdissected glomeruli and tubules. *Pflugers Arch* 1988; **412**: 604-612 [PMID: 3211711 DOI: 10.1007/BF00583761]
16 **Tiwari S**, Wade JB, Ecelbarger CA. Insulin receptor localization and regulation in rat kidney (Abstract). *FASEB J* 2006; **20**: A1169 [DOI: 10.1096/fasebj.20.5.a1169]
17 **Bates CM**, Merenmies JM, Kelly-Spratt KS, Parada LF. Insulin receptor-related receptor expression in non-A intercalated cells in the kidney. *Kidney Int* 1997; **52**: 674-681 [PMID: 9291186 DOI: 10.1038/ki.1997.381]
18 **Ozaki K**, Takada N, Tsujimoto K, Tsuji N, Kawamura T, Muso E, Ohta M, Itoh N. Localization of insulin receptor-related receptor in the rat kidney. *Kidney Int* 1997; **52**: 694-698 [PMID: 9291189 DOI: 10.1038/ki.1997.384]
19 **Tiwari S**, Sharma N, Gill PS, Igarashi P, Kahn CR, Wade JB, Ecelbarger CM. Impaired sodium excretion and increased blood pressure in mice with targeted deletion of renal epithelial insulin receptor. *Proc Natl Acad Sci USA* 2008; **105**: 6469-6474 [PMID: 18424559 DOI: 10.1073/pnas.0711283105]
20 **Tiwari S**, Singh RS, Li L, Tsukerman S, Godbole M, Pandey G, Ecelbarger CM. Deletion of the insulin receptor in the proximal tubule promotes hyperglycemia. *J Am Soc Nephrol* 2013; **24**: 1209-1214 [PMID: 23723425 DOI: 10.1681/ASN.2012060628]
21 **Whelton PK**, Appel LJ, Sacco RL, Anderson CA, Antman EM, Campbell N, Dunbar SB, Frohlich ED, Hall JE, Jessup M, Labarthe DR, MacGregor GA, Sacks FM, Stamler J, Vafiadis DK, Van Horn LV. Sodium, blood pressure, and cardiovascular disease: further evidence supporting the American Heart Association sodium reduction recommendations. *Circulation* 2012; **126**: 2880-2889 [PMID: 23124030 DOI: 10.1161/CIR.0b013e318279acbf]
22 **Lambers Heerspink HJ**, Holtkamp FA, Parving HH, Navis GJ, Lewis JB, Ritz E, de Graeff PA, de Zeeuw D. Moderation of dietary sodium potentiates the renal and cardiovascular protective effects of angiotensin receptor blockers. *Kidney Int* 2012; **82**: 330-337 [PMID: 22437412 DOI: 10.1038/ki.2012.74]
23 **Graudal NA**, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. *Cochrane Database Syst Rev* 2017; **4**: CD004022 [PMID: 28391629 DOI: 10.1002/14651858.CD004022.pub4]

24 **Wang X**, Armando I, Upadhyay K, Pascua A, Jose PA. The regulation of proximal tubular salt transport in hypertension: an update. *Curr Opin Nephrol Hypertens* 2009; **18**: 412-420 [PMID: 19654544 DOI: 10.1097/MNH.0b013e32832f5775]

25 **Doris PA**. Renal proximal tubule sodium transport and genetic mechanisms of essential hypertension. *J Hypertens* 2000; **18**: 509-519 [PMID: 10826552 DOI: 10.1097/00004872-200018050-00002]

26 **Capasso G**, Rizzo M, Garavaglia ML, Trepiccione F, Zacchia M, Mugione A, Ferrari P, Paulmichl M, Lang F, Loffing J, Carrel M, Damiano S, Wagner CA, Bianchi G, Meyer G. Upregulation of apical sodium-chloride cotransporter and basolateral chloride channels is responsible for the maintenance of salt-sensitive hypertension. *Am J Physiol Renal Physiol* 2008; **295**: F556-F567 [PMID: 18480177 DOI: 10.1152/ajprenal.00340.2007]

27 **Horita S**, Seki G, Yamada H, Suzuki M, Koike K, Fujita T. Insulin resistance, obesity, hypertension, and renal sodium transport. *Int J Hypertens* 2011; **2011**: 391762 [PMID: 21629870 DOI: 10.4061/2011/391762]

28 **Sechi LA**, Griffin CA, Schambelan M. Effect of dietary sodium chloride on insulin receptor number and mRNA levels in rat kidney. *Am J Physiol* 1994; **266**: F31-F38 [PMID: 8304482 DOI: 10.1152/ajprenal.1994.266.1.F31]

29 **Catena C**, Cavarape A, Novello M, Giacchetti G, Sechi LA. Insulin receptors and renal sodium handling in hypertensive fructose-fed rats. *Kidney Int* 2003; **64**: 2163-2171 [PMID: 14633139 DOI: 10.1046/j.1523-1755.2003.00313.x]

30 **Blazer-Yost BL**, Esterman MA, Vlahos CJ. Insulin-stimulated trafficking of ENaC in renal cells requires PI 3-kinase activity. *Am J Physiol Cell Physiol* 2003; **284**: C1645-C1653 [PMID: 12606308 DOI: 10.1152/ajpcell.00372.2002]

31 **Féraille E**, Carranza ML, Gonin S, Béguin P, Pedemonte C, Rousselot M, Caverzasio J, Geering K, Martin PY, Favre H. Insulin-induced stimulation of Na+,K(+)-ATPase activity in kidney proximal tubule cells depends on phosphorylation of the alpha-subunit at Tyr-10. *Mol Biol Cell* 1999; **10**: 2847-2859 [PMID: 10473631 DOI: 10.1091/mbc.10.9.2847]

32 **Fuster DG**, Bobulescu IA, Zhang J, Wade J, Moe OW. Characterization of the regulation of renal Na+/H+ exchanger NHE3 by insulin. *Am J Physiol Renal Physiol* 2007; **292**: F577-F585 [PMID: 17018843 DOI: 10.1152/ajprenal.00240.2006]

33 **Salvetti A**, Brogi G, Di Legge V, Bernini GP. The inter-relationship between insulin resistance and hypertension. *Drugs* 1993; **46** Suppl 2: 149-159 [PMID: 7512468 DOI: 10.2165/00003495-199300462-00024]

34 **Hall JE**. Mechanisms of abnormal renal sodium handling in obesity hypertension. *Am J Hypertens* 1997; **10**: 49S-55S [PMID: 9160781 DOI: 10.1016/S0895-7061(97)00075-7]

35 **Hall JE**, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. *Circ Res* 2015; **116**: 991-1006 [PMID: 25767285 DOI: 10.1161/CIRCRESAHA.116.305697]

36 **Soleimani M**. Insulin resistance and hypertension: new insights. *Kidney Int* 2015; **87**: 497-499 [PMID: 25723632 DOI: 10.1038/ki.2014.392]

37 **Nizar JM**, Walczak EM, Dong W, Bankir L, Bhalla V. Inducible Renal Tubule-specific Insulin Receptor Knockout Mice Have Decreased NCC-mediated Sodium Reabsorption and Reduced Sensitivity to Mineralocorticoid-induced Hypertension in Obesity and Insulin Resistance (Abstract). *FASEB J* 2016; **30**: 968

38 **Hayashi K**, Fujiwara K, Oka K, Nagahama T, Matsuda H, Saruta T. Effects of insulin on rat renal microvessels: studies in the isolated perfused hydronephrotic kidney. *Kidney Int* 1997; **51**: 1507-1513 [PMID: 9150466 DOI: 10.1038/ki.1997.207]

39 **Schmetterer L**, Müller M, Fasching P, Diepolder C, Gallenkamp A, Zanaschka G, Findl O, Strenn K, Mensik C, Tschernko E, Eichler HG, Wolzt M. Renal and ocular hemodynamic effects of insulin. *Diabetes* 1997; **46**: 1868-1874 [PMID: 9356038 DOI: 10.2337/diab.46.11.1868]

40 **Ter Maaten JC**, Bakker SJ, Serné EH, Moshage HJ, Donker AJ, Gans RO. Insulin-mediated increases in renal plasma flow are impaired in insulin-resistant normal subjects. *Eur J Clin Invest* 2000; **30**: 1090-1098 [PMID: 11122324 DOI: 10.1046/j.1365-2362.2000.00749.x]

41 **Scherrer U**, Sartori C. Insulin as a vascular and sympathoexcitatory hormone: implications for blood pressure regulation, insulin sensitivity, and cardiovascular morbidity. *Circulation* 1997; **96**: 4104-4113 [PMID: 9403636 DOI: 10.1161/01.CIR.96.11.4104]

42 **Erdely A**, Freshour G, Maddox DA, Olson JL, Samsell L, Baylis C. Renal disease in rats with type 2 diabetes is associated with decreased renal nitric oxide production. *Diabetologia* 2004; **47**: 1672-1676 [PMID: 15490111 DOI: 10.1007/s00125-004-1509-1]

43 **Elkayam U**, Cohen G, Gogia H, Mehra A, Johnson JV, Chandraratna PA. Renal vasodilatory effect of endothelial stimulation in patients with chronic congestive heart failure. *J Am Coll Cardiol* 1996; **28**: 176-182 [PMID: 8752811 DOI: 10.1016/0735-1097(96)00092-7]

44 **Ortiz PA**, Hong NJ, Garvin JL. NO decreases thick ascending limb chloride absorption by reducing Na(+)-K(+)-2Cl(-) cotransporter activity. *Am J Physiol Renal Physiol* 2001; **281**: F819-F825 [PMID: 11592939 DOI: 10.1152/ajprenal.2001.281.5.F819]

45 **Stoos BA**, Garcia NH, Garvin JL. Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. *J Am Soc Nephrol* 1995; **6**: 89-94 [PMID: 7579075 DOI: 10.1111/j.1464-410X.1995.tb07860.x]

46 **Li L**, Garikepati RM, Tsukerman S, Tiwari S, Ecelbarger CM. Salt sensitivity of nitric oxide generation and blood pressure in mice with targeted knockout of the insulin receptor from the renal tubule. *Am J Physiol Regul Integr Comp Physiol* 2012; **303**: R505-R512 [PMID: 22814664 DOI: 10.1152/ajpregu.00033.2012]

47 **Cowley AW Jr**, Mattson DL, Lu S, Roman RJ. The renal medulla and hypertension. *Hypertension* 1995; **25**: 663-673 [PMID: 7721413 DOI: 10.1161/01.HYP.25.4.663]

48 **Cowley AW Jr**, Mori T, Mattson D, Zou AP. Role of renal NO production in the regulation of medullary blood flow. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R1355-R1369 [PMID: 12736168 DOI: 10.1152/ajpregu.00701.2002]

49 **Wu F**, Park F, Cowley AW Jr, Mattson DL. Quantification of nitric oxide synthase activity in microdissected segments of the rat kidney. *Am J Physiol* 1999; **276**: F874-F881 [PMID: 10362776 DOI: 10.1152/ajprenal.1999.276.6.F874]

50 **Pandey G**, Makhija E, George N, Chakravarti B, Godbole MM, Ecelbarger CM, Tiwari S. Insulin

regulates nitric oxide production in the kidney collecting duct cells. *J Biol Chem* 2015; **290**: 5582-5591 [PMID: 25533472 DOI: 10.1074/jbc.M114.592741]
51 **Underwood PC**, Adler GK. The renin angiotensin aldosterone system and insulin resistance in humans. *Curr Hypertens Rep* 2013; **15**: 59-70 [PMID: 23242734 DOI: 10.1007/s11906-012-0323-2]
52 **Csibi A**, Communi D, Müller N, Bottari SP. Angiotensin II inhibits insulin-stimulated GLUT4 translocation and Akt activation through tyrosine nitration-dependent mechanisms. *PLoS One* 2010; **5**: e10070 [PMID: 20383279 DOI: 10.1371/journal.pone.0010070]
53 **Yavuz D**, Koç M, Toprak A, Akpinar I, Velioğlu A, Deyneli O, Haklar G, Akalin S. Effects of ACE inhibition and AT1-receptor antagonism on endothelial function and insulin sensitivity in essential hypertensive patients. *J Renin Angiotensin Aldosterone Syst* 2003; **4**: 197-203 [PMID: 14608527 DOI: 10.3317/jraas.2003.032]
54 **Andreozzi F**, Laratta E, Sciacqua A, Perticone F, Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. *Circ Res* 2004; **94**: 1211-1218 [PMID: 15044323 DOI: 10.1161/01.RES.0000126501.34994.96]
55 **Stumvoll M**, Meyer C, Mitrakou A, Nadkarni V, Gerich JE. Renal glucose production and utilization: new aspects in humans. *Diabetologia* 1997; **40**: 749-757 [PMID: 9243094 DOI: 10.1007/s001250050745]
56 **Stumvoll M**, Meyer C, Mitrakou A, Gerich JE. Important role of the kidney in human carbohydrate metabolism. *Med Hypotheses* 1999; **52**: 363-366 [PMID: 10416940 DOI: 10.1054/mehy.1997.0655]
57 **Cano N**. Bench-to-bedside review: glucose production from the kidney. *Crit Care* 2002; **6**: 317-321 [PMID: 12225606 DOI: 10.1186/cc1517]
58 **Meyer C**, Stumvoll M, Nadkarni V, Dostou J, Mitrakou A, Gerich J. Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. *J Clin Invest* 1998; **102**: 619-624 [PMID: 9691098 DOI: 10.1172/JCI2415]
59 **Stumvoll M**, Meyer C, Kreider M, Perriello G, Gerich J. Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. *Metabolism* 1998; **47**: 1227-1232 [PMID: 9781626 DOI: 10.1016/S0026-0495(98)90328-6]
60 **Cersosimo E**, Judd RL, Miles JM. Insulin regulation of renal glucose metabolism in conscious dogs. *J Clin Invest* 1994; **93**: 2584-2589 [PMID: 8200996 DOI: 10.1172/JCI117270]
61 **Bergman H**, Drury DR. The relationship of kidney function to the glucose utilization of the extra abdominal tissues. *Am J Physiol (legacy content)* 1938; **124**: 279-284 [DOI: 10.1152/ajplegacy.1938.124.2.279]
62 **Wilding JP**. The role of the kidneys in glucose homeostasis in type 2 diabetes: clinical implications and therapeutic significance through sodium glucose co-transporter 2 inhibitors. *Metabolism* 2014; **63**: 1228-1237 [PMID: 25104103 DOI: 10.1016/j.metabol.2014.06.018]
63 **Krebs HA**, Bennett DA, DE Gasquet P, Gasquet P, Gascoyne T, Yoshida T. Renal gluconeogenesis. The effect of diet on the gluconeogenic capacity of rat-kidney-cortex slices. *Biochem J* 1963; **86**: 22-27 [PMID: 14035641 DOI: 10.1042/bj0860022]
64 **Peres LA**, Bredt LC, Cipriani RF. Acute renal injury after partial hepatectomy. *World J Hepatol* 2016; **8**: 891-901 [PMID: 27478539 DOI: 10.4254/wjh.v8.i21.891]
65 **Coward RJ**, Welsh GI, Yang J, Tasman C, Lennon R, Koziell A, Satchell S, Holman GD, Kerjaschki D, Tavaré JM, Mathieson PW, Saleem MA. The human glomerular podocyte is a novel target for insulin action. *Diabetes* 2005; **54**: 3095-3102 [PMID: 16249431 DOI: 10.2337/diabetes.54.11.3095]
66 **Cersosimo E**, Garlick P, Ferretti J. Insulin regulation of renal glucose metabolism in humans. *Am J Physiol* 1999; **276**: E78-E84 [PMID: 9886953 DOI: 10.1016/S0303-7207(98)00201-9]
67 **Meyer C**, Dostou J, Nadkarni V, Gerich J. Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. *Am J Physiol* 1998; **275**: F915-F921 [PMID: 9843908 DOI: 10.1152/ajprenal.1998.275.6.f915]
68 **McGivan JD**, Pastor-Anglada M. Regulatory and molecular aspects of mammalian amino acid transport. *Biochem J* 1994; **299**: 321-334 [PMID: 8172590 DOI: 10.1042/bj2990321]
69 **Eid A**, Bodin S, Ferrier B, Delage H, Boghossian M, Martin M, Baverel G, Conjard A. Intrinsic gluconeogenesis is enhanced in renal proximal tubules of Zucker diabetic fatty rats. *J Am Soc Nephrol* 2006; **17**: 398-405 [PMID: 16396963 DOI: 10.1681/ASN.2005070742]
70 **Tejada T**, Catanuto P, Ijaz A, Santos JV, Xia X, Sanchez P, Sanabria N, Lenz O, Elliot SJ, Fornoni A. Failure to phosphorylate AKT in podocytes from mice with early diabetic nephropathy promotes cell death. *Kidney Int* 2008; **73**: 1385-1393 [PMID: 18385666 DOI: 10.1038/ki.2008.109]
71 **Barthel A**, Schmoll D. Novel concepts in insulin regulation of hepatic gluconeogenesis. *Am J Physiol Endocrinol Metab* 2003; **285**: E685-E692 [PMID: 12959935 DOI: 10.1152/ajpendo.00253.2003]
72 **Valenti L**, Rametta R, Dongiovanni P, Maggioni M, Fracanzani AL, Zappa M, Lattuada E, Roviaro G, Fargion S. Increased expression and activity of the transcription factor FOXO1 in nonalcoholic steatohepatitis. *Diabetes* 2008; **57**: 1355-1362 [PMID: 18316359 DOI: 10.2337/db07-0714]
73 **Michael MD**, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 2000; **6**: 87-97 [PMID: 10949030 DOI: 10.1016/S1097-2765(05)00015-8]
74 **DeFronzo RA**, Davidson JA, Del Prato S. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. *Diabetes Obes Metab* 2012; **14**: 5-14 [PMID: 21955459 DOI: 10.1111/j.1463-1326.2011.01511.x]
75 **Winzell MS**, Ahrén B. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. *Diabetes* 2004; **53** Suppl 3: S215-S219 [PMID: 15561913 DOI: 10.2337/diabetes.53.suppl_3.S215]
76 **Radziuk J**, Pye S. Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. *Diabetes Metab Res Rev* 2001; **17**: 250-272 [PMID: 11544610 DOI: 10.1002/dmrr.217]
77 **Pandey G**, Shankar K, Makhija E, Gaikwad A, Ecelbarger C, Mandhani A, Srivastava A, Tiwari S. Reduced Insulin Receptor Expression Enhances Proximal Tubule Gluconeogenesis. *J Cell Biochem* 2017; **118**: 276-285 [PMID: 27322100 DOI: 10.1002/jcb.25632]
78 **Horita S**, Nakamura M, Suzuki M, Satoh N, Suzuki A, Seki G. Selective Insulin Resistance in the

Kidney. *Biomed Res Int* 2016; **2016**: 5825170 [PMID: 27247938 DOI: 10.1155/2016/5825170]
79 **Biddinger SB**, Hernandez-Ono A, Rask-Madsen C, Haas JT, Alemán JO, Suzuki R, Scapa EF, Agarwal C, Carey MC, Stephanopoulos G, Cohen DE, King GL, Ginsberg HN, Kahn CR. Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab* 2008; **7**: 125-134 [PMID: 18249172 DOI: 10.1016/j.cmet.2007.11.013]
80 **Brown MS**, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab* 2008; **7**: 95-96 [PMID: 18249166 DOI: 10.1016/j.cmet.2007.12.009]
81 **Laplante M**, Sabatini DM. mTORC1 activates SREBP-1c and uncouples lipogenesis from gluconeogenesis. *Proc Natl Acad Sci USA* 2010; **107**: 3281-3282 [PMID: 20167806 DOI: 10.1073/pnas.1000323107]
82 **Sasaki M**, Sasako T, Kubota N, Sakurai Y, Takamoto I, Kubota T, Inagi R, Seki G, Goto M, Ueki K, Nangaku M, Jomori T, Kadowaki T. Dual Regulation of Gluconeogenesis by Insulin and Glucose in the Proximal Tubules of the Kidney. *Diabetes* 2017; **66**: 2339-2350 [PMID: 28630133 DOI: 10.2337/db16-1602]
83 **Yañez AJ**, Ludwig HC, Bertinat R, Spichiger C, Gatica R, Berlien G, Leon O, Brito M, Concha II, Slebe JC. Different involvement for aldolase isoenzymes in kidney glucose metabolism: aldolase B but not aldolase A colocalizes and forms a complex with FBPase. *J Cell Physiol* 2005; **202**: 743-753 [PMID: 15389646 DOI: 10.1002/jcp.20183]
84 **Mitrakou A**. Kidney: its impact on glucose homeostasis and hormonal regulation. *Diabetes Res Clin Pract* 2011; **93** Suppl 1: S66-S72 [PMID: 21864754 DOI: 10.1016/S0168-8227(11)70016-X]
85 **Wang Q**, Guo T, Portas J, McPherron AC. A soluble activin receptor type IIB does not improve blood glucose in streptozotocin-treated mice. *Int J Biol Sci* 2015; **11**: 199-208 [PMID: 25561902 DOI: 10.7150/ijbs.10430]
86 **Tesch GH**. Review: Serum and urine biomarkers of kidney disease: A pathophysiological perspective. *Nephrology (Carlton)* 2010; **15**: 609-616 [PMID: 20883281 DOI: 10.1111/j.1440-1797.2010.01361.x]
87 **Mogensen CE**, Christensen CK. Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 1984; **311**: 89-93 [PMID: 6738599 DOI: 10.1056/NEJM198407123110204]
88 **Russo LM**, Sandoval RM, Campos SB, Molitoris BA, Comper WD, Brown D. Impaired tubular uptake explains albuminuria in early diabetic nephropathy. *J Am Soc Nephrol* 2009; **20**: 489-494 [PMID: 19118149 DOI: 10.1681/ASN.2008050503]
89 **Tojo A**, Onozato ML, Ha H, Kurihara H, Sakai T, Goto A, Fujita T, Endou H. Reduced albumin reabsorption in the proximal tubule of early-stage diabetic rats. *Histochem Cell Biol* 2001; **116**: 269-276 [PMID: 11685557 DOI: 10.1007/s004180100317]
90 **Dickson LE**, Wagner MC, Sandoval RM, Molitoris BA. The proximal tubule and albuminuria: really! *J Am Soc Nephrol* 2014; **25**: 443-453 [PMID: 24408874 DOI: 10.1681/ASN.2013090950]
91 **Christensen EI**, Birn H. Megalin and cubilin: synergistic endocytic receptors in renal proximal tubule. *Am J Physiol Renal Physiol* 2001; **280**: F562-F573 [PMID: 11249847 DOI: 10.1152/ajprenal.2001.280.4.F562]
92 **Pilz S**, Rutters F, Nijpels G, Stehouwer CD, Højlund K, Nolan JJ, Balkau B, Dekker JM; RISC Investigators. Insulin sensitivity and albuminuria: the RISC study. *Diabetes Care* 2014; **37**: 1597-1603 [PMID: 24623021 DOI: 10.2337/dc13-2573]
93 **Coffey S**, Costacou T, Orchard T, Erkan E. Akt Links Insulin Signaling to Albumin Endocytosis in Proximal Tubule Epithelial Cells. *PLoS One* 2015; **10**: e0140417 [PMID: 26465605 DOI: 10.1371/journal.pone.0140417]
94 **Zeng B**, Chen GL, Garcia-Vaz E, Bhandari S, Daskoulidou N, Berglund LM, Jiang H, Hallett T, Zhou LP, Huang L, Xu ZH, Nair V, Nelson RG, Ju W, Kretzler M, Atkin SL, Gomez MF, Xu SZ. ORAI channels are critical for receptor-mediated endocytosis of albumin. *Nat Commun* 2017; **8**: 1920 [PMID: 29203863 DOI: 10.1038/s41467-017-02094-y]
95 **Mottl AK**, Divers J, Dabelea D, Maahs DM, Dolan L, Pettitt D, Marcovina S, Imperatore G, Pihoker C, Mauer M, Mayer-Davis EJ; SEARCH for Diabetes in Youth Study. The dose-response effect of insulin sensitivity on albuminuria in children according to diabetes type. *Pediatr Nephrol* 2016; **31**: 933-940 [PMID: 26754041 DOI: 10.1007/s00467-015-3276-2]
96 **Bryniarski MA**, Yee BM, Jaffri I, Chaves LD, Yu JA, Guan X, Ghavam N, Yacoub R, Morris ME. Increased Megalin Expression in Early Type 2 Diabetes: Role of Insulin Signaling Pathways. *Am J Physiol Renal Physiol* 2018 [PMID: 29949391 DOI: 10.1152/ajprenal.00210.2018]
97 **Salem ES**, Grobe N, Elased KM. Insulin treatment attenuates renal ADAM17 and ACE2 shedding in diabetic Akita mice. *Am J Physiol Renal Physiol* 2014; **306**: F629-F639 [PMID: 24452639 DOI: 10.1152/ajprenal.00516.2013]

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MINIREVIEWS

Dehydration and volume depletion: How to handle the misconceptions

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Abstract

Dehydration and volume depletion describe two distinct body fluid deficit disorders with differing pathophysiology, clinical manifestations and treatment approaches. However, the two are often confused or equated with each other. Here, we address a number of commonly encountered misconceptions about body-fluid deficit disorders, analyse their origins and propose approaches to overcome them.

Key words: Body fluids; Dehydration; Volume depletion; Misconceptions

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Core tip: The conceptual error of using the term "dehydration" as a non-specific generic term to represent any type of fluid deficit affecting any fluid compartment, or even worse, to imply extracellular fluid volume depletion remains disturbingly prevalent among medical students and doctors. Careless and casual use of the term "dehydration" for patients who, in fact, have intravascular "volume depletion" contaminates the medical language, creates misleading impressions and unfortunately, in some cases, leads to inappropriate management. We propose a multi-faceted approach that supplements real life clinical scenarios with reflective activities through active participation of students and helps remove these robust misconceptions and instigate conceptual restructuring.

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INTRODUCTION

Students often confuse concepts related to sodium and water balance. One concept that has received considerable attention in recent medical teaching is the notion that disorders of water balance are manifested as hyponatraemia or hypernatraemia, whilst disorders of sodium balance are manifested as disruption of extracellular fluid (ECF) volume. In this review, we focus on another key concept regarding dehydration and volume depletion, and how the two are completely different clinical syndromes with distinct pathophysiological mechanisms, clinical features, biochemical characteristics, and management strategies.

Assessment of body fluid status is an integral component of the physician's evaluation of most hospitalized patients. A competent fluid assessment requires sound knowledge of the dynamic interaction between body fluid compartments, as well as a skilful examination and careful biochemical analysis of serum and urine. In addition, a command of medical language and terminology is essential to precisely describe and categorize body-fluid status. Mange *et al*[1] highlighted the importance of recognizing dehydration and volume depletion as two completely different clinical entities. However, the conceptual error of using the term "dehydration" as a non-specific, generic term to represent any type of fluid deficit affecting any fluid compartment, or even worse, to imply ECF volume depletion, remains disturbingly prevalent among medical students and doctors. Careless and casual use of the term "dehydration" for patients who, in fact, have intravascular "volume depletion" contaminates the medical language, creates misleading impressions and unfortunately, in some cases, leads to inappropriate management. Considering the magnitude of the problem, in 2004 the International Classification of Diseases coordination and maintenance Committee made recommendations to modify the coding for body fluid disorders to uniquely identify dehydration and volume depletion[2]. For the sake of this review, we will use the term "students" to refer to medical students as well as to junior physicians who fall prey to the dehydration/volume depletion misconceptions.

Body fluid compartments – basic facts

Total body water (TBW) is estimated to be 50%-60% of body weight, varying with age, gender and race, and resides in three main fluid compartments of body (Figure 1)[3,4]. The bulk of the TBW (67%) is confined intracellularly; the remaining 33% is distributed between the two sub-compartments of the extracellular space: interstitial and intravascular (25% and 8% respectively)[5]. Hence, in a 70 kg man, TBW is approximately 42 L, out of which 28 L, 10.5 L and 3.5 L are distributed in the intracellular, interstitial and intravascular compartments, respectively. Another subcategory of ECF, albeit small, is transcellular fluid (not shown in the figure) that resides in pleural, pericardial, peritoneal, synovial, ocular and cerebrospinal spaces, although in some cases, its chemical composition and physical properties may differ from that of intravascular or interstitial fluid[6]. Fluid input and output from the body proceeds *via* the intravascular compartment.

Intravascular and interstitial compartments are separated solely by highly permeable capillary membranes. Hence, their ionic composition is almost identical; the major cation is sodium (Na^+) and the major anions are chloride and bicarbonate. In contrast, the major cation in the intracellular fluid (ICF) is potassium (K^+) and the major anions are inorganic phosphates. Sodium chloride is typically confined to the ECF compartment by virtue of the Na-K-ATPase pumps, anchored in the cell membranes, which pump Na^+ out and K^+ into the cells. This constant active transport of Na^+ and K^+ across the cell membrane makes the ECF rich in Na^+ and the ICF rich in K^+. Consequently, the osmolality of the ECF is largely dependent on sodium and chloride whereas the osmolality of the ICF is derived from potassium along with other intracellular osmoles. Water moves freely between all fluid compartments through highly water permeable cell membranes; therefore, the osmolality of the plasma is equal to the osmolality of other compartments.

Of the total plasma volume, 85% is in venous circulation and 15% is in arterial circulation. It is this small arterial volume (approximately 700 mL) that constitutes the effective circulating volume, which is responsible for tissue perfusion and regulation

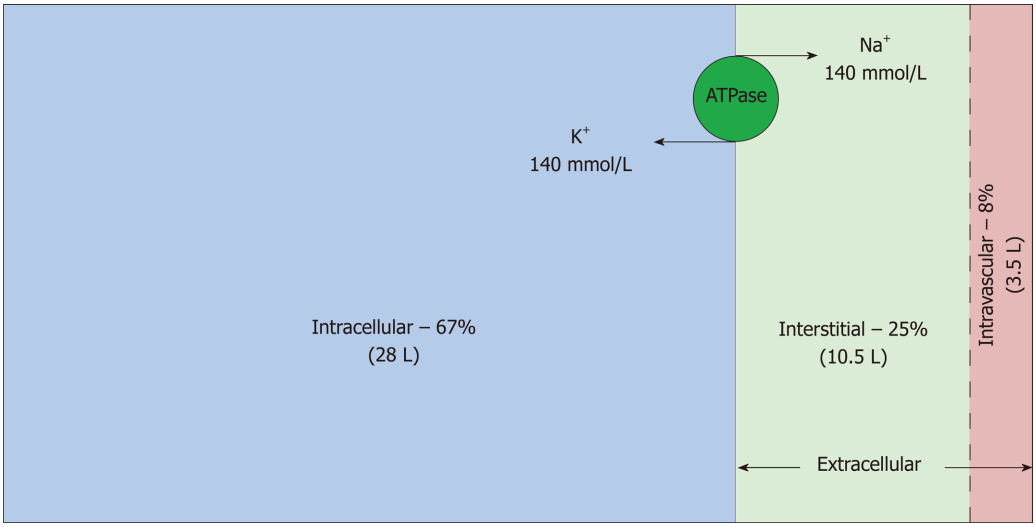


Figure 1 Distribution of total body water in various fluid compartments of a 70 kg man (see text for explanation).

of the body's salt and water balance[7,8].

Considering the differing permeability of the membranes that separate fluid compartments in the body, administration of different IV fluids will result in differing distribution amongst these compartments. Since water flows freely between all three compartments, infusion of one litre of 5% dextrose water (D5W) will lead to an increase in the volume of the intracellular compartment of approximately 670 mL (67% of one litre), that of the interstitial compartment of 250 mL (25% of one litre) and that of intravascular compartment of 80 mL (8% of one litre). On the other hand, infusion of one litre of normal saline (0.9% NS) will add approximately 750 mL to the interstitial space and 250 mL to the intravascular space due to the inhibition of Na+ entry into the cell by the aforementioned Na/K/ATPase pumps located in the cell membranes. Though the water content of both D5W and 0.9% NS solutions is equal (1000 mL), much more fluid will reside in the intravascular space if given in the form of 0.9% NS (as none enters the intracellular space). Hence, 0.9% NS is preferred over D5W if the aim is to correct intravascular volume depletion. Conversely, if the aim is to correct dehydration (pure water loss) then a fluid that flows to all the compartments, such as D5W is the preferred solution. Giving D5W is equivalent to giving free water because glucose is rapidly metabolized.

TBW and volume of each fluid compartment can be accurately measured by radionuclide and "indicator-dilution" methods or by bioelectrical impedance[9-11].

Dehydration and volume depletion – two distinct entities

As indicated by Mange *et al*[1], two distinct clinical syndromes can develop secondary to excessive body fluid losses: (1) Dehydration, which means pure water loss ("Hydro" originates from the ancient Greek word "hudōr", meaning "water"; to dehydrate means removing water). Loss of water reduces the distribution space of Na^+, thereby disturbing the Na^+ and water ratio, leading to hypernatremia and hypertonicity. Because cell membranes are freely permeable to water, this results in osmotic movement of water from the larger intracellular compartment to the extracellular compartment. There is a contraction of all body water compartments proportional to their share of TBW[12]. Since the intracellular compartment is the largest reservoir of body water, it suffers the largest water deficit. For instance, for each litre of water lost from the body, the intracellular compartment contributes 670 mL. In contrast, the intravascular compartment suffers a loss of only 80 mL; hence pure water loss rarely compromises the effective circulating volume or haemodynamic stability. Pure water loss results in hypernatremia and hypertonicity because Na^+ is a membrane-impermeant solute. This induces shrinkage of osmoreceptor cells in the anterior hypothalamus, stimulating the release of antidiuretic hormone (ADH) from the posterior pituitary gland. ADH promotes incorporation of water channels (aquaporin 2) in the distal nephron segments allowing increased water reabsorption. At the same time, the thirst mechanism is triggered leading to increased water ingestion. Renal conservation of water along with increased water intake act to reverse the osmolal changes brought about by the initial water loss by restoring normonatremia (Figure 2); (2) Volume depletion, which implies an ECF volume deficit secondary to the loss of both sodium and water.

Sodium is confined into the extracellular compartment by the Na-K-ATPase pumps in the cell membranes, which helps to hold water extracellularly[13]. Sodium and water loss lead to a reduction in the effective circulating volume. The human body orchestrates a number of homeostatic responses to combat hypovolemia that include activation of the renin-angiotensin-aldosterone system (receptors in renal afferent arterioles), stimulation of the sympathetic nervous system (aortic arch and carotid sinus receptors), suppression of ANP (atrial receptors) and stimulation of ADH release. All these lead to renal conservation of both salt and water, thereby restoring normovolemia. It is noteworthy that ADH release is stimulated in both dehydration (due to hypertonicity), and ECF volume depletion (due to decreased effective circulating volume).

ORIGINS OF MISCONCEPTIONS

Insufficient knowledge/faulty mental models of body fluid compartments and fluid assessment:

Though uncommon, some physicians have insufficient knowledge of body fluids due to a lack of factual information about body fluid compartments and differences in their composition. Most are aware that a patient with haemorrhagic shock has a depleted intravascular compartment, but only a few recognize which compartment suffers the most in a dehydrated patient with a serum sodium of 170 μmol/L. Suppose an elderly patient is admitted with community-acquired pneumonia. He has been rather drowsy for two days before admission with poor oral intake. He is tachypneic and pyrexial, but his blood pressure is normal with no postural change. Initial laboratory tests reveal a serum sodium of 170 μmol/L. He is receiving antibiotics and D5W infusion. When asked "What condition are you treating with D5W infusion?", most students reply "hypernatremia" rather than "dehydration", *i.e.*, they mention the biochemical derangement rather than the condition that produced it. Further probing reveals that some students do not recognize that hypernatremia in most instances represents loss of water in relation to Na^+ (not an excess of sodium) and is a manifestation of dehydration (hence we calculate the free water deficit to assess the amount of water replacement needed to correct hypernatremia). In other words, it is the water intake/excretion (rather than Na^+ handling) that regulates the ECF sodium concentration. It also appears that although some students have knowledge of the different fluid compartments, they fail to apply their knowledge to real life cases.

A number of students have a skewed understanding of body fluid compartments and harbour various misconceptions, the most common of which is erroneously referring to "ECF volume depletion" or "intravascular volume depletion" as "dehydration". The vast majority of doctors appreciate that patients who present with profuse diarrhoea and vomiting and are consequently hypotensive and tachycardic are intravascularly depleted. They also very appropriately resuscitate these patients with 0.9% NS rather than D5W infusion. However, when presenting such a case during the ward round, they say "this patient was severely dehydrated and resuscitated with 0.9% NS". So, although they correctly identify and treat the clinical syndrome of intravascular volume depletion, they use imprecise terminology.

Another common misbelief among students is that dehydration can be reliably diagnosed by physical signs such as sunken eyes, decreased skin turgor and dry mucous membranes. Contrarily, the predictive value of these individual clinical signs in diagnosing dehydration is limited in adult populations. Studies endorsing these physical signs were mostly carried out on paediatric and elderly patient populations[14-18]. Many of these patients in fact had ECF volume depletion rather than dehydration, as evidenced by haemodynamic compromise and normal serum sodium levels.

The term "sunken eyes" (enophthalmos) implies posterior displacement of the eyeballs within the orbits due to a decrease in volume of orbital soft tissues. However, exophthalmometry, the standard objective technique for measuring enophthalmos, is not used in general medicine leaving substantial variation in inter-observer agreement for this physical sign. Furthermore, normal anatomical variation amongst individuals and age-related changes (lipodystrophy of orbital fat with increasing age) make "sunken eyes" an unreliable physical sign of dehydration.

Reduced skin turgor means reduced elastic recoil of the skin to its normal contour after being pinched in a fold. As pointed out by Laron *et al*[19], it reflects contraction of the interstitial and intravascular space (both are subcategories of the extracellular compartment) rather than the loss of intracellular water. Skin turgor also correlates directly with the elastin content of the skin, which decreases significantly with

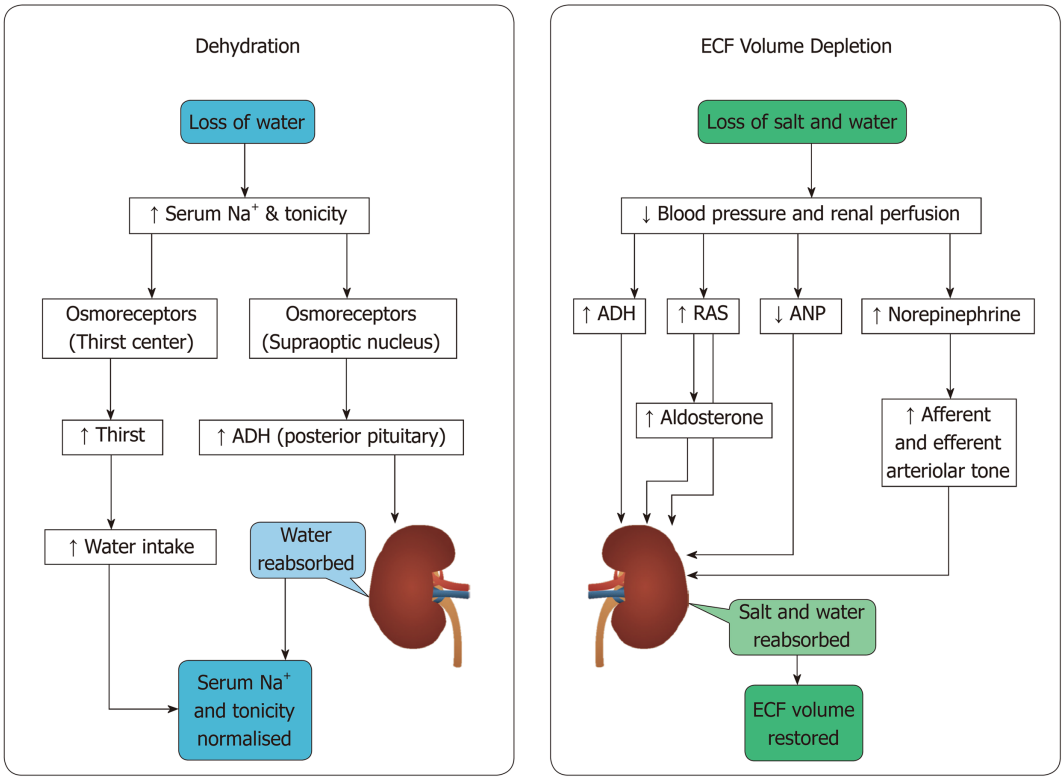


Figure 2 Compensatory responses to dehydration and extracellular fluid volume depletion. The kidneys retain what is lost from the body – water conserved in dehydration; salt and water are reabsorbed in extracellular fluid volume depletion. ADH: Antidiuretic hormone; ANP: Atrial natriuretic peptide; ECF: Extracellular fluid; RAS: Renin angiotensin system.

ageing[16,20].

"Isonatraemic, hyponatraemic, and hypernatraemic dehydration" – ancient concepts prevailing in modern doctors

Though it had long been known that primary loss or deprivation of water produces biological disturbances (thirst) dissimilar to those seen in primary loss or deprivation of salt (circulatory instability), both types of deficits were considered to be subcategories of dehydration in the early 20th century[21,22]. These ancient concepts have managed to exercise a strong pull on some modern doctors, who have persevered in using the term "dehydration" to refer to both intracellular water loss and ECF volume loss and to sub-classify dehydration into isonatraemic, hyponatraemic and hypernatraemic forms[23-27].

In fact, it is *volume depletion* that has isonatraemic, hyponatraemic and hypernatraemic subtypes determined by the tonicity of the fluid lost and the type of fluid ingested[28-31]. If the losses are isotonic, *i.e.*, proportionate quantities of water and sodium are lost (*e.g.*, blood loss), then serum sodium and tonicity will remain unchanged resulting in isonatraemic volume depletion. However, if more sodium relative to water is lost (or the patient takes plenty of salt-free fluids, for example tap water), hyponatraemic volume depletion ensues. Finally, if less sodium is lost relative to water (or if the patient does not drink water, or takes hypertonic soup), hypernatraemic volume depletion follows. In contrast to volume depletion, dehydration is always hypernatraemic (due to loss of pure water); the categories "hyponatraemic" and "isonatraemic" do not apply in dehydration.

Co-existence of dehydration and intravascular volume depletion in some patients

Some patients can present with features of both dehydration and intravascular volume depletion. The co-existence of these two different entities is partly responsible for some physicians misjudging them as a single disorder. Indeed, many patients in paediatric clinical studies with diarrhoeal illnesses were both dehydrated and ECF volume depleted[14,32]. This complex pathophysiological state was oversimplified as "dehydration" and the severity of body fluid losses categorized according to percentage of body weight loss. The "dehydration" assessment scales included the physical signs and laboratory parameters of both intracellular water loss and ECF volume depletion[14,32].

Clinically, it is not possible to establish whether hypernatremia in an intravascularly depleted patient is secondary to hypernatraemic intravascular depletion (water loss greater than sodium loss), severe dehydration (profuse water loss alone), or a combination of the two. This differentiation requires marker/tracer studies[9-11]. In clinical situations, there is hardly any need for this differentiation. As a first step, intravascular volume depletion is treated with 0.9% NS to support organ function. Once adequate haemodynamic stability is achieved, hyperosmolality is corrected with D5W.

Potential of dehydration to cause intravascular volume depletion

Usually, dehydration does not lead to intravascular volume depletion as the intravascular space contributes only a small percentage to the TBW loss; the major bulk is lost from the intracellular space, the largest reservoir of body water. As discussed earlier, a loss of 1 L from TBW removes only 80 ml from the intravascular space (2.3% of intravascular volume); consequently, no appreciable deleterious effects on haemodynamics are seen. Development of signs and symptoms of intravascular volume depletion usually require more than 0.5 L of intravascular volume deficit. For this intravascular volume deficit to develop in a 70 kg person with dehydration, a TBW deficit of more than 6 litres (more than 15% of TBW) will be required. By this time severe hypernatremia (serum Na^+ > 170 mmol/L) would have developed[12].

Oversimplification of medical terminology

Albert Einstein said, "everything should be made as simple as possible, but not simpler". When discussing a patient's condition, physicians commonly use shortened forms of legitimate medical words and phrases as a time-saving measure. The drawback of this is that these brief forms can lead to varied interpretations and thus confound medical personnel. The term "volume depletion" is used as a brief (though obscure) form for ECF volume depletion or intravascular volume depletion but might not adequately convey the intended meaning. It neither clarifies whether the loss of fluid is from intracellular or extracellular space, nor indicates the type of fluid lost (hypotonic or isotonic). Hence, for some it may imply depletion of TBW (*i.e.*, dehydration), while for others, depletion of the intravascular fluid alone (*i.e.*, isotonic fluid loss).

STRATEGIES TO OVERCOME MISCONCEPTIONS

An organized approach is imperative in correcting robust misconceptions related to body fluid deficit disorders. First, it is crucial that all faculty members develop a critical understanding of the body-fluids, as misconceptions acquired from faculty members and textbooks are very difficult to eliminate from the minds of young doctors later in their professional lives.

In the following section we present our approach to overcoming misconceptions in a manner that will create a lasting effect on students and prevent them from reverting to their preconceptions.

Identify misconceptions in the students

Although misconceptions about body fluids disorders are widespread, students are generally unaware that the knowledge they possess is faulty. Mere use of medical terminology is not sufficient evidence of students' knowledge; it needs to be ensured that these terms are used with accurate meaning in the context of body fluid compartments. We actively bring up the subject when encountering patients with body fluid deficits in order to probe students for the presence of misconceptions.

Tackle the misconceptions

Once identified, we try to make students discontent with their misconceptions. This provides a strong stimulus for refinement or replacement of the flawed concepts with intelligible and plausible ones. Utmost care is given to maintain a favourable learning environment where the students are not ridiculed for holding incorrect preconceptions.

We split teaching into short modules, each with a clear framework and objectives. Most of the modules remain "learner-centred" where students are engaged in meaningful activities which promote thought. Multiple teaching techniques are employed to cater to the diverse learning styles of the students and rekindle students' interest and participation.

Introductory presentation: We start with a 10-min introductory presentation using visual aids such as a white-board or PowerPoint presentation to orient the students to

body fluid compartments. Classifying the body fluid deficit disorder based on the nature of the fluid deficit (water alone *vs* water with salt) and the main body fluid compartment affected (intracellular *vs* extracellular) in each disorder generates uneasiness in the minds of those students who misconceive dehydration and volume depletion as one entity. This serves as an important turning point in the students' learning as they start feeling dissatisfied with their pre-conceptions.

We make a conscious effort to avoid ambiguous linguistic expressions. We use the term "dehydration" to specifically refer to a body-fluid disorder resulting from pure water depletion with consequent hypernatremia, rather than using it as a blanket term for any type of fluid deficit. Furthermore, since the term "volume depletion" gives rise to referential ambiguity (because it does not specify the referent body fluid compartment), we disambiguate this term by adding an adjective, for example, "ECF" or "Intravascular" to indicate the depleted body-fluid compartment. This also encourages students to abandon the habit of using misleading abbreviations.

Clinical encounter: The newly implanted concepts must be supplemented with real life applications within a patient care context ensuring the students do not merely learn the new rote information. Utilizing the "think-pair-share" technique, we invite students to form either pairs or small groups. One group evaluates a pre-selected patient with dehydration while the other group assesses a patient with ECF volume depletion.

Debriefing session 1: After seeing their respective patients, the two groups return to the "classroom". They are given a 2 min "reflection time" for formulation of ideas after which each group interacts with the other to compare the clinical, laboratory and therapeutic details of their patients. The instructor facilitates the learning process and highlights the contrasting features of the two patients. This session also provides an opportunity for the instructor to point out any common conceptual errors and offer constructive suggestions.

Instead of beginning with the presenting illness, which is the generally considered "norm", a unique way to provoke curiosity in the minds of the students is to start the discussion with the management of the two patients. This disrupts the students' expectations, captures their attention and makes them think and reflect retrospectively. An example of this could be asking: "Both the patients are receiving IV fluids because they have body fluid deficits; why is one patient being treated with slow infusion of D5W and the other with rapid administration of 0.9% NS or blood?" or "What factors have influenced the choice of fluid and the rate of infusion?" These queries will encourage the students to talk about the body fluid compartment from which the fluid has been lost in each condition and the concept of replacing "like with like" by choosing appropriate fluids for each condition. These questions also help cement the fact that dehydration and volume depletion are different disorders and hence necessitate different treatments. At this juncture, schematic illustrations of fluid compartments to demonstrate the effect of the addition of different types of fluids (blood, albumin, 0.9% NS, NaHCO3, D5W, *etc*.) on each body fluid compartment are utilized. Students are interested to note how each type of fluid initially expands the intravascular space but then distributes distinctively through different fluid compartments. This also helps them with the application of theoretical knowledge into direct patient care. The ECF volume maintains blood pressure and perfusion of organs, hence a fluid that is more likely to stay in the ECF compartment (0.9% NS) is administered as a rapid infusion in a volume depleted patient to ensure haemodynamic stability. Conversely, a fluid that predominantly restores the ICF compartment (D5W) is administered to a dehydrated patient in a controlled fashion to prevent the development of cerebral oedema. Again, throughout the session, the terms "ECF volume depletion" and "intravascular volume depletion" are used to dispel referential ambiguity.

Analysis of the laboratory values: After the debriefing session, we focus on the laboratory investigation results of the two patients. This is done either at the computer station on the ward or in the classroom where lab values from electronic medical records are projected on to the screen. It is important to emphasize the dissimilarity between the urinary and serum biochemical values of the two patients. In the ECF volume depleted patient, the urine becomes concentrated and contains very little Na^+ consequent to renal conservation of salt and water. In the dehydrated patient, although urine is concentrated (due to water absorption in the distal tubule), urinary Na^+ is not decreased. In fact, hypernatremia in a dehydrated patient can augment renal natriuresis by mechanisms that appear to be independent of changes in atrial natriuretic peptide[33]. In ECF volume depletion, BUN/creatinine ratio rises due to renal hypo-perfusion with variable effects on serum sodium (depending upon the

type of fluid loss). In contrast, there is no significant increase in serum urea or creatinine in dehydration; the gold standard is hypernatremia and consequent hypertonicity[1,34].

Back to the patients: After going through the laboratory results, students go back to the patients to demonstrate physical signs. If the physical signs have resolved consequent to the treatment, then students review the medical file to note the physical findings at time of presentation. Important findings include orthostatic hypotension, tachycardia, prolonged capillary refill time and decreased skin turgor. These are defined to students as signs of ECF volume depletion, as dehydration cannot be reliably determined by use of clinical examination. Finally, to complete the chain of events in reverse chronological order, we focus on the modes of presentation of the two patients by reviewing their medical records. ECF volume depleted patients usually present with a history of blood-loss or gastrointestinal fluid loss, conditions that cause third-spacing (*e.g.*, ileus or pancreatitis) or sepsis (vasodilatation induced relative hypovolemia). On the other hand, since intracellular hydration influences cellular function, severely dehydrated patients may present with altered cognitive and neuromuscular function. It is noteworthy that altered mentation can be both a cause (by affecting the patient's ability to access water) and a consequence (due to the resultant deranged neuro-cellular function) of dehydration. Critically ill patients in the ICU are prone to develop dehydration because they are unconscious, sedated or ventilated and are hence unable to voluntarily control their free water intake.

After going through all the aspects of care for their respective patients, the two groups switch and examine the other patient using the same system. This session is usually shorter as it simply affirms the pre-discussed differences between the two clinical conditions, cementing the newly acquired knowledge in the students' minds. We find that by the end of this session, most students can appreciate the key differences between dehydration and volume depletion and the clinical implications of each.

Home assignment: The above stated clinical and biochemical differences invariably prompt analysis of different homeostatic mechanisms that operate in ECF volume depletion and dehydration. We direct students to carry out further reading to explore pathophysiological differences between the two disorders (Figure 2). We also provide them with additional learning resources that allow them to adequately explore the whole subject at their own comfortable pace to build up their new conceptual frameworks.

Debriefing session 2: On the following day, the pathophysiology of dehydration and volume depletion are addressed in the final session. Representatives from both student groups are invited to draw simple diagrams on a white board illustrating the homeostatic mechanisms. As they do this, it highlights to the audience that the sensors and effectors of both conditions are different (Figure 2); in dehydration, the osmoregulatory mechanisms are activated whereas in volume depletion the ECF volume regulatory pathways are stimulated to restore a normal physiologic environment. Students should then be able to link up the pathophysiological changes to the symptoms, signs and treatment of dehydration and volume depletion. They appreciate that the therapeutic manoeuvres mimic the response of normal kidneys to these conditions – kidneys retain water in dehydration, so we treat dehydration with water administration; kidneys retain salt and water in volume depletion, hence we infuse 0.9% NS to combat volume depletion.

In the second half of this session, students may share their perspectives on the whole topic. They also reflect on how the new concepts will resolve the problems that previously led to dissatisfaction in the management of such patients, and how their new understanding will benefit them in their future clinical experiences.

Create opportunities for the students to apply the new knowledge into routine clinical practice

New concepts that are not reflected upon often fade away rapidly. Therefore, opportunities are tactfully created for the students to continuously apply the new knowledge into clinical practice; acute medical admissions' wards are good places to see patients with ECF volume depletion whereas ICUs are good avenues to evaluate intubated and ventilated patients who are prone to dehydration. Multiple encounters with volume depleted and dehydrated patients allow the students to revise new concepts and train them to identify and treat these two contrasting conditions appropriately.

Another effective strategy is to have the advanced students in the team (fellows or senior residents who has successfully demonstrated proficiency in the subject) teach

the subject to the junior residents or interns. During this process, the senior students invest more personal effort into learning and thus consolidate deeper understandings. The faculty member acts as a facilitator who designs and supervises the learning environment as well as provides on-the-spot advice when required.

Preventing relapse

Dehydration/volume depletion misconceptions are prone to relapse. We regularly revisit these misconceptions through lectures, case discussions, publications and board review programmes. In our training programme, most residents from general medical teams rotate to nephrology. This opportunity is used to advance-train these residents in the field of fluid balance so that they may act as surrogate teachers to complement professional teaching by faculty. Finally, questions on our in-house exams are crafted to carefully test the conceptual framework of students rather than their ability to memorize facts.

CONCLUSION

The terms dehydration and volume depletion represent two fundamentally different clinical disorders. Erroneous concepts about these body fluid disorders are worryingly prevalent among medical students and physicians. A multi-pronged approach is required to wipe out these robust misconceptions. We strongly believe that the most effective strategy to instigate conceptual restructuring is by supplementing real life clinical experiences with reflective activities through active participation of students. We recommend that dehydration/volume depletion misconceptions be included in medical school curricula and textbooks so that medical students become aware of the common pitfalls of fluid status evaluation.

REFERENCES

1 **Mange K**, Matsuura D, Cizman B, Soto H, Ziyadeh FN, Goldfarb S, Neilson EG. Language guiding therapy: the case of dehydration versus volume depletion. *Ann Intern Med* 1997; **127**: 848-853 [PMID: 9382413 DOI: 10.7326/0003-4819-127-9-199711010-00020]
2 **AHIMA**. Summary of October 2004 ICD-9-CM Coordination and Maintenance Committee Meeting. Available from: URL: http://library.ahima.org/doc?oid=62385#.W83gHlUzaUl
3 **Chumlea WC**, Guo SS, Zeller CM, Reo NV, Baumgartner RN, Garry PJ, Wang J, Pierson RN Jr, Heymsfield SB, Siervogel RM. Total body water reference values and prediction equations for adults. *Kidney Int* 2001; **59**: 2250-2258 [PMID: 11380828 DOI: 10.1046/j.1523-1755.2001.00741.x]
4 **Watson PE**, Watson ID, Batt RD. Total body water volumes for adult males and females estimated from simple anthropometric measurements. *Am J Clin Nutr* 1980; **33**: 27-39 [PMID: 6986753 DOI: 10.1093/ajcn/33.1.27]
5 **Edelman IS**, Leibman J. Anatomy of body water and electrolytes. *Am J Med* 1959; **27**: 256-277 [PMID: 13819266 DOI: 10.1016/0002-9343(59)90346-8]
6 **Guyton AC**, Hall JE. The Body Fluids and Kidney. In: Textbook of medical physiology. 11th ed. Philadelphia: Elsevier Saunders, 2006: 292.
7 **Berl T**, Schrier RW. Disorders of water metabolism. In: Schrier RW, editor. Renal and Electrolyte Disorders. 6th ed. Philadelphia: Lippincott Williams Wilkins. 2003; 1-63
8 **Freda BJ**, Davidson MB, Hall PM. Evaluation of hyponatremia: a little physiology goes a long way. *Cleve Clin J Med* 2004; **71**: 639-650 [PMID: 15449759 DOI: 10.1097/00000441-200408000-00010]
9 **Ritz P**; Source Study. Bioelectrical impedance analysis estimation of water compartments in elderly diseased patients: the source study. *J Gerontol A Biol Sci Med Sci* 2001; **56**: M344-M348 [PMID: 11382792 DOI: 10.1093/gerona/56.6.M344]
10 **O'Brien C**, Baker-Fulco CJ, Young AJ, Sawka MN. Bioimpedance assessment of hypohydration. *Med Sci Sports Exerc* 1999; **31**: 1466-1471 [PMID: 10527321 DOI: 10.1097/00005768-199910000-00017]
11 **Olde Rikkert MG**, Deurenberg P, Jansen RW, van't Hof MA, Hoefnagels WH. Validation of multi-frequency bioelectrical impedance analysis in detecting changes in fluid balance of geriatric patients. *J Am Geriatr Soc* 1997; **45**: 1345-1351 [PMID: 9361660 DOI: 10.1111/j.1532-5415.1997.tb02934.x]
12 **Feig PU**, McCurdy DK. The hypertonic state. *N Engl J Med* 1977; **297**: 1444-1454 [PMID: 337143 DOI: 10.1056/NEJM197712292972608]
13 **Rose BD**, Post TW. Introduction to disorders of osmolality. In: Clinical physiology of acid-base and electrolyte disorders. 5th ed. . New York: McGraw-Hill; 2001; 683
14 **Steiner MJ**, DeWalt DA, Byerley JS. Is this child dehydrated? *JAMA* 2004; **291**: 2746-2754 [PMID: 15187057 DOI: 10.1001/jama.291.22.2746]
15 **Gorelick MH**, Shaw KN, Murphy KO. Validity and reliability of clinical signs in the diagnosis of dehydration in children. *Pediatrics* 1997; **99**: E6 [PMID: 9113963 DOI: 10.1542/peds.99.5.e6]
16 **McGee S**, Abernethy WB 3rd, Simel DL. The rational clinical examination. Is this patient hypovolemic? *JAMA* 1999; **281**: 1022-1029 [PMID: 10086438 DOI: 10.1001/jama.281.11.1022]
17 **Gross CR**, Lindquist RD, Woolley AC, Granieri R, Allard K, Webster B. Clinical indicators of dehydration severity in elderly patients. *J Emerg Med* 1992; **10**: 267-274 [PMID: 1624737 DOI: 10.1016/0736-4679(92)90331-M]

18 **Thomas DR**, Tariq SH, Makhdomm S, Haddad R, Moinuddin A. Physician misdiagnosis of dehydration in older adults. *J Am Med Dir Assoc* 2003; **4**: 251-254 [PMID: 12959652 DOI: 10.1097/01.JAM.0000083444.46985.16]
19 **LARON Z**. Skin turgor as a quantitative index of dehydration in children. *Pediatrics* 1957; **19**: 816-822 [PMID: 13431306 DOI: 10.1016/S0022-3476(57)80319-9]
20 **Dorrington KL**. Skin turgor: do we understand the clinical sign? *Lancet* 1981; **1**: 264-266 [PMID: 6109910 DOI: 10.1016/S0140-6736(81)90519-5]
21 **Kerpel-Fronius E**. Über die Beziehungen zwischen Salz- und Wasserhaushalt bei experimentellen Wasserverlusten. *Ztschr f Kinderh* 1935; **57**: 489 [DOI: 10.1007/BF02253151]
22 **Nadal JW**, Pedersen S, Maddock WG. A comparison between dehydration from salt loss and from water deprivation. *J Clin Invest* 1941; **20**: 691-703 [PMID: 16694874 DOI: 10.1172/JCI101262]
23 **Ballestero Y**, Hernandez MI, Rojo P, Manzanares J, Nebreda V, Carbajosa H, Infante E, Baro M. Hyponatremic dehydration as a presentation of cystic fibrosis. *Pediatr Emerg Care* 2006; **22**: 725-727 [PMID: 17110865 DOI: 10.1097/01.pec.0000245170.31343.bb]
24 **Finberg L**, Bernstein J. Acute hyponatremic dehydration. *J Pediatr* 1971; **79**: 499-503 [PMID: 5567975 DOI: 10.1016/S0022-3476(71)80166-X]
25 **Caksen H**, Odabaş D, Sar S, Celebi V, Arslan S, Kuru M, Abuhandan M. Hyponatremic dehydration: an analysis of 78 cases. *Int Urol Nephrol* 2001; **33**: 445-448 [PMID: 12230268 DOI: 10.1023/a:1019563222488]
26 **Grisanti KA**, Jaffe DM. Dehydration syndromes. Oral rehydration and fluid replacement. *Emerg Med Clin North Am* 1991; **9**: 565-588 [PMID: 2070768]
27 **Moritz ML**, Ayus JC. Improving intravenous fluid therapy in children with gastroenteritis. *Pediatr Nephrol* 2010; **25**: 1383-1384 [PMID: 20309584 DOI: 10.1007/s00467-010-1505-2]
28 **Sarhill N**, Walsh D, Nelson K, Davis M. Evaluation and treatment of cancer-related fluid deficits: volume depletion and dehydration. *Support Care Cancer* 2001; **9**: 408-419 [PMID: 11585267 DOI: 10.1007/s005200100251]
29 **Berk L**, Rana S. Hypovolemia and dehydration in the oncology patient. *J Support Oncol* 2006; **4**: 447-54; discussion 455-7 [PMID: 17080733]
30 **Leaf A**. The clinical and physiologic significance of the serum sodium concentration. *N Engl J Med* 1962; **267**: 24-30 contd [PMID: 14463225 DOI: 10.1056/NEJM196207052670106]
31 **Leaf A**. The clinical and physiologic significance of the serum sodium concentration. *N Engl J Med* 1962; **267**: 77-83 concl [PMID: 14463226 DOI: 10.1056/NEJM196207122670206]
32 **Duggan C**, Refat M, Hashem M, Wolff M, Fayad I, Santosham M. How valid are clinical signs of dehydration in infants? *J Pediatr Gastroenterol Nutr* 1996; **22**: 56-61 [PMID: 8788288 DOI: 10.1097/00005176-199601000-00009]
33 **Andersen LJ**, Andersen JL, Pump B, Bie P. Natriuresis induced by mild hypernatremia in humans. *Am J Physiol Regul Integr Comp Physiol* 2002; **282**: R1754-R1761 [PMID: 12010758 DOI: 10.1152/ajpregu.00732.2001]
34 **Armstrong LE**. Hydration assessment techniques. *Nutr Rev* 2005; **63**: S40-S54 [PMID: 16028571 DOI: 10.1111/j.1753-4887.2005.tb00153.x]

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Observational Study

33 Associations of renal function with diabetic retinopathy and visual impairment in type 2 diabetes: A multicenter nationwide cross-sectional study

Kaewput W, Thongprayoon C, Rangsin R, Ruangkanchanasetr P, Mao MA, Cheungpasitporn W

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ORIGINAL ARTICLE

Observational Study

Associations of renal function with diabetic retinopathy and visual impairment in type 2 diabetes: A multicenter nationwide cross-sectional study

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Abstract

BACKGROUND

Diabetic retinopathy (DR) separately has been noted as a major public health problem worldwide as well. Currently, many studies have demonstrated an association between diabetic nephropathy and DR in type 1 diabetes mellitus (T1DM) patients, but this association is less strong in T2DM. The evidence for an association between renal function and DR and visual impairment among T2DM patients is limited, particularly in the Asian population.

AIM

To assess the association between glomerular filtration rate (GFR) and DR, severe DR, and severe visual impairment among T2DM patients in Thailand.

METHODS

We conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. This study evaluated adult T2DM patients from 831 public hospitals in Thailand in the year 2013. GFR was categorized into ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2. The association between GFR and DR, severe DR, and severe visual impairment

were assessed using multivariate logistic regression.

RESULTS

A total of 13192 T2DM patients with available GFR were included in the analysis. The mean GFR was 66.9 ± 25.8 mL/min/1.73 m^2. The prevalence of DR, proliferative DR, diabetic macular edema, and severe visual impairment were 12.4%, 1.8%, 0.2%, and 2.1%, respectively. Patients with GFR of 60-89, 30-59 and < 30 mL/min/1.73 m^2 were significantly associated with increased DR and severe DR when compared with patients with GFR of ≥ 90 mL/min/1.73 m^2. In addition, increased severe visual impairment was associated with GFR 30-59 and < 30 mL/min/1.73 m^2.

CONCLUSION

Decreased GFR was independently associated with increased DR, severe DR, and severe visual impairment. GFR should be monitored in diabetic patients for DR awareness and prevention.

Key words: Diabetic retinopathy; Visual impairment; Glomerular filtration rate; Type 2 diabetes

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Core tip: Currently, the associations of renal function with diabetic retinopathy (DR), its severity, and severe visual impairment in type 2 diabetes mellitus (DM) are inconclusive, especially in the Asian population. In this study, we conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. We demonstrated that decreased glomerular filtration rate (GFR) was independently associated with increased DR, severe DR, and severe visual impairment. GFR should be monitored in diabetic patients for DR awareness and prevention.

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INTRODUCTION

Type 2 diabetes mellitus (DM) is a common chronic disease worldwide[1] that poses a major crisis in the global health system[2]. The associated morbidity and mortality can be caused by the disease itself or its complications. Severe and not uncommon complications include chronic kidney disease (CKD) and diabetic retinopathy (DR). DR separately has been noted as a major public health problem worldwide as well[3,4]. The associated problems include disability, increased healthcare costs, and socioeconomic burdens[5-8].

Currently, many studies have demonstrated an association between diabetic nephropathy and DR in T1DM patients, but this association is less strong in T2DM[9,10]. Hence, currently the associations of renal function with DR, its severity, and severe visual impairment vision in T2DM are inconclusive[11], especially in the Asian population[12,13]. If such an association of renal function with DR and visual impairment existed, it would provide further support to the importance for regular monitoring of glomerular filtration rate (GFR) in T2DM patients. Furthermore, it would motivate future research on whether more intensive monitoring of T2DM patients may allow earlier detection and prevention of DR and visual impairment. Visual impairment secondary to DR can be corrected if early sight threatening disease is detected and treated with laser photocoagulation.

This study was thus aimed to determine whether such an association between GFR with DR and visual impairment exists, and if so the prevalence and magnitude of this association.

the Consortium of Thai Medical Schools to adjudicate that the process of data collection was compiled according to study protocol. The data management team was responsible for inquiries to study sites to verify data. Site monitoring was randomly performed in approximately 10% of study sites.

Informed consent statement: Patients were all patients were recruited from the outpatient clinic. Written informed consent was obtained from patients before enrolment.

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MATERIALS AND METHODS

Study design and population

This was a nationwide, multi-center, cross-sectional study in Thailand *via* secondary analysis of the DM/HT dataset in 2013[14]. This dataset was a nationwide survey conducted annually in Thailand to evaluate the status of medical care in T2DM patients who visited the public hospitals of the Thai Ministry of Public Health and the clinics in the Thailand National Health Security Office's program. The inclusion criteria of this DM/HT survey consisted of T2DM patients aged ≥ 35 years who received regular medical care in the targeted hospitals and clinics (n = 831) for at least 12 mo. Patients who received care at primary care units outside of Bangkok and university hospitals were excluded from the study. A two-stage stratified cluster sampling method was used to select a nationally and provincially representative sample of T2DM patients in Thailand. The first stage of sample collection consisted of the provinces that constituted 77 strata. The second stage of sample collection was the hospitals' levels in each province, which were stratified into 5 strata according to the size of the hospital. These 5 strata were regional (> 500 beds), provincial (200-500 beds), large community (80-120 beds), medium community (60 beds), and small community (10-30 beds) hospitals. All regional (n = 25), provincial (n = 70), and community (n = 736) hospitals were included. Of 736 community hospitals, 10%, 20%, and 70% were large, medium and small community hospitals, respectively. For the objectives of this study to assess the association between GFR and DR and visual impairment, we included only patients with available eye examination data in the analysis.

All patients were recruited from the outpatient clinic. Written informed consent was obtained from patients before enrolment. This study was approved by both the Institutional Review Board of the Royal Thai Army Medical Department and the Ethical Review Committee for Research in Human Subjects, the Ministry of Public Health of Thailand. Well-trained research nurses reviewed medical records and collected data into a case record form. Data entry into the case record form was then transferred to the central data management of the Medical Research Network of the Consortium of Thai Medical Schools to adjudicate that the process of data collection was compiled according to study protocol. The data management team was responsible for inquiries to study sites to verify data. Site monitoring was randomly performed in approximately 10% of study sites. This study was conducted by the Strengthening the Reporting of Observational Studies in Epidemiology[15].

Data collection

Clinical characteristics, demographic information, medication, and laboratory data were collected using manual data retrieval from the medical record as described above. The laboratory data consisted of the 12 mo results prior to the consent process. GFR was estimated based on age, sex, race and the most recent creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation[16]. Primary outcome was the diagnosis of DR. DR was diagnosed by ophthalmologists and was identified by ICD10 codes H36.0x. The technique for eye examination was fundus photography by digital camera with interpretation performed by ophthalmologists. The diagnosis of DR was then stratified into: (1) non-proliferative DR; (2) proliferative DR; (3) diabetic macular edema; and (4) non-specific DR. Non-specific DR were defined as DR without available staging data in the medical record. Severe DR was defined as both proliferative DR and macular edema. Visual impairment was based on visual acuity (VA) exam as documented by physicians. Severe visual impairment was defined as a VA exam that consisted of "counting fingers", "hand motions", "projection of light" and "no light perception".

Statistical analysis

Continuous variables were presented as mean ± SD. Categorical variables were presented as count with percentage. Clinical characteristics and outcomes were compared among different estimated GFR (eGFR) group, using analysis of variance for continuous variables and the chi-square test for categorical variables. eGFR was categorized into 4 groups; ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2. GFR of ≥ 90 mL/min/1.73 m^2 was selected as the reference group. Univariate and then multivariate logistic regression analysis, adjusting for priori-defined variables, was performed to assess the independent association between GFR and DR, severe DR (proliferative DR and macular edema), and severe visual impairment. Odds ratio (OR) with 95% confidence interval (CI) was reported. The adjusted variables were age, sex, smoking, waist circumference, duration of diabetes, comorbidities and medications. Comorbidities were hypertension, coronary artery disease, stroke, and peripheral artery disease. Medications were metformin, sulfonylurea, insulin, and antiplatelet

medication. A *P*-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL, United States).

RESULTS

Baseline characteristics

A total of 13192 adult T2DM patients with available GFR and eye examination data from Thailand public hospitals were included in this analysis. The clinical characteristics are summarized in Table 1. The mean age was 60.7 ± 10.3 years, and 30.4% were male. The mean diabetic duration was 7.4 ± 4.8 years. The mean eGFR was 67.4 ± 25.7 mL/min/1.73 m^2. Patients with lower GFR were significantly older, female, had longer diabetic duration, higher prevalence of hypertension, coronary artery disease, stroke, peripheral artery disease; and higher medication use of antiplatelets and insulin compared to patients with higher GFR. Lower GFR was additionally associated with lower smoking rates, waist circumference and medication use of metformin and sulfonylureas (Table 1).

The prevalence of all DR, non-proliferative DR, proliferative DR, diabetic macular edema, and severe visual impairment were 12.4%, 10.0%, 1.8%, 0.2%, and 2.1%, respectively (Table 2).

The association between GFR and DR

The prevalence of DR was 9.5%, 11.0%, 14.1%, and 20.9% in patients with GFR of ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2, respectively (*P* < 0.001) (Table 2). In adjusted analysis, GFR of 60-89, 30-59 and < 30 mL/min/1.73 m^2 were associated with an increased OR of 1.36 (95%CI: 1.12-1.65), 2.08 (95%CI: 1.70-2.55), and 2.38 (95%CI: 1.79-3.18), respectively, for DR (Table 3).

The association between GFR and severe DR

The prevalence of proliferative DR was 1.1%, 1.3%, 2.2%, and 5.2% in patients with GFR of ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2, respectively (*P* < 0.001) (Table 2). The prevalence of diabetic macular edema was 0.0%, 0.2%, 0.2%, and 0.4% in patients with GFR of ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2, respectively (*P* < 0.06) (Table 2). The prevalence of severe DR defined as proliferative DR with severe macular edema was 1.1%, 1.4%, 2.3%, and 5.5% in patients with GFR of ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2, respectively (*P* < 0.001) (Table 2). In adjusted analysis, GFR of 60-89, 30-59 and < 30 mL/min/1.73 m^2 was associated with an increased OR of 1.95 (95%CI: 1.14-3.33), 2.82 (95%CI: 1.61-4.93), and 4.89 (95%CI: 2.47-9.67), respectively for severe DR (Table 4).

The association between GFR and severe visual impairment

The prevalence of severe visual impairment was 0.8%, 1.5%, 3.2%, and 4.8% in patients with GFR of ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2, respectively (*P* < 0.001) (Table 2). In adjusted analysis, GFR of 30-59 and < 30 mL/min/1.73 m^2 was associated with an increased OR of 2.06 (95%CI: 1.22-3.46), and 2.50 (95%CI: 1.31-4.76), respectively for severe visual impairment (Table 4).

DISCUSSION

Analysis of the baseline patient characteristics showed that a lower GFR was associated with lower smoking rates, waist circumference and medication use of metformin and sulfonylureas. This finding could be explained by medical providers' intervention to slow the GFR decline in Thailand by recommending smoking cessation and weight reduction in the obese, or perhaps by the anorexia and associated reduced caloric intake in patients with advanced CKD and uremia. The decrease in metformin and sulfonylureas usage may reflect discontinuation of the medication as age progresses or from concerns for increased risk with renal impairment.

Prevalence of DR, severe DR (proliferative DR and macular edema) and severe visual impairment

Available prior studies have reported an overall prevalence of DR in Thailand ranging between 13.6%-31.2%[17,18], and they mostly involved non-proliferative DR. The previous results varied slightly according to the study setting and diagnostic criteria. Among the studies that used clinical fundoscopy, the prevalence of DR ranged from

Table 1 Baseline characteristics *n* (%)

Characteristics	All	eGFR (mL/min/1.73 m²)				*P*-value
		≥ 90	60-89	30-59	< 30	
N	13192	2979 (22.6)	4870 (36.9)	4397 (33.3)	946 (7.2)	
Age (yr), mean ± SD	60.7 ± 10.3	54.1 ± 8.5	59.4 ± 9.6	65.4 ± 9.2	66.0 ± 10.0	< 0.001
Male	4012 (30.4)	860 (28.9)	1587 (32.6)	1303 (29.6)	262 (27.7)	< 0.001
Duration of diabetes (yr), mean ± SD	7.4 ± 4.8	6.1 ± 4.1	7.0 ± 4.5	8.3 ± 5.1	9.1 ± 5.4	< 0.001
Waist circumference (cm), mean ± SD	88.6 ± 10.4	88.9 ± 10.6	88.7 ± 10.3	88.5 ± 10.3	87.4 ± 101.6	< 0.01
Hypertension	9741 (73.8)	1935 (65.0)	3466 (71.2)	3575 (81.3)	765 (80.9)	< 0.001
Smoking	467 (3.5)	127 (4.3)	203 (4.2)	117 (2.7)	20 (2.1)	< 0.001
CAD	621 (4.7)	76 (2.6)	205 (4.2)	269 (6.1)	71 (7.5)	< 0.001
CVD	273 (2.1)	42 (1.4)	94 (1.9)	104 (2.4)	33 (3.5)	< 0.001
PAD	24 (0.2)	5 (0.2)	5 (0.1)	8 (0.2)	6 (0.6)	< 0.01
Antiplatelets	8067 (61.4)	1569 (52.9)	2974 (61.3)	2923 (66.8)	601 (64.0)	< 0.001
Metformin	9756 (74.0)	2592 (87.0)	4066 (83.5)	2894 (65.8)	204 (21.6)	< 0.001
Sulfonylurea	8751 (66.3)	2070 (69.5)	3460 (71.0)	2882 (65.5)	339 (35.8)	< 0.001
Insulin	2866 (21.7)	493 (16.5)	765 (15.7)	1050 (23.9)	558 (59.0)	< 0.001

eGFR: Estimated glomerular filtration rate; CAD: Coronary artery diseases; CVD: Cerebrovascular diseases; PAD: Peripheral artery disease.

12.2%-37%[19,20]. Among the studies that used fundus photography, the prevalence of DR ranged from 10.1%-48.1%[21-24]. In newly diagnosed diabetes, the prevalence of DR ranged from 1.5%-28.6%[25]. Generally, the prevalence of DR at diagnosis of T1DM is reported low between 0-3% while those with newly diagnosed T2DM have a higher prevalence of DR 6.7%-30.2%[26]. In this study, the lower prevalence of DR, severe DR and severe visual impairment in Thailand found may be due to multiple reasons: limited access to ophthalmologists, inclusion of earlier diabetic cases, use of different diagnostic criteria, limitations in the technique of eye examination (such as the images produced were two-dimensional unlike the three-dimensional from indirect binocular ophthalmoscopy or the lower magnification and image clarity compared to indirect ophthalmoscopy), and exclusion of primary care units and university hospitals patients. The care for patients in university hospitals might be different from other public hospitals as these patients' medical conditions were likely more complicated. The tertiary and university hospitals may also possess greater availability of medical experts that allow a more comprehensive diagnosis and thus capture of these patients of interest. In public hospitals, this may have led to underdiagnosis of DR patients, resulting in the lower prevalence described.

The association between GFR and DR, severe DR and severe visual impairment

The present study showed that eGFR by using the CKD-EPI formula is independently associated with DR in adult T2DM patients. This study's results are similar to previous reports from non-Asian ethnicity; Penno *et al*[27] and Grunwald *et al*[28] studies, which found an independent inverse correlation between eGFR and DR. We found that patients with impaired renal function also were associated with an increased likelihood of DR compared to patients with normal renal function. The pathogenesis of DR and nephropathy is due to microvascular disease. Prior studies have demonstrated that DR and DN have a common pathological basis[29] and a similar course of elucidation. Hyperglycemia causes glomerular hyper perfusion leading to an increase in GFR during the early stages of T2DM[30]. The accumulation of advanced glycation end products (AGEs) due to hyperglycemia also promotes mesangial proliferation and basement membrane thickening in the glomerulus. Furthermore, multiple pathways such as the activation of the polyol, the protein kinase C, the pentose phosphate[31], oxidative stress, and various cytokines can cause a range of kidney pathology, which includes capillary obstruction, reduction of podocyte proliferation, podocyte death, and urinary protein loss. These can lead to a subsequent decline in renal function. The pathophysiology is similar to those observed in the retina. Hyperglycemia produces deleterious effects on the retina, such as apoptosis of Muller cells, ganglion cells[32], and pericytes; thickening of the capillary basement membrane, and proliferation of endothelial cells in the retina. These effects lead to the pathological changes in DR and threaten a patient's vision. Therefore, GFR not only may be an important clinical marker for DN, but could be correlated with

Table 2 Prevalence and severity of diabetic retinopathy stratified by estimated glomerular filtration rate level (mL/min/1.73 m^2) *n* (%)

Outcomes	All	eGFR (mL/min/1.73 m^2)				*P*-value
		≥ 90	60-89	30-59	< 30	
DR	1636 (12.4)	284 (9.5)	534 (11.0)	620 (14.1)	198 (20.9)	< 0.001
Non-proliferative DR	1313 (10.0)	242 (8.1)	438 (9.0)	498 (11.3)	135 (14.3)	< 0.001
Proliferative DR	240 (1.8)	33 (1.1)	63 (1.3)	95 (2.2)	49 (5.2)	< 0.001
Diabetic macular edema	21 (0.2)	1 (0.0)	8 (0.2)	8 (0.2)	4 (0.4)	0.06
Non-specific DR	84 (0.6)	9 (0.3)	33 (0.7)	27 (0.6)	15 (1.6)	< 0.001
Severe DR	258 (2.0)	34 (1.1)	70 (1.4)	102 (2.3)	52 (5.5)	< 0.001
Severe visual impairment	281 (2.1)	24 (0.8)	72 (1.5)	140 (3.2)	45 (4.8)	< 0.001
Counting finger	138 (1.0)	7 (0.2)	43 (0.9)	72 (1.6)	16 (1.7)	< 0.001
Hand movement	77 (0.6)	10 (0.3)	17 (0.3)	35 (0.8)	15 (1.6)	< 0.001
Projection of light	14 (0.1)	0 (0)	5 (0.1)	6 (0.1)	3 (0.3)	0.06
No light perception	56 (0.4)	7 (0.2)	8 (0.2)	30 (0.7)	11 (1.2)	< 0.001

DR: Diabetic retinopathy; eGFR: Estimated glomerular filtration rate.

DR[33]. It is worth noting that the prior studies that demonstrated a lack of significant independent association between eGFR and DR in T2DM (Chen *et al*[12] and Sabanayagam *et al*[13]) were conducted among participants of Asian ethnicity. Contrarily, Penno *et al*[27] and Grunwald *et al*[28] studies had reported an "inverse correlation between eGFR and DR" in a study population of non-Asian ethnicity. Our study is the first study that has shown a significant independent inverse correlation between eGFR and DR in Asian patients, and thus opposes the possibility of ethnic differences as previously suggested in the prior available literature[11,34].

This current study showed an association of GFR with severe stages of DR. Moreover, even patients with mild to moderate impaired renal function were more likely to have severe stages of DR compared to patients with normal renal function. This study's results are similar to Man *et al*[11], Pugliese *et al*[35] and Penno *et al*[27] studies which demonstrated an association of GFR with severe DR. This association is potentially due to a shared common pathogenesis as described above. These phenomena further confirm a likely causative relationship as strength of association and biologic gradient. The current study also showed the association of GFR by using CKD-EPI formula and severe visual impairment in T2DM patients. Moreover, patients with CKD also were more likely to have severe visual impairment compared to patients with normal renal function. The likelihood of severe visual impairment was higher with increasing severity of CKD in diabetic patients. The current study's results are similar to Wong *et al*[36]. This could also be explained by shared common risk factors for ocular and kidney disease [T2DM, age, smoking, hypertension (HTN), dyslipidemia and obesity] and shared common pathogenic mechanisms of sight-threatening conditions (any retinopathy and cataract) that are also present in persons with diabetes and CKD such as accumulation of AGEs[37-40], vitamin D deficiency[41], and vascular endothelial growth factor driven angiogenesis[42,43].

Strengths

The present study provides information from a large nationwide multicenter study consisting of adult T2DM Thailand patients. The study measures severe visual impairment by using a semi-quantitative clinical scale composed of bedside testing, which is easy to perform in the community setting. Our analysis for an association between eGFR and DR utilized a multiple logistic regression model that included several possible confounders such as age, gender, smoking, waist circumference, duration of diabetes, comorbidities and medications. Comorbidities consisted of HTN, coronary artery diseases, cerebrovascular diseases and peripheral artery disease. In the final model adjustment, medications adjusted for included sulfonylurea, metformin, insulin, and antiplatelets. Lastly, use of the CKD-EPI eGFR formula is applicable to real-world practice for renal function evaluation in Thailand. The CKD-EPI creatinine equation is also arguably superior to the Cockcroft-Gault formula[44] and the Modification of Diet in Renal Disease Study equation[45] and could replace them for routine clinical use.

Limitations

Table 3 Association of estimated glomerular filtration rate level and diabetic retinopathy

eGFR (mL/min/1.73m^2)	Crude OR	95%CI	*P*-value	Adjusted OR[1]	95%CI	*P*-value
≥ 90	1 (reference)			1 (reference)		
60-89	1.17	1.01-1.36	0.04	1.36	1.12-1.65	< 0.01
30-59	1.56	1.34-1.81	< 0.001	2.08	1.70-2.55	< 0.001
< 30	2.51	2.06-3.06	< 0.001	2.38	1.79-3.18	< 0.001

[1]Adjusted for age, gender, duration of diabetes, waist circumference, hypertension, smoking, coronary artery diseases, cerebrovascular diseases, peripheral artery disease, antiplatelet, metformin, sulfonylurea, and insulin. eGFR: Estimated glomerular filtration rate.

The limitations of this study include the following. First, the study population does not include patients from university hospitals. Consequently, the prevalence of DR may be significantly underestimated as noted above. Second, data collection was performed using a retrospective medical record review; therefore, incomplete data records with missing diagnoses cannot be verified. Third, this study measured severe visual impairment by using the semi-quantitative clinical scale. Variations in the technique for eye examinations may be confounding factors. Fourth, this study did not classify causes of visual impairment such as cataract, glaucoma and age-related macular degeneration[42,46]. Finally, urine albumin values from the parent dataset were assessed in markedly different laboratory methods, such as urine dipstick, 24 h urine protein measurement, and timed urine protein collection. Furthermore, some urine albumin values were missing. Accordingly, we did not include this variable in the analytic model. Several reports have demonstrated however that urine albumin is an independent association with DR in diabetic patients[12].

Implication

The eGFR by using CKD-EPI formula should be additionally utilized for monitoring risk for DR, severe DR and severe visual impairment in T2DM patients. The importance of timely and frequent eye screening in T2DM and CKD patients cannot be over-emphasized. The early intervention to preserve renal function and adequate control of diabetes is keys to the reduction of ocular disease and prevent irreversible visual loss.

In conclusion, renal function was independently associated with DR, severe DR and severe visual impairment in T2DM patients. Renal function by eGFR should be closely monitored in T2DM patients as it may provide the clinician an additional risk marker to earlier detect, prevent and treat DR. Further studies are needed to demonstrate whether this will translate to improved clinical outcomes.

Table 4 Subgroup analysis comparing the association of estimated glomerular filtration rate level with severe diabetic retinopathy and severe visual impairment

eGFR (mL/min/1.73 m²)	Crude OR	95%CI	*P*-value	Adjusted OR[1]	95%CI	*P*-value
Proliferative diabetic retinopathy and macular edema						
≥ 90		1 (reference)			1 (reference)	
60-89	1.26	0.84-1.91	0.27	1.95	1.14-3.33	0.02
30-59	2.06	1.39-3.04	< 0.001	2.82	1.61-4.93	< 0.001
< 30	5.04	3.25-7.81	< 0.001	4.89	2.47-9.67	< 0.001
Severe visual impairment						
≥ 90		1 (reference)			1 (reference)	
60-89	1.85	1.16-2.94	0.01	1.07	0.62-1.82	0.81
30-59	4.05	2.62-6.26	< 0.001	2.06	1.22-3.46	< 0.01
< 30	6.15	3.73-10.15	< 0.001	2.50	1.31-4.76	< 0.01

[1]Adjusted for age, gender, duration of diabetes, waist circumference, hypertension, smoking, coronary artery diseases, cerebrovascular diseases, peripheral artery disease, antiplatelet, metformin, sulfonylurea, and insulin. eGFR: Estimated glomerular filtration rate.

ARTICLE HIGHLIGHTS

Research background

The evidence for an association of renal function with diabetic retinopathy (DR) and visual impairment among type 2 diabetes mellitus (T2DM) patients is limited, particularly in the Asian population. Currently, many studies have demonstrated an association between diabetic nephropathy and DR in T1DM patients, but this association is less strong in T2DM. This study aimed to assess the association between glomerular filtration rate (GFR) and DR, severe DR, and severe visual impairment among T2DM patients in Thailand.

Research motivation

If such an association of renal function with DR and visual impairment existed, it would provide further support to the importance for regular monitoring of GFR in T2DM patients. Furthermore, it would motivate future research on whether more intensive monitoring of T2DM patients may allow earlier detection and prevention of DR and visual impairment. Visual impairment secondary to DR can be corrected if early sight threatening disease is detected and treated with laser photocoagulation. To further investigate the association between GFR and DR, severe DR, and severe visual impairment were assessed using multivariate logistic regression, the authors conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools.

Research objectives

We conducted this study to determine whether such an association between GFR with DR and visual impairment exists, and if so the prevalence and magnitude of this association.

Research methods

We conducted a nationwide cross-sectional study based on the DM/HT study of the Medical Research Network of the Consortium of Thai Medical Schools. This study evaluated adult T2DM patients from 831 public hospitals in Thailand in the year 2013. GFR was categorized into ≥ 90, 60-89, 30-59 and < 30 mL/min/1.73 m^2. The association between GFR and DR, severe DR, and severe visual impairment were assessed using multivariate logistic regression.

Research results

In this study, a total of 13192 T2DM patients with available GFR were included in the analysis. The mean GFR was 66.9 ± 25.8 mL/min/1.73 m^2. The prevalence of DR, proliferative DR, diabetic macular edema, and severe visual impairment were 12.4%, 1.8%, 0.2%, and 2.1%, respectively. Patients with GFR of 60-89, 30-59 and < 30 mL/min/1.73 m^2 were significantly associated with increased DR and severe DR when compared with patients with GFR of ≥ 90 mL/min/1.73 m^2. In addition, increased severe visual impairment was associated with GFR 30-59 and < 30 mL/min/1.73 m^2.

Research conclusions

We found that decreased GFR was independently associated with increased DR, severe DR, and severe visual impairment. GFR should be monitored in diabetic patients for DR awareness and prevention.

Research perspectives

This study demonstrated significantly associations of decreased GFR with increased DR, severe DR, and severe visual impairment among diabetic patients. This finding suggests the importance

of timely and frequent eye screening in T2DM and CKD patients cannot be over-emphasized. The early intervention to preserve renal function and adequate control of diabetes is keys to the reduction of ocular disease and prevent irreversible visual loss.

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REFERENCES

1 **Ogurtsova K**, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017; **128**: 40-50 [PMID: 28437734 DOI: 10.1016/j.diabres.2017.03.024]
2 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]
3 **Yau JW**, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012; **35**: 556-564 [PMID: 22301125 DOI: 10.2337/dc11-1909]
4 **Sivaprasad S**, Gupta B, Crosby-Nwaobi R, Evans J. Prevalence of diabetic retinopathy in various ethnic groups: a worldwide perspective. *Surv Ophthalmol* 2012; **57**: 347-370 [PMID: 22542913 DOI: 10.1016/j.survophthal.2012.01.004]
5 **Kapur A**, Harries AD. The double burden of diabetes and tuberculosis - public health implications. *Diabetes Res Clin Pract* 2013; **101**: 10-19 [PMID: 23305899 DOI: 10.1016/j.diabres.2012.12.001]
6 **Corriere M**, Rooparinesingh N, Kalyani RR. Epidemiology of diabetes and diabetes complications in the elderly: an emerging public health burden. *Curr Diab Rep* 2013; **13**: 805-813 [PMID: 24018732 DOI: 10.1007/s11892-013-0425-5]
7 **Laxy M**, Hunger M, Stark R, Meisinger C, Kirchberger I, Heier M, von Scheidt W, Holle R. The Burden of Diabetes Mellitus in Patients with Coronary Heart Disease: A Methodological Approach to Assess Quality-Adjusted Life-Years Based on Individual-Level Longitudinal Survey Data. *Value Health* 2015; **18**: 969-976 [PMID: 26686780 DOI: 10.1016/j.jval.2015.07.003]
8 **Costa AF**, Flor LS, Campos MR, Oliveira AF, Costa MF, Silva RS, Lobato LC, Schramm JM. Burden of type 2 diabetes mellitus in Brazil. *Cad Saude Publica* 2017; **33**: e00197915 [PMID: 28380131 DOI: 10.1590/0102-311X00197915]
9 **Pedro RA**, Ramon SA, Marc BB, Juan FB, Isabel MM. Prevalence and relationship between diabetic retinopathy and nephropathy, and its risk factors in the North-East of Spain, a population-based study. *Ophthalmic Epidemiol* 2010; **17**: 251-265 [PMID: 20642348 DOI: 10.3109/09286586.2010.498661]
10 **Wolf G**, Müller N, Mandecka A, Müller UA. Association of diabetic retinopathy and renal function in patients with types 1 and 2 diabetes mellitus. *Clin Nephrol* 2007; **68**: 81-86 [PMID: 17722706 DOI: 10.5414/CNP68081]
11 **Man RE**, Sasongko MB, Wang JJ, MacIsaac R, Wong TY, Sabanayagam C, Lamoureux EL. The Association of Estimated Glomerular Filtration Rate With Diabetic Retinopathy and Macular Edema. *Invest Ophthalmol Vis Sci* 2015; **56**: 4810-4816 [PMID: 26218909 DOI: 10.1167/iovs.15-16987]
12 **Chen YH**, Chen HS, Tarng DC. More impact of microalbuminuria on retinopathy than moderately reduced GFR among type 2 diabetic patients. *Diabetes Care* 2012; **35**: 803-808 [PMID: 22338100 DOI: 10.2337/dc11-1955]
13 **Sabanayagam C**, Foo VH, Ikram MK, Huang H, Lim SC, Lamoureux EL, Tai ES, Wong TY. Is chronic kidney disease associated with diabetic retinopathy in Asian adults? *J Diabetes* 2014; **6**: 556-563 [PMID: 24636277 DOI: 10.1111/1753-0407.12148]
14 **Medical Research Network of the Consortium of Thai Medical Schools: MedResNet (Thailand)**. Data Archival for Maximum Utilization System (DAMUS). DM/HT study (NHSO Research Project). 2013; Available from: URL: http://www.damus.in.th/damus/index.php
15 **von Elm E**, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *PLoS Med* 2007; **4**: e296 [PMID: 17941714 DOI: 10.1371/journal.pmed.0040296]
16 **Levey AS**, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604-612 [PMID: 19414839 DOI: 10.7326/0003-4819-150-9-200905050-00006]
17 **Reutrakul S**, Deerochanawong C. Diabetes in Thailand: Status and Policy. *Curr Diab Rep* 2016; **16**: 28 [PMID: 26894266 DOI: 10.1007/s11892-016-0725-7]
18 **Nitiyanant W**, Chetthakul T, Sang-A-kad P, Therakiatkumjorn C, Kunsuikmengrai K, Yeo JP. A survey study on diabetes management and complication status in primary care setting in Thailand. *J Med Assoc Thai* 2007; **90**: 65-71 [PMID: 17621735 DOI: 10.1186/1744-8603-9-11]
19 **Namperumalsamy P**, Kim R, Vignesh TP, Nithya N, Royes J, Gijo T, Thulasiraj RD, Vijayakumar V. Prevalence and risk factors for diabetic retinopathy: a population-based assessment from Theni District, south India. *Br J Ophthalmol* 2009; **93**: 429-434 [PMID: 19091856 DOI: 10.1136/bjo.2008.147934]
20 **Javadi MA**, Katibeh M, Rafati N, Dehghan MH, Zayeri F, Yaseri M, Sehat M, Ahmadieh H. Prevalence of diabetic retinopathy in Tehran province: a population-based study. *BMC Ophthalmol* 2009; **9**: 12

[PMID: 19835608 DOI: 10.1186/1471-2415-9-12]
21 **Bursell SE**, Fonda SJ, Lewis DG, Horton MB. Prevalence of diabetic retinopathy and diabetic macular edema in a primary care-based teleophthalmology program for American Indians and Alaskan Natives. *PLoS One* 2018; **13**: e0198551 [PMID: 29924846 DOI: 10.1371/journal.pone.0198551]
22 **Hapnes R**, Bergrem H. Diabetic eye complications in a medium sized municipality in southwest Norway. *Acta Ophthalmol Scand* 1996; **74**: 497-500 [PMID: 8950402 DOI: 10.1111/j.1600-0420.1996.tb00607.x]
23 **Varma R**, Macias GL, Torres M, Klein R, Peña FY, Azen SP; Los Angeles Latino Eye Study Group. Biologic risk factors associated with diabetic retinopathy: the Los Angeles Latino Eye Study. *Ophthalmology* 2007; **114**: 1332-1340 [PMID: 17306879 DOI: 10.1016/j.ophtha.2006.10.023]
24 **Zhang G**, Chen H, Chen W, Zhang M. Prevalence and risk factors for diabetic retinopathy in China: a multi-hospital-based cross-sectional study. *Br J Ophthalmol* 2017; **101**: 1591-1595 [PMID: 28855195 DOI: 10.1136/bjophthalmol-2017-310316]
25 **Ruta LM**, Magliano DJ, Lemesurier R, Taylor HR, Zimmet PZ, Shaw JE. Prevalence of diabetic retinopathy in Type 2 diabetes in developing and developed countries. *Diabet Med* 2013; **30**: 387-398 [PMID: 23331210 DOI: 10.1111/dme.12119]
26 **Williams R**, Airey M, Baxter H, Forrester J, Kennedy-Martin T, Girach A. Epidemiology of diabetic retinopathy and macular oedema: a systematic review. *Eye (Lond)* 2004; **18**: 963-983 [PMID: 15232600 DOI: 10.1038/sj.eye.6701476]
27 **Penno G**, Solini A, Zoppini G, Orsi E, Zerbini G, Trevisan R, Gruden G, Cavalot F, Laviola L, Morano S, Nicolucci A, Pugliese G; Renal Insufficiency And Cardiovascular Events (RIACE) Study Group. Rate and determinants of association between advanced retinopathy and chronic kidney disease in patients with type 2 diabetes: the Renal Insufficiency And Cardiovascular Events (RIACE) Italian multicenter study. *Diabetes Care* 2012; **35**: 2317-2323 [PMID: 23093684 DOI: 10.2337/dc12-0628]
28 **Grunwald JE**, Alexander J, Ying GS, Maguire M, Daniel E, Whittock-Martin R, Parker C, McWilliams K, Lo JC, Go A, Townsend R, Gadegbeku CA, Lash JP, Fink JC, Rahman M, Feldman H, Kusek JW, Xie D, Jaar BG; CRIC Study Group. Retinopathy and chronic kidney disease in the Chronic Renal Insufficiency Cohort (CRIC) study. *Arch Ophthalmol* 2012; **130**: 1136-1144 [PMID: 22965589 DOI: 10.1001/archophthalmol.2012.1800]
29 **Manaviat MR**, Afkhami M, Shoja MR. Retinopathy and microalbuminuria in type II diabetic patients. *BMC Ophthalmol* 2004; **4**: 9 [PMID: 15228626 DOI: 10.1186/1471-2415-4-9]
30 **Silveiro SP**, Friedman R, de Azevedo MJ, Canani LH, Gross JL. Five-year prospective study of glomerular filtration rate and albumin excretion rate in normofiltering and hyperfiltering normoalbuminuric NIDDM patients. *Diabetes Care* 1996; **19**: 171-174 [PMID: 8718441 DOI: 10.2337/diacare.19.2.171]
31 **Goldberg HJ**, Scholey J, Fantus IG. Glucosamine activates the plasminogen activator inhibitor 1 gene promoter through Sp1 DNA binding sites in glomerular mesangial cells. *Diabetes* 2000; **49**: 863-871 [PMID: 10905498 DOI: 10.2337/diabetes.49.5.863]
32 **Hammes HP**, Federoff HJ, Brownlee M. Nerve growth factor prevents both neuroretinal programmed cell death and capillary pathology in experimental diabetes. *Mol Med* 1995; **1**: 527-534 [PMID: 8529118 DOI: 10.1007/BF03401589]
33 **He F**, Xia X, Wu XF, Yu XQ, Huang FX. Diabetic retinopathy in predicting diabetic nephropathy in patients with type 2 diabetes and renal disease: a meta-analysis. *Diabetologia* 2013; **56**: 457-466 [PMID: 23232641 DOI: 10.1007/s00125-012-2796-6]
34 **Mottl AK**, Kwon KS, Garg S, Mayer-Davis EJ, Klein R, Kshirsagar AV. The association of retinopathy and low GFR in type 2 diabetes. *Diabetes Res Clin Pract* 2012; **98**: 487-493 [PMID: 23068959 DOI: 10.1016/j.diabres.2012.09.041]
35 **Pugliese G**, Solini A, Zoppini G, Fondelli C, Zerbini G, Vedovato M, Cavalot F, Lamacchia O, Buzzetti R, Morano S, Nicolucci A, Penno G; Renal Insufficiency and Cardiovascular Events (RIACE) Study Group. High prevalence of advanced retinopathy in patients with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study. *Diabetes Res Clin Pract* 2012; **98**: 329-337 [PMID: 23020932 DOI: 10.1016/j.diabres.2012.09.006]
36 **Wong CW**, Lamoureux EL, Cheng CY, Cheung GC, Tai ES, Wong TY, Sabanayagam C. Increased Burden of Vision Impairment and Eye Diseases in Persons with Chronic Kidney Disease - A Population-Based Study. *EBioMedicine* 2016; **5**: 193-197 [PMID: 27077127 DOI: 10.1016/j.ebiom.2016.01.023]
37 **Busch M**, Franke S, Rüster C, Wolf G. Advanced glycation end-products and the kidney. *Eur J Clin Invest* 2010; **40**: 742-755 [PMID: 20649640 DOI: 10.1111/j.1365-2362.2010.02317.x]
38 **Ortwerth BJ**, Chemoganskiy V, Mossine VV, Olesen PR. The effect of UVA light on the anaerobic oxidation of ascorbic acid and the glycation of lens proteins. *Invest Ophthalmol Vis Sci* 2003; **44**: 3094-3102 [PMID: 12824256 DOI: 10.1167/iovs.02-0857]
39 **Stitt AW**. AGEs and diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2010; **51**: 4867-4874 [PMID: 20876889 DOI: 10.1167/iovs.10-5881]
40 **Canning P**, Glenn JV, Hsu DK, Liu FT, Gardiner TA, Stitt AW. Inhibition of advanced glycation and absence of galectin-3 prevent blood-retinal barrier dysfunction during short-term diabetes. *Exp Diabetes Res* 2007; **2007**: 51837 [PMID: 17641742 DOI: 10.1155/2007/51837]
41 **Payne JF**, Ray R, Watson DG, Delille C, Rimler E, Cleveland J, Lynn MJ, Tangpricha V, Srivastava SK. Vitamin D insufficiency in diabetic retinopathy. *Endocr Pract* 2012; **18**: 185-193 [PMID: 21940279 DOI: 10.4158/EP11147.OR]
42 **Wong CW**, Wong TY, Cheng CY, Sabanayagam C. Kidney and eye diseases: common risk factors, etiological mechanisms, and pathways. *Kidney Int* 2014; **85**: 1290-1302 [PMID: 24336029 DOI: 10.1038/ki.2013.491]
43 **Wong CW**, Teo BW, Lamoureux E, Ikram MK, Wang JJ, Tai ES, Sethi S, Wong TY, Sabanayagam C. Serum Cystatin C, Markers of Chronic Kidney Disease, and Retinopathy in Persons with Diabetes. *J Diabetes Res* 2015; **2015**: 404280 [PMID: 26576434 DOI: 10.1155/2015/404280]
44 **Michels WM**, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT. Performance of the Cockcroft-Gault, MDRD, and new CKD-EPI formulas in relation to GFR, age, and body size. *Clin J Am Soc Nephrol* 2010; **5**: 1003-1009 [PMID: 20299365 DOI: 10.2215/CJN.06870909]
45 **Matsushita K**, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, Polkinghorne KR, Shankar A, Smith DH, Tonelli M, Warnock DG, Wen CP, Coresh J, Gansevoort RT, Hemmelgarn BR, Levey AS; Chronic Kidney Disease Prognosis Consortium. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA* 2012; **307**: 1941-1951 [PMID: 22570462 DOI: 10.1001/jama.2012.3954]

46 **Grunwald JE**, Alexander J, Maguire M, Whittock R, Parker C, McWilliams K, Lo JC, Townsend R, Gadegbeku CA, Lash JP, Fink JC, Rahman M, Feldman H, Kusek J, Ojo A; CRIC Study Group. Prevalence of ocular fundus pathology in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 2010; **5**: 867-873 [PMID: 20299372 DOI: 10.2215/CJN.08271109]

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MINIREVIEWS

Point of care renal ultrasonography for the busy nephrologist: A pictorial review

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Abstract

The application of bedside ultrasonography in routine clinical practice has dramatically evolved over the last few decades and will likely continue to grow as technological advances lead to enhanced portability and affordability of the equipment. Despite mounting interest, most nephrology fellowship training programs do not offer formal training in renal ultrasonography and there is inertia among practicing nephrologists to adopt this skill as a practice-changing advancement. Lack of familiarity with the topic is considered a key reason for this inertia. Understanding of basic ultrasound physics, instrumentation, principles of optimal image acquisition and interpretation is critical for enhanced efficiency and patient safety while using this tool. Herein, we provide a brief overview of the basic principles of diagnostic renal ultrasonography as well as introduction to common sonographic pathologies encountered in day-to-day nephrology practice with illustrative images.

Key words: Ultrasound; Kidney; Cyst; Hydronephrosis; Stone; Point of care

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Core tip: Point-of-care renal ultrasonography has emerged as a valuable bedside diagnostic tool for non-radiology physicians. It reduces fragmentation of care and quickly provides answers to simple clinical questions thereby enhancing patient care and satisfaction. In addition, using bedside ultrasound as the first line investigation may eliminate unnecessary radiation and contrast exposure. This pictorial review includes a simplified version of the basics of ultrasound instrumentation and image interpretation.

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INTRODUCTION

Over the past few decades, point-of-care ultrasonography (POCUS) has emerged as an invaluable adjunct to bedside diagnostic evaluation especially in the field of emergency medicine. Lack of ionizing radiation, non-invasive nature, and advances in portability and affordability of the equipment have made ultrasound a first-line imaging modality for a broad range of indications. While radiology-performed conventional ultrasonography is a comprehensive examination intended to fully explore the area of interest, POCUS performed at the bedside is primarily intended to answer focused questions such as, "does the patient have pericardial effusion?", "is hydronephrosis present?", "is the patient short of breath because of hypervolemia?", etc. and help narrow the differential diagnosis. POCUS has been found so useful in clinical practice that some authors describe it as the fifth pillar of bedside physical examination added to the traditional ones, namely, inspection, palpation, percussion and auscultation[1]. In recent years, an increasing number of internal medicine specialists and subspecialists have been incorporating POCUS into their practice to improve diagnostic timeliness and accuracy. With respect to nephrology practice, sonography of the kidneys is integral to the diagnostic evaluation of acute kidney injury (AKI) and chronic kidney disease (CKD); its performance by the nephrologist can expedite and enhance the consultation process[2]. Moreover, it reduces fragmentation of care and enhances patient satisfaction. Though not a difficult skill to learn, performance and interpretation of POCUS does require training in the form of didactics and hands-on instruction. We have previously reported our experience with development of a nephrology-oriented POCUS curriculum for internal medicine residents in our institution, which was received with great enthusiasm and resulted in increased confidence of the residents in identifying structural abnormalities of the kidneys[3]. In this review, we will briefly describe the basics of ultrasonography, technique and interpretation of a normal renal sonogram. In addition, we will discuss the common renal sonographic pathologies encountered in routine nephrology practice. Discussion of volume status assessment by inferior vena cava, lung and focused cardiac ultrasound as well as use of sonography for procedural guidance is beyond the scope of this article.

BASIC ULTRASOUND PHYSICS, MODES AND INSTRUMENTATION

Over the past several years, technological advancements have resulted in the emergence of miniaturized handheld ultrasound equipment that are compact and battery operated; using these devices, POCUS can be readily performed with decent image quality. However, the basic ultrasound physics, modes and principles of image acquisition are essentially unchanged. Sound is a longitudinal mechanical wave produced by vibration and requires a material medium (*e.g.*, air, tissue) to travel. The number of waves that passes through a fixed point in 1 second is defined as frequency and is measured in hertz (Hz). Audible sound has a frequency of 20 to 20000 Hz. Frequencies below this range are called infrasound and those above are called ultrasound. Medical ultrasound imaging uses sound waves of 1 to 20 megahertz (MHz), which is significantly above the limit of human hearing.

The key working principle of ultrasonography is as follows: When stimulated by an electric current, crystals located in the transducer or probe vibrate and produce ultrasound waves. These are called piezoelectric crystals and typically made up of lead zirconium titanate. The ultrasound waves are then transmitted into the tissues and the transducer now listens for the reflected ultrasound waves, or the echoes. The returning echoes are converted into electrical signals by the piezoelectric crystals and displayed as a 2-dimensional gray-scale image on the monitor[4]. Conversion of mechanical (*i.e.*, sound) energy into electrical energy is called the "piezoelectric effect" and the opposite is called "inverse piezoelectric effect". Therefore, generation of an ultrasound involves both of these principles (Figure 1).

There are various modes of ultrasound display. Examples of the modes that are routinely used in POCUS are B-mode, M-mode, color Doppler and pulsed wave Doppler (Figure 2). B-mode or the brightness mode is the term used to denote the

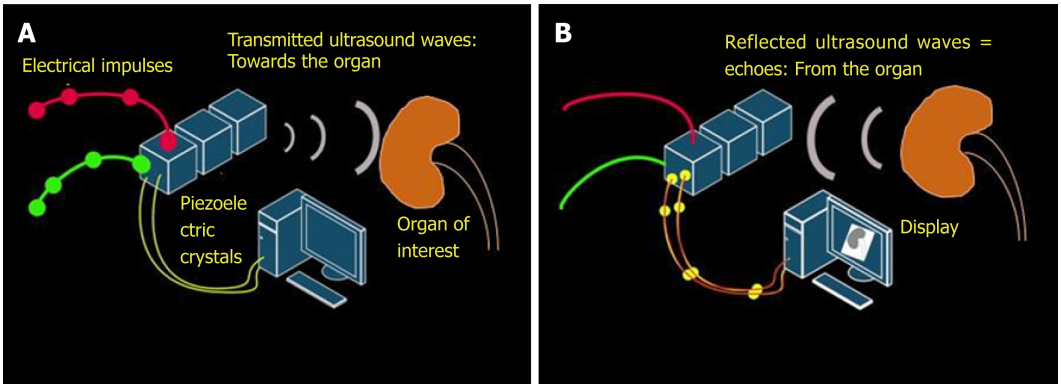


Figure 1 Working principles underlying generation of ultrasound image. A: Inverse piezoelectric effect – conversion of electrical energy into mechanical energy (ultrasound waves); B: Piezoelectric effect – conversion of mechanical energy into electrical energy by the piezoelectric crystals located in the transducer. These impulses are displayed on the monitor in the image format.

regular 2-dimensional gray-scale display. M-mode or the motion mode obtains an image in the B-mode and displays it graphically as changes over a period of time. It is unidimensional and useful to assess moving structures such as changes in diameter of the inferior vena cava with respiration, excursion of heart valves, pleural sliding etc. In the color Doppler mode, the velocity of mobile acoustic interfaces (typically blood cells) is measured as a shift in frequency, and represented as a range of colors: Red color denotes flow towards the transducer and blue away from the transducer. In pulsed wave Doppler, the velocity and direction of the blood flow are displayed graphically and audibly. If blood is moving away from the transducer, a lower frequency (negative deflection) is detected and if it is moving toward the transducer, a higher frequency (positive deflection) is detected[5]. In nephrology, doppler modality is helpful in assessing renal blood flow and measuring resistive indices in transplanted kidneys.

The probe used to perform abdominal sonography is the curvilinear or convex array probe, which has a lower frequency (typically 2 to 5 MHz) and provides a wide, fan-shaped scanning area on the monitor (Figure 3). Frequency of the probe determines depth of penetration and image resolution; higher frequency probes provide less penetration of the ultrasound waves through the tissue planes, but generate higher resolution images. Therefore, superficial applications such as dialysis catheter placement and evaluation of the arteriovenous fistula are performed using high frequency probes (typically 6 to 15 MHz). Conversely, low frequency probes provide deeper penetration and are ideal for abdominal and cardiac applications. Cardiac probe or the phased array probe is a low frequency probe as well, the main difference being that it has a smaller footprint or face of the probe, which allows better manipulation in the rib interspaces.

IMAGE ORIENTATION AND INTERPRETATION

Understanding image orientation is an important component of accurate interpretation. For instance, if a cyst is noted on one of the poles of the kidney, how do we know if it is superior pole or the inferior, especially if liver or spleen is not seen in the image? Every ultrasound transducer has a probe marker along one side of its head. This marker can be a light, dot, or a linear ridge and corresponds to indicator on the screen, which is typically a dot or letter denoting the manufacturer. During standard abdominal exam, the probe marker should be pointed toward patient's head when obtaining longitudinal images and to the right in the transverse plane. Therefore, if the cyst in above example is on the pole closer to the screen indicator, it is towards the head of the patient, *i.e.*, superior pole of the kidney in the longitudinal plane.

Now, what determines which structure is on the top of the image and which is at the bottom? The underlying principle is "time is equal to distance". Echoes returning from a structure that is farther from the probe takes longer time compared to those returning from the structure, that is closer to the probe and that farther structure is portrayed at the bottom of the screen, nearer one on the top. Therefore, the top part of the image represents "anterior" or "lateral" aspect of the abdomen depending on the scanning plane used (Figure 4).

The next concept of image interpretation is "relative echogenicity". Echogenicity refers to how bright or dark something appears in the gray scale ultrasound image;

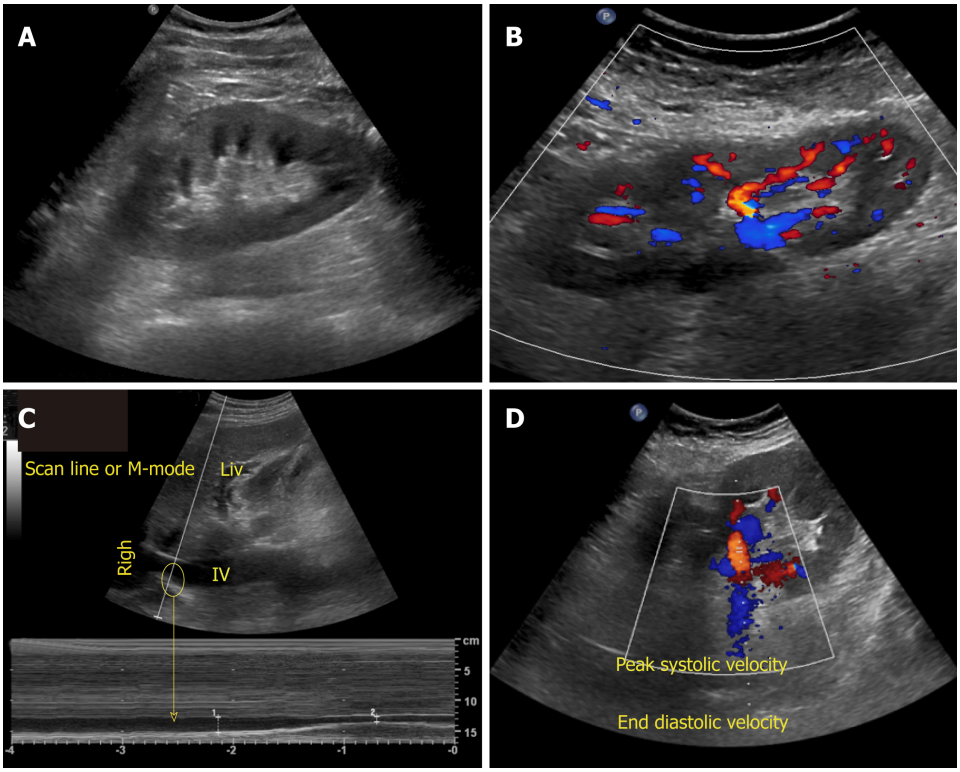


Figure 2 Commonly used modes in nephrology-oriented point of care ultrasonography. A: B-mode: Grey-scale image of the left kidney; B: Color Doppler mode: Blood flow inside the kidney is indicated by red and blue colors; C: M-mode: Variations in diameter of the inferior vena cava over time at the area of interest (circle); D: Pulsed wave Doppler: Transverse view of the kidney with graphic representation of flow in the renal artery.

the brighter something appears, the more echogenic it is. In other words, the brighter structure makes strong echoes or returning sound waves. Echogenicity is "relative" to the surrounding structures as the ultrasound image is typically composed of 256 different shades of grey and each shade can be both brighter and darker relative to other shades, unless it is completely "white" or "black" (Figure 5). The physical property of the tissue that determines its echogenicity is called "acoustic impedance", which is defined as the resistance for propagation of ultrasound waves. This varies according to the density of the material ultrasound passes through[6]. A structure that reflects most of the sound waves back will appear bright (hyperechoic); bone (*e.g.*, ribs), fibrous tissue (*e.g.*, diaphragm, renal capsule) and adipose tissue (*e.g.*, renal sinus fat) are very echogenic and therefore appear bright on abdominal ultrasonography images. Air is also a strong reflector of the ultrasound beam and appears bright, making it difficult to visualize structures behind it. Typically, the kidneys are easily identifiable because renal capsule (consisting of thin fibrous tissue) which is next to fat appears bright and makes the organ well demarcated. Solid organs, such as the liver and spleen, have intermediate echogenicity, and the kidney cortex is normally isoechoic (equal in brightness) or hypoechoic (darker) compared with the normal liver or normal spleen[7,8]. Fluid (*e.g.*, blood, urine, serous fluid in cysts) is an excellent transmitter of sound waves and appears black (anechoic).

In this context, an adjustable feature of the machine called "gain" is noteworthy. The sonographer can manipulate the brightness of the image by increasing or decreasing the gain. By increasing the gain, the ultrasound machine allows processing of more incoming echoes, creating a brighter image. Conversely, a darker image is obtained when the gain is decreased. When a part of the image is optimal and the rest of it is too bright or too dark, a feature known as "time gain compensation" allows the operator to adjust the image brightness at specific depths[5].

SONOGRAPHIC EVALUATION OF THE KIDNEYS AND THE BLADDER

Technique

As mentioned above, ultrasound examination of the kidney is usually performed with a low frequency curvilinear transducer. Higher frequencies may be needed while

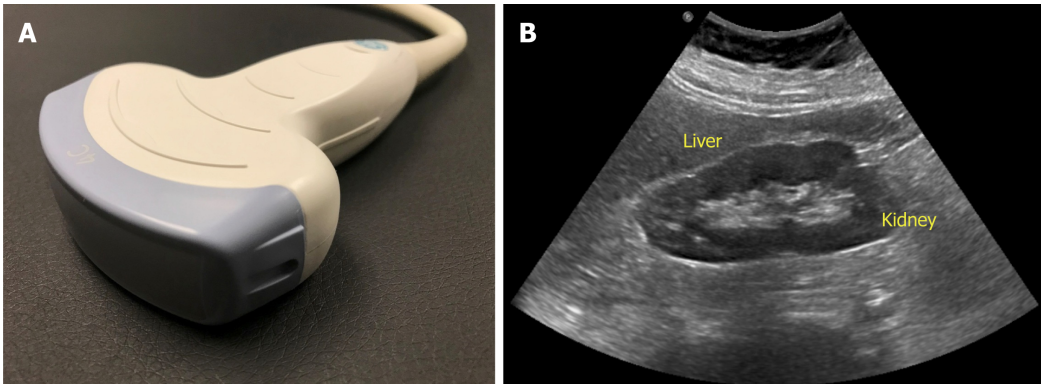


Figure 3 Curvilinear probe (A) used for abdominal applications (B) example of an image obtained using this probe.

evaluating renal transplant patients, when the allograft location is very superficial. Imaging of the right kidney is easier than that of the left kidney as the liver provides a large acoustic window (*i.e*., we are looking at the kidney "through" the liver using it as a window) in contrast to the spleen on the left. Examination is typically performed in the supine position, although lateral decubitus, oblique or even prone positions may be required for visualization of the left kidney due to overlying bowel gas[9]. To acquire longitudinal views of the kidney, the examination is usually started with the probe positioned in the mid axillary line at tenth rib interspace with the probe marker pointing towards patient's head. The probe may have to be slided superiorly or inferiorly depending on the patient's body habitus. Once the entire long axis of the kidney is in view, the probe should be fanned anteriorly and posteriorly to image it completely. The probe is then rotated 90 degrees counterclockwise (probe marker now faces right of the patient) to obtain a short axis or transverse view of the kidney. Now it is fanned to view the kidney from upper to lower pole to ensure complete examination. Comprehensive renal Doppler imaging consists of direct visualization of renal arteries and veins, measurement of blood velocity, and analysis of pulsed wave doppler waveforms (including resistive index calculation) in segmental arteries. Renal artery evaluation is technically difficult due to poor insonation angles (should be less than 60 degrees) overlying bowel gas, and the frequent occurrence of multiple arteries. It is of note that renal artery evaluation has a high failure rate (up to 30%) even when performed by expert sonographers[9]. When detailed evaluation is not possible or deemed unnecessary, we recommend obtaining at least one color doppler image in each plane as it would help differentiating vessels from dilated collecting system and also identify small stones. This will be discussed further in the latter sections of the manuscript.

With respect to ultrasound examination of the transplanted kidney, the surgical anatomy should be confirmed and comparison with prior studies be made whenever possible. The kidney is easily identifiable as it is usually palpable in the right or left pelvis. Longitudinal and transverse views of the transplanted kidney should be obtained and special attention be paid to perinephric space for any evidence of fluid collection. If a ureteral stent is in place, an attempt should be made to determine the proximal and distal extent of the stent[10]. Color Doppler images of the entire kidney provide a global assessment of transplant renal perfusion and assess for vascular abnormalities. Measurement of resistive indices using pulsed wave Doppler is important for transplanted kidneys as higher values may serve as a clue to graft rejection. Moreover, it has been shown that renal transplant recipients with high resistive index of at least 0.80 have higher mortality in the first 2 years post-transplant compared with those with lower values[11]. The resistive index measures the degree of intrarenal arterial impedance and is calculated as follows: [(PSV – EDV)/PSV], where PSV is peak systolic velocity and EDV is end diastolic velocity. Resistive index is calculated at the level of arcuate arteries at corticomedullary junction or the larger segmental and interlobar vessels (preferred); the value of 0.7 is used as the threshold to discriminate between normal and pathologic resistance to flow[12]. Too low values may indicate prerenal state or lack of good blood flow to the transplanted kidney. Figure 6 demonstrates resistive indices in arcuate arteries in a transplanted kidney.

Sonography is a simple and useful tool in the evaluation of the urinary bladder, and should be performed in any patient with dilated collecting system. The examination is performed with the patient in supine position with suprapubic area exposed. The probe is placed sagittally in the midline above the pubic symphysis with probe marker towards patient's head to obtain longitudinal view of the bladder. Then

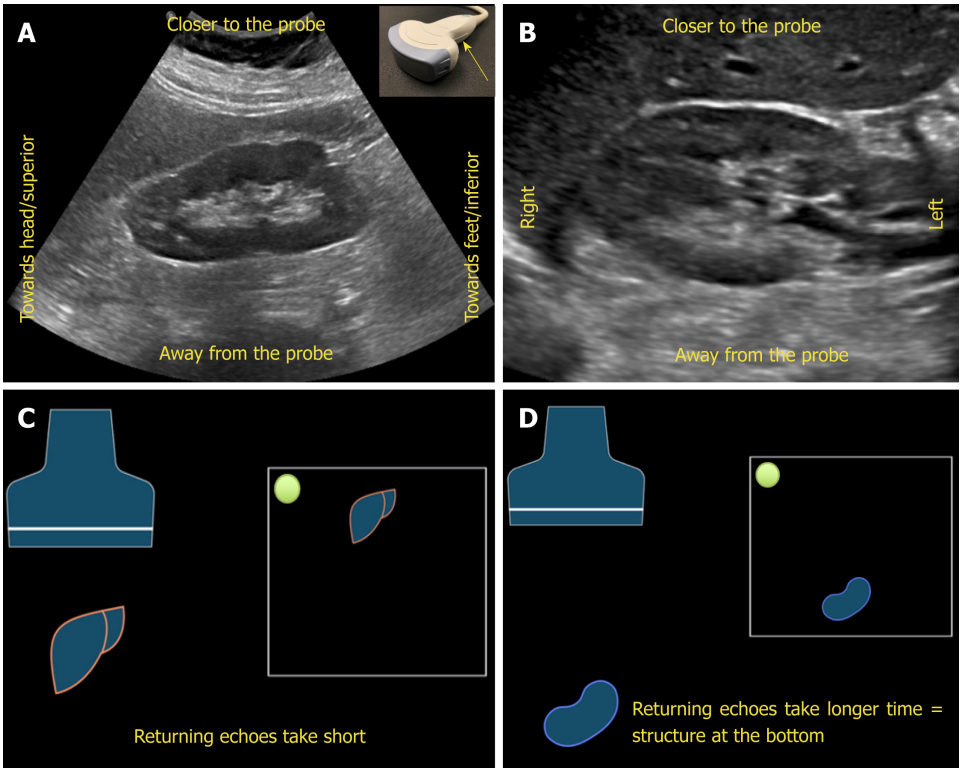


Figure 4 Orientation of the image. A, B: Ultrasound image of the (A) longitudinal and (B) transverse views of the right kidney demonstrating the image orientation; C, D: Cartoon illustrating that echoes returning from a structure that is farther from the probe take longer time compared to those returning from the structure that is closer to the probe and that farther structure is portrayed at the bottom of the screen and the nearer one on the top. Note that the arrow in **Figure 4A** points to the probe marker and the green dot represents indicator on the screen.

it should be angled laterally and swept to left and right to examine the lateral borders. The probe is then rotated 90 degrees counter clockwise to obtain the transverse view and swept superior to inferior to image the bladder completely. The volume of the bladder is estimated by orthogonal measurements, assuming it to be an ellipsoid (= 0.52 × the three orthogonal dimensions)[13] (Figure 7A).

Normal features

On the sonogram, renal capsule is echogenic and makes the bean shaped organ easily identifiable. Kidney length in adults is typically about 10–12 cm but varies with body size. Renal parenchyma is composed of two distinct zones, the outer cortex and the inner medulla, which is organized into pyramids. As mentioned before, the renal cortex is either isoechoic or hypoechoic (more commonly) compared with the normal liver or normal spleen. The medullary pyramids are hypoechoic or anechoic compared to cortex. The renal cortical tissue extends into the medulla separating pyramids in the form of columns called "columns of Bertin". It is important to make a note of this anatomical feature as these columns can sometimes hypertrophy and mimic a neoplasm[14]. Unlike a true neoplasm, the hypertrophied column of Bertin is in continuity with, and of similar appearance to normal cortex, and the renal outline is typically preserved. The sinus fat is echogenic and occupies major part of the inner kidney. The collecting system is usually not visualized unless distended owing to its small caliber and the surrounding echogenic sinus fat[15] (Figure 8). However, the collecting system of a well-functioning transplanted kidney is often slightly dilated, likely because of a combination of an increased volume of urine produced (*i.e.*, acting as the sole kidney) and loss of the ureter's tonicity from denervation[16]. In the transverse section, the mid portion of the kidney is C-shaped with the vessels entering and leaving through the hilum (Figure 2D) and the poles appear circular.

Urinary bladder appears as an anechoic fluid-filled structure (urine is black on ultrasound) located in the mid pelvis. A full bladder is necessary to comprehensively evaluate the characteristics of its walls, contents and pelvic organs. Ureteric orifices, if visible, appear as small echogenic protuberances on the posterior aspect. Urine passes down the ureters in pulses, which can be detected upon entry into the base of the bladder. These ureteral "jets" (Figure 7B) are best detected in the Doppler mode and provide evidence of ureteral patency[15]. While the presence of strong jets bilaterally goes against obstruction, their absence does not rule in obstructive uropathy. More-

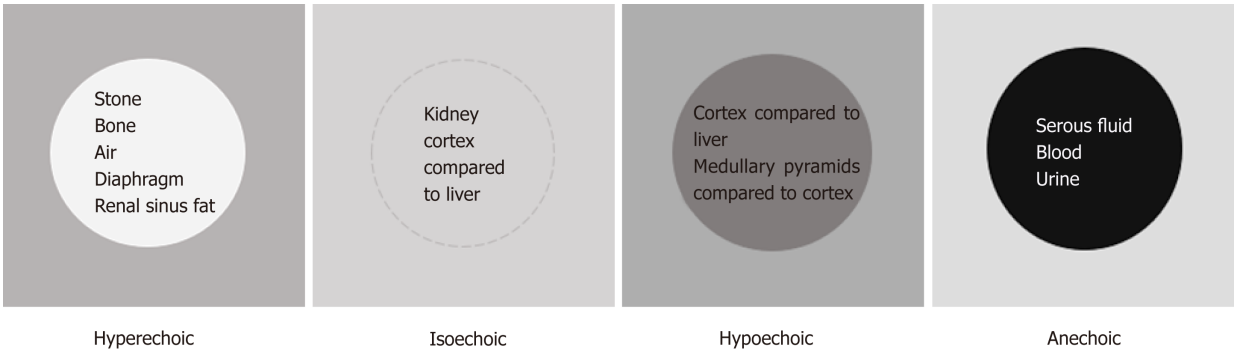


Figure 5 Illustration of "relative echogenicity". The circular area in the center is hyperechoic (brighter), isoechoic (similar brightness), hypoechoic (darker) and anechoic (black) compared to the surrounding area respectively. Echogenicity of the commonly encountered structures on renal sonography are listed inside the circles. Note that renal cortex can be either isoechoic or hypoechoic compared to that of normal liver.

over, their practical utility is limited and there is disagreement among various specialties about their necessity and clinical relevance[17].

COMMON SONOGRAPHIC ABNORMALITIES

Abnormalities of the renal size and echogenicity

Though the normal pole-to-pole kidney length in adults is 10-12 cm, it varies with body size. While nomograms based on large population studies are not available unlike in children[13,18], there exists a formula to estimate the kidney size: Kidney length (mm) = 49.18 + 0.21 × weight (kg) + 0.27 × height (cm). However, this was based on computed tomography measurements[19]. When measured appropriately, kidney length may help distinguish AKI from CKD as it is expected to decrease in patients with CKD[8], though patients with diabetic nephropathy are known to have relatively large kidneys. Large kidneys in AKI may result from infiltrative diseases (*e.g.*, multiple myeloma, amyloidosis) (Figure 9A), acute glomerulonephritis and acute interstitial nephritis (due to edema and inflammatory infiltrates) and renal vein thrombosis (edema from congestion)[7]. In addition to length, assessment of renal cortical and/or parenchymal thickness is helpful in distinguishing AKI from CKD. Cortical thickness is measured from the base of the medullary pyramid to the outer margin of the kidney. It is generally around 7-10 mm, being thicker at the poles[20,21]. Cortical thickness is expected to be reduced in CKD (Figure 9B). When the medullary pyramids are not clearly visible, which is not uncommon, parenchymal thickness should be measured from outer margin of the kidney to tip of the pyramid or till the sinus fat is visible, which is usually 1.5–2.0 cm[13].

Echogenicity of the renal cortex relative to liver or spleen can be evaluated both qualitatively and quantitatively[22], though qualitative method is commonly used. Increased cortical echogenicity is commonly attributed to CKD and has been correlated with interstitial fibrosis, tubular atrophy, and glomerulosclerosis in histologic studies[23]. However, increased echogenicity can also be seen in AKI where inflammatory infiltrates and proteinaceous casts reflect sound waves (*e.g.*, acute glomerulonephritis, acute tubular necrosis). Decreased cortical echogenicity usually results from interstitial edema in cases of inflammation or infection.

Hydronephrosis

Exclusion of urinary tract obstruction as a cause for AKI is one of the commonest indications for renal POCUS. Hydronephrosis appears as branching, "interconnected" areas of decreased echogenicity (anechoic in general, indicating the presence of fluid) in the renal collecting system area. The source of obstruction is usually located distal to the kidney, *e.g.*, a stone in the pelviureteric junction, ureter or ureterovesical junction or bladder outlet obstruction from a stone, mass or enlarged prostate. As the severity of hydronephrosis increases, the urine moves proximally into the kidney exerting pressure on the parenchyma. While there is no universally accepted grading system but in routine clinical practice, we classify hydronephrosis as mild moderate and severe. In mild hydronephrosis, there is dilatation of the renal pelvis and calyces but the pelvicalyceal pattern is retained. In moderate hydronephrosis, medullary pyramids start to flatten due to back pressure in addition to dilatation of pelvicalyceal system. In severe cases, renal pelvis and calyces appear ballooned and cortico-medullary differentiation is lost making the cortex thin (Figure 10).

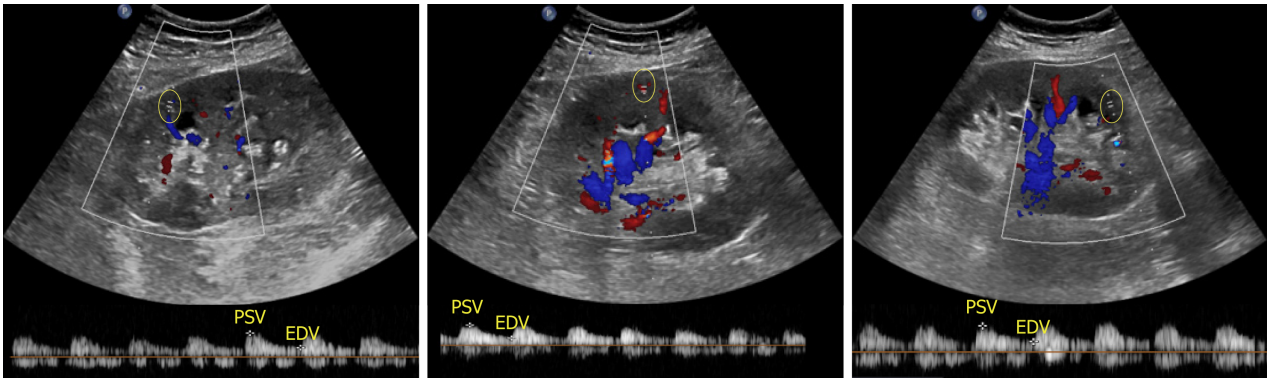


Figure 6 Images of the transplanted kidney demonstrating resistive indices in the superior, middle and inferior arcuate arteries (at corticomedullary junction; sample indicated by yellow circle), which were calculated as 0.61, 0.64 and 0.60 respectively and EDV = end diastolic velocity.

False-negative findings can occur in the setting of acute or partial obstruction, volume depletion and retroperitoneal fibrosis[24,25]. Conversely, presence of hydronephrosis does not always indicate pathologic urinary obstruction. For example, a large diuresis can distend the intrarenal collecting system as with congenital nephrogenic diabetes insipidus[26]. Pregnancy is another well-known example where dilatation of the collecting system and ureters begins in the first trimester and persists for a few weeks postpartum[27] due to smooth muscle relaxing effect of progesterone and compression of the gravid uterus on the ureters.

There are certain conditions that can mimic hydronephrosis, which physicians performing POCUS need to be aware of. Renal vessels or vascular malformations are common amongst those and can be easily differentiated using color Doppler imaging (Figure 11)[28]. Parapelvic cysts can also mimic hydronephrosis on a renal sonogram because of their hypoechoic nature and close proximity to the collecting system. Hydronephrosis appears as branching, "interconnected" anechoic area, while parapelvic cysts are seen as "noncommunicating" cystic masses close to the renal pelvis. In addition, a dilated pelvicalyceal system has a cauliflower appearance, whereas a parapelvic cyst is more spherical[29,30]. When the sonogram is not clear enough, a computed tomogram (CT scan) with contrast should be considered to differentiate between these two conditions (Figure 12), especially if the patient presents with renal dysfunction. Extrarenal pelvis is an anatomical variant that can be confused with hydronephrosis. It appears as a large hypoechoic mass just outside the renal sinus and unlike hydronephrosis, it is not associated with dilated calyces, parenchymal thinning, hydroureter, or enlarged kidney *per se*[31].

Obstructed urinary bladder

Evaluation of urinary tract obstruction is never complete without bladder examination. POCUS is a non-invasive way of measuring post void residual urine volume, which may be helpful when bladder outlet obstruction is suspected. It may also reveal abnormalities such as prostatomegaly, bladder mass, stone or even obstructed Foley catheter (Figure 13).

Kidney stones

Although sonography is less sensitive than CT for detecting kidney stones, it is the preferred initial imaging modality as there is no risk of radiation, is reproducible, inexpensive, and the outcome is not significantly different for patients with suspected urolithiasis undergoing initial ultrasound exam compared to those undergoing CT scan[32,33]. On gray-scale images, stones appear as hyperechoic or bright structures with a posterior "acoustic shadow". Acoustic shadowing is the black area or signal void seen beyond structures that do not transmit ultrasound waves[34]. In the Doppler mode, stones exhibit "twinkling sign" or artifact, which refer to a rapidly alternating focus of color Doppler signals mimicking turbulent flow and is more pronounced with rougher stones. It is of note that this sign is more sensitive than shadowing for detection of small stones[35], which we found to be very helpful in our practice (Figure 14).

Kidney cysts

Cysts in the kidney are other common abnormality encountered in clinical practice. They may be sporadic, hereditary (*e.g.*, autosomal dominant polycystic kidney disease, tuberous sclerosis complex), acquired cystic kidney disease, or occasionally

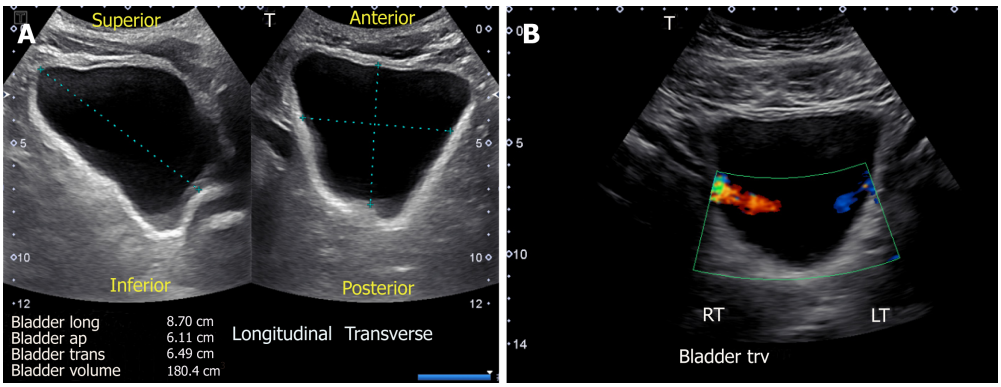


Figure 7 Urinary bladder ultrasound demonstrating (A) bladder volume measurement: Length, antero-posterior and transverse measurements are obtained and multiplied by 0.52 to obtain the approximate amount of urine volume and (B) color Doppler image of the bladder showing ureteral jets.

malignant[9,15]. Cysts can be simple or complex. The diagnosis of a simple benign renal cyst on sonography requires the presence of all the following findings: A well-defined, round, anechoic structure, imperceptible near wall and thin echogenic far wall, and increased through transmission manifested by "acoustic enhancement". Acoustic enhancement refers to the increased intensity of echoes (bright area) relative to surrounding tissues, distal to a low-attenuating structure such as a cyst[36]. This phenomenon is not specific to cysts and can be seen with any fluid containing space such as a blood vessel or urinary bladder. Any lesion that does not meet criteria for a simple cyst is considered a complex cyst and may be characterized by findings such as irregular thickened walls, septations, internal echoes, and calcifications (Figure 15). In this context, it is important to note that the utility of sonography in the Bosniak classification of cysts is limited, as the detection of neovascularization in malignant lesions, indicated by contrast enhancement of solid components, septa or walls, is an essential part of the classification. However, it is known that ultrasound may demonstrate internal septa better than CT and magnetic resonance imaging. Therefore, it has been suggested that simple and minimally complex (Bosniak I and II) cysts may be followed with sonography alone[37,38].

Kidney infections

Though commonly obtained, renal ultrasound is relatively an insensitive test for the diagnosis of acute pyelonephritis as sonographic abnormalities are found in only about 20%-24% of the patients[39,40]. The positive findings of pyelonephritis on sonography, if present, may include congenital anomalies and a variety of changes in the renal parenchyma such as hydronephrosis, unilateral increase in kidney size, loss of renal sinus fat due to edema, changes in echogenicity due to hemorrhage (hyperechoic) or edema (hypoechoic), abscess formation, and areas of hypoperfusion (visible on Doppler mode)[41]. However, POCUS may be helpful in evaluating for pyonephrosis (pus in the collecting system), which is characterized by the presence of low-level echoes within the collecting system, especially when iodinated contrast use for CT is contraindicated. We recently reported a case of pyonephrosis that was missed on non-contrast CT scan but diagnosed with renal sonography leading to positive patient outcome (Figure 16)[42].

BEYOND THE KIDNEY

In addition to diagnostic renal sonography, assessment of volume status is an important application of POCUS in the practice of nephrology. Lung, inferior vena cava and focused cardiac ultrasound are relatively easy to learn and immensely useful tools for nephrologists, especially in the care of end-stage renal disease (ESRD) patients[43]. For example, B-lines in the lung suggestive of increased lung water are detected in ESRD patients even in the absence of symptoms such as dyspnea or peripheral edema[44]. Similarly, data suggests that inferior vena cava measurements by sonography are accurate to predict high or low central venous pressure in dialysis patients[45]. Focused bedside echocardiography is another must-have skill for the nephrologists, which helps quick detection of life-threatening conditions such as pericardial effusion. Moreover, focused echo might detect dynamic abnormalities (*e.g.*, regional wall motion abnormalities) during hemodialysis, which would not be otherwise detected during elective procedure in the cardiology or radiology department[43].

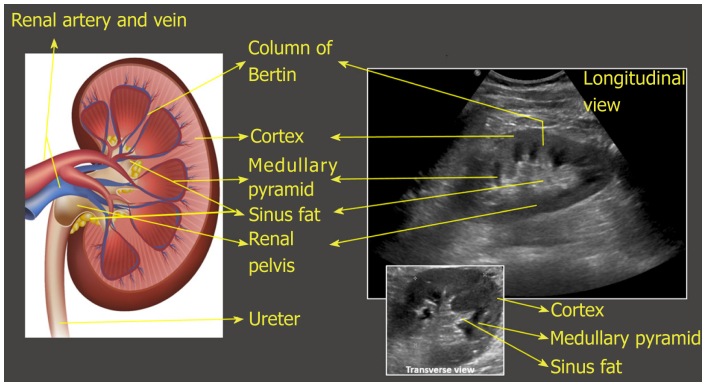


Figure 8 Illustration of longitudinal section of the kidney with corresponding ultrasound image. Transverse view of the kidney is also shown. Note that the most echogenic part of the kidney is sinus fat, and calyces and ureter are not usually visible unless distended. Renal pelvis is hypoechoic but not usually "black" (anechoic) unless there is hydronephrosis.

CONCLUSION

POCUS is quickly becoming an indispensable bedside diagnostic tool for non-radiology physicians. Having a basic understanding of the ultrasound physics, modes used for image generation and ability to distinguish normal from abnormal studies optimizes the scans being performed and enhances the use of ultrasound in patient care. At the same time, it is important to recognize that while POCUS alleviates the need for further investigations in some cases, it is not always a "replacement" for formal ultrasonography. Radiology consultation should be sought even when is slightest doubt about the diagnosis. Furthermore, as with any other skill, the use of POCUS requires dedicated education and hands-on training. Currently several national and local organizations offer training and certification for practicing physicians in various aspects of POCUS but the content and quality of these programs is not held to any universal standard. Therefore, it is imperative that we come up with subspecialty-specific guidelines defining the scope of practice and competency assessment to ensure quality control, which in turn is essential for safe and efficient patient care.

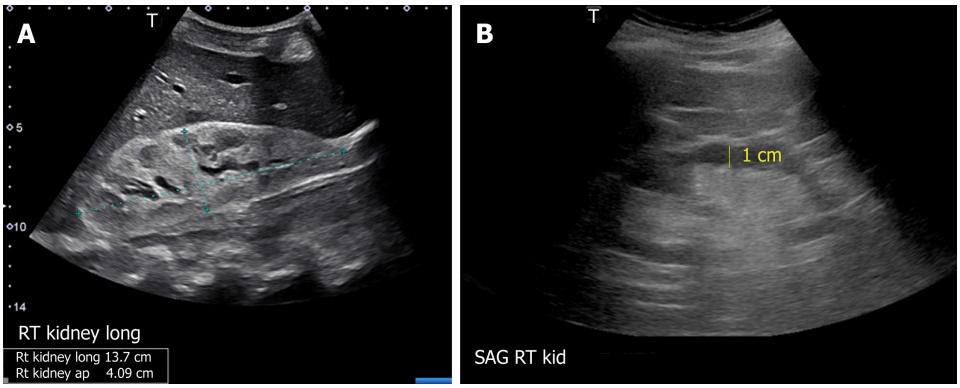


Figure 9 Renal sonogram demonstrating (A) large echogenic kidney in a patient with multiple myeloma and (B) thin parenchyma approximately 1 cm in a patient with chronic kidney disease.

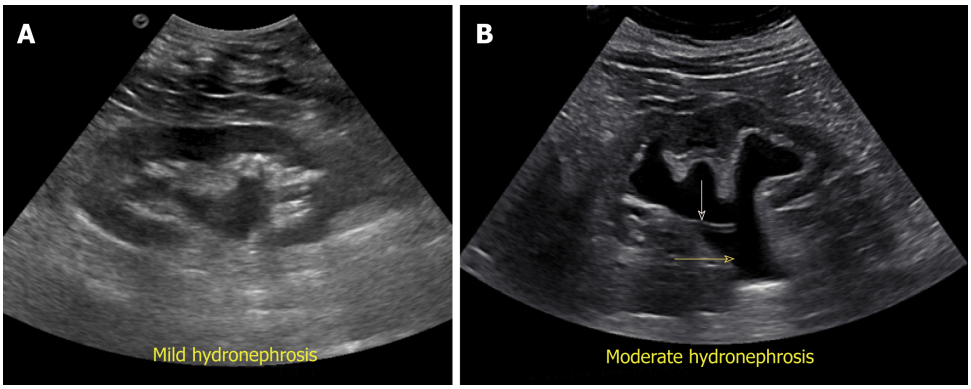


Figure 10 Grading of hydronephrosis. A: Note that as the severity of hydronephrosis increases, the urine (anechoic structure) moves proximally into the kidney exerting pressure on the parenchyma; B: White arrow points to ureteral stent and yellow arrow indicates dilated proximal ureter.

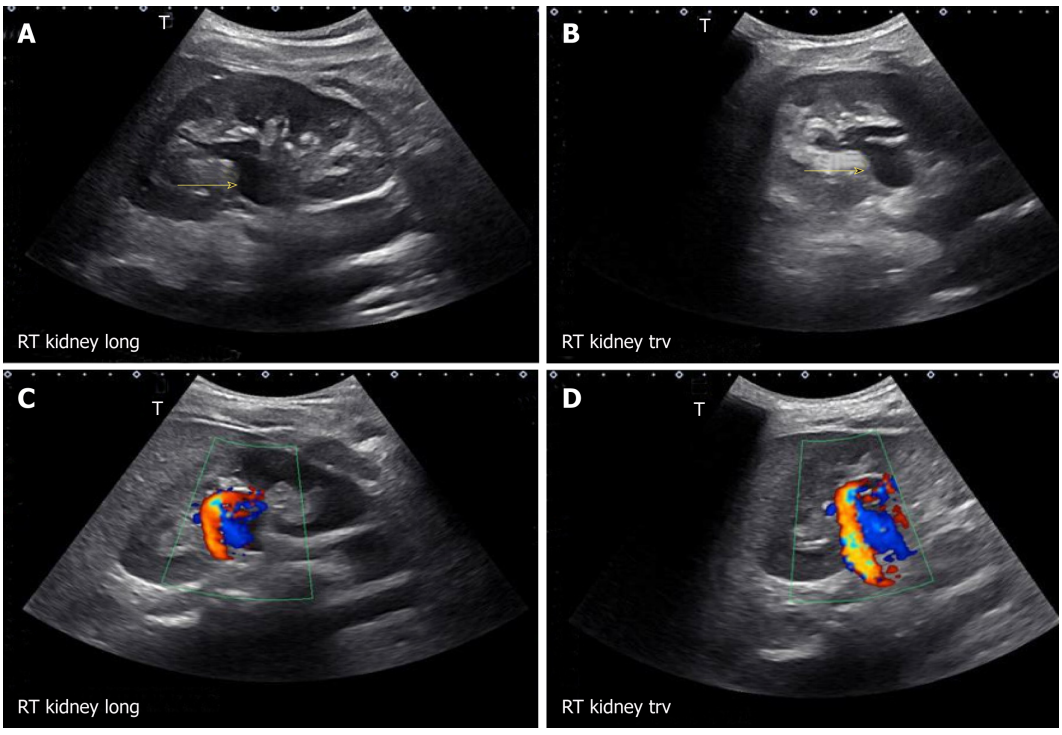


Figure 11 Longitudinal and transverse gray-scale renal ultrasound images demonstrating anechoic region in the mid-kidney suggestive of mild hydronephrosis in the top panel. Doppler images demonstrating prominent arteriovenous flow suggestive of vascular malformation are shown in the bottom panel. Adapted from reference No. 29, first author's previous work, published under CC BY-NC 4.0 license.

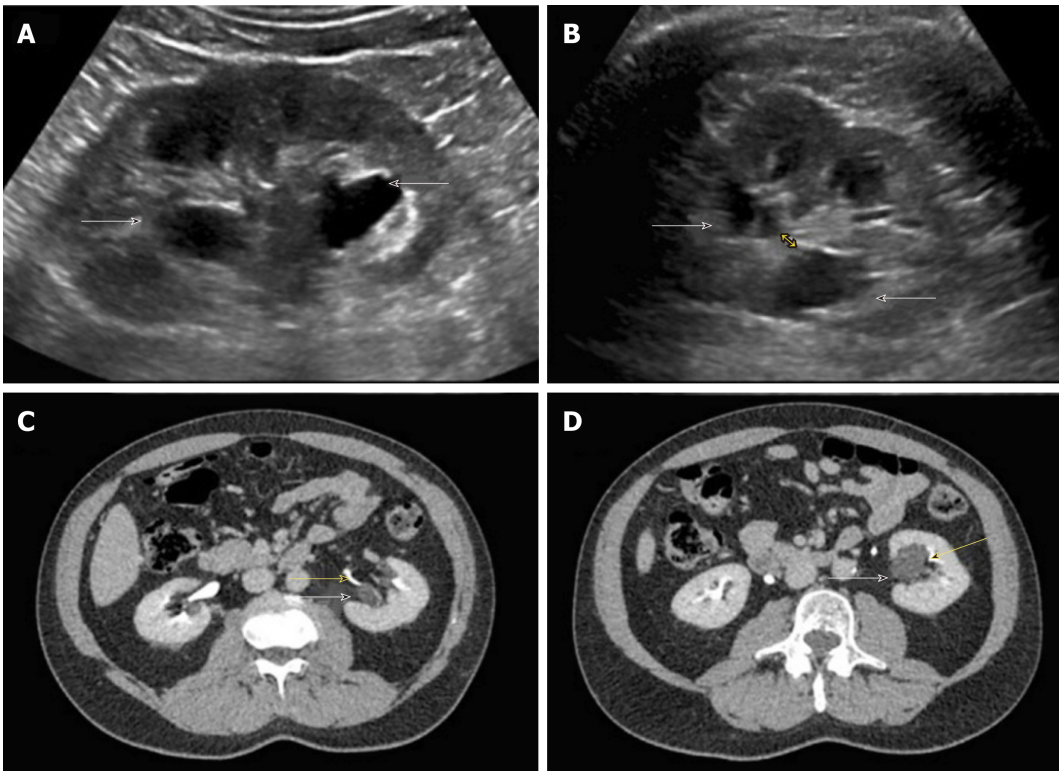


Figure 12 Renal sonogram. A, B: Sagittal (A) and transverse (B) views of the left kidney showing hypoechoic areas in the pelvic area (arrows) suggestive of hydronephrosis; C, D: However, on the transverse view, these areas do not seem to be connected with one another (doubleheaded arrow) and computed tomography (CT) scan with contrast demonstrating (C) and parapelvic cysts (arrows) that are separate from the contrastfilled collecting system (yellow arrows) (D). Adapted from reference no. 30, first author's previous work, published under CC BY-NC 4.0 license.

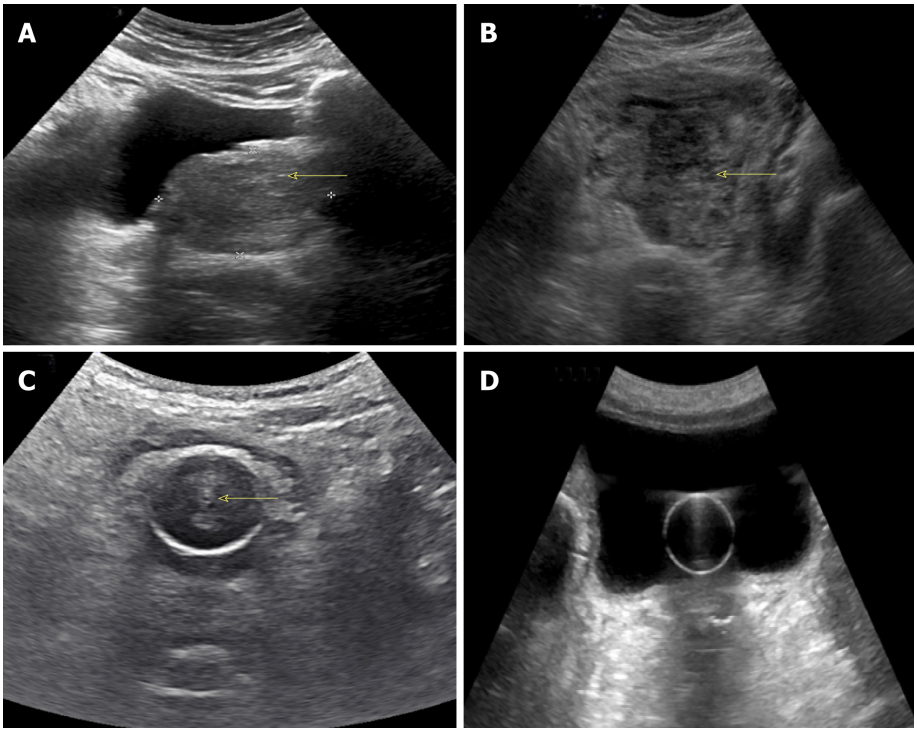


Figure 13 Urinary bladder ultrasound. A: Enlarged prostate (arrow) compressing the urinary bladder; B: Heterogeneous solid mass (arrow) occupying almost the entire bladder; C: Normal appearance of a decompressed bladder with Foley catheter: The empty bladder with functioning catheter is difficult to visualize, and the hypoechoic/anechoic fluid-filled balloon (arrow) is the only portion visible; D: Distended bladder filled with urine despite the presence of Foley catheter indicating catheter malfunction.

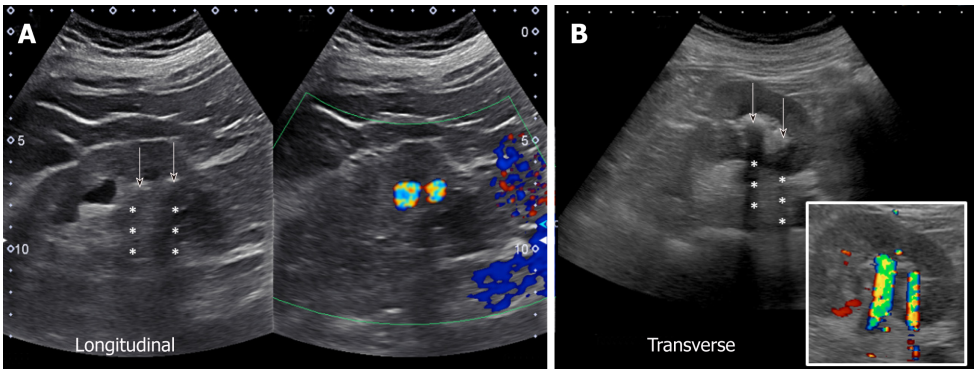


Figure 14 Renal sonogram demonstrating grey-scale (A) and corresponding color Doppler (B) images of the kidney demonstrating acoustic shadowing and twinkling sign respectively, which is diagnostic of stones. Arrows point to the hyperechoic stones and asterisks indicate shadowing.

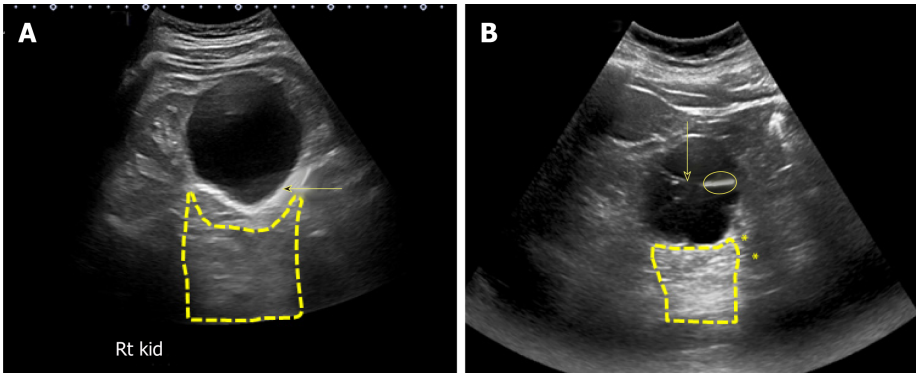


Figure 15 Renal sonogram demonstrating a large simple cyst in the lower pole of the right kidney. A: Arrow points to echogenic far wall and the contained area posterior to the cyst represents acoustic enhancement; B: Example of a complex cyst with septations (arrow). The image shows both acoustic enhancement from the cyst (contained area with yellow outline) and acoustic shadowing (asterisks) from calcifications in the septa (circle).

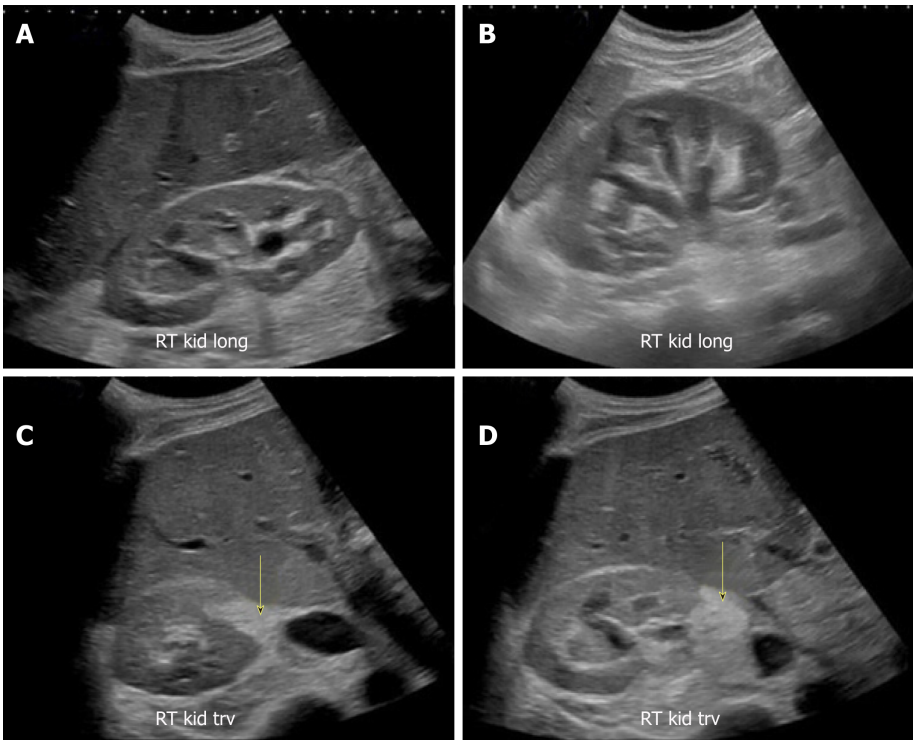


Figure 16 Renal sonogram images. A, B: Bilateral moderate hydronephrosis and hydroureter; C, D: Transverse views of the right kidney showing echogenic debris (arrows) in the right collecting system in addition to fluid–fluid levels, suggestive of pyonephrosis. Adapted from reference No. 43, first author's previous work, published under CC BY-NC 4.0 license.

REFERENCES

1 **Narula J**, Chandrashekhar Y, Braunwald E. Time to Add a Fifth Pillar to Bedside Physical Examination: Inspection, Palpation, Percussion, Auscultation, and Insonation. *JAMA Cardiol* 2018; **3**: 346-350 [PMID: 29490335 DOI: 10.1001/jamacardio.2018.0001]

2 **Kaptein MJ**, Kaptein EM. Focused Real-Time Ultrasonography for Nephrologists. *Int J Nephrol* 2017; **2017**: 3756857 [PMID: 28261499 DOI: 10.1155/2017/3756857]

3 **Koratala A**, Bhattacharya D, Kazory A. Helping patients and the profession: Nephrology-oriented point-of-care ultrasound program for internal medicine residents. *Clin Nephrol* 2018 [PMID: 30563609 DOI: 10.5414/CN109652]

4 **Lieu D**. Ultrasound physics and instrumentation for pathologists. *Arch Pathol Lab Med* 2010; **134**: 1541-1556 [PMID: 20923312 DOI: 10.1043/2009-0730-RA.1]

5 **Enriquez JL**, Wu TS. An introduction to ultrasound equipment and knobology. *Crit Care Clin* 2014; **30**: 25-45, v [PMID: 24295840 DOI: 10.1016/j.ccc.2013.08.006]

6 **Abu-Zidan FM**, Hefny AF, Corr P. Clinical ultrasound physics. *J Emerg Trauma Shock* 2011; **4**: 501-503 [PMID: 22090745 DOI: 10.4103/0974-2700.86646]

7 **Faubel S**, Patel NU, Lockhart ME, Cadnapaphornchai MA. Renal relevant radiology: use of ultrasonography in patients with AKI. *Clin J Am Soc Nephrol* 2014; **9**: 382-394 [PMID: 24235286 DOI: 10.2215/CJN.04840513]

8 **Ozmen CA**, Akin D, Bilek SU, Bayrak AH, Senturk S, Nazaroglu H. Ultrasound as a diagnostic tool to differentiate acute from chronic renal failure. *Clin Nephrol* 2010; **74**: 46-52 [PMID: 20557866 DOI: 10.2379/CN106407]

9 **Gulati M**, Cheng J, Loo JT, Skalski M, Malhi H, Duddalwar V. Pictorial review: Renal ultrasound. *Clin Imaging* 2018; **51**: 133-154 [PMID: 29477809 DOI: 10.1016/j.clinimag.2018.02.012]

10 **American College of Radiology (ACR)**; Society for Pediatric Radiology (SPR); Society of Radiologists in Ultrasound (SRU); American Institute of Ultrasound in Medicine (AIUM). AIUM practice guideline for the performance of an ultrasound examination of solid-organ transplants. *J Ultrasound Med* 2014; **33**: 1309-1320 [PMID: 24958421 DOI: 10.7863/ultra.33.7.1309]

11 **Naesens M**, Heylen L, Lerut E, Claes K, De Wever L, Claus F, Oyen R, Kuypers D, Evenepoel P, Bammens B, Sprangers B, Meijers B, Pirenne J, Monbaliu D, de Jonge H, Metalidis C, De Vusser K, Vanrenterghem Y. Intrarenal resistive index after renal transplantation. *N Engl J Med* 2013; **369**: 1797-1806 [PMID: 24195547 DOI: 10.1056/NEJMoa1301064]

12 **Granata A**, Fiorini F, Andrulli S, Logias F, Gallieni M, Romano G, Sicurezza E, Fiore CE. Doppler ultrasound and renal artery stenosis: An overview. *J Ultrasound* 2009; **12**: 133-143 [PMID: 23397022 DOI: 10.1016/j.jus.2009.09.006]

13 **O'Neill WC**. Renal relevant radiology: use of ultrasound in kidney disease and nephrology procedures. *Clin J Am Soc Nephrol* 2014; **9**: 373-381 [PMID: 24458082 DOI: 10.2215/CJN.03170313]

14 **Leekam RN**, Matzinger MA, Brunelle M, Gray RR, Grosman H. The sonography of renal columnar hypertrophy. *J Clin Ultrasound* 1983; **11**: 491-494 [PMID: 6417185 DOI: 10.1002/jcu.1870110907]

15 **Niyyar VD**, O'Neill WC. Point-of-care ultrasound in the practice of nephrology. *Kidney Int* 2018; **93**: 1052-1059 [PMID: 29477241 DOI: 10.1016/j.kint.2017.11.032]

16 **Kolofousi C**, Stefanidis K, Cokkinos DD, Karakitsos D, Antypa E, Piperopoulos P. Ultrasonographic features of kidney transplants and their complications: an imaging review. *ISRN Radiol* 2012; **2013**: 480862 [PMID: 24967275 DOI: 10.5402/2013/480862]

17 **Delair SM**, Kurzrock EA. Clinical utility of ureteral jets: disparate opinions. *J Endourol* 2006; **20**: 111-114 [PMID: 16509793 DOI: 10.1089/end.2006.20.111]

18 **Dinkel E**, Ertel M, Dittrich M, Peters H, Berres M, Schulte-Wissermann H. Kidney size in childhood. Sonographical growth charts for kidney length and volume. *Pediatr Radiol* 1985; **15**: 38-43 [PMID: 3881724 DOI: 10.1007/bf02387851]

19 **Harmse WS**. Normal variance in renal size in relation to body habitus. *SAJR* 2011; **15**: 355 [DOI: 10.4102/sajr.v15i4.355]

20 **Abrams HL**. Quantitative derivates of renal radiologic studies. An overview. *Invest Radiol* 1972; **7**: 240-279 [PMID: 4560927 DOI: 10.1097/00004424-197207000-00008]

21 **Miletić D**, Fuckar Z, Sustić A, Mozetic V, Stimac D, Zauhar G. Sonographic measurement of absolute and relative renal length in adults. *J Clin Ultrasound* 1998; **26**: 185-189 [PMID: 9572380 DOI: 10.1002/(sici)1097-0096(199805)26:43.0.co;2-9]

22 **Manley JA**, O'Neill WC. How echogenic is echogenic? Quantitative acoustics of the renal cortex. *Am J Kidney Dis* 2001; **37**: 706-711 [PMID: 11273869 DOI: 10.1016/S0272-6386(01)80118-9]

23 **Page JE**, Morgan SH, Eastwood JB, Smith SA, Webb DJ, Dilly SA, Chow J, Pottier A, Joseph AE. Ultrasound findings in renal parenchymal disease: comparison with histological appearances. *Clin Radiol* 1994; **49**: 867-870 [PMID: 7828393 DOI: 10.1016/s0009-9260(05)82877-6]

24 **Koratala A**, Chamarthi G, Loy JL, Olaoye OA. Illusion, Suspicion, and Obstruction: An Intriguing Case of Acute Kidney Injury. *Am J Med* 2018; **131**: e489-e491 [PMID: 30138598 DOI: 10.1016/j.amjmed.2018.08.003]

25 **Esprit DH**, Koratala A, Chornyy V, Wingo CS. Obstructive Nephropathy Without Hydronephrosis: Suspicion Is the Key. *Urology* 2017; **101**: e9-e10 [PMID: 28011273 DOI: 10.1016/j.urology.2016.11.041]

26 **Morin ME**, Baker DA. The influence of hydration and bladder distension on the sonographic diagnosis of hydronephrosis. *J Clin Ultrasound* 1979; **7**: 192-194 [PMID: 110840 DOI: 10.1002/jcu.1870070306]

27 **Peake SL**, Roxburgh HB, Langlois SL. Ultrasonic assessment of hydronephrosis of pregnancy. *Radiology* 1983; **146**: 167-170 [PMID: 6849041 DOI: 10.1148/radiology.146.1.6849041]

28 **Koratala A**, Bhattacharya D, Chamarthi G. Point-of-care renal ultrasound: Interpret with care. *Clin Case Rep* 2018; **7**: 233-235 [PMID: 30656051 DOI: 10.1002/ccr3.1934]

29 **Koratala A**, Alquadan KF. Parapelvic cysts mimicking hydronephrosis. *Clin Case Rep* 2018; **6**: 760-761 [PMID: 29636957 DOI: 10.1002/ccr3.1431]

30 **Tarzamni MK**, Sobhani N, Nezami N, Ghiasi F. Bilateral parapelvic cysts that mimic hydronephrosis in two imaging modalities: a case report. *Cases J* 2008; **1**: 161 [PMID: 18801196 DOI: 10.1186/1757-1626-1-161]

31 **Koratala A**, Bhattacharya D. Extrarenal pelvis mimicking hydronephrosis: a case for caution. *Clin Case Rep* 2017; **5**: 1720-1721 [PMID: 29026582 DOI: 10.1002/ccr3.1143]

32 **Hansen KL**, Nielsen MB, Ewertsen C. Ultrasonography of the Kidney: A Pictorial Review. *Diagnostics (Basel)* 2015; **6**: pii: E2 [PMID: 26838799 DOI: 10.3390/diagnostics6010002]

33 **Smith-Bindman R**, Aubin C, Bailitz J, Bengiamin RN, Camargo CA, Corbo J, Dean AJ, Goldstein RB, Griffey RT, Jay GD, Kang TL, Kriesel DR, Ma OJ, Mallin M, Manson W, Melnikow J, Miglioretti DL, Miller SK, Mills LD, Miner JR, Moghadassi M, Noble VE, Press GM, Stoller ML, Valencia VE, Wang J, Wang RC, Cummings SR. Ultrasonography versus computed tomography for suspected nephrolithiasis. *N Engl J Med* 2014; **371**: 1100-1110 [PMID: 25229916 DOI: 10.1056/NEJMoa1404446]

34 **Koratala A**, Vangapalli A, Bhattacharya D, Lee Loy J. Bladder stone: "Must know" ultrasonographic signs. *Clin Case Rep* 2018 [DOI: 10.1002/ccr3.1977]

35 **Gliga ML**, Chirila CN, Podeanu DM, Imola T, Voicu SL, Gliga MG, Gliga PM. Twinkle, twinkle little stone: an artifact improves the ultrasound performance! *Med Ultrason* 2017; **19**: 272-275 [PMID: 28845492 DOI: 10.11152/mu-984]

36 **Baad M**, Lu ZF, Reiser I, Paushter D. Clinical Significance of US Artifacts. *Radiographics* 2017; **37**: 1408-1423 [PMID: 28777700 DOI: 10.1148/rg.2017160175]

37 **Park BK**, Kim B, Kim SH, Ko K, Lee HM, Choi HY. Assessment of cystic renal masses based on Bosniak classification: comparison of CT and contrast-enhanced US. *Eur J Radiol* 2007; **61**: 310-314 [PMID: 17097844 DOI: 10.1016/j.ejrad.2006.10.004]

38 **Muglia VF**, Westphalen AC. Bosniak classification for complex renal cysts: history and critical analysis. *Radiol Bras* 2014; **47**: 368-373 [PMID: 25741120 DOI: 10.1590/0100-3984.2013.1797]

39 **Vourganti S**, Agarwal PK, Bodner DR, Dogra VS. Ultrasonographic evaluation of renal infections. *Radiol Clin North Am* 2006; **44**: 763-775 [PMID: 17147985 DOI: 10.1016/j.rcl.2006.10.001]

40 **June CH**, Browning MD, Smith LP, Wenzel DJ, Pyatt RS, Checchio LM, Amis ES. Ultrasonography and computed tomography in severe urinary tract infection. *Arch Intern Med* 1985; **145**: 841-845 [PMID: 3888134 DOI: 10.1016/s0022-5347(17)47485-4]

41 **Craig WD**, Wagner BJ, Travis MD. Pyelonephritis: radiologic-pathologic review. *Radiographics* 2008; **28**: 255-77; quiz 327-8 [PMID: 18203942 DOI: 10.1148/rg.281075171]

42 **Kamboj M**, Lee Loy J, Koratala A. Renal ultrasonography: a reliable diagnostic tool for pyonephrosis. *Clin Case Rep* 2018; **6**: 1176-1178 [PMID: 29881590 DOI: 10.1002/ccr3.1512]

43 **Beaubien-Souligny W**, Bouchard J, Denault A. Point-of-care ultrasound in end-stage kidney disease: beyond lung ultrasound. *Curr Opin Nephrol Hypertens* 2018; **27**: 487-496 [PMID: 30188387 DOI: 10.1097/MNH.0000000000000453]

44 **Mallamaci F**, Benedetto FA, Tripepi R, Rastelli S, Castellino P, Tripepi G, Picano E, Zoccali C. Detection of pulmonary congestion by chest ultrasound in dialysis patients. *JACC Cardiovasc Imaging* 2010; **3**: 586-594 [PMID: 20541714 DOI: 10.1016/j.jcmg.2010.02.005]

45 **Sekiguchi H**, Seaburg LA, Suzuki J, Astorne WJ, Patel AS, Keller AS, Gajic O, Kashani KB. Central venous pressure and ultrasonographic measurement correlation and their associations with intradialytic adverse events in hospitalized patients: A prospective observational study. *J Crit Care* 2018; **44**: 168-174 [PMID: 29132056 DOI: 10.1016/j.jcrc.2017.10.039]

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ORIGINAL ARTICLE

Observational Study

Static pressures, intra-access blood flow and dynamic Kt/V profiles in the prediction of dialysis access function

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Author contributions: Koratala A and Dass B have contributed equally to this work. Koratala A, Dass B and Ejaz AA designed research, drafted the manuscript; Koratala A, Dass B and Alquadan KF collected the data; Koratala A, Sharma S, Singhania G and Ejaz AA analyzed the data; Ejaz AA served as the mentor and critically reviewed and revised the manuscript.

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Conflict-of-interest statement: The authors have no conflict of interest to declare.

STROBE statement: The STROBE statement has been adopted.

Open-Access: This article is an

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Abstract

BACKGROUND

Hemodialysis machine-generated circuit pressures and clearance profiles are potential predictors of quality assurances. In our practice, we previously we observed that elevated static access pressures were associated with abnormal Kt/V values, high access recirculation and deviation of the Kt/V profile (Abnormal Kt/V profile) from normally expected values (Normal Kt/V profile).

AIM

To hypothesize that static or derived access pressures would correlate with direct intra-access blood flow rates and that clearance (Kt/V) profiles would correlate with measured Kt/V values.

METHODS

Static access pressures, real-time adequacy of dialysis and intra-access blood flow were investigated in end stage renal disease patients undergoing hemodialysis. Wilcoxon-Mann-Whitney test, chi-square test or Fisher's exact test was used to investigate differences between the groups; Spearman's rank correlation test to investigate relationships between static pressures, direct intra-access pressures and Kt/V profiles; and multinomial logistic regression models to identify the independent effect of selected variables on Kt/V profiles. Odds ratio were calculated to measure the association between the variables and Kt/V profiles.

RESULTS

One hundred and seven patients were included for analysis. There were no significant differences between genders, and types of vascular access between the normal vs. abnormal clearance (Kt/V) profile groups. No significant correlation

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could be demonstrated between static access pressures and Kt/V profiles, static access pressures and intra-access blood flow, intra-access blood flow and Kt/V profiles, measured Kt/V and Kt/V profiles or recirculation and Kt/V profiles.

CONCLUSION
In this study utilizing measured versus estimated data, we could not validate that dialysis machine generated elevated static pressures predict intra-access blood flow disturbances or that abnormal Kt/V profiles predict access recirculation or inadequate dialysis. These parameters, though useful estimates, cannot be accepted as quality assurance for dialysis adequacy or access function without further evidences.

Key words: Static; Intra-access; Pressures; Hemodialysis; Kt/V

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Core tip: Hemodialysis machine-generated circuit pressures and clearance profiles are potential predictors of quality assurances. We hypothesized that static or derived access pressures would correlate with direct intra-access blood flow rates and that Kt/V profiles would correlate with measured Kt/V values. However, we could not validate that dialysis machine generated elevated static pressures predict intra-access blood flow disturbances or that abnormal Kt/V profiles predict access recirculation or inadequate dialysis. While future larger studies are needed to confirm these findings, we believe our study serves as a basis for them.

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INTRODUCTION

On-line monitoring of ultraviolet (UV) absorption of spent dialysate during routine hemodialysis allows continuous measurement of removed solutes from spent dialysate. The continuous calculation of the clearances-adequacy of dialysis (Kt/V) - throughout therapy is graphically displayed and allows clinicians to monitor the efficacy of the dialysis treatment in real time, the so-called dynamic Kt/V profile. Previously we observed that elevated static access pressures were associated with abnormal Kt/V values, high access recirculation and deviation of the Kt/V profile (Abnormal Kt/V profile) from normally expected values (Normal Kt/V profile). These observations suggested the possibility that dialysis machine-generated real-time Kt/V profiles may provide a non-invasive, inexpensive and quick assessment of adequacy of dialysis and access function in hemodialysis patients. To validate our observations, we performed a larger, prospective observational study. We hypothesized that static or derived access pressures would correlate with direct intra-access blood flow rates and that Kt/V profiles would correlate with measured Kt/V values.

MATERIALS AND METHODS

End stage renal disease patients undergoing maintenance hemodialysis at the outpatient dialysis unit of an academic medical center were included in the study. All patients were dialyzed with Dialog (B Braun AG)™ dialysis machine with a built-in UV-Spectrophotometer (Option Adimea, B Braun AG™). The machine continually calculates the adequacy of dialysis (Kt/V) throughout therapy and graphically displays Kt/V profile and an indication of how close it is to the target (Figure 1). Fluctuations in the predicted curve caused by changes in variables affecting Kt/V are visible at a glance. Abnormal Kt/V profile was defined as major fluctuations from the predicted curve displayed. The standard dialysis prescription was high-flux polysulfone dialyzers (F160; Fresenius, Worcester, MA, United States), blood flow rate

of 350-450 mL/min, dialysate flow rate of 500-800 mL/min and ultrafiltration rates of 5-10 mL/kg·h as appropriate. Anticoagulation was not used in patients per divisional protocol. Only patients with functional arteriovenous fistula or graft were included in the study.

The ESRD program in this academic center employed an Access Surveillance Program and the team members included nephrologists, nephrology nurses, technicians, interventional radiologists, surgeons, primary care physicians and patients. Functional access was defined as blood flow > 600 mL/min[1]. Vascular access dysfunction was defined as elevated static or derived venous pressures (PV), elevated negative arterial pre-pump pressures (PA) that prevent increasing to acceptable blood flow rates, or unexplained decreases in the measured amount of hemodialysis delivered (Kt/V). Critical intra-access blood flow was defined as AVF blood flow < 600 mL/min, AVG blood flow < 800 mL/min, or decreased intra-access blood flow by > 20% compared to previous values[2,3]. Direct intra-access flow measurements were performed with ultrasound dilution (Transonic Systems Inc.™). Clinically significant access recirculation was defined as values more than 5%. The demographic information was obtained from the chart, the static pressures and Kt/V profiles were obtained by direct visualization of the dialysis machine display screen, the Kt/V values obtained from biochemical measurements, and intra-access blood flow data were obtained from Transonic measurement data. The study was approved by the Institutional Review Board at the University of Florida.

Statistical analysis

Results are presented as mean ± SE with *P*-value. Wilcoxon-Mann-Whitney test was used to compare independent groups not normally distributed, χ^2 test or Fisher's exact test for comparison between two categorical variables, and *P* value < 0.05 was considered statistically significant. Associations between static pressures, direct intra-access pressures and Kt/V profiles were investigated using Spearman's rank correlation test (r_s) and regression analysis where applicable. These predictors were included in a univariate and subsequent multinomial logistic regression model to identify their independent effect on Kt/V profiles. The strength of the association of each variable with Kt/V profiles were summarized by calculating an odds ratio (OR) and a corresponding 95%CI. All analyses were conducted using SPSS version 20 (Chicago, IL, United States). The study conception, design, execution, data collection, analysis, and manuscript preparation were formed in its entirety and independently by the investigators. Every author had independent access to the analysis.

RESULTS

Baseline characteristics

The analysis included 107 patients. The baseline characteristics of the patients are shown in Table 1. There were no significant differences between age, gender, types of vascular access, PA, intra-access flow rates or percentage of patients with abnormal access recirculation between the Normal vs. Abnormal Kt/V profile groups. However, the Abnormal Kt/V profile group had significantly lower PV and higher measured Kt/V values. Twenty-one patients underwent access recirculation studies, twelve in the Normal Kt/V group and nine in the Abnormal Kt/V group. Access recirculation test was positive in 12/12 patients studied in the Normal Kt/V profile group and 8/9 patients in the Abnormal Kt/V profile group. The percentages in their respective groups are shown in Table 1.

Relationship of static access pressures and Kt/V profiles

No significant association could be demonstrated between PA and Kt/V profile groups, nor between PA and absolute Kt/V values (Table 2). However, significant associations were noted between PV and Kt/V profiles. When analyzed for highest quartile of PV values (PV_{QTRL}), no significant associations were found between PV_{QTRL} and Kt/V profiles or Kt/V values.

Relationship of static access pressures and intra-access blood flow

No significant association could be demonstrated between PA or PV and intra-access blood flow, even when analyzed separately for AVF and AVF. When plotted against PA or PV, intra-access blood flow exhibited a quadratic relationship (Figure 2A,B). Similar patterns were revealed when plotted separately for Kt/V profiles (Figure 2C-F) and access types (Figures not shown).

Relationship of intra-access blood flow and Kt/V profiles

No significant association could be demonstrated between intra-access blood flow and

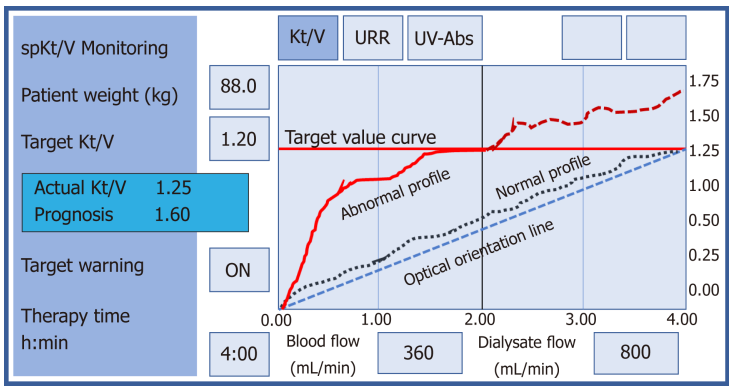


Figure 1 Depiction of continuous display of adequacy of dialysis throughout therapy (Kt/V profile) as seen on a hemodialysis machine monitor. Optical orientation line depicts the ideal Kt/V profile. Normal Kt/V profile represents real-time values that closely mimic the optical orientation line. Abnormal Kt/V profile depicts major fluctuations from the optical orientation line.

Kt/V profiles.

Relationship of Critical intra-access blood flow and static pressures and Kt/V profiles

No significant association could be demonstrated between critical intra-access blood flow and static pressures or Kt/V profiles.

Relationship of measured Kt/V and Kt/V profiles

No significant association could be demonstrated between Kt/V and Kt/V profiles.

Relationship of recirculation and Kt/V profiles

No significant association could be demonstrated between access recirculation and Kt/V profiles. The odds ratio for positive access recirculation associated with Abnormal Kt/V profile was 2.5 (95%CI: 1-5-4.2; P = 0.429).

Risks for Abnormal Kt/V profile

In univariate analysis, age (OR = 1.02, 95%CI: 0.99-1.04, P = 0.186), gender (OR = 1.10, 95%CI: 0.50-2.42, P = 0.813), type of access (OR = 1.78, 95%CI: 0.76-4.16, P = 0.182), PA (OR = 1.00, 95%CI: 0.90-1.00, P = 0.874) and intra-access blood flow rates (OR = 1.00, 95%CI: 0.99-1.00, P = 0.075) were not significant risk factors for Abnormal Kt/V profile. However, PV (OR = 1.01, 95%CI: 1.00-1.02, P = 0.033) was a significant risk factor. In the multivariate regression model that included all of these variables irrespective of their statistical significance in the univariate model, none of them were significant predictors of Abnormal Kt/V profile.

DISCUSSION

UV absorption technology has enabled dynamic monitoring of dialysis efficiency in real time and the potential for non-invasive and quick assessment of access function. In this study we investigated the validity of these observations by analyzing the association between machine-generated data and measured data in patients undergoing maintenance dialysis.

The major finding of the study was the lack of significant association between static access pressures and direct intra-access blood flow, Kt/V profiles and direct intra-access blood flow or Kt/V profiles and Kt/V values. The significant associations seen between PV and Kt/V profile and PA and intra-access blood flow could not be demonstrated in the adjusted regression model. The findings are better understood when considering that static access pressures are arterial and venous circuit pressures that are generated by transducers inside the machine. The arterial pump effect is measured as the arterial pressure and is dependent on the blood flow within the tubule, which is dependent on the internal diameter of the tubing segment within the pump. Effective blood flow deviates significantly from calculated blood flow when PA ≤ −150 mmHg and can lead to loss of treatment efficiency[4]. PV are monitored distal to the dialyzers and usually located before the air bubble chamber. Low PV may be due to low PA secondary to poor arterial flow. High PV indicate an obstruction in the venous limb distal to this point and can be due to venous needle blow out or clots

Table 1 Baseline patient characteristics

Variables	Full cohort (*n* = 107)	Normal Kt/V profile (*n* = 68)	Abnormal Kt/V profile (*n* = 39)	*P*-value
Demographics				
Age (yr)	54.9 ± 1.5	56.5 ± 1.8	52.3 ± 2.8	0.210
Male gender (%)	52.3	46.1	53.8	0.843
Access types				
AV fistula (%)	63.5	58.8	71.8	1.000
AV graft (%)	36.5	41.2	28.2	1.000
Access pressures and flow rates				
Static PA (mmHg)	-196.7 ± 4.1 (-203)[1]	-196.2 ± 4.6 (-202)	-197.5 ± 7.9 (-206)	0.884
Static PV (mmHg)	240.8 ± 5.5 (244)	249.9 ± 7.2 (254)	224.8 ± 7.9 (235)	0.021
Intra-access flow rate	1369.5 ± 82.8 (1070)	1256.3 ± 87.4 (1035)	1566.8 ± 165.6 (1170)	0.103
Adequacy of dialysis				
Measured Kt/V	1.42 ± 0.04 (1.46)	1.40 ± 0.04 (1.44)	1.45 ± 0.07 (1.51)	< 0.001
Access recirculation > 5% (*n* = 21) (%)	18.7	17.4	20.5	0.429

[1]Median values in parenthesis.

in the air trap, kinks in the tubing or a stenosis within the vascular access. Equating elevated static pressures to vascular access problems maybe deceptive as suggested by our findings.

Data was also analyzed according to access types as the sites of stenosis vary accordingly. In AVF, problems occur in the arterial inflow (forearm AVF 55%, upper arm AVF 17%) or between the needles (forearm AVF 19%, upper arm AVF 17%). In contrast, majority of stenosis in AV grafts are in the venous outflow (93%)[5,6]. The validity of the major findings of this study of no significant association between study parameters persisted in this subgroup. This was also true when analyzed for critical intra-access blood flow in the two types of vascular access group. We did not determine absolute static intra-access pressure ratios due to its reported limitations in detecting flow limiting problems on the arterial inflow or between the dialysis needles where most problems occur in AVFs.

Close concordance between the blood-based and UV-based Kt/V values has been reported[7,8], however the relationship between Kt/V profiles and Kt/V has hitherto not been studied. In our investigations we could not demonstrate significant correlation between Kt/V profile and Kt/V values. Technical factors including volume of distribution and mass transfer coefficients affect dialysate absorbance and confound clearance results. A dialyzer membrane has different filtration rate for molecules of different compounds and for this reason concentrations of these substances, both in blood and in the dialysis do not change according to one pattern. While the UV-absorbance correlates well to the concentration of small solutes such as urea, creatinine and uric acid in the spent dialysate and in the blood at a fixed wavelength of 285 nm, the lowest correlations exist for sodium, calcium, glucose, vitamin B_{12} and albumin[9]. Medications and fluid administration during dialysis may therefore affect clearance outputs.

Determining the percentage of access recirculation is used to evaluate adequacy of dialysis clearance. Access recirculation decreases adequacy of dialysis and has been used to indicate access issues. Despite the high percentage of positive access recirculation present in the patients studied in both groups, no correlation was demonstrated with Kt/V profiles. Thus, in our current investigation utilizing a surveillance measure and a clinical monitoring measure simultaneously, we could not validate that dialysis machine generated elevated static pressures predict intra-access blood flow disturbances or that abnormal Kt/V profiles predict access recirculation or inadequate dialysis. These parameters, though useful estimates, cannot be accepted as quality assurance for dialysis adequacy or access function without further studies.

Table 2 Relationships between static access pressures, access blood flow and Kt/V profiles

Strength of association		
Variables	r_s	*P*-value
Static PA – Kt/V profiles	-0.05	0.636
Static PA – Kt/V	-0.14	0.155
Static PV – Kt/V profiles	0.19	0.047
Static PV_{QTRL} – Kt/V profiles	-0.17	0.078
Static PA – Intra-access blood flow	-0.25	0.009
Static PA – Intra-AVF blood flow	-0.22	0.069
Static PA – Intra-AVG blood flow	-0.16	0.319
Static PV – Intra-access blood flow	0.04	0.666
Intra-access blood flow - Kt/V profile	-0.12	0.208
Intra-AVF blood flow - Kt/V profile	0.17	0.174
Intra-AVG blood flow - Kt/V profile	0.05	0.783
Critical intra-access blood flow - PA	0.24	0.226
Critical intra-access blood flow - PV	0.14	0.482
Critical intra-access blood flow – Kt/V profile	0.01	0.962
20% reduced intra-access blood flow – Static PA	-0.15	0.262
20% reduced intra-access blood flow – Static PV	-0.03	0.851
20% reduced intra-access blood flow – Kt/V profile	0.14	0.294
Access recirculation > 5% - Kt/V profile	0.20	0.389
Kt/V – Kt/V profile	-0.08	0.397

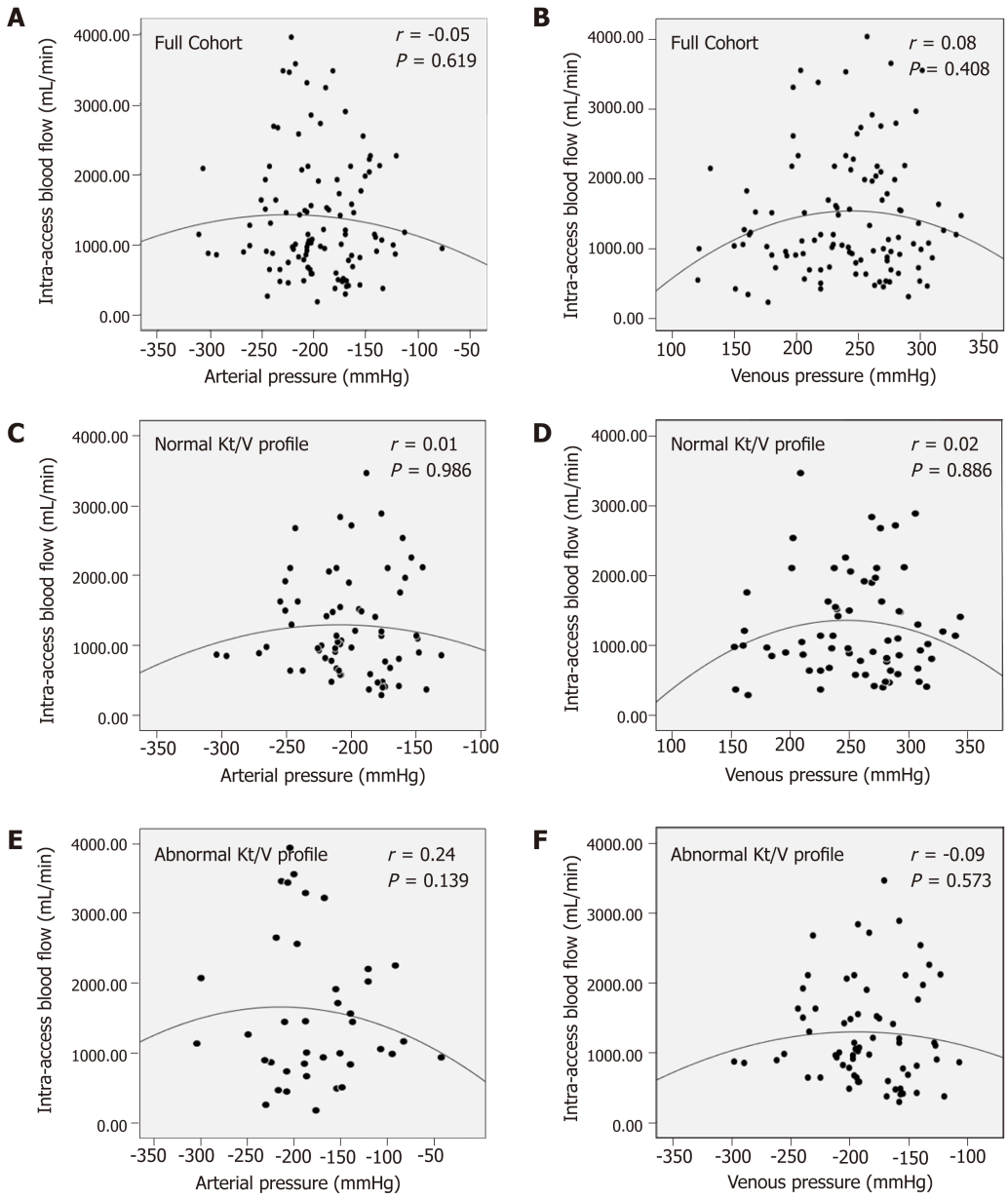


Figure 2 Relationship between intra-access blood flow and access (arterial and venous) pressures in the full cohort (A and B); normal Kt/V profile cohort (C and D) and the abnormal Kt/V profile cohort (E and F).

ARTICLE HIGHLIGHTS

Research background

The monitoring of dialysate ultraviolet absorbance and hemodialysis machine generated clearance profiles is a validated technology to measure dialysis adequacy (Kt/V) and allows for continuous and real-time tracking during each session. The Kt/V profile displayed by the machine includes the delivered-dose curve superimposed on the anticipated trajectory.

Research motivation

In our practice, we noted that that elevated static access pressures were associated with abnormal Kt/V values, high access recirculation and deviation of the Kt/V profile (Abnormal Kt/V profile) from normally expected values (Normal Kt/V profile).

Research objectives

Based on the above observation, we hypothesized that static or derived access pressures would correlate with intra-access blood flow rates and that clearance (Kt/V) profiles would correlate with measured Kt/V values.

Research methods

We investigated static access pressures, real-time adequacy of dialysis and intra-access blood

flow in patients undergoing hemodialysis. Appropriate statistical methods were used to investigate differences between the groups; relationships between static pressures, direct intra-access pressures and Kt/V profiles; and identify the independent effect of selected variables on Kt/V profiles. Odds ratio were calculated to measure the association between the variables and Kt/V profiles.

Research results

Among the 107 participants included, no significant correlation could be demonstrated between static access pressures and Kt/V profiles, static access pressures and intra-access blood flow, intra-access blood flow and Kt/V profiles, measured Kt/V and Kt/V profiles or recirculation and Kt/V profiles.

Research conclusions

We could not validate that dialysis machine generated elevated static pressures predict intra-access blood flow disturbances or that abnormal Kt/V profiles predict access recirculation or inadequate dialysis.

Research perspectives

The investigated hemodialysis machine-generated parameters and access flows, though useful estimates can't be accepted as quality assurance for dialysis adequacy or access function without further evidences. Larger studies are needed to formulate protocol-driven modifications to treatment parameters, which may serve as valuable nursing tools for optimizing patient therapy outcomes.

REFERENCES

1 **National Kidney Foundation, Inc**. K/DOQI Guidelines–Updates 2006. New York: National Kidney Foundation, Inc; 2001; Available from: http://www.kidney.org/PROFESSIONALS/kdoqi/guideline_upHD_PD_VA/index.htm

2 **Lee T**, Mokrzycki M, Moist L, Maya I, Vazquez M, Lok CE; North American Vascular Access Consortium. Standardized definitions for hemodialysis vascular access. *Semin Dial* 2011; **24**: 515-524 [PMID: 21906166 DOI: 10.1111/j.1525-139X.2011.00969.x]

3 **Hurst FP**, Lee RE, Thompson AM, Pullin BD, Silverstein DM. FDA Regulatory Perspectives for Studies on Hemodialysis Vascular Access. *Clin J Am Soc Nephrol* 2018; **13**: 513-518 [PMID: 28739575 DOI: 10.2215/CJN.02900317]

4 **Twardowski ZJ**, Haynie JD, Moore HL. Blood Flow, Negative Pressure, and Hemolysis During Hemodialysis. *Home Hemodial Int* 1999; **3**: 45-50 [PMID: 28455857 DOI: 10.1111/hdi.1999.3.1.45]

5 **Turmel-Rodrigues L**, Pengloan J, Baudin S, Testou D, Abaza M, Dahdah G, Mouton A, Blanchard D. Treatment of stenosis and thrombosis in haemodialysis fistulas and grafts by interventional radiology. *Nephrol Dial Transplant* 2000; **15**: 2029-2036 [PMID: 11096150 DOI: 10.1093/ndt/15.12.2029]

6 **Tessitore N**, Bedogna V, Lipari G, Melilli E, Mantovani W, Baggio E, Lupo A, Mansueto G, Poli A. Bedside screening for fistula stenosis should be tailored to the site of the arteriovenous anastomosis. *Clin J Am Soc Nephrol* 2011; **6**: 1073-1080 [PMID: 21441125 DOI: 10.2215/CJN.06230710]

7 **Castellarnau A**, Werner M, Günthner R, Jakob M. Real-time Kt/V determination by ultraviolet absorbance in spent dialysate: technique validation. *Kidney Int* 2010; **78**: 920-925 [PMID: 20631678 DOI: 10.1038/ki.2010.216]

8 **Mohamed A**, Davenport A. Comparison of methods to estimate haemodialysis urea clearance. *Int J Artif Organs* 2018; **41**: 371-377 [PMID: 29642725 DOI: 10.1177/0391398818766832]

9 **Fridolin I**, Magnusson M, Lindberg LG. On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: technique description. *Int J Artif Organs* 2002; **25**: 748-761 [PMID: 12296459 DOI: 10.1177/039139880202500802]

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CASE REPORT

A rare presentation of spontaneous atheroembolic renal disease: A case report

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Author contributions: Piranavan P, Rajan A, and Verma A provided direct patient care, collected data, and performed follow-up. Piranavan P and Rajan A performed the literature review and wrote the manuscript. Verma A and Vishal Jindal helped locate the relevant literature and draft the manuscript. All authors read and approved the final manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this case report and any accompanying histological images. A copy of the written consent is available for the review of the journal's editor-in-chief.

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Abstract

BACKGROUND

Atheroembolic renal disease (AERD) is caused by occlusion of the small renal arteries from embolized cholesterol crystals arising from ulcerated atherosclerotic plaques. This usually manifests as isolated renal disease or involvement from systemic atheroembolic disease. Here we report a case of AERD that responded well to steroid therapy.

CASE SUMMARY

A 62-year-old woman with a history of hypertension and stage IIIa chronic kidney disease was referred for rapidly worsening renal function over a 4-mo period. She complained of swollen legs, dyspnea on exertion, and two episodes of epistaxis about a month prior to admission. She reported no history of invasive vascular procedures, use of radio contrast agents, or treatment with anticoagulants or thrombolytic agents. Urinalysis showed a few red blood cells and granular casts. Serology was positive for cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA). Non-contrast-enhanced computed tomography of the chest, abdomen, and pelvis showed diffuse atherosclerotic changes in the aortic arch. Thus, c-ANCA-associated vasculitis was suspected, and the patient was started on pulse intravenous methylprednisolone. Her renal biopsy showed evidence of AERD. She was discharged with oral prednisone, and her renal function continued to improve during the initial follow-up.

CONCLUSION

In cases of non-vasculitis-associated ANCA, a high degree of clinical suspicion is required to pursue the diagnosis of spontaneous AERD in patients with clinical or radiological evidence of atherosclerotic burden. Although no specific treatment is available, the potential role of statins and steroids requires

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exploration.

Key words: Atheroembolic renal disease; Antineutrophil cytoplasmic antibodies associated vasculitis; Chronic kidney disease; Case report

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Core tip: Spontaneous atheroembolic renal disease (AERD) is a rare clinical entity. The role of antineutrophil cytoplasmic antibodies (ANCA) in atheroembolic diseases remains to be elucidated. Here, we report a case of rapidly progressive renal failure initially managed as cytoplasmic-ANCA associated renal disease but subsequently diagnosed as AERD with renal biopsy that responded surprisingly well to steroid therapy in a 62-year-old female patient. The patient was discharged with oral prednisone, and her renal function continued to improve during the initial follow-up. Although no specific treatment is available, the potential role of steroids requires exploration.

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INTRODUCTION

Atheroembolic renal disease (AERD) is an important yet underdiagnosed kidney disease that remains in need of further research. It can manifest as isolated renal disease or as a part of systemic atheroembolic disease[1]. AERD is caused by occlusion of the small arteries in the kidneys due to embolizing cholesterol crystals arising from ulcerated atherosclerotic plaques[2]. AERD generally occurs in patients aged > 60 years and generally complicates widespread atherosclerosis[3]. Increased invasive procedures, awareness, and patient longevity with atherosclerotic vascular disease as well as routine use of thrombolytic and anticoagulants in clinical practice are some of the main reasons behind the increase in incidence of AERD[4,5].

Although 60%-80% of cases occur following invasive procedures like angiography or vascular surgery, spontaneous cases are not uncommon[3,6]. Studies have shown poor renal outcomes and patient survival rates associated with AERD[3]. The dialysis requirement in AERD is variable per several studies as 37%-61%[2,7]. Moreover, the 1-year reported mortality rate associated with AERD is very high and variable at 13%-81%[8,9]. Here we report a case of rapidly progressive renal failure initially managed as cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA) associated renal disease but subsequently diagnosed as AERD that responded surprisingly well to steroid therapy.

CASE PRESENTATION

Chief complaints

A 62-year-old woman with a history of stage IIIa chronic kidney disease (CKD) was referred to our hospital for rapidly worsening renal function with hyperkalemia and metabolic acidosis. Her baseline creatinine level was 1.5 mg/dL but had increased to 5.62 mg/dL over a period of 4 mo.

History of present illness

She has noticed bilateral lower-extremity edema and dyspnea on exertion 2 wk prior to the presentation. She denied any hemoptysis, chest pain, nausea, or vomiting. She had two episodes of epistaxis about a month prior to admission. She had two admissions over the prior 6 mo for hypertensive encephalopathy and influenza pneumonia with hypoxemic respiratory failure. There was no history of any invasive vascular procedures, use of radio-contrast agents, or treatment with anticoagulants or thrombolytic agents.

History of past illness

Her relevant medical history included poorly controlled hypertension, hyperlipidemia, transient ischemic attack (TIA), diastolic heart failure, reactive airway disease, nephrolithiasis status post-lithotripsy, and osteoarthritis with nonsteroidal anti-inflammatory drug usage.

Personal and family history

She reported no family history of renal disease or thrombosis. She was a non-smoker and not a current alcohol user.

Physical examination upon admission

Her vitals were within normal limits and physical exam was normal except for bilateral lower-extremity edema and diminished breath sounds at the lung bases.

Laboratory examinations

Laboratory examination revealed normocytic normochromic anemia, serum eosinophilia (10%), hyperkalemia, metabolic acidosis, elevated creatinine (5.2 mg/dL), and elevated blood urea nitrogen (69 mg/dL). Urinalysis showed few red blood cells (RBCs), granular casts, and microalbuminuria and was negative for eosinophil and red cell casts. Serology was positive for antineutrophil antibody, anti-cardiolipin IgM antibody, and c-ANCA; 1:320.

Liver function tests, troponins, creatinine kinase, serum magnesium, calcium, HbA1c, complement levels (C3, C4), serum immunoglobulin levels, coagulation studies including prothrombin time, protein C, S levels/activity, antithrombin III activity, and lupus anticoagulant were within normal limits. Additionally, D-dimer was positive, and factor V Leiden mutation was negative.

Her perinuclear ANCA, atypical pANCA, anti-histone antibodies, anti-double-stranded DNA antibodies, anti-glomerular basement membrane antibodies, myeloma panel (serum and urine protein electrophoresis and immunofluorescence), viral panel (hepatitis B, C; HIV serology), and urine toxicology tests were negative.

Imaging examinations

A retroperitoneal ultrasound showed diffuse cortical thinning suggestive of medical renal disease but negative for obstructive uropathy. Non-contrast-enhanced computed tomography of the chest, abdomen, and pelvis was unremarkable except for diffuse atherosclerotic changes in the aortic arch. An electrocardiogram showed normal sinus rhythm, without any significant ischemic changes. A transthoracic echocardiogram showed a normal ejection fraction and was negative for atrial myxoma, vegetation, or intracardiac thrombi.

Initial management

She was clinically volume overloaded and had no oliguria. She responded well with good urine output to intravenous diuretics. Hyperkalemia and metabolic acidosis were improved upon initial medical management. In the background of rapidly worsening renal function with a positive titer of ANCA and history of epistaxis, ANCA-associated vasculitis was suspected; thus, she was started on pulse therapy of IV methylprednisolone 1 g/d. A renal biopsy was postponed to day 4 after admission due to relative contraindications such as aspirin usage, poorly controlled hypertension, and the patient's inability to assume a prone position due to body habitus. Once she was medically optimized, an interventional radiologist performed the renal biopsy under anesthesia. She was discharged with prednisone 60 mg because the biopsy report was not yet available at the time of discharge.

FINAL DIAGNOSIS

AERD. The biopsy showed major pathology of atheroembolic kidney with minimal acute tubular necrosis (ATN) that was negative for glomerulonephritis (Figure 1-4).

TREATMENT

There is no specific treatment for AERD; rather, care is generally supportive. The aspirin and statin that she was on for risk factor control for prior TIA were continued upon discharge. Due to her steroid responsiveness as shown in isolated case reports in the past, prednisone 40 mg was continued with the intention of a slow taper.

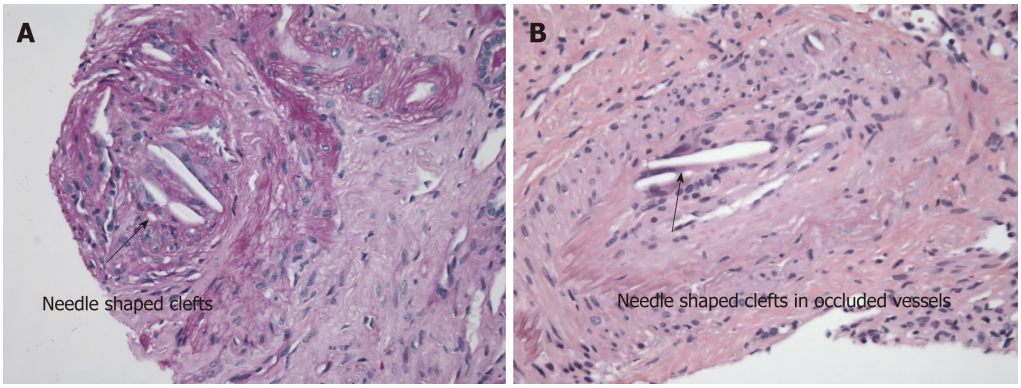


Figure 1 Atheroembolic disease of the kidney. A, B: Several small arteries and arterioles are affected and show needle-shaped clefts in occluded and recanalized vessels (see arrow).

OUTCOME AND FOLLOW-UP

Her renal function continued to improve, and the serum creatinine level had declined to 2.9 mg/dL at the 1-mo follow up. An ANCA test repeated twice after her 1-mo follow-up visit was negative.

DISCUSSION

Rapidly worsening renal function in a patient with pre-existing CKD can be challenging, especially when the differentials are broad. In our patient, the differentials were broad, initially including all pre-, intra-, and post-renal causes. The normal retroperitoneal ultrasound excluded post-renal obstructive uropathies. She had a recent hospital admission within the previous few months for pneumonia, but there were no documented hypotensive, sepsis, or cardiorenal episodes to suggest a pre-renal cause. Intra-renal etiologies include glomerular disease, tubular disease, interstitial pathologies, and vascular disease including medium-vessel vasculitis and thrombotic microangiopathies. History and physical exam findings were not strongly supportive of any of the above.

Although ANA tested positive, she had no other manifestations of lupus including renal biopsy evidence. She had a positive c-ANCA titer with a history of recurrent hematemesis but no significant hematuria, proteinuria, or dysmorphic RBCs; urinalysis revealed bland sediments. However, based on a high degree of clinical suspicion of ANCA-associated vasculitis, she was started on steroid pulse therapy. The biopsy confirmed predominant AERD pathology, and the presence of serum eosinophilia supported the above findings. In addition, computed tomography scans showed a significant atherosclerotic burden from the aortic arch to the abdominal aorta. However, she had no history of invasive vascular procedures, anticoagulation, or hemodynamic instability prior to the initial presentation.

Although the impact of technology upon health care is immense, diagnosing AERD remains challenging[9,10]. The association between atheroembolism and ANCA positivity is exceedingly rare[11]. A recent literature review performed by Zhang *et al*[11] mentioned 12 cases (3 with c-ANCA, 6 with p-ANCA, 1 with both, 2 positive for ANCA by early indirect immunofluorescence) of cholesterol embolism with ANCA positivity. Of those 12 patients, the mean age was 69 years; 10 were males with multiple medical comorbidities[11]. One-third of the patients had spontaneous atheroembolism like our patient[11]. The role of ANCA in atheroembolic diseases remains to be elucidated. A positive c-ANCA result without any systemic evidence of vasculitis at the time of testing was reported previously in the literature attributed to various reasons like cross-reactivity, non-specific neutrophil-activating properties, and analytical false values[12].

Two forms of AERD have been reported[1]. Acute or sub-acute AERD manifests from a single episode or recurrent episodes of massive showering of cholesterol emboli from ruptured unstable plaques[1]. Conversely, the slow erosion of atherosclerotic plaques leads to chronic AERD[13]. Although aortic atherosclerosis is essential for diagnosis, few patients with aortic atherosclerosis develop AERD[14,15]. Invasive angiography and vascular surgeries are the usual triggering factors for iatrogenic AERD[3,9]. Hemodynamic instability and anticoagulation contribute to spontaneous cases of AERD[6,16]. Anticoagulation leads to bleeding inside the plaque and

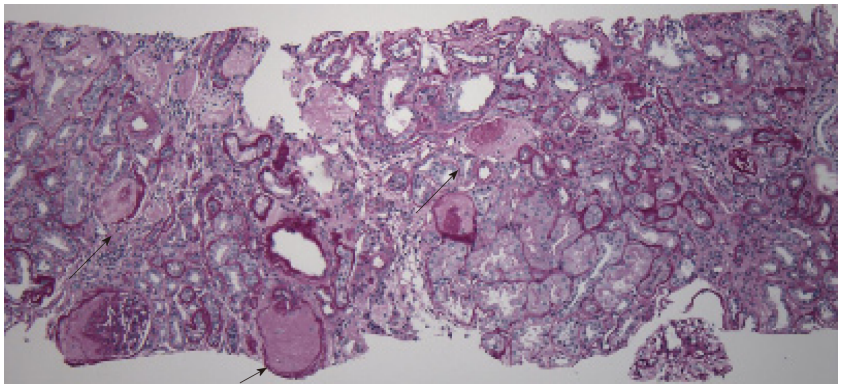


Figure 2 Focal global and segmental glomerulosclerosis (50% of glomeruli). Arrow: Acute tubular injury, mild.

subsequent rupture[5,17]. Mechanical trauma plays a key role, while angiography, particularly coronary angiography, is the most common iatrogenic cause[1,16]. In a good proportion (4%-13%) of cases, the disease remains idiopathic[4].

Cholesterol crystal embolization affects the skin, gastrointestinal system, kidneys, retina, lower-extremity skeletal muscles, and brain[18-20]. Two large renal biopsy studies[21,22] revealed an AERD frequency of 1%, while other studies based on autopsies of elderly patients who died after aortography or aortic surgery reported a frequency of 12%-77%[15]. Risk factors include older age, male, diabetes, hypertension, hyperlipidemia, and smoking[4]. It is generally associated with other atherosclerotic diseases. Contrast-induced nephropathy (CIN) and ATN are associated with AERD[2]. In CIN, renal function declines 1-2 d after contrast exposure[23] Conversely, in AERD, the decline in renal function is delayed, varying from days to weeks, except in a few rare cases of massive large showers of emboli[23]. Serum eosinophilia is noted up to 80% of patients with atheroembolic disease[24]. It is very challenging to differentiate atheroembolic disease from vasculitis, particularly with multi-organ involvement including skin manifestations like livedo reticularis and purple toe syndrome[2]. Chronic forms are confused with ischemic nephropathy and hypertensive nephropathy[2].

When the clinical triad of a precipitating event, acute or subacute renal failure, and typical skin findings is present, a biopsy is not required[9]. The presence of eosinophilia generally supports the diagnosis of AERD[1]. Histopathology usually reveals cholesterol crystal emboli as biconvex needle-shaped empty clefts identified in the lumen of arcuate and interlobular arteries[1,2]. These cells are referred to as ghost cells since they dissolve during processing[15]. Ischemic and inflammatory changes are observed in the lesions distal to the embolization[15].

Treatment is usually supportive and aims to restrict ischemic damage and prevent recurrent embolization[2]. Preventive management aims to avoid further precipitating factors, while medical intervention includes the aggressive treatment of hypertension, cardiac, renal failure, or optimal dialysis type[2]. No randomized controlled clinical trials of AERD treatment have been performed [2]. Belenfant and co-workers showed improvement in symptoms and nutritional intake with a low-dose steroid (0.3 mg/kg) in 18 patients with relapsing disease[25]. Dahlberg *et al*[26] and a few other studies showed the role of high-dose steroids[26]. Conversely, a prospective study of 354 patients with AERD showed no improvement in renal or patient outcomes[1]. Thus, steroid usage could play a role and may feature multi-system involvement, recurrent and progressive disease, and systemic inflammation[2]. Statins were justified with favorable outcomes in a few studies[3], and isolated reports have shown success with iloprost, pentoxifylline, and low-density lipoprotein apheresis[2] Antiplatelet agents and low molecular weight dextran have not shown a benefit[16].

Although the role of steroids is controversial, we decided to continue the steroids at a lower dose since our patient's renal function showed significant improvement. One could argue alternative explanations like spontaneous resolution of the disease in the setting of ATN (especially when her repeat ANCA was negative at the 1-month follow-up), but further studies are needed to confirm this in the future. The high-intensity statin was continued. We have limited data regarding the sequelae of AERD and renal outcome, requirements for dialysis, and survival rates[2]. Future research is needed regarding the potential benefits of steroids and statins[2].

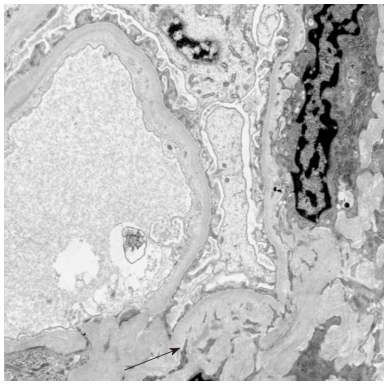


Figure 3 Electron microscopy showing sparsely distributed mesangial and glomerular capillary wall deposits (electron dense) (see arrow).

CONCLUSION

In summary, AERD has become a recognizable cause of acute or chronic renal failure. In cases like the above in which non-vasculitis was associated with ANCA tests, a high degree of clinical suspicion is required to pursue the diagnosis of spontaneous AERD in patients with clinical or radiological evidence of the atherosclerotic burden. No specific treatment is available, and the potential role of statins and steroids requires exploration.

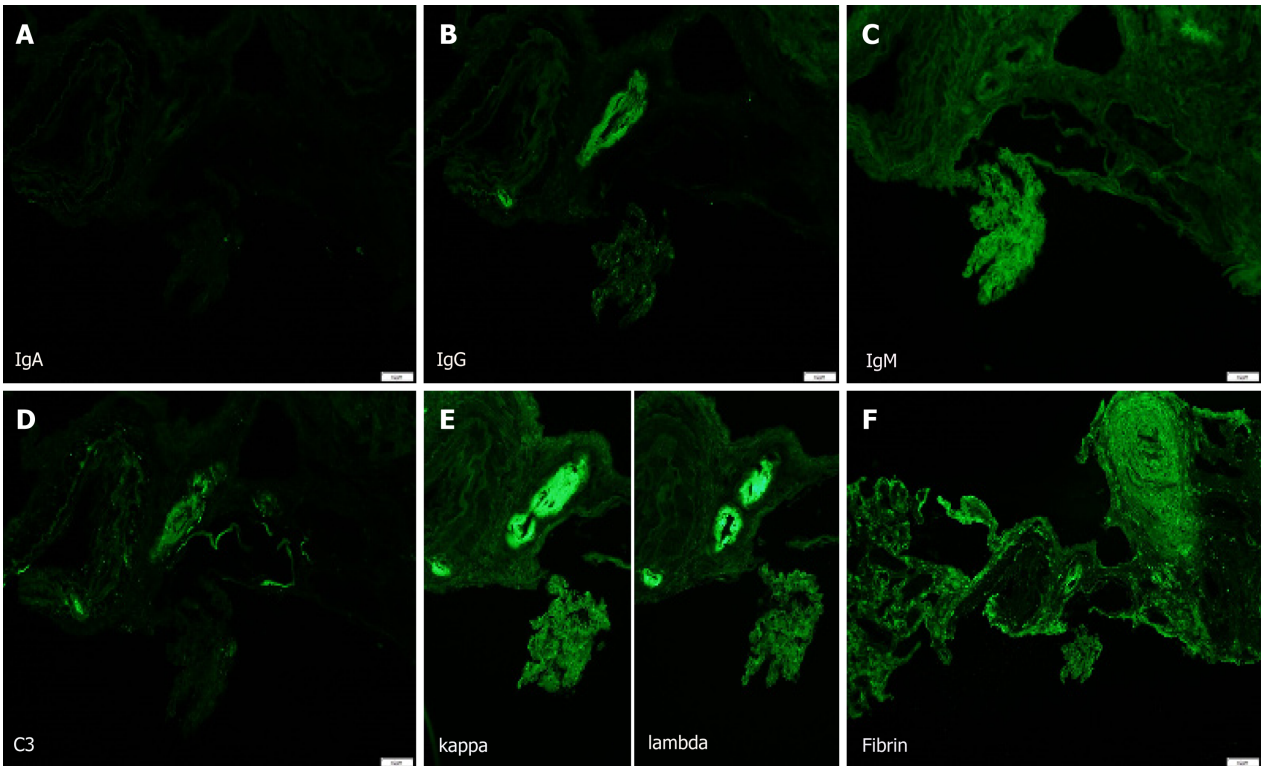


Figure 4 Ultra structural features suggestive of a mild and currently quiescent or inactive IgA nephropathy or sequelae of an IgA-dominant infection-associated glomerulonephritis. There are sparsely distributed mesangial and glomerular capillary wall deposits reactive for IgA, IgM, C3, and with equal expression of kappa and lambda light chains. There are no signs of a currently active glomerulitis. A: Mild IgA deposition; B: IgG deposition; C: IgM deposition; D: C3 deposition; E: Kappa and lambda chain deposition; F: Fibrin deposition.

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REFERENCES

1 **Scolari F**, Ravani P, Gaggi R, Santostefano M, Rollino C, Stabellini N, Colla L, Viola BF, Maiorca P, Venturelli C, Bonardelli S, Faggiano P, Barrett BJ. The challenge of diagnosing atheroembolic renal disease: clinical features and prognostic factors. *Circulation* 2007; **116**: 298-304 [PMID: 17606842 DOI: 10.1161/circulationaha.106.680991]
2 **Scolari F**, Ravani P. Atheroembolic renal disease. *Lancet* 2010; **375**: 1650-1660 [PMID: 20381857 DOI: 10.1016/S0140-6736(09)62073-0]
3 **Scolari F**, Ravani P, Pola A, Guerini S, Zubani R, Movilli E, Savoldi S, Malberti F, Maiorca R. Predictors of renal and patient outcomes in atheroembolic renal disease: a prospective study. *J Am Soc Nephrol* 2003; **14**: 1584-1590 [PMID: 12761259 DOI: 10.1097/01.ASN.0000069220.60954.F1]
4 **Modi KS**, Rao VK. Atheroembolic renal disease. *J Am Soc Nephrol* 2001; **12**: 1781-1787 [PMID: 11461954]
5 **Pasupala U**, Soare M, Dianne S, Paixao R, Fromkin B, Berho M, Braun M. Atheroembolic Renal Disease and Anticoagulants use: A Case Report and Literature Review. *World J Nephrol Urol* 2012; **I**: 115-117 [DOI: 10.4021/wjnu37w]
6 **Mittal BV**, Alexander MP, Rennke HG, Singh AK. Atheroembolic renal disease: a silent masquerader. *Kidney Int* 2008; **73**: 126-130 [PMID: 17667989 DOI: 10.1038/sj.ki.5002433]
7 **Ravani P**, Gaggi R, Rollino C, Santostefano M, Stabellini N, Colla L, Dallera N, Ravera S, Bove S, Faggiano P, Scolari F. Lack of association between dialysis modality and outcomes in atheroembolic renal disease. *Clin J Am Soc Nephrol* 2010; **5**: 454-459 [PMID: 20019115 DOI: 10.2215/CJN.06590909]
8 **Lye WC**, Cheah JS, Sinniah R. Renal cholesterol embolic disease. Case report and review of the literature. *Am J Nephrol* 1993; **13**: 489-493 [PMID: 8141186 DOI: 10.1159/000168669]
9 **Fine MJ**, Kapoor W, Falanga V. Cholesterol crystal embolization: a review of 221 cases in the English literature. *Angiology* 1987; **38**: 769-784 [PMID: 3310742 DOI: 10.1177/000331978703801007]
10 **Scoble JE**, O'Donnell PJ. Renal atheroembolic disease: the Cinderella of nephrology? *Nephrol Dial Transplant* 1996; **11**: 1516-1517 [PMID: 8856200 DOI: 10.1093/ndt/11.8.1516]
11 **Zhang J**, Zhang HY, Chen SZ, Huang JY. Anti-neutrophil cytoplasmic antibodies in cholesterol embolism: A case report and literature review. *Exp Ther Med* 2016; **12**: 1012-1018 [PMID: 27446313 DOI: 10.3892/etm.2016.3349]
12 **Knight A**, Ekbom A, Brandt L, Askling J. What is the significance in routine care of c-ANCA/PR3-

ANCA in the absence of systemic vasculitis? A case series. *Clin Exp Rheumatol* 2008; **26**: S53-S56 [PMID: 18799054]
13 **Polu KR**, Wolf M. Clinical problem-solving. Needle in a haystack. *N Engl J Med* 2006; **354**: 68-73 [PMID: 16394304 DOI: 10.1056/NEJMcps051939]
14 **Flory CM**. Arterial Occlusions Produced by Emboli from Eroded Aortic Atheromatous Plaques. *Am J Pathol* 1945; **21**: 549-565 [PMID: 19970827]
15 **ThurlbeckWM**, Castleman B. Atheromatous emboli to the kidneys after aortic surgery. *N Engl J Med* 1957; **257**: 442-447 [PMID: 13464955 DOI: 10.1056/NEJM195709052571002]
16 **Meyrier A**. Cholesterol crystal embolism: diagnosis and treatment. *Kidney Int* 2006; **69**: 1308-1312 [PMID: 16614719 DOI: 10.1038/sj.ki.5000263]
17 **Arroyo LH**, Lee RT. Mechanisms of plaque rupture: mechanical and biologic interactions. *Cardiovasc Res* 1999; **41**: 369-375 [PMID: 10341836 DOI: 10.1016/S0008-6363(98)00308-3]
18 **Donohue KG**, Saap L, Falanga V. Cholesterol crystal embolization: an atherosclerotic disease with frequent and varied cutaneous manifestations. *J Eur Acad Dermatol Venereol* 2003; **17**: 504-511 [PMID: 12941082 DOI: 10.1046/j.1468-3083.2003.00710.x]
19 **Jucgla A**, Moreso F, Muniesa C, Moreno A, Vidaller A. Cholesterol embolism: still an unrecognized entity with a high mortality rate. *J Am Acad Dermatol* 2006; **55**: 786-793 [PMID: 17052483 DOI: 10.1016/j.jaad.2006.05.012]
20 **Liew YP**, Bartholomew JR. Atheromatous embolization. *Vasc Med* 2005; **10**: 309-326 [PMID: 16444859 DOI: 10.1191/1358863x05vm640ra]
21 **Jones DB**, Iannaccone PM. Atheromatous emboli in renal biopsies. An ultrastructural study. *Am J Pathol* 1975; **78**: 261-276 [PMID: 1115220]
22 **Lie JT**. Cholesterol atheromatous embolism. The great masquerader revisited. *Pathol Annu* 1992; **27** Pt 2: 17-50 [PMID: 1584626]
23 **Stratta P**, Bozzola C, Quaglia M. Pitfall in nephrology: contrast nephropathy has to be differentiated from renal damage due to atheroembolic disease. *J Nephrol* 2012; **25**: 282-289 [PMID: 22419233 DOI: 10.5301/jn.5000093]
24 **Kasinath BS**, Corwin HL, Bidani AK, Korbet SM, Schwartz MM, Lewis EJ. Eosinophilia in the diagnosis of atheroembolic renal disease. *Am J Nephrol* 1987; **7**: 173-177 [PMID: 3631147 DOI: 10.1159/000167459]
25 **Belenfant X**, Meyrier A, Jacquot C. Supportive treatment improves survival in multivisceral cholesterol crystal embolism. *Am J Kidney Dis* 1999; **33**: 840-850 [PMID: 10213638 DOI: 10.1016/S0272-6386(99)70415-4]
26 **Dahlberg PJ**, Frecentese DF, Cogbill TH. Cholesterol embolism: experience with 22 histologically proven cases. *Surgery* 1989; **105**: 737-746 [PMID: 2727901]



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MINIREVIEWS

The role of mycophenolate in the treatment of antineutrophil cytoplasmic antibody-associated vasculitis

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Author contributions: Koukoulaki M designed the study, acquired data, analyzed and interpreted data, drafted the article, and approved the final version submitted for review Iatrou C proposed the study, analyzed and interpreted data, critically revised the article, and approved the final version submitted for review.

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Abstract

Mycophenolic acid, the active metabolite for mycophenolate mofetil and mycophenolic sodium, is a strong, noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase, the key enzyme in *de novo* synthesis of guanosine nucleotides leading to selective inhibition of lymphocyte proliferation. Mycophenolic acid has been evaluated as induction and remission maintenance agent in the treatment of antineutrophil cytoplasmic antibody-associated vasculitis (AAV). Since the course of disease of AAV usually requires long term immunosuppression, mycophenolate has been explored as a less toxic agent compared to cyclophosphamide and azathioprine. Mycophenolate is a potent immunosuppressive agent in the therapy of AAV, non-inferior to other available drugs with comparable side effect profile. Therefore, it could be a valuable alternative in cases of toxicity with life threatening side effects or intolerance to cyclophosphamide or azathioprine, in cases with high cumulative dose of cyclophosphamide, but also in cases with insufficient response. Several studies have shown a higher relapse rate following discontinuation of mycophenolate or in mycophenolate treated subjects that raises concerns about its usefulness in the treatment of AAV. This review describes the efficacy of mycophenolate in AAV as remission induction agent, as remission maintenance agent, and as therapeutic option in relapsing AAV disease, the relapse rate following discontinuation of mycophenolate, and the adverse events related to mycophenolate treatment.

Key words: Mycophenolic acid; Mycophenolate mofetil; Mycophenolate sodium; Antineutrophil cytoplasmic antibody-associated vasculitis; Microscopic polyangiitis; Granulomatosis with polyangiitis; Induction; Remission; Relapse

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Core tip: Antineutrophil cytoplasmic antibody-associated vasculitis is characterized by a remitting - relapsing course of disease that requires long term immunosuppression.

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Mycophenolic acid has been evaluated as induction and remission maintenance agent in the treatment of antibody-associated vasculitis as a less toxic agent compared to cyclophosphamide and azathioprine. Mycophenolate has been proven a potent immunosuppressive agent and non-inferior to other available drugs with comparable side effect profile, but several studies have shown a higher relapse rate following discontinuation of mycophenolate or in mycophenolate treated subjects. In this review, the role of mycophenolate in treatment of antibody-associated vasculitis is further discussed.

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INTRODUCTION

Mycophenolic acid was initially introduced successfully as a therapeutic agent of solid organ transplantation, reducing the incidence of acute rejection in renal transplant recipients[1]. The encouraging efficacy of mycophenolate prompted the assessment of its potency in the treatment of antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV), both as induction and maintenance therapy. ANCA-AAV includes granulomatosis with polyangiitis (GPA) (formerly Wegener's granulomatosis, WG), microscopic polyangiitis (MPA), and eosinophilic GPA (formerly Churg-Strauss syndrome, CSS). AAV has a variable course of disease activity with phases of remission and relapse requiring therapeutic regimens initially for induction of remission and subsequently for maintenance of remission. Historically, the standard induction treatment plan included high doses of corticosteroids and cyclophosphamide (CYC) that resulted in complete remission rates around 70% with increased percentage of relapse after lowering or discontinuing immunosuppression[2]. Azathioprine was usually employed as maintenance of remission agent. However, despite their efficacy, both CYC and azathioprine were accompanied with significant side effects regarding infection and malignancies that imposed a hindrance in long term or repeated therapies that are frequently necessary in AAV. Therefore, other agents less toxic but with similar efficacy were explored, such as mycophenolate acid, rituximab, deoxyspergualin, infliximab, or etanercept.

For the purposes of this review, we searched published clinical studies of mycophenolate in AAV and more specifically GPA and MPA as induction agent and maintenance treatment.

MYCOPHENOLIC ACID MECHANISM OF ACTION

Mycophenolic acid is the active metabolite for mycophenolate mofetil and mycophenolic sodium. It is a strong, non-competitive, reversible inhibitor of inosine monophosphate dehydrogenase, the key enzyme in *de novo* synthesis of guanosine nucleotides leading to selective inhibition of lymphocyte proliferation[3]. Purine synthesis could be also achieved *via* the salvage pathway in the majority of eukaryotic cells but not in lymphocytes that are more dependent on *de novo* pathway than on the salvage pathway. Therefore, the administration of mycophenolic inhibits DNA synthesis in the S phase of cell cycle and subsequently lymphocyte proliferation. Experimental models have confirmed that mycophenolic acid reduces the production of lymphocyte-derived cytokines such as interferon-gamma and tumor necrosis factor alpha, proinflammatory cytokines produced by monocytes, along with inhibition of primary humoral responses.

Mycophenolate mofetil and mycophenolic sodium are usually orally administered, undergo rapid absorption, and metabolized to the active metabolite mycophenolic acid. Due to the enterohepatic recirculation, the inactive phenolic glucuronide of mycophenolic acid is converted back to mycophenolic acid. A minor percentage of acylglucuronide of mycophenolic acid is also formed that is an active metabolite and could be responsible for side effects like diarrhea or leucopenia. It should be further noted that monitoring plasma mycophenolic acid levels is not routinely performed[4]. Several measurements are required over a period of 12 hours in order to calculate the

area under the curve, which is not realistic in every day practice.

In regard to drug interactions, it is important to mention that antacids and proton pump inhibitors, which are commonly used, decrease exposure to mycophenolic but without any effect on transplant rejection rates. However, it is suggested that these two drug categories should avoid co-administration with mycophenolic[5]. Further significant drug interactions concern colestyramine, sevelamer, ciclosporin A, and medicinal products that interfere with the enterohepatic circulation, and for that reason it is suggested to administer their drugs at different times.

MYCOPHENOLIC ACID AS INDUCTION TREATMENT

Mycophenolate has been evaluated as induction treatment in patients with relapsing AAV who have been exposed to significantly high doses of CYC or had contraindication to CYC (Table 1). Joy *et al*[6] reported their experience in a limited number of 12 patients with relapsing or grumbling AAV that required induction therapy. The majority were proteinase 3 ANCA (PR3-ANCA) positive (75%). In a 6-mo induction phase, 60% achieved remission at least for a short period of time with negative Birmingham Vasculitis Activity Score, leading to sustained remission in 30% but also to relapse in 30% while five patients failed to show any signs of positive response.

Three prospective randomized studies comparing CYC to mycophenolate have been conducted as induction of remission treatment[7-9]. Two published clinical trials have been performed in China and a third was led by the European Vasculitis Study (EUVAS) group. Hu *et al*[7] examined the efficacy of mycophenolate *versus* CYC as 6-mo induction therapy in 34 patients diagnosed with MPA and one patient with GPA. After excluding subjects lost during follow-up, the mycophenolate group showed superior remission rates than the CYC group. More specifically 77.8% (14/17) of the mycophenolate group and 61.5% (8/13) of the CYC group achieved remission. The other Chinese trial randomized 41 patients, all tested myeloperoxidase ANCA (MPO-ANCA) positive, with active disease to receive CYC or mycophenolate[8]. Remission rates were higher though not statistically significant in the mycophenolate group (78.9%) than in CYC group (63.5%). A limitation of both studies is the short period of 6 mo follow-up, so no data regarding relapse rates are included in the published results.

The EUVAS group designed an international randomized study in 140 patients with newly-diagnosed AAV to be treated with mycophenolate or CYC as induction of remission[9]. This clinical trial concluded that remission was induced in 67% (47/70) mycophenolate *versus* 61% (43/70) CYC treated subjects. Even though the primary remission induction end-point of non-inferiority of mycophenolate was achieved, the mycophenolate treated group displayed more relapses (33% *vs* 19%) during the follow-up period along with shorter relapse-free survival. Further, the authors comment that in the mycophenolate group, higher relapse rate was accounted in PR3-ANCA patients but not in MPO-ANCA patients.

MYCOPHENOLIC ACID AS MAINTENANCE TREATMENT

Mycophenolate was initially administered as a remission maintenance agent (Table 2). The first report was published in 1999 by Nowack *et al*[10] describing the efficacy of mycophenolate in nine patients with WG and two patients with MPA as maintenance therapy after achieving remission with standard protocol with CYC, corticosteroids and plasma exchange. Remission was maintained with 2 g/d of mycophenolate and low dose of corticosteroids (less than 7.5 mg/d) during the follow-up period of 15 mo with the exception of one relapse at 14 mo. In 2004, another small cohort of 14 WG patients evaluated mycophenolate as remission maintenance agent following initial treatment with CYC and corticosteroids[11]. Relapse rate was reported in 43% (6/14 patients) at a median time from remission to relapse of 10 mo (range 1-25 mo).

A study by Koukoulaki and Jayne[12] in 2005 reviewed retrospectively the efficacy of mycophenolate during the maintenance phase in 29 AAV patients. A relapse rate of 48.3% (14/29 patients) was reported in a period of 12 mo with mean time to relapse of 14.1 ± 13.9 mo.

Iatrou *et al*[13] reported their experience of mycophenolate as maintenance therapy in 22 newly diagnosed patients with AAV and renal involvement. The majority of patients were diagnosed with MPA (16 patients) followed by WG (four patients) along with one patient with renal limited vasculitis and one with CSS with renal vasculitis. Induction protocol consisted of intravenous CYC and corticosteroids that

Table 1 Mycophenolic acid for induction treatment in antineutrophil cytoplasmic antibody-associated vasculitis

Authors	Yr	Study design	No Pt	Diagnosis	ANCA	Follow-up in mo	Remission rate
Joy *et al*[6]	2005	Case series	12	WG: 7, MPA: 2, Renal Limited: 2; CS:1	MPO: 3, PR3: 9	12	6/10 - 60%
Koukoulaki *et al*[12]	2006	Case series	22	N/A	N/A	24	18/22 – 81.8%
Stassen *et al*[15]	2007	Cohort	32	MPA: 3; WG: 29	MPO: 3; PR3: 29	48	Complete remission: 25/32 - 78%; Partial remission: 6/32 - 19%
Hu *et al*[7]	2008	Randomized	35	N/A	MPO: 28; PR3: 2	6	Mycophenolate: 77.8% (14/18); CYC: 61.5% (8/13)
Silva *et al*[16]	2010	Cohort	17	MPA: 17	MPO: 17	18	Remission at 6 mo: 13/17 - 76%; Sustained remission at 18 mo: 12/17 - 70%
Han *et al*[8]	2011	Randomized	42	N/A	MPO: 42	6	Mycophenolate: 78.9% (14/18); CYC: 63.6% (8/13)
Jones *et al*[9]	2018	Randomized	140	MPA: 49; GPA: 91	MPO: 53; PR3: 82	18	Mycophenolate: 67% (47/70); CYC:61% (43/70)
Chen *et al*[17]	2016	Cohort	34	MPA: 34	MPO: 34	86	31/34 - 91.2%

ANCA: Antineutrophil cytoplasmic antibody; AZA: Azathioprine; CYC: Cyclophosphamide; MPA: Microscopic polyangiitis; MPO: Myeloperoxidase; No: Number; N/A: Not available; PR3: Proteinase 3; Pt: Patients; WG: Wegener's granulomatosis.

were withdrawn at the end of the induction phase, followed by remission protocol that consisted of mycophenolate monotherapy for 18 mo. After the 18 mo of maintenance treatment, mycophenolate was only given in case of relapse and the mean follow-up was 42 mo (range 24-101 mo). However, until the end of the follow-up period 31.58% of the patients had relapsed, and 15.8% progressed to end stage renal disease. The extension of the study, with the addition of new cases (in total 34 patients) and a median follow-up of 97.5 mo (range 66-128), showed significantly declining efficacy of mycophenolate as a maintenance treatment in patients with AAV during long-term follow-up. During the follow-up period, 13 patients (38.2%) had relapsed (5/13 had more than one relapse: Three patients had two relapses, one patient - three relapses, and one patient four relapses), and at the end of the follow-up period 18 patients (53%) were undergoing chronic hemodialysis and 9/34 (26.4%) had died (unpublished data presented in the 19th PanHellenic Congress of Nephrology).

The International Mycophenolate Mofetil Protocol to Reduce Outbreaks of Vasculitides study was an open label randomized controlled trial in AAV, including 156 newly diagnosed patients with either WG (n = 100) or MPA (n = 56)[14]. CYC and corticosteroids were administered for induction of remission. For maintenance of remission, patients were randomized to receive azathioprine (n = 80) or mycophenolate (n = 76). It should be noted that prednisolone dose was 15 mg/d beginning the remission regimen, tapered to 5 mg/d at 12 mo, and discontinued at 24 mo. Follow-up was extended up to 42 mo. The overall relapse rate was 55% (42/76) in the mycophenolate group compared to 37.5% (30/80) in the azathioprine group. Correspondingly major relapses were more common in the mycophenolate treated patients (n = 18) *versus* the azathioprine group (n = 10).

MYCOPHENOLATE FOR INDUCTION AND MAINTENANCE OF REMISSION

In addition to the evaluation of mycophenolate either as induction agent or as remission maintenance agent after induction of remission with CYC, there is a series of studies evaluating mycophenolate both as induction (Table 1) and maintenance

Table 2 Mycophenolic acid for remission maintenance treatment in antineutrophil cytoplasmic antibody-associated vasculitis

Authors	Yr	Study design	No Pt	Diagnosis	ANCA	Follow-up in mo	Relapse rate
Nowack *et al*[10]	1999	Cohort	11	WG: 9; MPA: 2	PR3: 9; MPO: 2		1/11 – 9% time to relapse 14 mo
Langford *et al*[11]	2004	Cohort	14	WG	cANCA: 13; pANCA: 1	Median 18 (range 1-50)	6/14 - 43% median time to relapse 10 mo
Koukoulaki *et al*[12]	2006	Case series	29	N/A	N/A	24	14/29 – 48.3%
Stassen *et al*[15]	2007	Cohort	32	WG: 29; MPA: 3	PR3: 29; MPO: 3	48	Median time to relapse: 16 mo; Relapse free survival 1-yr: 63%, 3-yr: 38%, 5-yr: 27%
Iatrou *et al*[13]	2009	Cohort	22	MPA: 16; WG: 4, RENAL LIMITED: 1; CSS: 1	MPO: 18; PR3: 4	18	6/19 - 31.58% - median time to relapse 21.5 mo (range 18-60 mo)
Silva *et al*[16]	2010	Cohort	13	MPA: 13	MPO: 13	18	1/13 – 7.7%
Hiemstra *et al*[14]	2010	Randomized	156	WG: 90; MPA: 56	MPO: 51; PR3: 90	39	MYCOPHENOLATE GROUP 42/76 - 55% (18 major, 24 minor relapses); AZA 30/80 - 37.5% (10 major, 20 minor relapses)
Draibe *et al*[18]	2015	Case series	20	N/A	MPO: 18	N/A	3/20 – 16% (time to relapse 18-23 mo)
Chen *et al*[17]	2016	Cohort	31	MPA: 31	MPO: 31	86	7/31 – 22.6% median time to relapse 43 mo (IQR 10-128 mo)

ANCA: Antineutrophil cytoplasmic antibody; AZA: Azathioprine; CYC: Cyclophosphamide; IQR: Interquartile range; MPA: Microscopic polyangiitis; MPO: Myeloperoxidase; CSS: Churg-Strauss syndrome; No: Number; N/A: Not available; PR3: Proteinase 3; Pt: Patients; WG: Wegener's granulomatosis.

treatment (Table 2).

A cohort of 32 AAV patients mostly with GPA (29/32) with active disease were treated with oral prednisolone in combination with mycophenolate for 12 mo as induction of remission and subsequently with tapered dose of mycophenolate until 24 mo[15]. Complete or partial remission was induced in 78% (25/32) and 19% (6/32), respectively. The authors report 1-, 3-, and 5-year free survival of 63%, 38%, and 27%, accordingly correlating the rates of relapse to previous exposure to CYC.

Likewise, another cohort of 17 MPA patients reported induction of remission with mycophenolate based treatment protocol in 76% (13/17) with prolonged remission at the end of follow-up at 18 mo in 70% (12/17)[16].

A retrospective study by Chen *et al*[17] evaluated mycophenolate as induction and maintenance treatment in 34 MPA patients with extended follow-up up to 7 years. Chen *et al*[17] administered mycophenolate both for induction and remission regimen. Patients who achieved remission with mycophenolate continued treatment with mycophenolate and corticosteroids for a median period of 24 mo (interquartile range 15-53 mo). Relapse rate was 22.6% (7/31), with a median time to relapse of 43 mo (interquartile range 20-128 mo).

A Spanish group reported their experience with mycophenolate as induction therapy in 29 patients and as maintenance treatment in 67 patients with AAV, the majority MPO-ANCA positive[18]. Treatment plan consisted of 3 mo induction phase and then maintenance phase up to 24 mo while patients remained on low dose of prednisolone along with reduced dose of mycophenolate depending on disease activity. During the follow-up period six patients experienced renal relapse and two patients relapsed with cutaneous manifestations (in total 8/67, 11.9%). In terms of initial regimen, three subjects had been treated with CYC and three with mycophenolate. During the maintenance period, four patients progressed to end stage renal disease requiring renal replacement therapy.

MYCOPHENOLIC ACID IN RELAPSING OR REFRACTORY VASCULITIS

A pilot study of efficacy of mycophenolate in relapsing or resistant AAV included 10 patients with active disease evaluated with Birmingham Vasculitis Activity Score. Transient complete remission was accomplished in six patients (60%), while just three of them maintained long-lasting remission[6].

A retrospective analysis of 22 patients with active or partially controlled AAV who received mycophenolate while on relatively low dose of prednisolone showed 81.8% (18/22) induction of remission[12]. However, the initial response was temporary with more than 50% relapse rate (55.6%, 10/18) through a period of 24 mo.

ADVERSE EVENTS WITH MYCOPHENOLATE TREATMENT

Adverse events related to mycophenolate treatment are associated with its mechanism of action. Therefore, rapidly regenerated cells such as the gastrointestinal track are affected, along with the leucocytes that are the main site of drug's action. Certainly, as an immunosuppressant agent, mycophenolate leads to increased risk of infections. In our case-series of 34 AAV patients treated with mycophenolate in accordance with published experience, mild gastrointestinal intolerance was reported in two patients, increased serum amylase in three patients, leucopenia in two patients, and herpes zoster infections in three patients. All adverse events resolved by reducing the dose of mycophenolate mofetil.

Among the commonest adverse events related to mycophenolate treatment regarding the gastrointestinal tract are diarrhea, nausea, bloating, vomiting, abdominal pain, and rectum bleeding. Another frequent side effect is leucopenia and seldom pancytopenia. Like all immunosuppressive agents, mycophenolate increases the possibility of infections through re-activation of latent viruses, like herpes zoster or cytomegalovirus, and more commonly in transplanted population and respiratory infections. Further cognitive or psychological side effects have been reported in some studies[6,12,16]. A small number of cases with sleeping difficulties or insomnia, poor concentration, headache, and depression have been described.

All the studies conclude that most of the adverse events are temporary and reversible with dose reduction or rarely discontinuation of mycophenolate, which is considered a well tolerated with low toxicity immunomodulating drug.

CONCLUSION

This is a mini-review to summarize published clinical studies with mycophenolate in AAV and describe the experience of our center. Mycophenolate was introduced in the therapeutics of AAV in the middle of the 1990s[10]. Relatively few clinical studies have been published, usually as cohorts of patients, describing single-center experience. The EUVAS study group has led important clinical trials, two of which concern the efficacy of mycophenolate as induction and as remission maintenance agent in AAV and include a considerable number of patients[9,14]. Taken into account published studies, mycophenolate is an effective treatment for induction and maintenance of remission of AAV. In 2016, the European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association recommendations for the management of AAV state that mycophenolate could be used as remission induction of non-organ threatening vasculitis and as remission maintenance agent[19]. For both indications mycophenolate is not the first choice as supported by published clinical trials. Published experience could not provide plausible explanations for the reasons that mycophenolate, even though a potent immunosuppressive with great and superior efficacy in organ transplantation, was not superior to other treatment options in AAV. The hypothesis of inadequate dosing or the type of positivity of ANCA, PR3 or MPO, vasculitis was not proven or supported by clinical data. Mycophenolate's efficacy in AAV is not doubted. So far it has been proven non-inferior to other options like CYC or azathioprine and should not be considered obsolete. Therefore, mycophenolate could still be a valuable alternative in cases of toxicity with life threatening side effects or intolerance to CYC or azathioprine, in cases with high cumulative dose of CYC, and also in cases with insufficient response. Several studies have shown a higher relapse rate following discontinuation of mycophenolate or in mycophenolate treated subjects. The complicated course of AAV with relapsing-remitting course requires treatments with long-term efficacy without significant side effects in order to minimize exposure to immunosuppression. Among

the available options, rituximab may have potential both as induction and maintenance of remission agent[20-22]. Despite the recognized increased cost of rituximab, its efficacy could be proven superior and ultimately cost-effective; and, therefore, its role should be further investigated in AAV.

REFERENCES

1 **Halloran P**, Mathew T, Tomlanovich S, Groth C, Hooftman L, Barker C. Mycophenolate mofetil in renal allograft recipients: a pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. The International Mycophenolate Mofetil Renal Transplant Study Groups. *Transplantation* 1997; **63**: 39-47 [PMID: 9000658 DOI: 10.1097/00007890-199701150-00008]

2 **Jayne D**, Rasmussen N, Andrassy K, Bacon P, Tervaert JW, Dadonienė J, Ekstrand A, Gaskin G, Gregorini G, de Groot K, Gross W, Hagen EC, Mirapeix E, Pettersson E, Siegert C, Sinico A, Tesar V, Westman K, Pusey C; European Vasculitis Study Group. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 2003; **349**: 36-44 [PMID: 12840090 DOI: 10.1056/NEJMoa020286]

3 **Allison AC**, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* 2000; **47**: 85-118 [PMID: 10878285 DOI: 10.1016/S0162-3109(00)00188-0]

4 **Gu Z**, Chen B, Song Y, Shen B, Zhu Z, Zhang W, Xie J, Deng X, Peng C, Fan Q, Chen H. Pharmacokinetics of free mycophenolic acid and limited sampling strategy for the estimation of area under the curve in liver transplant patients. *Eur J Pharm Sci* 2012; **47**: 636-641 [PMID: 22917640 DOI: 10.1016/j.ejps.2012.08.001]

5 **Sherwin CM**, Fukuda T, Brunner HI, Goebel J, Vinks AA. The evolution of population pharmacokinetic models to describe the enterohepatic recycling of mycophenolic acid in solid organ transplantation and autoimmune disease. *Clin Pharmacokinet* 2011; **50**: 1-24 [PMID: 21142265 DOI: 10.2165/11536640-000000000-00000]

6 **Joy MS**, Hogan SL, Jennette JC, Falk RJ, Nachman PH. A pilot study using mycophenolate mofetil in relapsing or resistant ANCA small vessel vasculitis. *Nephrol Dial Transplant* 2005; **20**: 2725-2732 [PMID: 16188901 DOI: 10.1093/ndt/gfi117]

7 **Hu W**, Liu C, Xie H, Chen H, Liu Z, Li L. Mycophenolate mofetil versus cyclophosphamide for inducing remission of ANCA vasculitis with moderate renal involvement. *Nephrol Dial Transplant* 2008; **23**: 1307-1312 [PMID: 18065810 DOI: 10.1093/ndt/gfm780]

8 **Han F**, Liu G, Zhang X, Li X, He Q, He X, Li Q, Wang S, Wang H, Chen J. Effects of mycophenolate mofetil combined with corticosteroids for induction therapy of microscopic polyangiitis. *Am J Nephrol* 2011; **33**: 185-192 [PMID: 21311184 DOI: 10.1159/000324364]

9 **Jones RB**, Hiemstra TF, Ballarin J, Blockmans DE, Brogan P, Bruchfeld A, Cid MC, Dahlsveen K, de Zoysa J, Espigol-Frigolé G, Lanyon P, Peh CA, Tesar V, Vaglio A, Walsh M, Walsh D, Walters G, Harper L, Jayne D; European Vasculitis Study Group (EUVAS). Mycophenolate mofetil versus cyclophosphamide for remission induction in ANCA-associated vasculitis: a randomised, non-inferiority trial. *Ann Rheum Dis* 2019; **78**: 399-405 [PMID: 30612116 DOI: 10.1136/annrheumdis-2018-214245]

10 **Nowack R**, Göbel U, Klooker P, Hergesell O, Andrassy K, van der Woude FJ. Mycophenolate mofetil for maintenance therapy of Wegener's granulomatosis and microscopic polyangiitis: a pilot study in 11 patients with renal involvement. *J Am Soc Nephrol* 1999; **10**: 1965-1971 [PMID: 10477149]

11 **Langford CA**, Talar-Williams C, Sneller MC. Mycophenolate mofetil for remission maintenance in the treatment of Wegener's granulomatosis. *Arthritis Rheum* 2004; **51**: 278-283 [PMID: 15077273 DOI: 10.1002/art.20240]

12 **Koukoulaki M**, Jayne DR. Mycophenolate mofetil in anti-neutrophil cytoplasm antibodies-associated systemic vasculitis. *Nephron Clin Pract* 2006; **102**: c100-c107 [PMID: 16286784 DOI: 10.1159/000089667]

13 **Iatrou C**, Zerbala S, Revela I, Spanou E, Marinaki S, Nakopoulou L, Boletis J. Mycophenolate mofetil as maintenance therapy in patients with vasculitis and renal involvement. *Clin Nephrol* 2009; **72**: 31-37 [PMID: 19640385 DOI: 10.5414/CNP72031]

14 **Hiemstra TF**, Walsh M, Mahr A, Savage CO, de Groot K, Harper L, Hauser T, Neumann I, Tesar V, Wissing KM, Pagnoux C, Schmitt W, Jayne DR; European Vasculitis Study Group (EUVAS). Mycophenolate mofetil vs azathioprine for remission maintenance in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized controlled trial. *JAMA* 2010; **304**: 2381-2388 [PMID: 21060104 DOI: 10.1001/jama.2010.1658]

15 **Stassen PM**, Tervaert JW, Stegeman CA. Induction of remission in active anti-neutrophil cytoplasmic antibody-associated vasculitis with mycophenolate mofetil in patients who cannot be treated with cyclophosphamide. *Ann Rheum Dis* 2007; **66**: 798-802 [PMID: 17179175 DOI: 10.1136/ard.2006.060301]

16 **Silva F**, Specks U, Kalra S, Hogan MC, Leung N, Sethi S, Fervenza FC. Mycophenolate mofetil for induction and maintenance of remission in microscopic polyangiitis with mild to moderate renal involvement--a prospective, open-label pilot trial. *Clin J Am Soc Nephrol* 2010; **5**: 445-453 [PMID: 20093349 DOI: 10.2215/CJN.06010809]

17 **Chen Y**, Gao E, Yang L, Liu X, Li K, Liu Z, Zeng C, Zhang H, Liu Z, Hu W. Long-term outcome of mycophenolate mofetil treatment for patients with microscopic polyangiitis: an observational study in Chinese patients. *Rheumatol Int* 2016; **36**: 967-974 [PMID: 27169414 DOI: 10.1007/s00296-016-3492-5]

18 **Draibe J**, Poveda R, Fulladosa X, Vidaller A, Zulberti C, Gomà M, Pujol R, Ripoll È, Torras J, Grinyó JM. Use of mycophenolate in ANCA-associated renal vasculitis: 13 years of experience at a university hospital. *Nephrol Dial Transplant* 2015; **30** Suppl 1: i132-i137 [PMID: 25805744 DOI: 10.1093/ndt/gfv061]

19 **Yates M**, Watts RA, Bajema IM, Cid MC, Crestani B, Hauser T, Hellmich B, Holle JU, Laudien M, Little MA, Luqmani RA, Mahr A, Merkel PA, Mills J, Mooney J, Segelmark M, Tesar V, Westman K, Vaglio A, Yalçındağ N, Jayne DR, Mukhtyar C. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis* 2016; **75**: 1583-1594 [PMID: 27338776 DOI: 10.1136/annrheumdis-2016-209133]

20 **Jones RB**, Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, Savage CO, Segelmark M, Tesar V, van Paassen P, Walsh D, Walsh M, Westman K, Jayne DR; European Vasculitis Study Group. Rituximab

versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010; **363**: 211-220 [PMID: 20647198 DOI: 10.1056/NEJMoa0909169]

21 **Stone JH**, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, Kallenberg CG, St Clair EW, Turkiewicz A, Tchao NK, Webber L, Ding L, Sejismundo LP, Mieras K, Weitzenkamp D, Ikle D, Seyfert-Margolis V, Mueller M, Brunetta P, Allen NB, Fervenza FC, Geetha D, Keogh KA, Kissin EY, Monach PA, Peikert T, Stegeman C, Ytterberg SR, Specks U; RAVE-ITN Research Group. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010; **363**: 221-232 [PMID: 20647199 DOI: 10.1056/NEJMoa0909905]

22 **Guillevin L**, Pagnoux C, Karras A, Khouatra C, Aumaître O, Cohen P, Maurier F, Decaux O, Ninet J, Gobert P, Quémeneur T, Blanchard-Delaunay C, Godmer P, Puéchal X, Carron PL, Hatron PY, Limal N, Hamidou M, Ducret M, Daugas E, Papo T, Bonnotte B, Mahr A, Ravaud P, Mouthon L; French Vasculitis Study Group. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. *N Engl J Med* 2014; **371**: 1771-1780 [PMID: 25372085 DOI: 10.1056/NEJMoa1404231]



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MINIREVIEWS

WNT/β-catenin signaling in urothelial carcinoma of bladder

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Abstract

Urothelial carcinoma of bladder is the second most prevalent genitourinary disease. It is a highly heterogeneous disease as it represents a spectrum of neoplasms, including non-muscle invasive bladder cancer (NMIBC), muscle invasive bladder cancer (MIBC) and metastatic lesions. Genome-wide approaches and candidate gene analysis suggest that malignant transformation of the bladder is multifactorial and a multitude of genes are involved in the development of MIBC or NMIBC phenotypes. Wnt signaling is being examined to control and maintain balance between stemness and differentiation in adult stem cell niches. Owing to its participation in urothelial development and maintenance of adult urothelial tissue homeostasis, the components of Wnt signaling are reported as an important diagnostic and prognostic markers as well as novel therapeutic targets. Mutations/epigenetic alterations in the key molecules of Wnt/β-catenin canonical pathway have been linked with tumorigenesis, development of drug resistance and enhanced survival. Present review extends our understanding on the functions of key regulatory molecules of canonical Wnt/β-catenin pathway in urothelial tumorigenesis by inducing cancer stem cell phenotype (UCSCs). UCSCs may be responsible for tumor heterogeneity, high recurrence rates and complex biological behavior of bladder cancer. Therefore, understanding the role of UCSCs and the regulatory mechanisms that are responsible for high relapse rates and metastasis could help to develop pathway inhibitors and augment current therapies. Potential implications in the treatment of urothelial carcinoma of bladder by targeting this pathway primarily in UCSCs as well as in bulk tumor population that are responsible for high relapse rates and metastasis may facilitate potential therapeutic avenues and better prognosis.

Key words: Chemoresistance; Therapeutic approaches; Urothelial carcinoma of bladder; Urothelial cancer stem cells; Wnt/β-catenin

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Core tip: Wnt/β-catenin signaling pathway plays significant role in maintaining balance between stemness and differentiation in adult stem cell niches. Mutations/epigenetic alterations in the regulatory components of Wnt/β-catenin signaling lead to acquisition of

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urothelial cancer stem cell phenotype, chemoresistance and enhanced survival. Key regulatory molecules of Wnt/β-catenin pathways are being examined as diagnostic/prognostic markers as well as novel therapeutic targets in urothelial tumorigenesis.

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NTRODUCTION

Bladder cancer is the second most prevalent genitourinary disease and ranks ninth most common cause of deaths due to cancer worldwide. Nearly 90% of bladder cancers are diagnosed as urothelial carcinoma of bladder (UCB). Industrial and occupational exposure to carcinogens/aromatic amines, prolonged use of arsenic or chlorine contaminated drinking water, use of tobacco, anatomical, hormonal, genetical, and socio-economical status are considered as major risk factors for bladder cancer[1]. Schistosomiasis infections are identified as the main cause for non-urothelial cancer and are prevalent in many parts of the world including Africa and Middle East.

UCB is highly heterogeneous disease as it represents a spectrum of neoplasms, including non-muscle invasive bladder cancer (NMIBC), muscle invasive bladder cancer (MIBC) and metastatic lesions. Around 80% of UCB are papillary bladder tumors that arise through the non-invasive papillary pathway (primary tumor stage: pTa-T1). Approximately 20%-30% of patients are initially presented with more aggressive muscle invasive disease (primary tumor stage: pT2-T4) which evolves from severe dysplasia or carcinoma *in situ*. Genome-wide approaches and candidate gene analysis suggest that malignant transformation of the bladder is multifactorial and a multitude of genes are involved in the development of MIBC or NMIBC phenotypes[2,3]. Recent experimental studies validate the involvement of oncogenic mutations in telomerase reverse transcriptase, fibroblast growth factor (FGF) receptor, Harvey rat sarcoma viral oncogene, and phosphoinositide 3'-kinase (PI3K); activation of mitogen-activated protein kinases; and increased expression of cyclin D1 in the development of papillary cancer[4,5]. A strong cooperation of Ras pathway activation with dysregulated Wnt/β-catenin signaling to drive UCB in mouse bladder has been reported[6]. Gene expression, whole genome array-comparative genomic hybridization and mutation analyses validate frequent alterations in loss of tumor protein 53 function; increased instability of chromosomes; inactivation of phosphatase tensin homolog; severe disturbances in cell cycle regulators; loss in functions of retinoblastoma and cyclin dependent kinase inhibitor 2A (CDKN2A)/p16INK4A; and the effect of altered DNA methylation on the expression of cell cycle regulators, in muscle invasive disease[4,7]. Castillo-Martin *et al*[8] report the presence of mutations in PI3K, tumor suppressor genes, deleted in bladder cancer, tuberous sclerosis 1, CDKN2A and patched in both papillary and invasive tumors.

Transurethral resection of the bladder tumor is the treatment of choice in NMIBC, however, 50%-90% of patients presented with pTa (primary tumor) suffer frequent recurrences, whereas 20%-25% of patients with pT1 tumors progress to high grade MIBC[9,10]. MIBCs are treated with radical cystectomy, chemo- and radiotherapies. Despite these therapies, about 50% of MIBC patients eventually develop metastasis[5]. Besides, possible toxic effects of adjuvant/neo-adjuvant chemo-radiotherapies may adversely affect the quality of life and overall survival of the patients[11]. Despite of significant advancements in treatment modalities and prognostic methods, bladder cancer continues to be an extremely common disease with high mortality rates and thus this disease still remains a challenge for genito-urinary surgeons.

Recently identified concept of cancer stem cell theory can be explained by the presence of subset of cells with the self-renewal/ differentiation ability into hierarchical cells, known as urothelial stem cells (USCs), and their transformation into malignant counterparts, known as urothelial cancer stem cells (UCSCs). UCSCs may be responsible for tumor heterogeneity, high recurrence rates and complex biological behavior of bladder cancer. Therefore, understanding the role of UCSCs and the regulatory mechanisms that are responsible for high relapse rates and metastasis may facilitate potential therapeutic avenues and better prognosis.

Evolutionarily conserved Wnt signaling initiates and regulates diverse range of cellular activities including stem cell self-renewal, degree of differentiation and tissue homeostasis. Activated Wnt signaling has been linked with tumorigenesis of many cancer types and development of drug resistance. The present review summarizes our understanding on detailed molecular insights of Wnt signaling into the complex biological pathology of UCB. The paper presents an overview on the components of Wnt signaling and their participation in urothelial stemness, tumorigenesis and drug resistance. Understanding the mechanisms of Wnt signaling dysregulation in the development and progression of UCB could help to develop pathway inhibitors and augment current therapies by targeting UCSCs and bulk tumor progenies.

UROTHEILAL STEM CELLS AND UROTHELIAL CANCER STEM CELLS

Epithelial lining of bladder wall/urothelium also known as transitional epithelium, is composed of highly differentiated, specialized, giant, frequently multinucleated single layered umbrella cells towards the bladder lumen and is in direct contact with urinary space. Single layer of small polygonal basal cells with the highest proliferative capacity due to presence of subset of basal stem cells, is organized at the stromal edge of the basement membrane. Variable number of multilayered intermediate cells is organized in between two layers.

Long life span; high regenerative potential; high colony-forming efficiency; high nuclear-cytoplasmic ratio; and low granularity are the properties of slow cycling basal USCs[12-15]. The subset of USCs lying in basal layer is characterized to express CD44, β1/β4 integrins, cytokeratins (CK-5/14, CK-17) and laminin receptor (LR). Nevertheless, studies on p63-null mice that lack basal/intermediate cells support the origin of umbrella cells by another independent pool of USCs[15]. Urothelium provides anatomical and functional microenvironment to USCs and protect them from loss of self-renewal ability, differentiation and apoptosis. Regenerative potential of USCs diminishes with its differentiation into transit-amplifying cells of intermediate cell layer and fully differentiated umbrella cells. Differential/maximal distribution of USCs in the caudal region of the rat bladder may be correlated with relatively higher incidence rate of carcinoma per surface area in this region[15].

Epigenetic changes, genomic instability, altered tumor microenvironment, and mutations in proto-oncogenes/tumor suppressor genes lead to aberrant molecular pathways in either adult stem cells or differentiated tumor progenies and thus transform them into a subset of unique UCSCs. These subsets of cells, also known as side-population, are characterized by their high tumor-initiating, self-renewal, clonogenic and proliferative potential; and enriched ability to conserve cellular heterogeneity by inducing xenograft tumors *in vivo*[16]. These cells are examined to express cell surface markers which are also shared by adult stem cells and mature urothelial cells. USCs specific markers were used to identify and isolate UCSCs for the first time in 2009[16]. Subset of these markers included early progenitor Nestin (NES); low carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6^{low}); 67LR at the tumor-stromal interface (upregulated in 80% of invasive cancer); aldehyde dehydrogenase 1A1 (ALDH1A1^{hi}); CD44, CD44 splice variant 6 (CD44v6); CD133; and cytokeratins (CK5, CK7, CK17). *In vivo* and *in vitro* studies identify upregulation of host of oncogenes including cadherin-associated protein beta 1, also known as β-catenin; B lymphoma Moloney murine leukemia virus insertion region 1 homolog (BMI1); GLI1; NANOG; POU domain class 5 transcription factor 1 (POU5F1)/Oct4; and signal transducer and activator of transcription 3. Efflux of vital dye Hoechst 33342 and DyeCycle violet due to higher expression of ATP-binding cassette transporters and multidrug resistance pumps provide evidences for their survival and preservation of cancer stem cell phenotype[17].

Wnt signaling pathway crosstalks and regulates diverse set of cellular activities including embryonic development, wound healing, stemness maintenance, calcium and tissue homeostasis, cell proliferation, and cell polarity of USCs and UCSCs[18]. Wnt signaling is classified into two intersecting signaling networks: (1) The canonical Wnt (or β-catenin-dependent); and (2) Non-canonical Wnt (or β-catenin-independent) pathways. Present review organizes collection of studies that extends our understanding on canonical Wnt/β-catenin pathway, highlights the functions of major components involved in maintenance of UCSCs properties, and their therapeutic implications in the treatment of UCB.

WNT SIGNALING IN UROTHELIAL CARCINOMA OF BLADDER

Wnt signaling is being examined to play central role in urothelial development as well as in the maintenance of adult urothelial tissue homeostasis[19]. The human Wnt gene family encodes 19 evolutionarily conserved 40 kDa glycoproteins with several glycosylation sites. Besides, Wnt ligands contain 22 or 24 conserved cysteine (Cys) residues that participate in lipidation, impart hydrophobic character to it and maintain their activity. Binding of secreted Wnt ligands to the members of the seven transmembrane family G-protein coupled receptors of proteins with cysteine-rich domains, called Frizzleds (Fzds), and their associated co-receptors, stimulates complex network of events including, non-canonical and canonical pathways.

CANONICAL WNT SIGNALING

Canonical Wnt signaling is initiated with the activation of Dishevelled (DVL) protein, a key cytoplasmic partner of Wnt signaling, which directly interacts with FZD at the cytoplasmic side; promotes the phosphorylation of single-pass transmembrane proteins of the low-density lipoprotein family called LRP5 and LRP6; and facilitates the formation of FZD-LRP5/6 heterotrimeric complex. Recruitment of AXIN to the inner membrane leaflet *via* interactions with DVL and LRP-5/6 disrupts the β-catenin destruction complex. In Wnt "OFF" state, casein kinase 1 phosphorylation at Ser45 primes β-catenin for subsequent phosphorylation by glycogen synthase kinase-3β (GSK-3β) on Thr41, Ser37 and Ser33[20,21]. Phosphorylation at N-terminal serine and threonine residues creates a binding site for the ubiquitin ligase, SCFb-TrCP and targets β-catenin for proteasomal degradation and consequently maintains β-catenin in cytoplasm at a low level[22]. In Wnt "ON" state, binding of receptors to Wnt activates the downstream signaling cascade; initiates the disassembly of the destruction complex consisting of DVL/AXIN/APC (adenomatous polyposis coli)/GSK-3β/CK1; facilitates the release of the stabilized β-catenin to cytoplasm and its translocation to nucleus. Nuclear β-catenin binds with T-cell factor/lymphoid enhancing factors (TCF/LEF) family of DNA-binding factors. It then displaces Groucho to create a transcriptional activator complex (Figure 1). It further recruits co-activators including histone acetyl transferases, cyclic AMP response element binding protein, p300 and the chromatin remodeling factor Brg1. Following chromatin remodeling, many target genes including c-myc, stromelysin, FGF, epidermal growth factor (EGF), cyclin D1, c-Myc, CD44 and ALDH are transactivated[23].

Other receptor proteins with known Wnt-binding domains, single-pass receptor tyrosine kinase–like orphan receptor-1 and -2 (Ror1/Ror2: Structurally distinct from Fz receptors) mediate the Wnt5a signal and inhibit β-catenin-TCF signaling. Another receptor protein, tyrosine kinase, Ryk, with Wnt inhibitory factor (WIF) ligand binding domain, is known to control Wnt3a-mediated canonical Wnt signaling. Wnt/receptor tyrosine kinase signaling is known to be involved in human cancer recurrence and therapeutic resistance, partly through activation of PI3K-AKT signaling[24].

NON-CANONICAL WNT SIGNALING

Two non-canonical pathways (a) planar cell polarity (PCP) pathway and (b) Wnt/Ca^{2+} pathways are best-characterized and do not require the involvement of β-catenin. PCP pathway regulates actin cytoskeletal dynamics and maintains apical-basal polarity of cells or polarity of cells within an epithelial layer. Non-canonical Wnt ligand binding to FZD and co-localization of a co-receptor facilitate activation and localization of DVL to the inner membrane leaflet. This is followed by subsequent activation of actin-binding protein Profilin and small GTPases, RHO and RAC through parallel pathways. RHO and Profilin activation occurs through a DVL associated activator of morphogenesis 1 (DAAM1)-dependent manner while RAC activation is in DAAM1-independent manner. RHO-associated kinase (ROCK) and myosin downstream of RHO activation, participate in modification and rearrangement of the actin cytoskeleton (Figure 1). Functions of RacGTPase activated JNK in cytoskeletal modification are remain poorly characterized[25,26].

Activation of Wnt/Ca^{2+}pathway is mediated by binding of Wnt ligand to FZD, followed by activation of G-proteins and DVL. Activated DVL on binding with phosphodiesterase inhibits protein kinase G, increases intracellular calcium, as well as phospholipase C (PLC). Membrane-bound phospholipid phosphatidyl inositol 4,5-

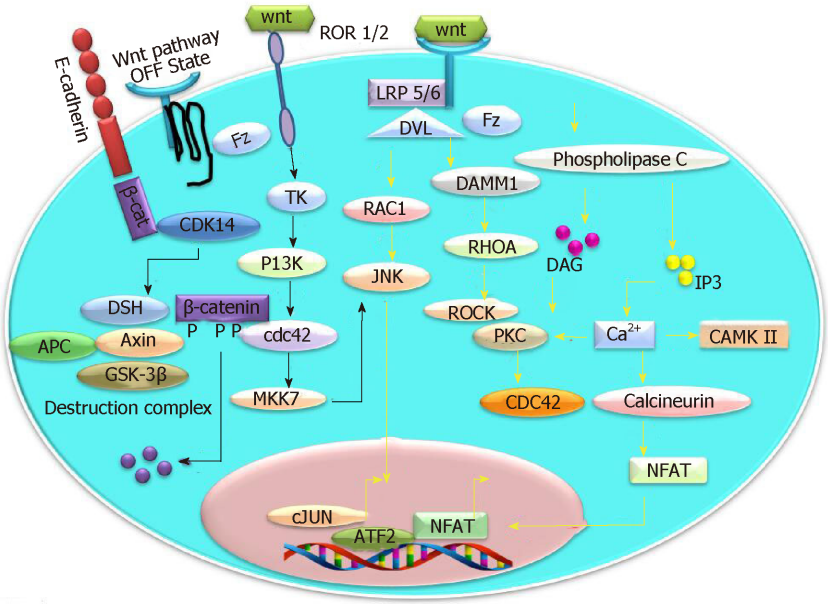


Figure 1 Wnt signaling with two intersecting networks: Canonical Wnt (or β-catenin-dependent) and non-canonical Wnt (or β-catenin-independent) pathways. Canonical Wnt pathway is shown in black color arrows whereas non-canonical pathway is shown in yellow color arrows.

bisphosphate produces inositol triphosphate (IP3) and 1,2-diacylglycerol (DAG) by activated PLC. IP3 triggers release of intracellular Ca^{2+}in the endoplasmic reticulum and activates downstream targets, including calcineurin, calmodulin-dependent protein kinase II (CamKII), and protein kinase C (PKC). Calcineurin and calmodulin activation result in dephosphorylation and stimulation of the nuclear factor associated with T cells transcription factors, thereby result in transcription of target genes which are critically involved in immune response and influence ventral cell fates in vertebrate embryos. PKC activation requires DAG, phosphorylates and activates GTPase [cell division control protein 42 (Cdc42)]. Activation of CamKII antagonizes β-catenin/TCF regulated transcription (Figure 1)[27,28].

Components of Wnt/β-catenin signaling pathway are by far the best characterized among Wnt pathways. Complexity of Wnt signals and their functional role in cell differentiation, polarization and migration are crucial during embryogenesis. Nevertheless, their aberrant activation results in the development of many human tumors and others diseases. One of the recent studies has shown the increased expression of LRP 5/6 in many tissues and its important role in the progression of many cancers including osteosarcoma, prostate cancer, lung squamous cell carcinoma, primary chronic lymphocytic leukemia, triple negative breast cancer, non small cell lung cancer and hepatocellular carcinoma[29]. Overexpression of WNT ligands, impaired cytosolic β-catenin degradation, modulated activities of the TCF/LEF transcription factors and epigenetic repression of WNT antagonists contribute to the pathogenesis of malignant neoplasms of the hematopoietic system[30]. Aberrant Wnt/β-catenin signaling due to mutations in APC and β-catenin genes is reported in colorectal cancer[31]. Study by Nagahata *et al*[32] reports the tumorigenic implication of upregulated DVL in 50% of ductal breast cancers. Deregulation of canonical Wnt signaling and its functions in promoting DNA damage repair and inhibiting apoptosis has been examined to be associated with radioresistance of multiple cancers[33].

Signaling proteins of canonical Wnt/β-catenin being the most prominent regulatory molecules of the urothelial stem cell signaling pathways may hold promise as an early diagnostic/ prediction markers. Genomic mutations and epigenetic changes are being examined to regulate the cancer stem cell phenotype by controlling the expression levels of Wnt/β-catenin components. Single nucleotide polymorphisms in about forty genes that participate in Wnt/β-catenin stem cell pathway are genotyped and the genomic variants have been reported to be associated with the etiology of the bladder cancer[34]. Aberrant Wnt signaling due to mutations in β-catenin in CSCs results in induced tumorigenic proliferation[35]. Missense/ inactivating mutations in APC tumor suppressor (in 13% of tumors) and frameshift deletions (in 3% of tumors) in regions adjacent to β-catenin binding sites result in β-catenin accumulation, dysregulation of Wnt/β-catenin in cancer cells or UCSCs, induce neoplastic proliferation and increase invasion potential[36]. Activated stromal

cells provide appropriate microenvironment to USCs, signalize urothelium with the canonical Wnt/ β-catenin pathway, thereby mediate proliferation in basal urothelial cells[37]. Differential expression of many Wnt signaling components between UCSCs and non-tumorigenic cancer cells including elevated protein levels of β-catenin, c-Myc, and Wnt10a ligand in UCSCs, while Wnt7 in papillary non-invasive carcinomas hypothesizes the involvement of stimulated Wnt signaling in urothelial differentiation, stem cell homeostasis and cancer stem cell maintenance[38-40]. Number of endogenous Wnt inhibitors and Wnt ligand antagonists are known which include: (1) WIF1: Binds to Wnt ligands and prevents them from triggering signaling; (2) Secreted frizzled-related proteins (SFRPs: Interact with Wnt proteins and prevent the latter from binding with FZD receptors); (3) Differentially expressed in ovarian carcinoma-2/disabled homolog 2 (DOC-2/DAB2: Associates with Axin and prevents its interaction with LRP5 co-receptor); and (4) Dickkopf-related proteins (DKKs: Isolate LRP6 co-receptor and antagonize Wnt/β-catenin pathway by reducing β-catenin levels). Epigenetic silencing of these antagonists due to DNA promoter methylation is well known mechanism in urothelial carcinogenesis. Methylation levels of six Wnt-antagonist genes including sFRP-1, sFRP-2, sFRP-4, sFRP-5, Wif-1, and Dkk-3 have been marked as M score and thus considered as novel epigenetic biomarker panel for UCC[19]. Study by Urakami *et al*[41] validates the methylation levels of sFRP-2 and Dkk-3 as significant independent predictors of UCC.

Multiple studies report the cooperativity between Wnt/β-catenin and other signaling networks in induction, maintenance of stem cell properties, cancer cell proliferation, invasion and metastasis. Aberrant Wnt/β-catenin mediated inactivation of GSK-3β, nuclear localization of β-catenin, and transcriptional activation of c-Myc and cyclinD1 drive human carcinogenesis through the induction of CSC features, bulk tumor proliferation and epithelial-to-mesenchymal transition (EMT)-like changes. EMT, a trans-differentiation process, is characterized by the loss of cell-cell adherence/loss of apico-basal polarity; transition of cells with epithelial (E) phenotype to mesenchymal (M) phenotype; and increased motility of cells. Mounting evidences suggest that lower levels of E-cadherin (epithelial marker protein) due to promoter hypermethylation may induce inappropriate responsiveness to Wnt factors. E-cadherin acts as an inhibitor of β-catenin/TCF-mediated transcription by sequestering β-catenin at the plasma membrane[42]. Recent studies report the association of specific genotype of E-cadherin with susceptibility to UCB and a worse clinical prognosis[43]. Prognostic molecular switching to an invasive phenotype is evidenced by the loss of E-cadherin and novel gain of N-cadherin during microarray analysis of 825 UCC samples from 572 patients[44]. Comparable expressions of E-cadherin and p63 vary with tumoral degree of differentiation and the tumoral depth of invasion in UCB. Transcriptional inactivation of p53 and TAp63 (an isoform of p63 with N-terminal transactivation domain) and loss of ΔNp63 (an isoform of p63 with truncated N-terminal) correlate with poor prognosis of invasive urothelial cancer. Loss of ΔNp63α followed by increased levels of N-cadherin and ERK (extracellular signal regulated kinase) signaling activation is proposed as one of the molecular mechanisms in the progression of invasive UCB[45]. Overexpression of GSK-3β was examined for attenuated cigarette smoke extract/tobacco smoke-triggered activation of Wnt/β-catenin, EMT induction, suppressed expression of CSCs markers including CD44, Nanog, Oct4 and ALDH1; reduced migration and invasion capacities of SV-HUC-1 cells[46].

Identification of key regulatory molecules in mechanistic regulation of Wnt/β-catenin signaling and current knowledge on Wnt antagonists would offer potential opportunities to develop new therapies by targeting this pathway primarily in UCSCs as well as in bulk tumor population.

THERAPEUTIC INTERVENTIONS BY TARGETING WNT/-CATENIN AND FUTURE PERSPECTIVES

Major mechanisms of chemoresistance are experienced by UCSCs and these include: (1) Enzymatic breakdown of drugs; (2) Efflux of drugs; (3) Inhibition of anti-tumor response; and (4) Inhibition of apoptosis. CSCs targeting treatment although is far from clinical use, but it is still widely believed to be promising approach for better curative effects. Hence, it is extremely important to comprehensively examine the genomic profile of CSCs and decipher the associated genes in order to establish intervention targets[47]. Global gene expression analysis provides detailed profiling of UCSCs and associates aggressive late stages of bladder tumor with the presence of activated gene signature. Given the important roles and pleiotropic effects of canonical Wnt signaling in cell self-renewal/maintenance of stemness, plasticity

regulation (differentiation/dedifferentiation) and tissue homeostasis, loss of Wnt/β-catenin pathway has been examined to be associated with the inhibition of stemness of UCSCs. Expression studies examine that dysregulated Wnt/β-catenin support dedifferentiation, EMT and hypoxia which might be responsible for the transformation of early bladder cancer cells into cancer stem cells. Role of Wnt/β-catenin pathway alone in regulating long-term tobacco smoke exposure triggered EMT and acquisition of CSCs properties (increased expression of CD44, ALDH1, Nanog and Oct4) has been well-demonstrated in SV-HUC-1 cells and the mice bladder[46].

Growing number of studies highlight the potential of Wnt/β-catenin as a druggable target for sensitizing the small subset of UCSCs as well as bulk tumor cells. Conserved small noncoding RNAs (about 22 nucleotides in length), known as microRNAs or miRs, negatively regulate the expression of target genes by binding to the 3′ untranslated region of target gene. Molecular functions of most of the miRs are still unclear, yet many miRs are established with tumor suppressive or oncogenic functions in bladder tumor proliferation, survival and resistance. Mass spectrum analyses revealed the overexpression of Wnt7a (Wnt family protein) in 5637 HMI cells with high invasion capabilities than in 5637 NMI cells with low invasion capabilities. Its higher levels are being associated with metastasis and worse clinical outcome in UBC patients. Re-introduction of tumor suppressive miR-370-3p, which is frequently lost in bladder cancer in 5637 HMI cells, lead to inhibition of Wnt7a and suppression of 5637 HMI cell invasion[48]. Significant association between higher levels of miR-374a (anti-tumor small non-coding RNA) and longer survival/ recurrence free survival of patients documents its inhibitory effect on tumor metastasis and aggressive biological behavior. Anti-tumor effects of miR-374a may be further explained by abrogating the invasive/metastatic potential, and promoting apoptosis after cisplatin treatment[49]. *In vitro* experiments in T24 and TCCSUP human bladder cancer cells determined miR-374a mediated inhibition of Wnt/β-catenin pathway by targeting Wnt5a. Antitumor effect of miR-532-5p overexpression is being reported by targeting high-mobility group protein B3 (HMGB3), downregulating Wnt/β-catenin signaling, and inhibiting the proliferation and invasion of bladder cancer cells[50]. Lower expression of miR-1826 in bladder cancer tissues as well as in J82, T24 and TCCSUP cell lines and its negative correlation with clinical tumor stage and tumor grade concludes its tumor suppressor role in bladder cancer. MiR-1826-transfected cancer cells exhibited increased apoptosis and G_1 cell cycle arrest *via* β-catenin/ MEK1/ VEGFC downregulation in bladder cancer[51].

In vitro studies validate the functional roles of oncomiRs, miR-23a and miR-27a in targeting and downregulating SFRP1 protein, a negative regulator of Wnt signaling pathway. Oncosuppression of miR-23a and miR-27a based therapies might be effective in reducing levels of β-catenin and suppressing proliferation, migration, invasion of cancer cells by sensitizing them to radiotherapies or chemotherapeutic drugs[52]. Target prediction and luciferase reporter assays were performed to determine the regulatory relationship between miR-940 and the components of Wnt/β-catenin pathway. Overexpression of miR-940 has been studied to be associated with increased levels of c-Myc, cyclin D1, and β-catenin and reduced expression levels of p27 and p-β-catenin. Experiments on miR-940 mimics/miR-940 inhibitor small interference RNA mediated knockdown of inositol polyphosphate-4-phosphatase type 1 A or GSK-3β resulted in regulation of malignant behavior of bladder cancer cells[53]. Down-expression of miR-940 *via* oncosuppressive approaches may affect the levels of direct targets and result in suppressed cell proliferation, migration, invasion and induced cell apoptosis. Oncogenic property of miR-135 in invasion and migration of bladder cancer has been determined by inducing EMT and suppressing GSK-3β *via* Wnt/β-catenin signaling. Therapeutic efficacy of miR-135a may be based on blocking its expression in cancer cells to control the levels of β-catenin, GSK-3β, c-Myc, cyclinD1, matrix metalloproteinase-7 (MMP-7), E-cadherin and Vimentin[54]. Wang *et al*[55] reported the cancer cell survival function of miR-92 *via* activated Wnt/c-Myc/MMP7 signaling by targeting GSK-3β. It would be interesting to examine its therapeutic potential by downregulating the miR-92 levels in bladder cancer cells.

Mediator complex subunit19 (Med19) is one of components of the mediator multiprotein complex which is known to increase DNA binding affinity of transcription factors to RNA polymerase II, thereby transcriptionally regulates target signaling molecules[56]. Wen and coworkers elucidated the critical role of Med19 in promoting invasive behavior and bone metastasis of bladder cancer cells by stimulating bone morphogenetic protein 2 (BMP-2)[57]. Short hairpin RNA mediated knockdown of Med19 was examined to remarkably reduce the expression of Wnt2, active β-catenin, cyclin D1 and MMP9, and increase the levels of GSK-3β and E-cadherin. Suppression of cell proliferation and migration in T24, UM-UC3 cells and 5637 *in vitro*, and inhibition of bladder tumor growth *in vivo* highlight the potential

role of Med19 as therapeutic target in the treatment of bladder cancer[58].

Curcumin with antioxidant, anti-inflammatory, anticancer and antifibrotic properties, is considered as one of the most promising chemopreventive agents. Interventional effects of curcumin in chronic tobacco smoke exposure-mediated urocystic EMT and acquisition of CSC like properties are validated *via* inhibition of activated Wnt/β-catenin. Suppressive effects of curcumin are further evidenced by increased GSK-3β, decreased β-catenin, weak expression of c-Myc, cyclin D1 both *in vitro* and *in vivo*[46]. In another study, a novel natural product analogue CYD 6-17 has been discovered to target β-catenin gene transcription by decreasing the binding of X-box binding protein 1 to the promoter region. CYD 6-17 with an IC50 at nM range, exhibits potent inhibitory effect on chemoresistant bladder cancer cells, thus offers rational therapeutic regimen to MIBC[59]. Novel mechanism for silibinin (a nontoxic natural flavonoid) in targeting or suppressing bladder cancer metastasis has been elucidated. Silibinin treatment to highly metastatic T24-L cell model suppressed cell migration/*in vitro* invasion, inhibited bladder cancer lung metastasis and prolonged animal survival *in vivo*. Dual block of EMT and stemness were identified as important therapeutic functions of silibinin mediated inactivation of β-catenin/zinc finger E-box binding homeobox 1 (ZEB1) signaling. Important findings of research study by Wu *et al*[60] on anticancer effects of silibinin include: (1) Blocking of GSK-3β phosphorylation and nuclear translocation/transactivation of β-catenin; (2) Downregulation of Zeb-1, cytokeratins, Vimentin and MMP2; and (3) Suppression of CSCs traits as evidenced by decreased side population, reduced spheroid colony formation, and lower expression of stem cell factor CD44 (Figure 2)[60]. Table 1 enlists the potential therapeutic agents/drugs/microRNA molecules which are being exploited in targeting the Wnt/β-catenin signaling components in UCSCs as well as bulk tumor progenies.

In vitro and *in vivo* studies provide detailed analysis on cross talk of Wnt signaling with other niche derived signals such as BMP, Hedgehog, TGF-β, Notch, FGF, VEGFC (vascular endothelial growth factor C). These integrated molecular pathways may act in a hierarchy, regulate each other and thus maintain organ specific tissue homeostasis by controlling stem cell signaling, proliferation or differentiation of progenitor cells. Nevertheless, stimulated Wnt/β-catenin and its regulatory circuitry have been deciphered to influence the cancer stem cell phenotype and invasive potential and are well characterized in many cancer types.

Understanding the coordinated activity of signaling pathways derived from bladder organ/tissue to elucidate the *in vivo* control of USC and UCSCs under various physiological and pathological conditions is urgently required. Unraveling the complexity of Wnt/β-catenin by investigating the hierarchical stimulation of signaling molecules and their effects on cancer phenotype may lead to discover potential therapeutic targets in the clinical management of Wnt-associated benign and malignant UCB.

Table 1 Therapeutic agents/ drugs/ microRNA molecules targeting Wnt/β-catenin signaling components in the treatment of urothelial carcinoma of bladder

Therapeutic molecules	Mechanism of action	Ref.
Therapeutic agents/ drugs		
Oridonin analogue CYD6-17	Targets β-catenin gene transcription by decreasing the binding affinity of X-box binding protein 1 to the promoter region	Chen *et al*[59]
Silibinin	Inhibits glycogen synthase kinase-3β (GSK-3β) phosphorylation, β-catenin nuclear translocation and transactivation, and zinc finger E-box binding homeobox 1 gene transcription	Wu *et al*[60]
Curcumin	Reverses tumor smoke-elicited activation of Wnt/β-catenin	Liang *et al*[46]
Mediator complex subunit 19 (Med19)	Med19 knockdown reduces the activity of Wnt/β-catenin pathway, and its target genes, including Wnt2, β-catenin, Cyclin-D1 and matrix metalloproteases 9	Yuan *et al*[58]
MicroRNAs		
Onco-microRNAs		
miR-135a	Downregulation of GSK-3β expression	Mao *et al*[54]
miR-940	Inhibits cell apoptosis *via* targeting inositol polyphosphate-4-phosphatase type 1 A/GSK-3β	Wang *et al*[53]
miR-27a	Targets and downregulates secreted frizzled-related proteins 1 protein, a negative regulator of Wnt signaling pathway	Meng *et al*[52]
miR-23a	Upregulation of β-catenin	Meng *et al*[52]
miR-92	Activates Wnt/cellular myelocytomatosis (c-Myc)/matrix metalloproteases 7 signaling by targeting GSK-3β	Wang *et al*[55]
Tumor supressor-microRNAs		
miR-532-5p	Targets high mobility protein B3 and downregulates Wnt/β-catenin signaling	Xie *et al*[50]
mir-139-5p	Blocks self-renewal of Urothelial cancer stem cells by inhibiting B lymphoma Moloney murine leukemia virus insertion region 1 homolog	Luo *et al*[61]
miR-200a	Directly interacts with 3'untranslated region of β-catenin and suppresses Wnt/β-catenin signaling	Su *et al*[62]
miR-374a	Downregulation of Wnt5a in T24 and TCCSUP bladder cancer cell lines	Chen *et al*[49]
miR-370-3p	Downregulation of Wnt7a in 5637 HM1 bladder cancer cell lines	Huang *et al*[48]
miR-1826	G1 cell cycle arrest *via* β-catenin/mitogen activated protein kinase 1/vascular endothelial growth factor C downregulation	Hirata *et al*[51]
miR-3619-5p	Downregulates β-catenin and cyclin-dependent kinase 2 and activates tumor protein 21	Zhang *et al*[63]

GSK-3β: Glycogen synthase kinase-3β.

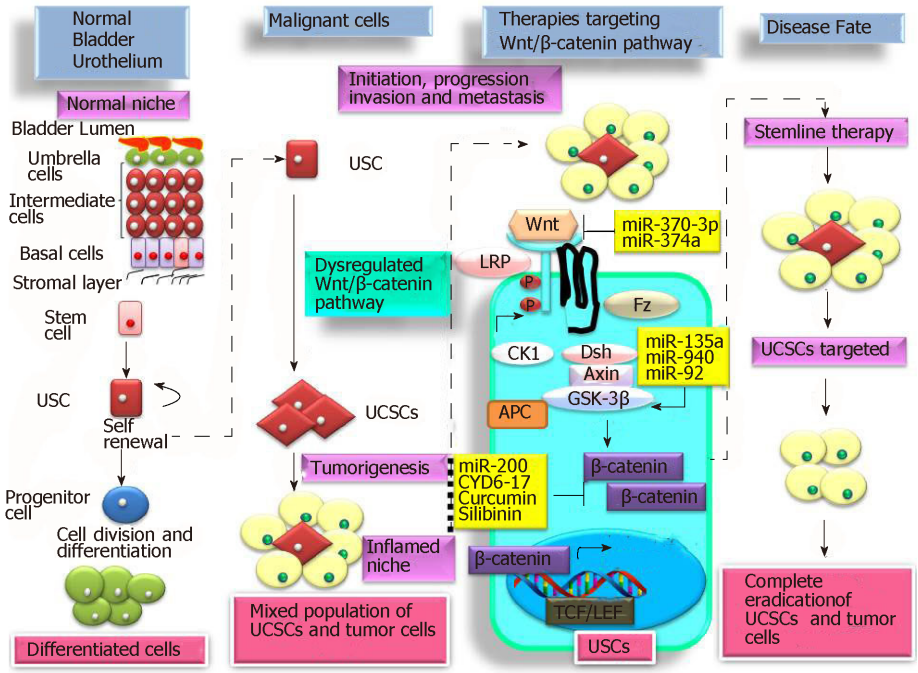


Figure 2 Potential therapies based on targeting the key regulatory molecules of canonical Wnt /β-catenin signaling in urothelial tumorigenesis.

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REFERENCES

1 **Burger M**, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and risk factors of urothelial bladder cancer. *Eur Urol* 2013; **63**: 234-241 [PMID: 22877502 DOI: 10.1016/j.eururo.2012.07.033]
2 **Di Pierro GB**, Gulia C, Cristini C, Fraietta G, Marini L, Grande P, Gentile V, Piergentili R. Bladder cancer: a simple model becomes complex. *Curr Genomics* 2012; **13**: 395-415 [PMID: 23372425 DOI: 10.2174/138920212801619232]
3 **Xiao J**, Yiqing Q. Bioinformatics analysis of the gene expression profile in Bladder carcinoma. *Genet Mol Biol* 2013; **36**: 287-291 [PMID: 23885213 DOI: 10.1590/S1415-47572013005000015]
4 **Schulz WA**. Understanding urothelial carcinoma through cancer pathways. *Int J Cancer* 2006; **119**: 1513-1518 [PMID: 16557569 DOI: 10.1002/ijc.21852]
5 **Garg M**. *Recent advancements towards molecular insights and therapeutic targets in bladder cancer*. In: Research Advances and Clinical Challenges 2018; 1-31
6 **Ahmad I**, Patel R, Liu Y, Singh LB, Taketo MM, Wu XR, Leung HY, Sansom OJ. Ras mutation cooperates with β-catenin activation to drive bladder tumourigenesis. *Cell Death Dis* 2011; **2**: e124 [PMID: 21368895 DOI: 10.1038/cddis.2011.7]
7 **Sauter G**, Moch H, Wagner U, Bubendorf L, Gasser TC, Mihatsch MJ. [Genomic changes in urinary bladder cancer]. *Verh Dtsch Ges Pathol* 1997; **81**: 287-296 [PMID: 9474882]
8 **Castillo-Martin M**, Domingo-Domenech J, Karni-Schmidt O, Matos T, Cordon-Cardo C. Molecular pathways of urothelial development and bladder tumorigenesis. *Urol Oncol* 2010; **28**: 401-408 [PMID: 20610278 DOI: 10.1016/j.urolonc.2009.04.019]
9 **Wallace DM**, Bryan RT, Dunn JA, Begum G, Bathers S; West Midlands Urological Research Group. Delay and survival in bladder cancer. *BJU Int* 2002; **89**: 868-878 [PMID: 12010230 DOI: 10.1046/j.1464-410X.2002.02776.x]
10 **van Rhijn BW**, Burger M, Lotan Y, Solsona E, Stief CG, Sylvester RJ, Witjes JA, Zlotta AR. Recurrence and progression of disease in non-muscle-invasive bladder cancer: from epidemiology to treatment strategy. *Eur Urol* 2009; **56**: 430-442 [PMID: 19576682 DOI: 10.1016/j.eururo.2009.06.028]
11 **Garg M**. Epithelial plasticity in urothelial carcinoma: Current advancements and future challenges. *World J Stem Cells* 2016; **8**: 260-267 [PMID: 27621760 DOI: 10.4252/wjsc.v8.i8.260]
12 **Barker N**, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007; **449**: 1003-1007 [PMID: 17934449 DOI: 10.1038/nature06196]
13 **Kurzrock EA**, Lieu DK, Degraffenried LA, Chan CW, Isseroff RR. Label-retaining cells of the bladder: candidate urothelial stem cells. *Am J Physiol Renal Physiol* 2008; **294**: F1415-F1421 [PMID: 18367656 DOI: 10.1152/ajprenal.00533.2007]
14 **Hatina J**, Schulz WA. Stem cells in the biology of normal urothelium and urothelial carcinoma. *Neoplasma* 2012; **59**: 728-736 [PMID: 22862174 DOI: 10.4149/neo_2012_089]

15 **Ho PL**, Kurtova A, Chan KS. Normal and neoplastic urothelial stem cells: getting to the root of the problem. *Nat Rev Urol* 2012; **9**: 583-594 [PMID: 22890301 DOI: 10.1038/nrurol.2012.142]

16 **Chan KS**, Espinosa I, Chao M, Wong D, Ailles L, Diehn M, Gill H, Presti J, Chang HY, van de Rijn M, Shortliffe L, Weissman IL. Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proc Natl Acad Sci U S A* 2009; **106**: 14016-14021 [PMID: 19666525 DOI: 10.1073/pnas.0906549106]

17 **Garg M**. Urothelial cancer stem cells and epithelial plasticity: current concepts and therapeutic implications in bladder cancer. *Cancer Metastasis Rev* 2015; **34**: 691-701 [PMID: 26328525 DOI: 10.1007/s10555-015-9589-6]

18 **Mohammed MK**, Shao C, Wang J, Wei Q, Wang X, Collier Z, Tang S, Liu H, Zhang F, Huang J, Guo D, Lu M, Liu F, Liu J, Ma C, Shi LL, Athiviraham A, He TC, Lee MJ. Wnt/β-catenin signaling plays an ever-expanding role in stem cell self-renewal, tumorigenesis and cancer chemoresistance. *Genes Dis* 2016; **3**: 11-40 [PMID: 27077077 DOI: 10.1016/j.gendis.2015.12.004]

19 **Dimov I**, Visnjic M, Stefanovic V. Urothelial cancer stem cells. *ScientificWorldJournal* 2010; **10**: 1400-1415 [PMID: 20661533 DOI: 10.1100/tsw.2010.138]

20 **Nusse R**, Varmus H. Three decades of Wnts: a personal perspective on how a scientific field developed. *EMBO J* 2012; **31**: 2670-2684 [PMID: 22617420 DOI: 10.1038/emboj.2012.146]

21 **Niehrs C**. The complex world of WNT receptor signalling. *Nat Rev Mol Cell Biol* 2012; **13**: 767-779 [PMID: 23151663 DOI: 10.1038/nrm3470]

22 **Rubinfeld B**, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 1996; **272**: 1023-1026 [PMID: 8638126 DOI: 10.1126/science.272.5264.1023]

23 **Li Y**, Lin K, Yang Z, Han N, Quan X, Guo X, Li C. Bladder cancer stem cells: clonal origin and therapeutic perspectives. *Oncotarget* 2017; **8**: 66668-66679 [PMID: 29029546 DOI: 10.18632/oncotarget.19112]

24 **Zhang S**, Chen L, Cui B, Chuang HY, Yu J, Wang-Rodriguez J, Tang L, Chen G, Basak GW, Kipps TJ. ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. *PLoS One* 2012; **7**: e31127 [PMID: 22403610 DOI: 10.1371/journal.pone.0031127]

25 **Huelsken J**, Behrens J. The Wnt signalling pathway. *J Cell Sci* 2002; **115**: 3977-3978 [PMID: 12356903 DOI: 10.1242/jcs.00089]

26 **Komiya Y**, Habas R. Wnt signal transduction pathways. *Organogenesis* 2008; **4**: 68-75 [PMID: 19279717 DOI: 10.4161/org.4.2.5851]

27 **Ishitani T**, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, Shibuya H, Moon RT, Ninomiya-Tsuji J, Matsumoto K. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca(2+) pathway to antagonize Wnt/beta-catenin signaling. *Mol Cell Biol* 2003; **23**: 131-139 [PMID: 12482967 DOI: 10.1128/MCB.23.1.131-139.2003]

28 **De A**. Wnt/Ca2+ signaling pathway: a brief overview. *Acta Biochim Biophys Sin (Shanghai)* 2011; **43**: 745-756 [PMID: 21903638 DOI: 10.1093/abbs/gmr079]

29 **Roslan Z**, Muhamad M, Selvaratnam L, Ab-Rahim S. The Roles of Low-Density Lipoprotein Receptor-Related Proteins 5, 6, and 8 in Cancer: A Review. *J Oncol* 2019; **2019**: 4536302 [PMID: 31031810 DOI: 10.1155/2019/4536302]

30 **Fetisov TI**, Lesovaya EA, Yakubovskaya MG, Kirsanov KI, Belitsky GA. Alterations in WNT Signaling in Leukemias. *Biochemistry (Mosc)* 2018; **83**: 1448-1458 [PMID: 30878020 DOI: 10.1134/S0006297918120039]

31 **Cheng X**, Xu X, Chen D, Zhao F, Wang W. Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. *Biomed Pharmacother* 2019; **110**: 473-481 [PMID: 30530050 DOI: 10.1016/j.biopha.2018.11.082]

32 **Nagahata T**, Shimada T, Harada A, Nagai H, Onda M, Yokoyama S, Shiba T, Jin E, Kawanami O, Emi M. Amplification, up-regulation and over-expression of DVL-1, the human counterpart of the Drosophila disheveled gene, in primary breast cancers. *Cancer Sci* 2003; **94**: 515-518 [PMID: 12824876 DOI: 10.1111/j.1349-7006.2003.tb01475.x]

33 **Zhao Y**, Tao L, Yi J, Song H, Chen L. The Role of Canonical Wnt Signaling in Regulating Radioresistance. *Cell Physiol Biochem* 2018; **48**: 419-432 [PMID: 30021193 DOI: 10.1159/000491774]

34 **Pierzynski JA**, Hildebrandt MA, Kamat AM, Lin J, Ye Y, Dinney CP, Wu X. Genetic Variants in the Wnt/β-Catenin Signaling Pathway as Indicators of Bladder Cancer Risk. *J Urol* 2015; **194**: 1771-1776 [PMID: 26173102 DOI: 10.1016/j.juro.2015.07.032]

35 **Teng Y**, Wang X, Wang Y, Ma D. Wnt/beta-catenin signaling regulates cancer stem cells in lung cancer A549 cells. *Biochem Biophys Res Commun* 2010; **392**: 373-379 [PMID: 20074550 DOI: 10.1016/j.bbrc.2010.01.028]

36 **Kastritis E**, Murray S, Kyriakou F, Horti M, Tamvakis N, Kavantzas N, Patsouris ES, Noni A, Legaki S, Dimopoulos MA, Bamias A. Somatic mutations of adenomatous polyposis coli gene and nuclear b-catenin accumulation have prognostic significance in invasive urothelial carcinomas: evidence for Wnt pathway implication. *Int J Cancer* 2009; **124**: 103-108 [PMID: 18844223 DOI: 10.1002/ijc.23917]

37 **Shin K**, Lee J, Guo N, Kim J, Lim A, Qu L, Mysorekar IU, Beachy PA. Hedgehog/Wnt feedback supports regenerative proliferation of epithelial stem cells in bladder. *Nature* 2011; **472**: 110-114 [PMID: 21389986 DOI: 10.1038/nature09851]

38 **Bui TD**, O'Brien T, Crew J, Cranston D, Harris AL. High expression of Wnt7b in human superficial bladder cancer vs invasive bladder cancer. *Br J Cancer* 1998; **77**: 319-324 [PMID: 9461004 DOI: 10.1038/bjc.1998.49]

39 **Urakami S**, Shiina H, Enokida H, Kawakami T, Tokizane T, Ogishima T, Tanaka Y, Li LC, Ribeiro-Filho LA, Terashima M, Kikuno N, Adachi H, Yoneda T, Kishi H, Shigeno K, Konety BR, Igawa M, Dahiya R. Epigenetic inactivation of Wnt inhibitory factor-1 plays an important role in bladder cancer through aberrant canonical Wnt/beta-catenin signaling pathway. *Clin Cancer Res* 2006; **12**: 383-391 [PMID: 16428476 DOI: 10.1158/1078-0432.CCR-05-1344]

40 **He X**, Marchionni L, Hansel DE, Yu W, Sood A, Yang J, Parmigiani G, Matsui W, Berman DM. Differentiation of a highly tumorigenic basal cell compartment in urothelial carcinoma. *Stem Cells* 2009; **27**: 1487-1495 [PMID: 19544456 DOI: 10.1002/stem.92]

41 **Urakami S**, Shiina H, Enokida H, Kawakami T, Kawamoto K, Hirata H, Tanaka Y, Kikuno N, Nakagawa M, Igawa M, Dahiya R. Combination analysis of hypermethylated Wnt-antagonist family genes as a novel epigenetic biomarker panel for bladder cancer detection. *Clin Cancer Res* 2006; **12**: 2109-2116 [PMID: 16609023 DOI: 10.1158/1078-0432.CCR-05-2468]

42 **Thievessen I**, Seifert HH, Swiatkowski S, Florl AR, Schulz WA. E-cadherin involved in inactivation of WNT/beta-catenin signalling in urothelial carcinoma and normal urothelial cells. *Br J Cancer* 2003; **88**: 1932-1938 [PMID: 12799639 DOI: 10.1038/sj.bjc.6601031]

43 **Tsukino H**, Kuroda Y, Nakao H, Imai H, Inatomi H, Kohshi K, Osada Y, Katoh T. E-cadherin gene polymorphism and risk of urothelial cancer. *Cancer Lett* 2003; **195**: 53-58 [PMID: 12767511 DOI: 10.1016/S0304-3835(03)00130-7]

44 **Baumgart E**, Cohen MS, Silva Neto B, Jacobs MA, Wotkowicz C, Rieger-Christ KM, Biolo A, Zeheb R, Loda M, Libertino JA, Summerhayes IC. Identification and prognostic significance of an epithelial-mesenchymal transition expression profile in human bladder tumors. *Clin Cancer Res* 2007; **13**: 1685-1694 [PMID: 17363521 DOI: 10.1158/1078-0432.CCR-06-2330]

45 **Fukushima H**, Koga F, Kawakami S, Fujii Y, Yoshida S, Ratovitski E, Trink B, Kihara K. Loss of DeltaNp63alpha promotes invasion of urothelial carcinomas via N-cadherin/Src homology and collagen/extracellular signal-regulated kinase pathway. *Cancer Res* 2009; **69**: 9263-9270 [PMID: 19934319 DOI: 10.1158/0008-5472.CAN-09-1188]

46 **Liang Z**, Lu L, Mao J, Li X, Qian H, Xu W. Curcumin reversed chronic tobacco smoke exposure induced urocystic EMT and acquisition of cancer stem cells properties via Wnt/β-catenin. *Cell Death Dis* 2017; **8**: e3066 [PMID: 28981096 DOI: 10.1038/cddis.2017.452]

47 **Mashima T**. Cancer Stem Cells (CSCs) as a Rational Therapeutic Cancer Target, and Screening for CSC-targeting Drugs. *Yakugaku Zasshi* 2017; **137**: 129-132 [PMID: 28154319 DOI: 10.1248/yakushi.16-00229-1]

48 **Huang X**, Zhu H, Gao Z, Li J, Zhuang J, Dong Y, Shen B, Li M, Zhou H, Guo H, Huang R, Yan J. Wnt7a activates canonical Wnt signaling, promotes bladder cancer cell invasion, and is suppressed by miR-370-3p. *J Biol Chem* 2018; **293**: 6693-6706 [PMID: 29549123 DOI: 10.1074/jbc.RA118.001689]

49 **Chen W**, Zhang L, Zhou ZQ, Ren YQ, Sun LN, Man YL, Ma ZW, Wang ZK. Effects of Long Non-Coding RNA LINC00963 on Renal Interstitial Fibrosis and Oxidative Stress of Rats with Chronic Renal Failure via the Foxo Signaling Pathway. *Cell Physiol Biochem* 2018; **46**: 815-828 [PMID: 29627834 DOI: 10.1159/000488739]

50 **Xie X**, Pan J, Han X, Chen W. Downregulation of microRNA-532-5p promotes the proliferation and invasion of bladder cancer cells through promotion of HMGB3/Wnt/β-catenin signaling. *Chem Biol Interact* 2019; **300**: 73-81 [PMID: 30639441 DOI: 10.1016/j.cbi.2019.01.015]

51 **Hirata H**, Hinoda Y, Ueno K, Shahryari V, Tabatabai ZL, Dahiya R. MicroRNA-1826 targets VEGFC, beta-catenin (CTNNB1) and MEK1 (MAP2K1) in human bladder cancer. *Carcinogenesis* 2012; **33**: 41-48 [PMID: 22049531 DOI: 10.1093/carcin/bgr239]

52 **Meng W**, Efstathiou J, Singh R, McElroy J, Volinia S, Cui R, Ibrahim A, Johnson B, Gupta N, Mehta S, Wang H, Miller E, Nguyen P, Fleming J, Wu CL, Haque SJ, Shipley W, Chakravarti A. MicroRNA Biomarkers for Patients With Muscle-Invasive Bladder Cancer Undergoing Selective Bladder-Sparing Trimodality Treatment. *Int J Radiat Oncol Biol Phys* 2019; **104**: 197-206 [PMID: 30583038 DOI: 10.1016/j.ijrobp.2018.12.028]

53 **Wang R**, Wu Y, Huang W, Chen W. MicroRNA-940 Targets INPP4A or GSK3β and Activates the Wnt/β-Catenin Pathway to Regulate the Malignant Behavior of Bladder Cancer Cells. *Oncol Res* 2018; **26**: 145-155 [PMID: 28337959 DOI: 10.3727/096504017X14902261600566]

54 **Mao XW**, Xiao JQ, Li ZY, Zheng YC, Zhang N. Effects of microRNA-135a on the epithelial-mesenchymal transition, migration and invasion of bladder cancer cells by targeting GSK3β through the Wnt/β-catenin signaling pathway. *Exp Mol Med* 2018; **50**: e429 [PMID: 29350680 DOI: 10.1038/emm.2017.239]

55 **Wang H**, Ke C, Ma X, Zhao Q, Yang M, Zhang W, Wang J. MicroRNA-92 promotes invasion and chemoresistance by targeting GSK3β and activating Wnt signaling in bladder cancer cells. *Tumour Biol* 2016 [PMID: 27830467 DOI: 10.1007/s13277-016-5460-9]

56 **Allen BL**, Taatjes DJ. The Mediator complex: a central integrator of transcription. *Nat Rev Mol Cell Biol* 2015; **16**: 155-166 [PMID: 25693131 DOI: 10.1038/nrm3951]

57 **Wen H**, Feng CC, Ding GX, Meng DL, Ding Q, Fang ZJ, Xia GW, Xu G, Jiang HW. Med19 promotes bone metastasis and invasiveness of bladder urothelial carcinoma via bone morphogenetic protein 2. *Ann Diagn Pathol* 2013; **17**: 259-264 [PMID: 23276457 DOI: 10.1016/j.anndiagpath.2012.11.004]

58 **Yuan H**, Yu S, Cui Y, Men C, Yang D, Gao Z, Zhu Z, Wu J. Knockdown of mediator subunit Med19 suppresses bladder cancer cell proliferation and migration by downregulating Wnt/β-catenin signalling pathway. *J Cell Mol Med* 2017; **21**: 3254-3263 [PMID: 28631286 DOI: 10.1111/jcmm.13229]

59 **Chen W**, Zhou J, Wu K, Huang J, Ding Y, Yun EJ, Wang B, Ding C, Hernandez E, Santoyo J, Chen H, Lin H, Sagalowsky A, He D, Zhou J, Hsieh JT. Targeting XBP1-mediated β-catenin expression associated with bladder cancer with newly synthetic Oridonin analogues. *Oncotarget* 2016; **7**: 56842-56854 [PMID: 27472396 DOI: 10.18632/oncotarget.10863]

60 **Wu K**, Ning Z, Zeng J, Fan J, Zhou J, Zhang T, Zhang L, Chen Y, Gao Y, Wang B, Guo P, Li L, Wang X, He D. Silibinin inhibits β-catenin/ZEB1 signaling and suppresses bladder cancer metastasis via dual-blocking epithelial-mesenchymal transition and stemness. *Cell Signal* 2013; **25**: 2625-2633 [PMID: 24012496 DOI: 10.1016/j.cellsig.2013.08.028]

61 **Luo H**, Yang R, Li C, Tong Y, Fan L, Liu X, Xu C. MicroRNA-139-5p inhibits bladder cancer proliferation and self-renewal by targeting the Bmi1 oncogene. *Tumour Biol* 2017; **39**: 1010428317718414 [PMID: 28720065 DOI: 10.1177/1010428317718414]

62 **Su J**, Zhang A, Shi Z, Ma F, Pu P, Wang T, Zhang J, Kang C, Zhang Q. MicroRNA-200a suppresses the Wnt/β-catenin signaling pathway by interacting with β-catenin. *Int J Oncol* 2012; **40**: 1162-1170 [PMID: 22211245 DOI: 10.3892/ijo.2011.1322]

63 **Zhang Q**, Miao S, Han X, Li C, Zhang M, Cui K, Xiong T, Chen Z, Wang C, Xu H. MicroRNA-3619-5p suppresses bladder carcinoma progression by directly targeting β-catenin and CDK2 and activating p21. *Cell Death Dis* 2018; **9**: 960 [PMID: 30237499 DOI: 10.1038/s41419-018-0986-y]

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ORIGINAL ARTICLE

Case Control Study

Complement activation and long-term graft function in ABO-incompatible kidney transplantation

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Author contributions: van Sandwijk MS, Klooster A, Bemelman FJ, and Sanders JS designed the study; van Sandwijk MS and Klooster A collected and analyzed the clinical data; Diepstra A, Florquin S, and Bemelman FJ collected and analyzed the biopsy data; van Sandwijk MS wrote the first draft of the article; all other authors reviewed the draft, provided expertise for revisions, and approved the final version of the manuscript.

Institutional review board statement: In this study, anonymized patient information was retrospectively obtained and analyzed. This information was available from the Dutch Organ Transplantation Registry. All subjects consented to being included in this Registry at the time of transplantation. All subjects of this study were treated according to standard clinical practice, and their treatment or clinical outcomes were not in any way affected by their retrospective inclusion in this study. As the subjects of this study were not subjected to procedures nor were

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Abstract

BACKGROUND

ABO-incompatible and ABO-compatible kidney transplantation are equivalent in terms of short-term graft and patient survival. This is thought to be the result of ABO-incompatible graft accommodation, which occurs when anti-blood group antibodies re-occur after transplantation but somehow do not yield their detrimental effect. The underlying mechanism is unclear, but one of the hypotheses is that this is the result of complement inhibition. Since virtually all ABO-incompatible graft biopsies are C4d positive, this complement inhibition must occur somewhere in the complement cascade after the formation of C4d has already taken place, but where exactly is unclear. It is also unclear whether complement inhibition is complete. Incomplete accommodation could explain why recent studies have shown that long-term graft function in ABO-incompatible transplantation is somewhat inferior to ABO-compatible kidney transplantation.

AIM

To unravel the relationship between pre-transplant anti-ABO antibodies,

required to follow rules of behaviour, Institutional Review Board approval was not required according to Dutch law.

Informed consent statement: Patient consent was not obtained but the presented data are anonymized and risk of identification is low.

Conflict-of-interest statement: All authors declare no potential conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at m.s.vansandwijk@amsterdamumc.nl.

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complement activation, and long-term graft function.

METHODS
We included all 27 ABO-incompatible transplantations that were performed between 2008 and 2013 at the Academic Medical Center Amsterdam and the University Medical Center Groningen. For each ABO-incompatible transplantation, we included four ABO-compatible controls matched by age, sex, and transplantation date.

RESULTS
Graft and patient survival were not significantly different. The slope of kidney function during five-year follow-up was also not significantly different, but ABO-incompatible recipients did have a lower kidney function at three months (creatinine clearance 58 *vs* 69 mL/min, $P = 0.02$, Modification of Diet in Renal Disease 46 *vs* 52 mL/min/1.73 m^2, $P = 0.08$), due to a high rate of early rejection (33% *vs* 15%, $P = 0.03$), mostly T-cell mediated. Pre-transplant anti-ABO IgG titers were positively correlated with C5b-9 staining, which itself was positively correlated with the occurrence of T-cell mediated rejection. This may be the result of concurrent C5a formation, which could function as a costimulatory signal for T-cell activation.

CONCLUSION
Co-stimulation of T-cell activation by ongoing complement activation by anti-ABO antibodies may be responsible for an impaired long-term graft function in ABO-incompatible kidney transplantation.

Key words: ABO-incompatible; Kidney transplantation; Complement; Graft function; Rejection

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Core tip: This retrospective case-control study was designed to unravel the relationship between complement activation, pre-transplant anti-ABO antibodies, and renal graft function. In this study, the slope of kidney function during a five-year follow up was not significantly different compared to matched ABO-compatible transplant recipients, but ABO-incompatible kidney transplant recipients did have a lower kidney function at three months, due to a high rate of early T-cell mediated rejection. Based on several complement staining results, we argue that this may be due to co-stimulation of T-cell activation by ongoing complement activation by anti-ABO antibodies.

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INTRODUCTION

To combat the long waiting times of kidney transplantation there is a push to increase deceased donation, for example by using organs from marginal donors, as well as living donation[1]. Based on the prevalence of ABO blood groups of donors and recipients, approximately 30% of willing and otherwise appropriate kidney donor-recipient pairs are estimated to be blood type incompatible and do not proceed to living donor transplantation[2]. Paired kidney exchange is a strategy that helps some ABO-incompatible patients to find a suitable donor, although the probability of a match varies greatly depending on the blood type combination and sensitization status[3]. For example, the chances for ABO-incompatible and sensitized non-O recipients to find a possible match are around 60%, whereas ABO-incompatible O recipients only have a 15% chance for a match[2]. It is estimated that an additional 10%-20% of living donor kidney transplantations can be performed through the implementation of an ABO-incompatible kidney transplantation program[4,5].

ABO-incompatible kidney transplantation is precluded by the presence of isohemagglutinins –antibodies formed against blood group A and/or B- leading to hyperacute rejection and allograft loss. Blood group antigens are present not only in blood, but are also expressed on renal tubular cells and endothelium[6]. As first described by Alexandre *et al*[7], removal of isohemagglutinins through plasmapheresis prior to ABO-incompatible kidney transplantation can prevent hyperacute rejection. Their work on ABO-incompatible kidney transplantation in the early 1980s was greatly expanded on in Japan, a country with very low rates of postmortal kidney donation due to religious objections[8]. In Japan, it was customary to perform a splenectomy prior to ABO-incompatible transplantation, but this practice has mostly been abandoned after the introduction of rituximab in the last decade[9]. The introduction of rituximab has also led to a more widespread adoption of ABO-incompatible kidney transplant programs outside Japan. In a large series of 101 European, Australian and New Zealand centers 1420 ABO-incompatible transplantations were compared to 1:1 matched ABO-compatible transplantations[10]. Three-year death-censored graft survival (89.9%) and three-year patient survival (95.6%) were not significantly different, but there was a slightly lower one-year ABO-incompatible patient survival due to a higher rate of infections. In a large American series comparing 738 ABO-incompatible kidney transplantations to 1:5 matched ABO-compatible transplantations, a lower ten-year death-censored graft survival (76.1% *vs* 72.9% was found in ABO-incompatible kidney transplantations[11]. Ten-year patient survival was comparable for both groups (75.1% *vs* 74.5%).

ABO-incompatible kidney transplantation results in superior long-term graft survival when compared to kidney transplantation in HLA-sensitized recipients[12-15]. This is thought to be caused by accommodation, which occurs when anti-blood group antibodies re-occur after transplantation but somehow do not yield their detrimental effect[16]. The pathophysiology of accommodation is poorly understood. Proposed mechanisms can be divided into adaptations in the graft and in the host. Possible adaptations in the graft include diminished blood type expression in the graft[17] and expression of protective genes[18]. Possible adaptations in the host include IgG subclass switching[19], an increase in regulatory B and/or T cells[20], and complement inhibition[21]. The complement inhibition hypothesis is especially intriguing in light of the well-known fact that almost all ABO-incompatible kidney biopsies are C4d positive, but that C4d-positivity – in contrast to ABO-compatible kidney transplantation – is not a surrogate marker of antibody-mediated rejection[22].

C4d is a split product without known biological function that is produced when activation of the classical or the lectin pathway results in the conversion of C4 into C3. Because it forms a stable bond to the cells in the tissue where it is deposited, it remains visible as a footprint while antibodies dissociate over time[23]. Complement inhibition in ABO-incompatible transplantation is thought to occur more distally in the complement cascade, *i.e.*, after the formation of C4d has already taken place, but the exact mechanism is unknown.

The case-control study presented here was designed to elucidate the relationship between the rate of kidney function decline, pre-transplant anti-ABO antibodies, and complement activation in ABO-incompatible renal transplantation. Firstly, we investigated whether ABO-incompatible kidney transplantation is equivalent to ABO-compatible kidney transplantation not only in terms of graft and patient survival, but also in terms of kidney function and long-term rate of kidney function decline. Full accommodation would imply that both groups are indistinguishable. Secondly, we studied various markers of complement activation (C1q, C3c, C4d, and C5b-9) in ABO-incompatible kidney biopsies.

MATERIALS AND METHODS

Study design

We conducted a retrospective case-control study in which we included all ABO-incompatible transplantations performed at the Amsterdam University Medical Centers, location AMC, and the University Medical Center Groningen between 2008 and 2013. For each ABO-incompatible transplantation, we included four matched ABO-compatible controls. The matching procedure was done as follows: from all 469 ABO-compatible kidney transplantations that were performed between 2008 and 2013, we selected the transplantations scheduled six months prior or after an ABO-incompatible transplantation in the same transplantation center. Within this group, we selected the four kidney transplant recipients of the same gender who had the smallest age difference with their ABO-incompatible counterpart.

All patients were treated according to standard clinical practice. All patient data

were obtained and analyzed retrospectively after being anonymized. Patient treatment and clinical outcomes were not in any way affected by their retrospective inclusion in this study, implying that Institutional Review Board approval was not required according to Dutch law.

ABO-compatible transplantation immunosuppression

ABO-compatible kidney transplant recipients received basiliximab (20 mg on day 0 and day 4) and intravenous prednisolone (50 mg twice daily on day 0 and day 1) as induction therapy. Post-transplantation maintenance immunosuppression consisted of tacrolimus (target levels 10-15 ng/mL in the first six weeks and 6-10 ng/mL thereafter), prednisolone 10 mg once daily, and mycophenolic acid (1000 mg twice daily), with graduate tapering of immunosuppression after one year.

ABO-incompatible transplantation requirements and procedure

In order to be eligible for ABO-incompatible transplantation, patients had to have a negative (HLA)-crossmatch with an ABO-incompatible donor and anti-ABO IgG titers (measured twice) not exceeding 1:256. All ABO-incompatible kidney transplant recipients received a single dose of rituximab of 375 mg/m^2 30 d prior to their scheduled transplantation. Maintenance immunosuppression consisted of prednisolone, tacrolimus and mycophenolic acid, in identical dosages as in ABO-compatible transplantation. However, whereas ABO-compatible recipients started maintenance immunosuppression on the day of transplantation, ABO-incompatible recipients did so two weeks before kidney transplantation. Immuno-adsorptions were started one week before transplantation using an antigen-specific carbohydrate column (Glycosorb®). At least four immuno-adsorption sessions were scheduled, with the option of additional sessions until the target anti-ABO titer ≤ 1:8 was reached. Intravenous immunoglobulins (500 mg/kg) were given just before transplantation. After transplantation, three more immuno-adsorption sessions were performed.

Infection prophylaxis

Patients with a cytomegalovirus mismatch (recipient IgG negative and donor IgG positive) received six months of valgancyclovir prophylaxis. In addition, all patients were prescribed at least four months of Pneumocystis Jirovecii prophylaxis (trimethoprim/sulfamethoxazole).

Complement activation in ABO-incompatible indication and protocol biopsies

Protocol biopsies were performed one year after ABO-incompatible kidney transplantation according to standard clinical practice. Indication biopsies were performed at the discretion of the treating physician. Immunofluorescence staining for C4d, C1q, C3c and C5b-9 on fresh frozen sections was made available for all ABO-incompatible indication and protocol biopsies (15 each).

Endpoints

The primary endpoint of this study was rate of kidney function decline, measured as the slope of estimated glomerular filtration (eGFR) by means of the Modification of Diet in Renal Disease (MDRD) equation and the slope of creatinine clearance. Secondary endpoints were proteinuria, graft and patient survival, biopsy proven rejections, infections, malignancies and complement activation on all indication and protocol biopsies of ABO-incompatible transplants.

Statistical analysis

For the primary endpoint (rate of kidney function decline), linear mixed models were used to estimate the slope of the MDRD and creatinine clearance for both ABO-incompatible and ABO-compatible kidney transplant recipients. In case of graft loss, an eGFR of 10 mL/min/1.73 m^2 and a creatinine clearance of 10 mL/min were imputed. Sensitivity analyses were run both with and without these imputations. Graft and patient survival and rejection episodes were compared using Kaplan-Meier analyses. Infections and malignancies were compared using chi-square tests. The statistical methods of this study were reviewed by H. Peters Sengers, senior epidemiologist at Amsterdam University Medical Centers, location AMC.

RESULTS

Between 2008 and 2013, 27 ABO-incompatible transplantations were performed at both centers. For each ABO-incompatible transplantation, we included 4 matched ABO-compatible transplantations at both centers, so that the total number of patients included in our analysis equals 135.

The baseline characteristics are described in Table 1. The groups were well-matched, except for a lower percentage of related donors in the ABO-incompatible group, which did not result in a higher number of HLA-mismatches. Panel-reactive antibodies were low and not significantly different between both groups. Tacrolimus trough levels were also not significantly different between the two groups except for a slightly higher trough level at week six (Figure 1).

Kidney function

Linear mixed models were used to estimate the slope of eGFR and creatinine clearance with and without imputation of 10 mL/min for graft loss. As Figure 2 indicates, ABO-incompatible kidney transplant recipients had a slightly but significantly lower kidney function at three months, but the slope over time was not significantly different. There was also no significant difference in proteinuria, which was 0.20 g/d at three months and at five years in both groups (P = 0.94 and 0.86, respectively).

Graft and patient survival

Graft and patient survival (Figure 3) were not significantly different, although there was a trend toward a slightly lower death-censored graft survival in the ABO-incompatible group (one-year graft survival 92% *vs* 99%, P = 0.65, compared to 83% *vs* 91% five-year graft survival, P = 0.12).

Biopsy results

Table 2 shows the results of both the indication and the protocol biopsies. ABO-incompatible kidney transplant recipients had a higher incidence of rejection (33% *vs* 15%, P = 0.03), mostly T-cell mediated. Antibody-mediated rejection rates were low in both groups. Donor-specific antibodies were low and not significantly different between both groups. Figure 4 (left-hand panel) shows that all ABO-incompatible rejections occurred very early after transplantation, whereas ABO-compatible rejections occurred more gradually over time. In the right-hand panel of Figure 4, ABO-incompatible and ABO-compatible transplantations are split based on whether they experienced a rejection episode or not. This graph clearly indicates that early rejection results in a lower kidney function at three months in ABO-incompatible transplantation (P < 0.001). The slopes of all curves are not significantly different, although there was a trend toward steeper curves for both ABO-compatible and ABO-incompatible recipients with a rejection.

Figure 5A-C contain the results of immunofluorescence staining for C4d, C1q, C3c, and C5b-9. As can be seen by comparing Figure 5A and B, the complement staining results were quite equivocal for indication and protocol biopsies, except for C5b-9 staining, which was significantly more intense in indication biopsies (P = 0.04). In addition, there was a significant correlation between pretransplant anti-ABO IgG titers and peritubular C5b-9 in indication biopsies (correlation coefficient 0.72, P = 0.002), as well as a significant correlation between C5b-9 and the occurrence of rejection (correlation coefficient 0.52, P = 0.016). As expected in ABO-incompatible biopsies, there were no significant correlations between C4d and the occurrence of rejection.

Infections and malignancies

Bacterial and viral infections occurred more frequently in the ABO-incompatible group (1.7 compared to 1.0 median infections per patient, P = 0.004, Table 3). As shown in Table 4, malignancies were also more likely to occur, with 22.2% of ABO-incompatible patients diagnosed with a (pre)malignancy compared to 7.4% in the ABO-compatible group (P = 0.01). This number should be interpreted with caution however, as ABO-incompatible patients with a malignancy were on average 10 years older than their counterparts without a malignancy. Also, ABO-compatible patients tended to have more severe malignancies, with 38% of ABO-compatible patients diagnosed with a malignancy dying from their disease compared to 0% in the ABO-incompatible group.

DISCUSSION

In this two-center case-control study, ABO-incompatible kidney transplant recipients had a lower kidney function three months after transplantation compared to ABO-compatible recipients, but the slope of kidney function over time was not significantly different. We have shown that the reason for this lower kidney function at three months was a higher rate of early mostly T-cell mediated rejections, all occurring very early after ABO-incompatible transplantation. Remarkably, antibody-mediated

Table 1 Patient and donor characteristics

	ABO-incompatible (*n* = 27)	ABO-compatible (*n* = 108)	*P* value
Patient and donor characteristics			
Recipient age (yr)	51.6 ± 12.3	51.7 ± 11.6	0.95
Donor age (yr)	53.6 ± 12.1	52.7 ± 12.4	0.73
Recipient gender (% male)	63	63	1.00
Donor gender (% male)	63	44	0.07
Related/unrelated donors (% related)	26	51	0.02
Donor MDRD (mL/min/1.73m^2)	96.6 ± 20.5	93.3 ± 18.4	0.42
HLA mismatches (A/B/DR)	3.5 ± 1.6	3.2 ± 1.7	0.48
Mean follow-up patient (yr)	3.7 ± 1.7	3.7 ± 1.6	0.98
Underlying renal disease			0.57
Glomerulonephritis, (%)	30	27	
Hypertensive disease, (%)	7	18	
Diabetic nephropathy, (%)	4	7	
Cystic kidney disease, (%)	33	19	
Urological disease, (%)	7	8	
Other, (%)	19	21	
Blood type combination			
A→O, (%)	59		
B→O, (%)	15		
AB → O, (%)	0		
B →A, (%)	11		
A→ B, (%)	7		
AB→ A, (%)	4		
AB → B, (%)	4		
Median pre-treatment IgG anti-A/B titer	1:64 (1:6-1:128)		
Median pre-treatment IgM anti-A/B titer	1:8 (1:2-1:28)		
Panel reactive antibodies (% positive)	9	7	0.60
Previous renal replacement therapy			
First transplantation (%)	89	94	0.30
Pre-emptive transplantation (%)	44	53	0.44
Non pre-emptive (%)	56	47	0.83
Haemodialysis (%)	34	26	
Peritoneal dialysis (%)	22	21	
Months on dialysis	22 (9-26)	16 (11-27)	0.41
Ischemia times			
Cold ischemia time (min)	161 ± 84	155 ± 59	0.49
Second warm ischemia time (min)	41 ± 27	40 ± 22	0.77
Delayed graft function (%)	8	4	0.37

All values as percentages, mean ± standard deviation or median and interquartile range. *P* values calculated with *t*-test, Mann-Whitney *U* test or chi-square test where applicable. Panel reactive antibodies > 8% were considered positive. HLA: Human leukocyte antigen; IgG: Immunoglobulin G; IgM: Immunoglobulin M; MDRD: Modification of diet in renal disease.

rejection rates were low in both groups. Tacrolimus levels were not significantly different for most of the follow-up period and are thus not responsible for the difference in kidney function.

The high rate of early mostly T-cell mediated rejection in our study is not a common finding in ABO-incompatible studies. It may be due to the fact that our standard protocol for ABO-incompatible transplantation does not include basiliximab, which is widely used as induction therapy to prevent T-cell mediated rejection in ABO-compatible transplantation[24]. There is no conclusive evidence available whether adding basiliximab to ABO-incompatible induction protocols improves outcomes. In the large series published by Opelz *et al*[10], 39% of centers had added basixilimab, 1% gave anti-thymocyte globulin, and 60% gave neither. Death-censored graft survival was not affected by the addition of basiliximab.

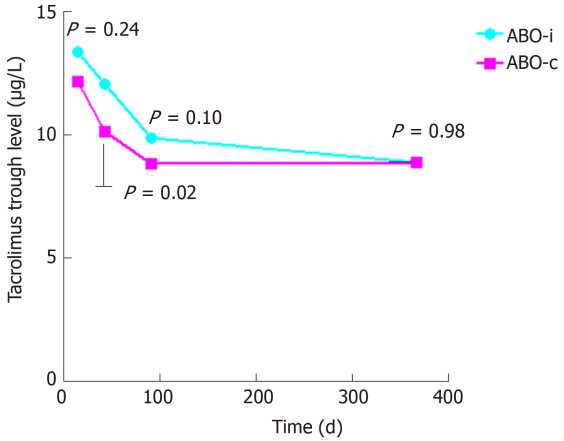


Figure 1 Tacrolimus trough levels. Tacrolimus trough levels at 2 wk, 6 wk, 3 mo, and 1 year after transplantation.

Another explanation for the high rate of early mostly T-cell mediated rejection could be complement activation. As discussed above, higher pre-transplant anti-ABO IgG titers resulted in more intense C5b-9 staining in indication biopsies. C5b-9 is the membrane attack complex, which is formed as a final common pathway of all complement cascades and causes cell lysis. C5b-9 formation implies that complement inhibition, which is one of the mechanisms thought to be responsible for accommodation in ABO-incompatible kidney transplantation, is incomplete. Incomplete complement inhibition also results in C5a still being formed in small quantities. C5a is an anaphylotoxin that has long been known be able to stimulate neutrophils, mast cells and macrophages. There is emerging evidence that C5a can also function as a costimulatory signal for T-cell activation[25-29]. One could postulate therefore that high pre-transplant anti-ABO IgG titers result in high levels of complement activation resulting in C5a-stimulated T-cell activation.

In summary, in our study ABO-incompatible and ABO-compatible renal transplantation have a similar 5-year graft and patient survival, but in ABO-incompatible transplant recipients, kidney function is inferior compared to well-matched ABO-compatible transplant recipients. This may be due to anti-ABO antibody driven ongoing complement activation resulting in a C5a-stimulated increase in early T-cell mediated rejection. In other words, high pretransplant anti-ABO antibody titers could result in incomplete accommodation of the ABO-incompatible graft, especially in the absence of induction therapy with agents like basiliximab. Further studies are needed to confirm this hypothesis.

Table 2 Biopsy results

	ABO-incompatible (*n* = 27)	ABO-compatible (*n* = 108)	*P* value
Treated rejections, (%)	33	15	**0.03**
Acute antibody mediated rejection, (%)	4	1	0.36
Acute T-cell mediated rejection, (%)	30	14	0.05
Grade IA, (%)	7	7	
Grade IB, (%)	4	3	
Grade IIA, (%)	15	3	
Grade IIB, (%)	4	1	
Grade III, (%)	0	0	
Borderline acute T-cell mediated rejection	4	7	0.50
Donor specific antibodies (% positive)	0	6	0.17

P values calculated with chi-square test. Donor specific antibodies were measured according to local practice, *i.e.*, at the discretion of the treating physician, and routinely at 8 wk after transplantation for Groningen patients and yearly for Amsterdam patients.

Table 3 Infectious complications

	ABO-incompatible (*n* = 27)	ABO-compatible (*n* = 108)	*P* value
Total no. of infectious complications per patient	1.7 (1-2.3)	1.0 (0-2)	0.004
CMV infections, (%)	12	11	
EBV infections, (%)	0	3	
BK infections, (%)	15	6	
Other viral infections, (%)	31	9	
First urinary tract infection, (%)	38	30	
Recurrent urinary tract infections, (%)	8	11	
Other bacterial infections, (%)	23	7	
Other infections, not otherwise specified, (%)	31	20	

All values as percentages or median and interquartile range. *P* values calculated Mann Whitney *U* test or chi-square test where applicable. CMV: Cytomegalovirus; EBV: Epstein Barr virus.

Table 4 Malignancies

	ABO-incompatible (*n* = 27)	ABO-compatible (*n* = 108)	*P* value
% of patients with a (pre)malignancy	22.2	7.4	0.01
Solid organ, (%)	11	2.8	
Lymphoma, (%)	0	1.9	
Skin malignancy, (%)	3.7	1.9	
Melanoma, (%)	3.7	0	
Premalignant lesion, (%)	3.7	0.9	
% of patients dying from their malignancy	0	38	0.09

P values calculated with chi-square test.

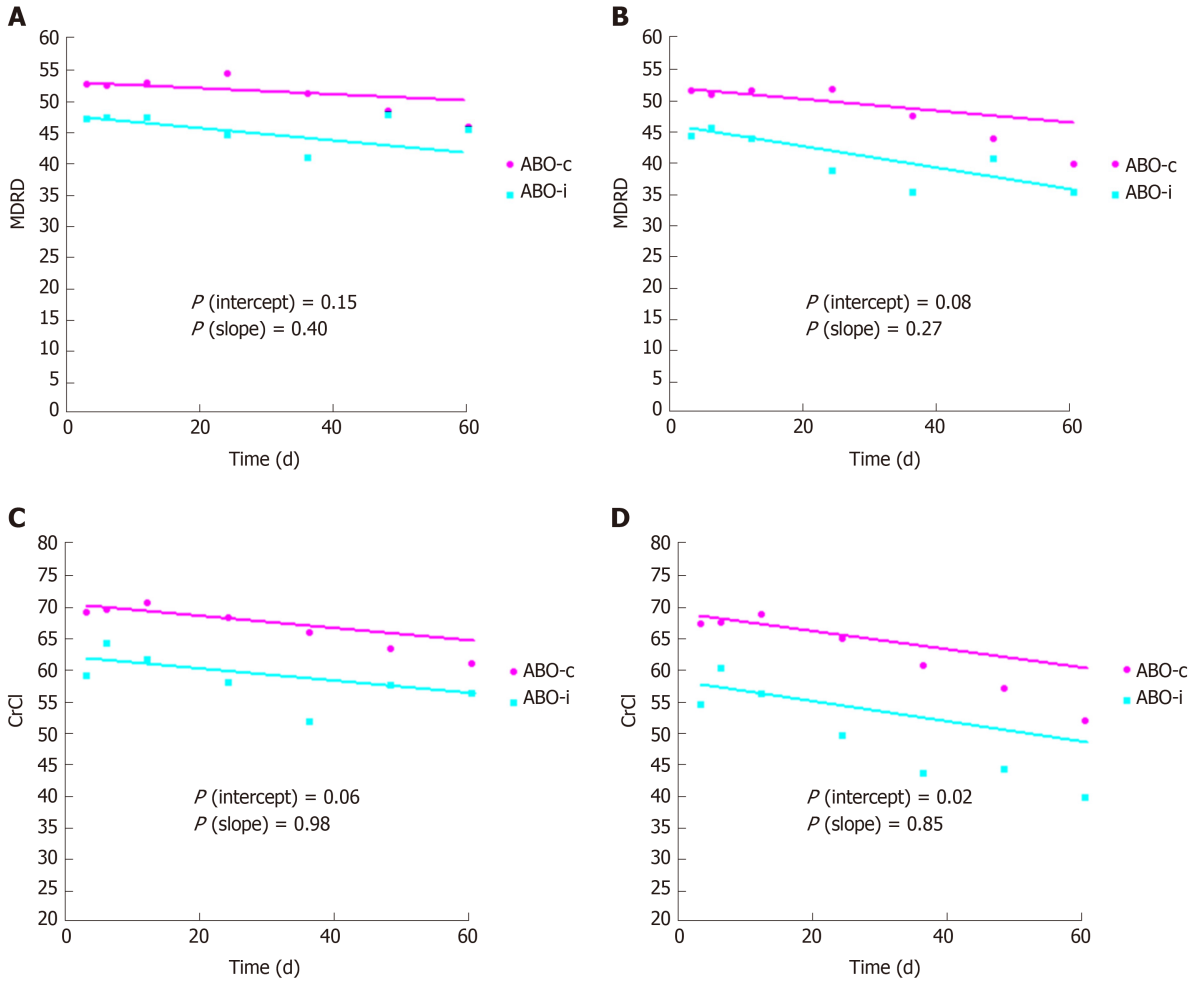


Figure 2 Kidney function. A: Estimated glomerular filtration rate (Modification of Diet in Renal Disease) without imputation in case of graft loss; B: Estimated glomerular filtration rate (Modification of Diet in Renal Disease) with imputation of 10 mL/min/1.73 m^2 in case of graft loss; C: Creatinine clearance without imputation in case of graft loss; D: Creatinine clearance with imputation of 10 mL/min/1.73 m^2 in case of graft loss. Curves were estimated using linear mixed models. The dots indicate point estimates at 3, 6, 12, 24, 36, 48 and 60 months. CrCl: Creatinine clearance; MDRD: Modification of Diet in Renal Disease.

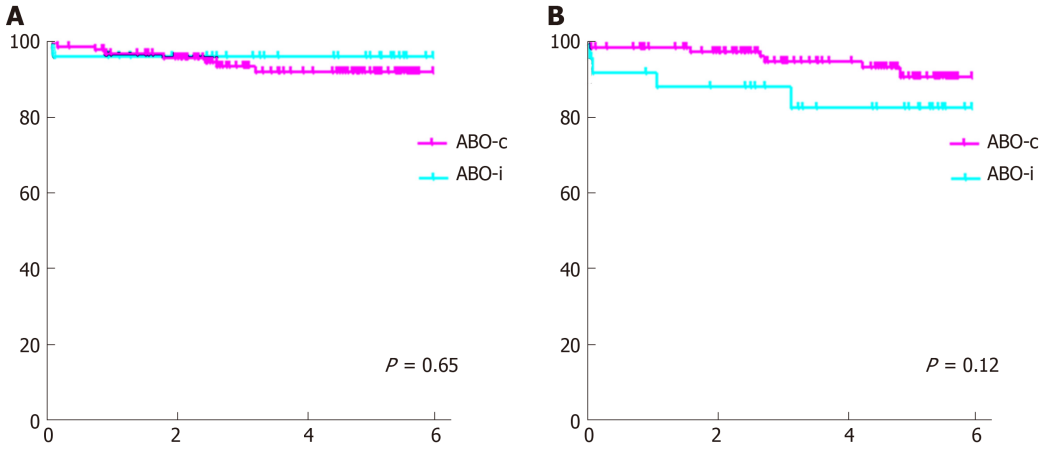


Figure 3 Patient and graft survival. A: Patient survival; B: Death-censored graft survival.

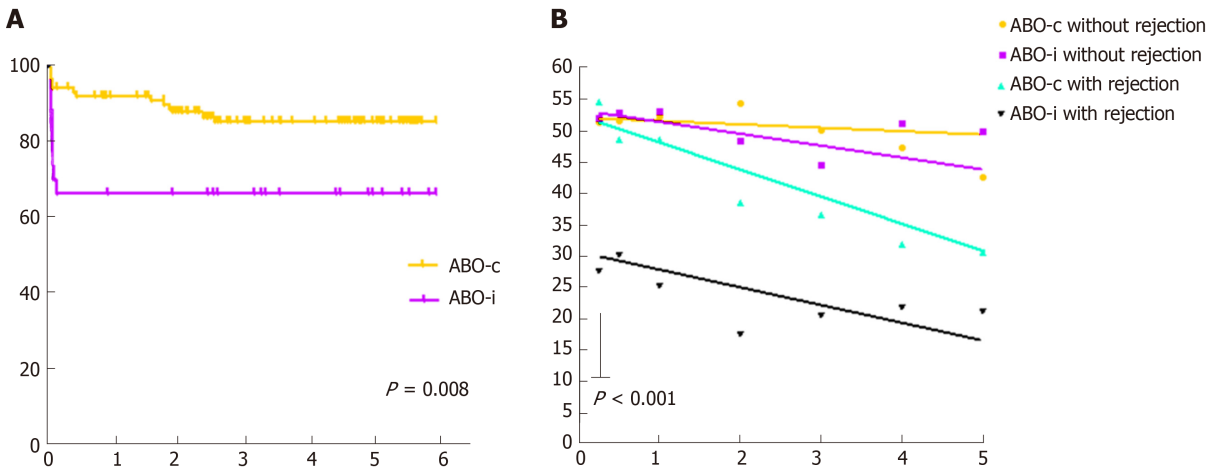
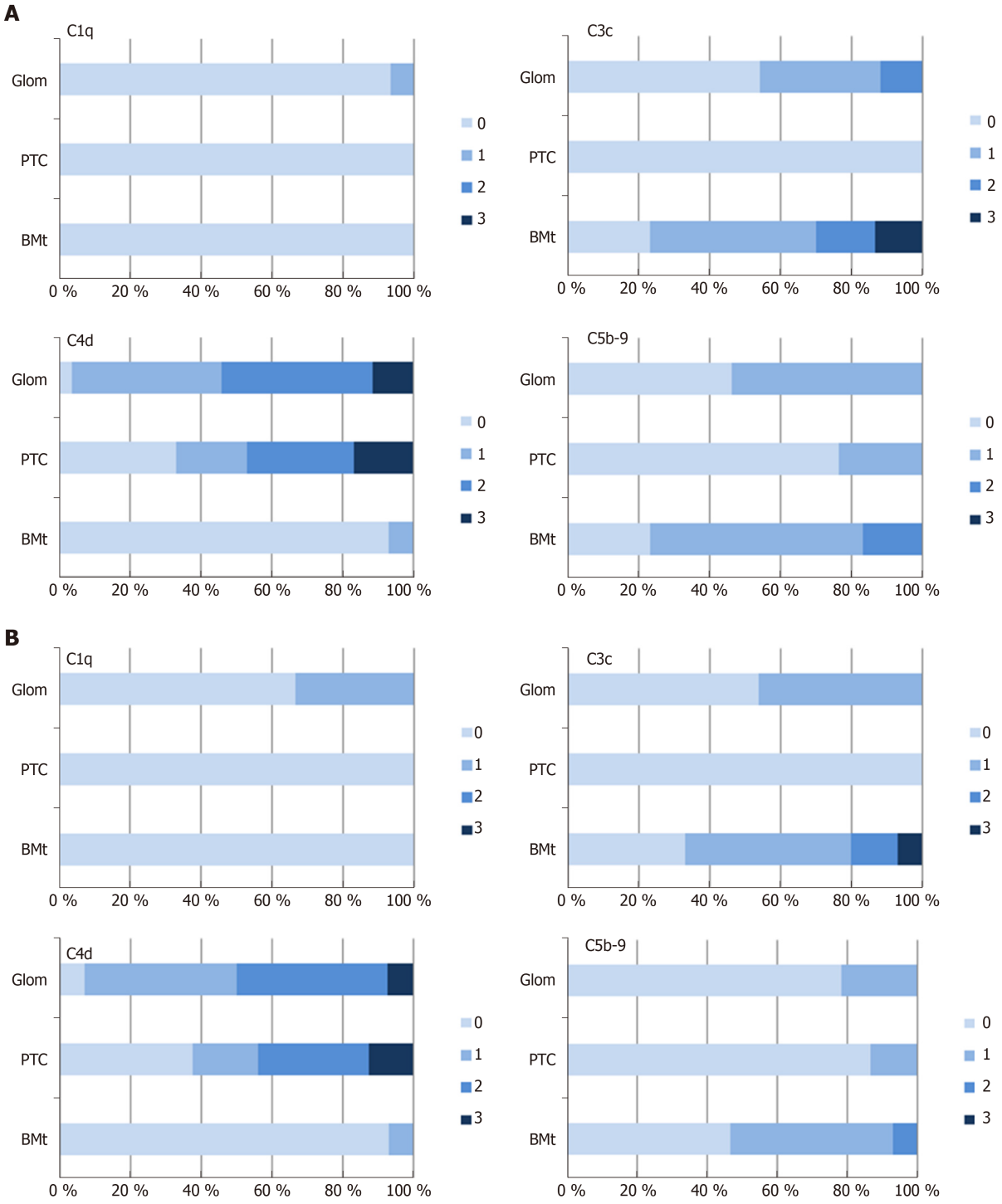


Figure 4 Rejection-free survival and kidney function split by occurrence of rejection. A: Rejection-free survival; B: Estimated glomerular filtration rate (Modification of Diet in Renal Disease). *P* value calculated for intercept of ABO-i recipients with rejection (black line) compared to all other groups.


A
C1q
C3c
C4d
C5b-9
Glom
PTC
BMt
0 %
20 %
40 %
60 %
80 %
100 %
0
1
2
3
B
C1q
C3c
C4d
C5b-9
Glom
PTC
BMt
0 %
20 %
40 %
60 %
80 %
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0
1
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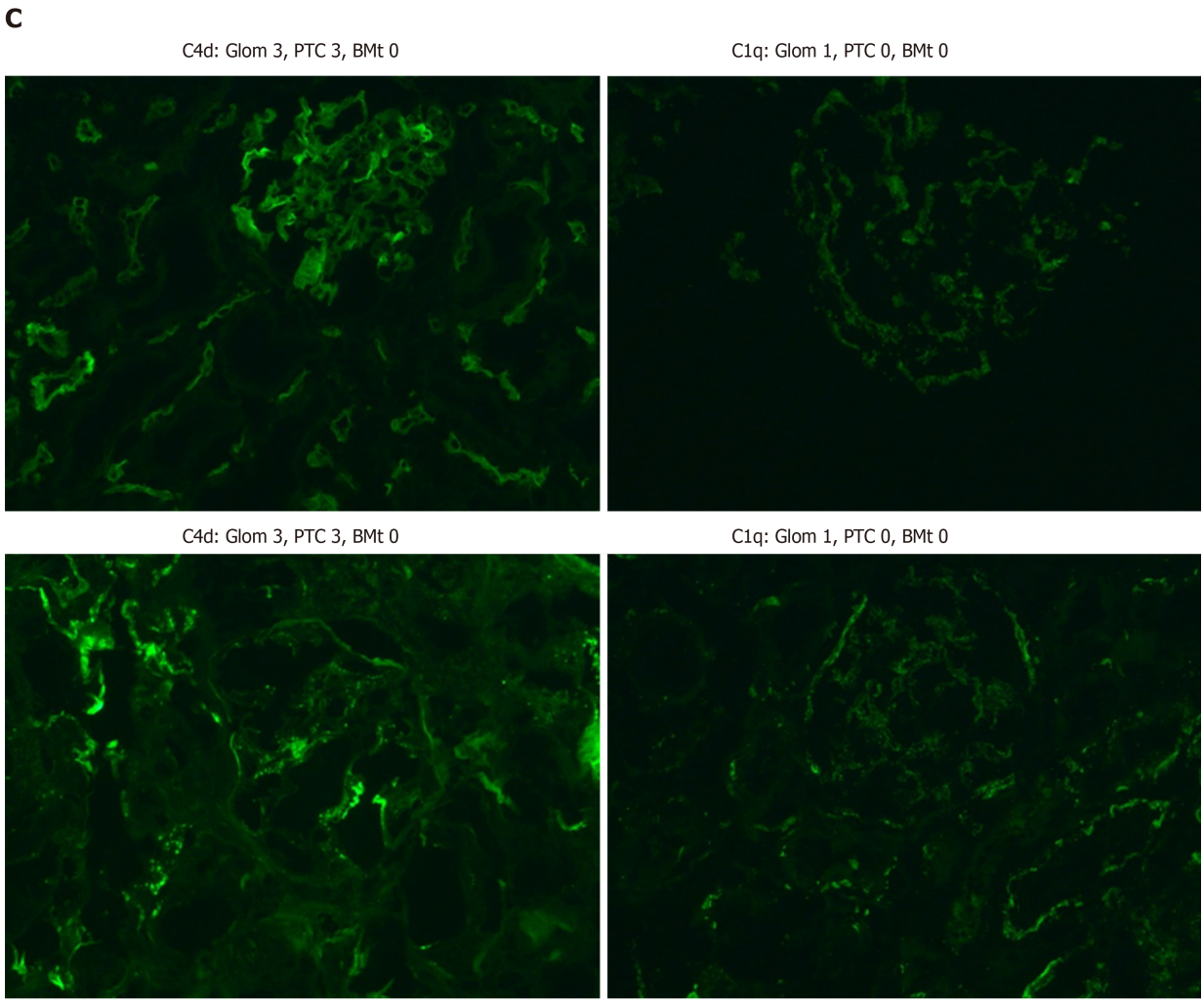


Figure 5 Complement activation in ABO-incompatible. A: Complement activation in ABO-incompatible indication biopsies; B: Complement activation in ABO-incompatible protocol biopsies; C: Digital photographs of complement activation. Intensity of staining ranges from 0-3. Figures indicate the percentage of biopsies with each intensity score. Glom: Glomerular; PTC: Peritubular capillaries; BMt: Basal membrane of the tubuli.

ARTICLE HIGHLIGHTS

Research background

Short-term graft and patient survival in ABO-incompatible kidney transplantation are equivalent to ABO-compatible kidney transplantation, but in ABO-incompatible kidney transplantation, ongoing activation of complement by anti-ABO antibodies might adversely affect long-term graft function.

Research motivation

We aimed to investigate whether ongoing complement activation in ABO-incompatible kidney transplantation could explain why long-term graft function is impaired in ABO-incompatible kidney transplantation compared to ABO-compatible kidney transplantation.

Research objectives

To measure long-term graft function in all ABO-incompatible kidney transplantation recipients at the Academic Medical Center Amsterdam and the University Medical Center Groningen, compare this to long-term graft function in matched ABO-compatible kidney transplantation recipients and relate this to various markers of complement activation.

Research methods

We used linear mixed models to estimate long-term graft function decline in both ABO-incompatible and matched ABO-compatible kidney transplantation recipients. Matching criteria were age, sex, and transplantation date. AB O-incompatible kidney biopsies were stained for various markers of complement activation.

Research results

The slope of kidney function during five-year follow-up was not significantly different between

ABO-incompatible and ABO-compatible kidney transplantation, but ABO-incompatible kidney transplant recipients did have a lower function at three months after transplantation due to a high rate of early mostly T-cell mediated rejection. Rejection and C5b-9 activation were positively correlated.

Research conclusions

Ongoing complement activation adversely affects long-term graft function in ABO-incompatible kidney transplantation. We hypothesize that this may be due to concurrent C5a formation, which functions as a costimulatory signal for T-cell activation.

Research perspectives

Further studies are needed to confirm whether ongoing C5a formation is responsible for T-cell mediated rejection in ABO-incompatible kidney transplantation.

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REFERENCES

1 **Maggiore U**, Oberbauer R, Pascual J, Viklicky O, Dudley C, Budde K, Sorensen SS, Hazzan M, Klinger M, Abramowicz D; ERA-EDTA-DESCARTES Working Group. Strategies to increase the donor pool and access to kidney transplantation: an international perspective. *Nephrol Dial Transplant* 2015; **30**: 217-222 [PMID: 24907023 DOI: 10.1093/ndt/gfu212]

2 **Wilpert J**, Fischer KG, Pisarski P, Wiech T, Daskalakis M, Ziegler A, Neumann-Haefelin E, Drognitz O, Emmerich F, Walz G, Geyer M. Long-term outcome of ABO-incompatible living donor kidney transplantation based on antigen-specific desensitization. An observational comparative analysis. *Nephrol Dial Transplant* 2010; **25**: 3778-3786 [PMID: 20466677 DOI: 10.1093/ndt/gfq229]

3 **Gentry SE**, Montgomery RA, Segev DL. Kidney paired donation: fundamentals, limitations, and expansions. *Am J Kidney Dis* 2011; **57**: 144-151 [PMID: 21184921 DOI: 10.1053/j.ajkd.2010.10.005]

4 **Stegall MD**, Dean PG, Gloor JM. ABO-incompatible kidney transplantation. *Transplantation* 2004; **78**: 635-640 [PMID: 15371661 DOI: 10.1097/01.tp.0000136263.46262.0d]

5 **Montgomery RA**, Locke JE, King KE, Segev DL, Warren DS, Kraus ES, Cooper M, Simpkins CE, Singer AL, Stewart ZA, Melancon JK, Ratner L, Zachary AA, Haas M. ABO incompatible renal transplantation: a paradigm ready for broad implementation. *Transplantation* 2009; **87**: 1246-1255 [PMID: 19384174 DOI: 10.1097/TP.0b013e31819f2024]

6 **Breimer ME**, Mölne J, Nordén G, Rydberg L, Thiel G, Svalander CT. Blood group A and B antigen expression in human kidneys correlated to A1/A2/B, Lewis, and secretor status. *Transplantation* 2006; **82**: 479-485 [PMID: 16926591 DOI: 10.1097/01.tp.0000231697.15817.51]

7 **Alexandre GP**, Squifflet JP, De Bruyère M, Latinne D, Reding R, Gianello P, Carlier M, Pirson Y. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. *Transplant Proc* 1987; **19**: 4538-4542 [PMID: 3321614 DOI: 10.1109/10.552251]

8 **Tanabe K**. Japanese experience of ABO-incompatible living kidney transplantation. *Transplantation* 2007; **84**: S4-S7 [PMID: 18162988 DOI: 10.1097/01.tp.0000296008.08452.4c]

9 **Macklin PS**, Morris PJ, Knight SR. A systematic review of the use of rituximab for desensitization in renal transplantation. *Transplantation* 2014; **98**: 794-805 [PMID: 25321163 DOI: 10.1097/TP.0000000000000362]

10 **Opelz G**, Morath C, Süsal C, Tran TH, Zeier M, Döhler B. Three-year outcomes following 1420 ABO-incompatible living-donor kidney transplants performed after ABO antibody reduction: results from 101 centers. *Transplantation* 2015; **99**: 400-404 [PMID: 25050471 DOI: 10.1097/TP.0000000000000312]

11 **Montgomery JR**, Berger JC, Warren DS, James NT, Montgomery RA, Segev DL. Outcomes of ABO-incompatible kidney transplantation in the United States. *Transplantation* 2012; **93**: 603-609 [PMID: 22290268 DOI: 10.1097/TP.0b013e318245b2af]

12 **Couzi L**, Manook M, Perera R, Shaw O, Ahmed Z, Kessaris N, Dorling A, Mamode N. Difference in outcomes after antibody-mediated rejection between abo-incompatible and positive cross-match transplantations. *Transpl Int* 2015; **28**: 1205-1215 [PMID: 26095452 DOI: 10.1111/tri.12621]

13 **Ko EJ**, Yu JH, Yang CW, Chung BH; Korean Organ Transplantation Registry Study Group. Clinical outcomes of ABO- and HLA-incompatible kidney transplantation: a nationwide cohort study. *Transpl Int* 2017; **30**: 1215-1225 [PMID: 28493630 DOI: 10.1111/tri.12979]

14 **Marfo K**, Lu A, Ling M, Akalin E. Desensitization protocols and their outcome. *Clin J Am Soc Nephrol* 2011; **6**: 922-936 [PMID: 21441131 DOI: 10.2215/CJN.08140910]

15 **Pankhurst L**, Hudson A, Mumford L, Willicombe M, Galliford J, Shaw O, Thuraisingham R, Puliatti C, Talbot D, Griffin S, Torpey N, Ball S, Clark B, Briggs D, Fuggle SV, Higgins RM. The UK National Registry of ABO and HLA Antibody Incompatible Renal Transplantation: Pretransplant Factors Associated With Outcome in 879 Transplants. *Transplant Direct* 2017; **3**: e181 [PMID: 28706984 DOI: 10.1097/TXD.0000000000000695]

16 **Takahashi K**. A new concept of accommodation in ABO-incompatible kidney transplantation. *Clin Transplant* 2005; **19** Suppl 14: 76-85 [PMID: 15955174 DOI: 10.1111/j.1399-0012.2005.00392.x]

17 **Tanabe T**, Ishida H, Horita S, Yamaguchi Y, Toma H, Tanabe K. Decrease of blood type antigenicity over the long-term after ABO-incompatible kidney transplantation. *Transpl Immunol* 2011; **25**: 1-6 [PMID: 21616149 DOI: 10.1016/j.trim.2011.05.002]

18 **Park WD**, Grande JP, Ninova D, Nath KA, Platt JL, Gloor JM, Stegall MD. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am J Transplant* 2003; **3**: 952-960 [PMID: 12859529 DOI: 10.1034/j.1600-6143.2003.00179.x]

19 **Ishida H**, Tanabe K, Ishizuka T, Furusawa M, Miyamoto N, Ishikawa N, Shirakawa H, Shimmura H, Ishii D, Nozaki D, Setoguchi K, Toma H. Differences in humoral immunity between a non-rejection group and a rejection group after ABO-incompatible renal transplantation. *Transplantation* 2006; **81**: 665-671 [PMID: 16534466 DOI: 10.1097/01.tp.0000185193.77929.96]

20 **Dehoux JP**, Gianello P. Accommodation and antibodies. *Transpl Immunol* 2009; **21**: 106-110 [PMID: 18973811 DOI: 10.1016/j.trim.2008.10.002]

21 **King KE**, Warren DS, Samaniego-Picota M, Campbell-Lee S, Montgomery RA, Baldwin WM. Antibody, complement and accommodation in ABO-incompatible transplants. *Curr Opin Immunol* 2004; **16**: 545-549 [PMID: 15341997 DOI: 10.1016/j.coi.2004.07.004]

22 **Setoguchi K**, Ishida H, Shimmura H, Shimizu T, Shirakawa H, Omoto K, Toki D, Iida S, Setoguchi S, Tokumoto T, Horita S, Nakayama H, Yamaguchi Y, Tanabe K. Analysis of renal transplant protocol biopsies in ABO-incompatible kidney transplantation. *Am J Transplant* 2008; **8**: 86-94 [PMID: 18021283 DOI: 10.1111/j.1600-6143.2007.02036.x]

23 **Cohen D**, Colvin RB, Daha MR, Drachenberg CB, Haas M, Nickeleit V, Salmon JE, Sis B, Zhao MH, Bruijn JA, Bajema IM. Pros and cons for C4d as a biomarker. *Kidney Int* 2012; **81**: 628-639 [PMID: 22297669 DOI: 10.1038/ki.2011.497]

24 **Webster AC**, Ruster LP, McGee R, Matheson SL, Higgins GY, Willis NS, Chapman JR, Craig JC. Interleukin 2 receptor antagonists for kidney transplant recipients. *Cochrane Database Syst Rev* 2010; CD003897 [PMID: 20091551 DOI: 10.1002/14651858.CD003897.pub3]

25 **Cravedi P**, Leventhal J, Lakhani P, Ward SC, Donovan MJ, Heeger PS. Immune cell-derived C3a and C5a costimulate human T cell alloimmunity. *Am J Transplant* 2013; **13**: 2530-2539 [PMID: 24033923 DOI: 10.1111/ajt.12405]

26 **Lalli PN**, Strainic MG, Yang M, Lin F, Medof ME, Heeger PS. Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. *Blood* 2008; **112**: 1759-1766 [PMID: 18567839 DOI: 10.1182/blood-2008-04-151068]

27 **Strainic MG**, Liu J, Huang D, An F, Lalli PN, Muqim N, Shapiro VS, Dubyak GR, Heeger PS, Medof ME. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. *Immunity* 2008; **28**: 425-435 [PMID: 18328742 DOI: 10.1016/j.immuni.2008.02.001]

28 **Dunkelberger JR**, Song WC. Role and mechanism of action of complement in regulating T cell immunity. *Mol Immunol* 2010; **47**: 2176-2186 [PMID: 20603023 DOI: 10.1016/j.molimm.2010.05.008]

29 **Heeger PS**, Kemper C. Novel roles of complement in T effector cell regulation. *Immunobiology* 2012; **217**: 216-224 [PMID: 21742404 DOI: 10.1016/j.imbio.2011.06.004]



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CASE REPORT

Broad spectrum of interferon-related nephropathies-glomerulonephritis, systemic lupus erythematosus-like syndrome and thrombotic microangiopathy: A case report and review of literature

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Author contributions: All authors designed the study and wrote the protocol; Gianassi I and Caroti L identified studies and independently reviewed each for eligibility in a two-step based process; Gianassi I extracted the data and assessed the quality of selected studies using the checklist developed by Downs and Black for both randomized and non-randomized studies; Allinovi M supervised the data by using an ad hoc developed data extraction spreadsheet; Gianassi I, Allinovi M and Caroti L were involved in drafting of the manuscript; Cirami LC revised the manuscript; Allinovi M has given final approval of the version to be published; all authors read and approved the final manuscript.

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Abstract

BACKGROUND

Interferons (IFNs) are characterized by a wide range of biological effects, which justifies their potential therapeutic use in several pathologies, but also elicit a wide array of adverse effects in almost every organ system. Among them, renal involvement is probably one of the most complex to identify.

CASE SUMMARY

We describe four cases of kidney damage caused by different IFN formulations: IFN-β-related thrombotic microangiopathy, IFN-β-induced systemic lupus erythematosus, and two cases of membranous nephropathy secondary to pegylated-IFN-α 2B. In each case, we carefully excluded any other possible cause of renal involvement. Once suspected as the casual relationship between drug and kidney damage, IFN treatment was immediately discontinued. In three cases, we observed a complete and persistent remission of clinical and laboratory abnormalities after IFN withdrawal, while the patient who developed thrombotic microangiopathy, despite IFN withdrawal and complement-inhibitor therapy with eculizumab, showed persistent severe renal failure requiring dialysis.

CONCLUSION

This case series highlights the causal relationship between IFN treatment and different types of renal involvement and enables us to delineate several peculiarities of this association.

Key words: Interferon; Thrombotic microangiopathy; Systemic lupus erythematosus; Eculizumab; Nephrotoxicity

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Core tip: Different patterns of kidney damage can occur in patients treated with different interferon types, even after years of well-tolerated therapy. Interferon can cause renal dysfunction which ranges from subclinical to severe dysfunction and, regardless the mechanism of renal failure, the usual pathological finding includes either podocytopathies, interstitial nephritis, systemic lupus erythematosus-like disease or thrombotic microangiopathy. We describe four cases of interferon-related nephropathies and for each case we highlight clinical features, laboratory work-up, histological findings, treatment and follow-up. Moreover, we report for the first time a case of drug-induced systemic lupus erythematosus with renal involvement after the use of interferon-β.

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INTRODUCTION

Interferons (IFNs) are a family of pleiotropic mediators of the immune system which collaborate in the genesis of numerous physiological processes, such as anti-viral, immunomodulatory and anti-proliferative activities[1]. This wide range of biological effects justifies their therapeutic use in several pathologies (Table 1). Class I IFNs (IFN-α and -β), in accordance to their huge biological effects, are the most widely used, while class II IFNs (IFN-γ) is mainly oriented to the macrophage response but have been less used. A wide array of adverse effects of IFNs have been described in almost every organ system[2]. Among them, renal involvement is probably one of the most complex to identify. Two potential mechanisms can be involved in IFN-related kidney injury: (1) Dose-dependent, caused by increasing cumulative drug doses and likely reversible at dose reduction; or (2) Idiosyncratic, which in general is not directly correlated with the drug dose and not necessarily reversible upon drug discontinuation[3]. Therefore, kidney damage can occur shortly after IFN-treatment beginning or after a long period of apparently well-tolerated therapy.

In preclinical surveys and in clinical trials, IFN-induced renal side effects were not infrequently reported but usually limited to mild proteinuria in about 20% of patients, mild abnormalities in urinalysis, and transient increase in serum creatinine[4,5]. Different types of renal toxicity have been associated with IFN therapy, mostly in case reports and including glomerulonephritis, tubulo-interstitial nephritis, systemic lupus erythematosus (SLE)-like syndrome or thrombotic microangiopathy (TMA)[6,7] (Figure 1). The causal relationship between IFN and several nephropathies is still underestimated and the incidence of renal involvement during IFN therapy remains undetermined. We herein report our case series of four patients affected by a broad spectrum of IFN-related nephropathies, among whom we report the first case with IFN-β-related SLE-like syndrome to be described in the literature.

We describe four cases of renal involvement during IFN therapy, diagnosed at our Nephrology Department during the last 10 years. Two patients were affected by relapsing-remitting multiple sclerosis (RRMS) and two patients by hepatitis C virus (HCV)-related chronic hepatitis, treated with different IFN formulation: IFN-β and pegylated (Peg)-IFN-α 2B, respectively. All patients were regularly followed up by the Multiple Sclerosis Centre of the Department of Neurosciences and Hepatitis Centre of the Department of Gastroenterology, at the Careggi University Hospital, Florence. Information on our patients, including age at onset, duration of the disease, type of IFN treatment, physical examination at the time of diagnosis, laboratory and clinical data were retrieved from medical records. We also reviewed the cases characterized by IFN-related kidney damage reported in the literature. A PubMed literature search was conducted to identify the publications discussing renal involvement in patients on IFN therapy from 2010 to 2013, using the medical subject heading terms: “interferon”, “beta-interferon”, "alpha-interferon", “nephritis”, “glomerulonephritis”,

Table 1 Therapeutic indications of different IFN formulations

Type of IFN	Hepatological indications	Immunological indications	Neurological indications	Haematological indications	Neoplastic indications
IFN-α 2A and IFN-α 2B	HBV-CH, HCV-CH, HDV-CH	BS, ECD	No current indications	ET, PV, CML, NHL, HCL, CTCL, WM	aRCC, MM stage II, AIDS-associated Kaposi sarcoma
IFN-β 1A and IFN-β 1B	No current indications	No current indications	RRSM	No current indications	No current indications

aRCC: Advanced renal cell carcinoma; BS: Behçet syndrome; CML: Chronic myeloid leukaemia; CTCL: T-cell cutaneous lymphoma; ECD: Erdheim-Chester disease; ET: Essential thrombocythemia; HBC-CH: Hepatitis B virus-related chronic hepatitis; HCL: Hair-cell leukaemia; HCV-CH: Hepatitis C virus-related chronic hepatitis; HDV-CH: Hepatitis D virus-related chronic hepatitis in patients with hepatitis B virus co-infection; IFN: Interferon; MM: Malignant melanoma stage II (Tumour node metastasis classification, American Joint Committee on Cancer 2010); NHL: Non-Hodgkin's lymphoma; PV: Polycythaemia vera; RRSM: Relapsing-remitting multiple sclerosis; WM: Waldenstrom macroglobulinemia.

"nephropathy", "proteinuria", "acute renal failure", "thrombotic microangiopathy", "haemolytic uremic syndrome", "thrombotic thrombocytopenic purpura," and "malignant hypertension." We excluded cases in whom other possible etiological factors or triggers of kidney damage were present. In cases of diabetes mellitus, monoclonal gammopathy or uncertain significance and HCV infection, which are potential causes of nephropathies but very prevalent in the elderly population, we have carefully investigated their causal role or simply their random coexistence.

CASE PRESENTATION

Patient 1

Chief complaint and history of present illness: A 56-year-old woman was referred to the emergency department of our hospital due to hypertensive crisis (blood pressure 210/120 mmHg) and headache.

History of past illness: Past medical history revealed RRMS treated with IFN-β for 10 years with good response and no complications.

Physical examination: The patient presented with dyspnoea, oedema of lower limbs and purpura.

Laboratory examinations: On admission, her laboratory workup showed normocytic anaemia, thrombocytopenia, haemolysis signs and severe acute kidney injury (serum creatinine 4.5 mg/dL). Furthermore, we observed an activation of the alternative complement pathway, with low C3 (79 mg/dL; n.v. 90-180 mg/dL) and normal C4 levels. Immunological tests [cryoglobulins, anti-nuclear antibody (ANA), anti-dsDNA, antineutrophil cytoplasmic antibody (ANCA), anti-phospholipid antibodies, complement levels] as well as infectious disease tests (hepatitis B virus, HCV, human immunodeficiency virus, cytomegalovirus, Epstein–Barr virus, parvovirus B19, *Escherichia coli* serotype O157:H) and cancer markers were normal.

Imaging examinations: Chest x-ray showed mild pulmonary congestion, while ultrasonography of the kidney revealed a normal kidney size but increased echogenicity in the renal cortex.

Final diagnosis: The clinical and laboratory findings were compatible with the diagnosis of TMA (Table 2). Subsequent tests, carried out for suspected TMA, showed no mutations in genes of regulatory complement factors (CFH, CFI, CFB, MCP, C3, THBD) and normal ADAMTS-13 activity. The patient underwent a kidney biopsy that confirmed our first hypothesis of TMA [IF: Minimal alone nonspecific entrapment of C3 (+)].

Treatment: IFN-β was discontinued and eculizumab therapy was then started. The patient was given intravenous eculizumab dose of 900 mg weekly for 4 wk, followed by 1200 mg 1 wk later as the 5th.

Outcome and follow-up: Despite IFN withdrawal and complement-inhibitor therapy, the severe renal failure persisted. Despite persistent normal haematological values, (3 years later) our patient is still undergoing a thrice-weekly intermittent dialysis.

Patient 2

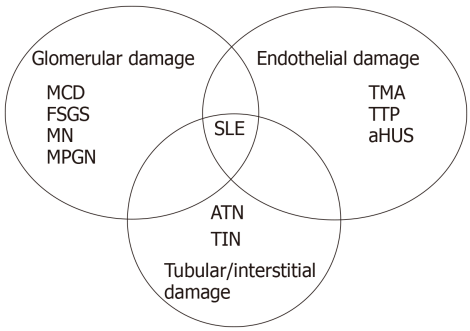


Figure 1 Possible histopathological patterns of kidney damage related to interferon treatment. aHUS: Atypical haemolytic uremic syndrome; ATN: Acute tubular necrosis; FSGS: Focal segmental glomerulosclerosis; MCD: Minimal-change disease; MN: Membranous nephropathy; MPGN: Membranoproliferative glomerulonephritis; SLE: Systemic lupus erythematosus; TIN: Tubular interstitial necrosis; TMA: Thrombotic microangiopathy; TTP: Thrombotic thrombocytopenic purpura.

Chief complaint and history of present illness: A 56-year-old woman was referred to our department due to dyspnoea that had started few hours before.

History of past illness: The patient was treated with IFN-β for 2 mo because of RRMS, which was recently diagnosed.

Physical examination: The patient reported moderate dyspnoea and positional chest pain. Her physical examination was otherwise unremarkable.

Laboratory examinations: Laboratory tests showed mild thrombocytopenia, inflammation (C-reactive protein of 31 mg/dL), acute kidney injury (serum creatinine of 2.5 mg/dL) and red cell casts on urine examination. Immunological tests showed ANA (1:640), anti-dsDNA (1:320) and anti-ENA (anti-RNP/sm, anti-nucleosome, anti-SM, anti-histone) positivity, low C3 and C4 levels (Table 2).

Imaging examinations: Echocardiography and chest-x ray showed pleural and pericardial effusions.

Final diagnosis: We performed kidney biopsy, that demonstrated a pattern of diffuse proliferative nephritis, involving more than 50% of the total number of glomerula with active and chronic lesions [class IV–S(A/C) lupus nephritis (LN)]. The electron microscopy showed tubuloreticular inclusions widespread, typically identified in LN as expression of endogenous IFNs but also suspected for being linked to the exposure of excess exogenous IFNs.

Treatment: We started treatment with corticosteroids (methylprednisolone 3 intravenous pulses) and oral corticosteroids, followed by maintenance therapy (azathioprine at 2 mg/kg per os) and concomitantly; IFN-β therapy was discontinued.

Outcome and follow-up: The patient showed a rapid clinical recovery, with progressive improvement of her renal function. Immunological tests exhibited a total remission. After 6 mo, we progressively suspended immunosuppressive treatment, without any clinical and laboratory relapse. Actually, 4 and ½ years later, she is regularly followed up at our nephrology centre, exhibiting a normal kidney function and a total immunological remission without steroids or immunosuppressive drugs.

Patient 3

Chief complaint and history of present illness: A 65-year-old man was admitted to the emergency department due to progressive symptomatic fatigue.

History of past illness: The patient had an HCV-related chronic hepatitis, previously treated with IFN-α 2B and ribavirin for 4 years, shifted to Peg-IFN-α 2B 1 year before.

Physical examination: At presentation, the patient had fatigue, oedema of lower limbs and oliguria.

Laboratory examinations: Laboratory examinations showed acute kidney injury (serum creatinine of 3 mg/dL). Urinary tests detected nephrotic proteinuria (24 g/die) and microhaematuria. Immunological tests did not show any pathological findings, in particular cryoglobulins and anti-PLA2R antibodies were negative. Furthermore blood exams revealed a stable hepatic status, with no abnormalities suspected for

Table 2 Clinical and laboratory findings of our case series: Medical history, diagnosis, treatment and outcome

	Case 1	Case 2	Case 3	Case 4
Age in yr	56	56	65	66
Sex	F	F	F	M
Type of IFN and time of exposure	IFN-β, 10 yr	IFN-β, 2 mo	IFN-α 2B, 4 yr; Peg-IFN-α 2B, 1 yr	Peg-IFN-α 2B, 2 yr
Indication for IFN treatment	RRMS	RRMS	HCV-related chronic hepatitis	HCV-related chronic hepatitis
Clinical presentation	Dyspnoea, severe hypertension, oedema of lower limbs, purpura	Chest pain and dyspnoea	Oedema of lower limbs and oliguria	Dyspnoea, lower limbs oedema, peripheral purpura, and itch
Serum creatinine at onset in mg/dL	4.5	2.5	3	0.83
Need for dialysis	Yes	No	No	No
Proteinuria in g/24 h	4	1.5	24	3.5
Renal biopsy	Thrombotic microangiopathy	Class IV-S (A/C) Lupus nephritis	Membranous nephropathy	Membranous nephropathy
Treatment, in addition to IFN discontinuation	Eculizumab	IV steroids, azathioprine	ACTH, RASS-B	No
Recurrence after IFN withdrawal	No	No	No	No
Outcome	ESRD	Remission	Remission	Remission
Length of follow-up in yr	3	4.5	12.5	5.5

ACTH: Adrenocorticotropic hormone; ESRD: End-stage renal disease; HCV: Hepatitis C virus; IFN: Interferon; IV: Intra-venous; RAAS-B: Renin-angiotensin-aldosterone system blockers; RRMS: Relapsing-remitting multiple sclerosis.

HCV-related disease, and virology tests excluded any sign of HCV viremia (HCV PCR RNA-negative) (Table 2).

Imaging examinations: Chest x-ray showed minimal basal bilateral opacities, while abdominal echography was unremarkable.

Final diagnosis: We performed a kidney biopsy, which was compatible with membranous nephropathy (MN) stage I/II.

Treatment: After IFN withdrawal, the patient started double renin-angiotensin-aldosterone system (commonly known as RAAS)-blockade and immunosuppressive therapy with long-acting adrenocorticotropic hormone, which was interrupted early due to appearance of complications (hyperaldosteronism and meta-steroid diabetes) after about 1 mo.

Outcome and follow up: After 12 and ½ years, our patient is still under conservative treatment with double RAAS-block and has had no recurrence of MN.

Patient 4

Chief complaint, history of present illness, and relapse of clinical manifestation: A 66-year-old man presented to the emergency department with purpura and itch, which progressively appeared 1 wk before. Despite the initial clinical and laboratory recovery, 2 mo later, after IFN dose increase, our patient experienced fatigue, dyspnoea and lower limbs' oedema.

History of past illness: The patient had HCV-related chronic hepatitis and had been treated with Peg-IFN-α 2B and ribavirin for the past 2 years with good response and no complications.

Physical examination: His physical examination revealed lower limbs' oedema, in addition to peripheral purpura.

Laboratory examinations: Laboratory workup showed normal liver function with no signs of HCV viremia (HCV PCR RNA-negative). Nephrotic-range proteinuria (3.5 g/die) was detected at urinalysis. The patient was transferred to our nephrology ward, where we performed secondary tests that showed nephrotic syndrome (nephrotic-range proteinuria, hypoalbuminemia, lipid abnormalities, thyroid dysfunction) with normal serum creatinine (0.83 mg/dL) (Table 2). Immunological tests were all normal and, in particular, cryoglobulins and anti-PLA2R antibodies

were negative.

Imaging examinations: Chest x-ray, echocardiography, and abdominal echography were unremarkable.

Diagnosis and treatment: At the beginning, the IFN daily dose was reduced because an IFN overdose had been hypothesized. Therefore, we performed kidney biopsy, which demonstrated a typical pattern of MN stage I. After IFN withdrawal, our patient showed a rapid kidney recovery with a complete remission, without immunosuppressive treatment. The patient definitively stopped IFN treatment, according with our gastroenterological centre.

Outcome and follow up: Currently, 5 and ½ years later, our patient is regularly followed up at the hepatitis centre of the department of gastroenterology, showing normal kidney function.

DISCUSSION

The case series we have presented here, together with the previously published cases on IFN-related nephropathies we reported, highlight the causal relationship between IFN treatment and different types of renal involvement, ruling out the hypothesis of a fortuitous association. Two patients with HCV-related chronic hepatitis were treated with Peg-IFN-α 2B and two patients with RRMS were treated with IFN-β, showing potentially early or late onset renal toxicity after IFN initiation. Evidence now supports the concept that IFNs contribute to several types of kidney diseases[8]. A causal association between IFN and renal toxicity was often recognized late or not diagnosed in the past. In many of these cases, the cause of this missed diagnosis was the presence of other possible etiological factors or triggers of kidney damage. This study enables us to delineate several peculiarities of this association.

IFN treatment and thrombotic microangiopathy

IFN has been widely described to be associated with development of TMA in the scientific literature[9]. It is well known that direct toxic reactions in the course of type I IFN treatment are typically dose-dependent[10]. In fact, quantitative histopathological analyses identified a dose-dependent spectrum of microvascular disease, including endothelial hypertrophy, intimal thickening, microaneuryms, perivascular inflammatory infiltrates and reduced intracellular nitric oxide generation[10,11]. Our patient developed TMA after 10 years of IFN treatment, confirming the dose-dependent toxic effect and also indicating that long-term IFN therapy can induce late-onset TMA. The development of renal lesions after several years of well-tolerated IFN therapy may be the result of a cumulative effect in renal and systemic microvessels.

A recent review analysed 24 cases of TMA occurring in multiple sclerosis patients treated with IFN-β and the authors observed TMA-onset after a mean time of 7 years[12]. We ruled out any other possible cause of TMA, such as primary forms (typical and atypical haemolytic-uremic syndrome, thrombotic thrombocytopenic purpura) and other secondary forms (malignant hypertension, cancers, autoimmune conditions, other causative drugs). In this context, an extensive list of drugs has been associated with TMA[13]. The first case of TMA associated with IFN-α was reported in 1993[14], and the first case associated with IFN-β was reported in 1998[15]. Since then, many cases of both IFN-α- and IFN-β-related TMA have been reported in the literature, mostly as individual case reports[16-20].

First line therapy in drug-related TMA is drug discontinuation and supportive treatments. Plasma exchange, immunosuppressive drugs and also eculizumab have been described as effective therapies on refractory forms of IFN-related TMA, although mainly in case reports. Nevertheless, the prognosis of drug-related TMA and particularly IFN-related TMA is particularly severe. It is generally characterized by fulminating presentation, even after years of well-tolerated IFN treatment, with severe clinical course, poor response to therapy and bad renal prognosis. In fact, despite treatment with eculizumab, our patient developed renal dysfunction that progressed to end-stage renal disease requiring dialysis.

IFN treatment and SLE

Several drugs can induce SLE, a condition collectively referred as drug-induced lupus (DIL)[21]. It generally shows only some manifestations of full-blown SLE (*e.g.*, sicca syndrome, Raynaud's phenomenon, arthritis and serositis) and tends to resolve, requiring weeks to months, after drug withdrawal. Less frequently, DIL shows major organ involvement (*e.g.*, LN, central nervous system disease, severe haematologic

abnormalities), but if these are present drug cessation should be combined with systemic immunosuppressive therapies. Idiopathic SLE and DIL differ not only for the extent of organ involvement and for the frequency and type of disease manifestations but also for the serological autoimmune profile[22]. The main immunological pattern in DIL is ANA and anti-histone antibodies positivity and, only in some cases, anti-dsDNA are present[23], depending on the type of the causative drugs (*e.g.*, TNFα inhibitors).

IFN-related DIL is generally associated with silent SLE or lupus-like syndromes, neither with systemic symptoms nor major organ involvement[24-26]. It is well known that type I IFNs, and particularly IFN-α, play a major role in SLE pathogenesis[27] and a proportion of patients displays increased serum levels of IFN-α or an upregulation of the IFN-regulated genes[28]. Type I IFNs prime an adaptive immune response and, if IFN sensitivity or its production are increased, a self-reactive immune response may be favoured. Neutrophils can respond to exposure to type I IFNs and immune complexes with extrusion of nucleic acids and their associated proteins concurring to disease flares. In most of the cases, DIL was caused by IFN-α treatment and less frequently by IFN-γ treatment[29]. Our patient was treated with IFN-β because of RRMS and, to the best of our knowledge, our case is the first report of LN-induced by IFN-β therapy. Interestingly, in our patient the immunological tests showed anti-dsDNA (1:320), ANA (1:640) and anti-histone positivity, an immunological pattern similar to idiopathic SLE and TNFα inhibitors-related SLE. Furthermore, even if not completely understood, type I IFNs and TNFα cross-regulation is well accepted in immune-mediated inflammatory diseases[30].

IFN treatment and glomerulonephritis

Our two patients developed nephrotic syndrome with a histological pattern of membranous nephropathy while on Peg-IFN-α therapy. Cases of nephritic or nephrotic syndrome have been reported during treatment with IFN associated with different underling nephropathies, such as focal segmental glomerulosclerosis[31-34], minimal change disease[35,36], IgA nephropathy[37], and membranoproliferative glomerulonephritis[38,39]. Among these, MN is an uncommon but important and previously described pattern[40,41]. Different clinical features at onset have been described, varying from a full-blown nephritic or nephrotic syndrome, to a severe oligoanuria requiring dialysis[42,43].

In our patients, Peg-IFN-α was used as HCV-related chronic hepatitis treatment. Certainly, kidney damage is one of the most important HCV-related extra-hepatic manifestations and membranoproliferative glomerulonephritis is the most considerable histopathological pattern. However, we have to consider a broad spectrum of histopathological features, with possible glomerular and/or interstitial lesions[44]. For these reasons, to diagnose a drug-induced nephritis in patients with HCV can be difficult. In addition, IFN treatment may produce an exacerbation of a previous nephropathy associated with the HCV infection[45], making the diagnosis even more difficult. We found these two possible pathogenic explanations (HCV as a direct cause or a trigger of nephropathy) very unlikely in our patients; in fact, laboratory workup did not show any sign of viremia (HCV PCR RNA-negative at different time points) and immunological tests (cryoglobulins, complement) did not exhibit any pathological finding.

As largely known, the pathogenetic mechanism of HCV-related kidney damage includes direct invasion of pathogens (justified by HCV PCR RNA positivity) or deposition of antigen-antibody complex by immunological reaction[44,46], neither detected in our cases. Moreover, after IFN withdrawal and with no immunosuppressive drugs, we observed a clinical and laboratory complete remission, persistent after a very long follow-up. For this reason, we excluded a form of HCV-related kidney injury, suggesting a causal relationship between IFN treatment and the appearance/disappearance of the kidney damage in the context of a casual HCV coexistence.

CONCLUSION

Different pattern of kidney damage can occur in patients treated with different IFN types, even after years of well-tolerated therapy. IFN can cause renal dysfunction which ranges from subclinical to severe dysfunction and, whatever the mechanism of renal failure, the usual pathological finding includes either podocytopathies, acute tubular necrosis, interstitial nephritis, SLE-like picture, TMA, or some features of focal sclerosis. This is the first report of drug-induced SLE with renal involvement after the use of IFN-β. We recommend that a new onset of severe headache, hypertension,

proteinuria or renal dysfunction in patients on IFN therapy should be identified early and evaluated thoroughly, also with consideration of a renal biopsy. Once it is suspected, interruption of IFN treatment is warranted, while the role of additional immunosuppressive therapy remains undefined. Despite IFN withdrawal, renal prognosis is bad in IFN-related TMA and kidney damage can persist and also evolve to end-stage renal disease.

REFERENCES

1 **Gresser I**. Biologic effects of interferons. *J Invest Dermatol* 1990; **95**: 66S-71S [PMID: 1701811 DOI: 10.1111/1523-1747.ep12874776]
2 **Raanani P**, Ben-Bassat I. Immune-mediated complications during interferon therapy in hematological patients. *Acta Haematol* 2002; **107**: 133-144 [PMID: 11978934 DOI: 10.1159/000057631]
3 **Nerrant E**, Charif M, Ramay AS, Perrochia H, Patrier L, de Champfleur NM, Renard D, Labauge P. Hemolytic uremic syndrome: an unusual complication of interferon-β treatment in a MS patient. *J Neurol* 2013; **260**: 1915-1916 [PMID: 23708674 DOI: 10.1007/s00415-013-6961-8]
4 **Kurschel E**, Metz-Kurschel U, Niederle N, Aulbert E. Investigations on the subclinical and clinical nephrotoxicity of interferon alpha-2B in patients with myeloproliferative syndromes. *Ren Fail* 1991; **13**: 87-93 [PMID: 1957045 DOI: 10.3109/08860229109022152]
5 **Quesada JR**, Talpaz M, Rios A, Kurzrock R, Gutterman JU. Clinical toxicity of interferons in cancer patients: a review. *J Clin Oncol* 1986; **4**: 234-243 [PMID: 2418169 DOI: 10.1200/JCO.1986.4.2.234]
6 **Markowitz GS**, Bomback AS, Perazella MA. Drug-induced glomerular disease: direct cellular injury. *Clin J Am Soc Nephrol* 2015; **10**: 1291-1299 [PMID: 25862776 DOI: 10.2215/CJN.00860115]
7 **Kundra A**, Wang JC. Interferon induced thrombotic microangiopathy (TMA): Analysis and concise review. *Crit Rev Oncol Hematol* 2017; **112**: 103-112 [PMID: 28325251 DOI: 10.1016/j.critrevonc.2017.02.011]
8 **Anders HJ**, Lichtnekert J, Allam R. Interferon-alpha and -beta in kidney inflammation. *Kidney Int* 2010; **77**: 848-854 [PMID: 20237459 DOI: 10.1038/ki.2010.71]
9 **Ben-Amor AF**, Trochanov A, Fischer TZ. Cumulative Review of Thrombotic Microangiopathy, Thrombotic Thrombocytopenic Purpura, and Hemolytic Uremic Syndrome Reports with Subcutaneous Interferon β-1a. *Adv Ther* 2015; **32**: 445-454 [PMID: 25991578 DOI: 10.1007/s12325-015-0212-6]
10 **Kavanagh D**, McGlasson S, Jury A, Williams J, Scolding N, Bellamy C, Gunther C, Ritchie D, Gale DP, Kanwar YS, Challis R, Buist H, Overell J, Weller B, Flossmann O, Blunden M, Meyer EP, Krucker T, Evans SJ, Campbell IL, Jackson AP, Chandran S, Hunt DP. Type I interferon causes thrombotic microangiopathy by a dose-dependent toxic effect on the microvasculature. *Blood* 2016; **128**: 2824-2833 [PMID: 27663672 DOI: 10.1182/blood-2016-05-715987]
11 **Jia H**, Thelwell C, Dilger P, Bird C, Daniels S, Wadhwa M. Endothelial cell functions impaired by interferon in vitro: Insights into the molecular mechanism of thrombotic microangiopathy associated with interferon therapy. *Thromb Res* 2018; **163**: 105-116 [PMID: 29407621 DOI: 10.1016/j.thromres.2018.01.039]
12 **Allinovi M**, Cirami CL, Caroti L, Antognoli G, Farsetti S, Amato MP, Minetti EE. Thrombotic microangiopathy induced by interferon beta in patients with multiple sclerosis: three cases treated with eculizumab. *Clin Kidney J* 2017; **10**: 625-631 [PMID: 28980667 DOI: 10.1093/ckj/sfw143]
13 **Reese JA**, Bougie DW, Curtis BR, Terrell DR, Vesely SK, Aster RH, George JN. Drug-induced thrombotic microangiopathy: Experience of the Oklahoma Registry and the BloodCenter of Wisconsin. *Am J Hematol* 2015; **90**: 406-410 [PMID: 25639727 DOI: 10.1002/ajh.23960]
14 **Stratta P**, Canavese C, Dogliani M, Thea A, Degani G, Mairone L, Vercellone A. Hemolytic-uremic syndrome during recombinant alpha-interferon treatment for hairy cell leukemia. *Ren Fail* 1993; **15**: 559-561 [PMID: 8210571 DOI: 10.3109/08860229309054974]
15 **Ubara Y**, Hara S, Takedatu H, Katori H, Yamada K, Yoshihara K, Matsushita Y, Yokoyama K, Takemoto F, Yamada A, Takagawa R, Endo Y, Hara M, Koida I, Kumada H. Hemolytic uremic syndrome associated with beta-interferon therapy for chronic hepatitis C. *Nephron* 1998; **80**: 107-108 [PMID: 9730725 DOI: 10.1159/000045147]
16 **Demoulin N**, Jadoul M. Interferon-α-induced thrombotic microangiopathy in patients with chronic myelogenous leukemia. *Kidney Int* 2014; **85**: 213-214 [PMID: 24380910 DOI: 10.1038/ki.2013.409]
17 **Zuber J**, Martinez F, Droz D, Oksenhendler E, Legendre C; Groupe D'stude Des Nephrologues D'ile-de-France (GENIF). Alpha-interferon-associated thrombotic microangiopathy: a clinicopathologic study of 8 patients and review of the literature. *Medicine (Baltimore)* 2002; **81**: 321-331 [PMID: 12169887 DOI: 10.1097/00005792-200207000-00008]
18 **Vosoughi R**, Marriott JJ. Thrombotic microangiopathy in Interferon Beta treated multiple sclerosis patients: Review of literature and report of two new cases. *Mult Scler Relat Disord* 2014; **3**: 321-325 [PMID: 25876469 DOI: 10.1016/j.msard.2013.12.007]
19 **Hunt D**, Kavanagh D, Drummond I, Weller B, Bellamy C, Overell J, Evans S, Jackson A, Chandran S. Thrombotic microangiopathy associated with interferon beta. *N Engl J Med* 2014; **370**: 1270-1271 [PMID: 24670186 DOI: 10.1056/NEJMc1316118]
20 **Yam C**, Fok A, Mclean C, Butler E, Kempster P. Interferon-beta in multiple sclerosis causing thrombotic microangiopathy. *Intern Med J* 2019; **49**: 274-276 [PMID: 30754081 DOI: 10.1111/imj.14204]
21 **Borchers AT**, Keen CL, Gershwin ME. Drug-induced lupus. *Ann N Y Acad Sci* 2007; **1108**: 166-182 [PMID: 17893983 DOI: 10.1196/annals.1422.019]
22 **Vaglio A**, Grayson PC, Fenaroli P, Gianfreda D, Boccaletti V, Ghiggeri GM, Moroni G. Drug-induced lupus: Traditional and new concepts. *Autoimmun Rev* 2018; **17**: 912-918 [PMID: 30005854 DOI: 10.1016/j.autrev.2018.03.016]
23 **Bonanni A**, Vaglio A, Bruschi M, Sinico RA, Cavagna L, Moroni G, Franceschini F, Allegri L, Pratesi F, Migliorini P, Candiano G, Pesce G, Ravelli A, Puppo F, Martini A, Tincani A, Ghiggeri GM. Multi-antibody composition in lupus nephritis: isotype and antigen specificity make the difference. *Autoimmun Rev* 2015; **14**: 692-702 [PMID: 25888464 DOI: 10.1016/j.autrev.2015.04.004]
24 **Morris LF**, Lemak NA, Arnett FC, Jordon RE, Duvic M. Systemic lupus erythematosus diagnosed during interferon alfa therapy. *South Med J* 1996; **89**: 810-814 [PMID: 8701383 DOI:

10.1097/00007611-199608000-00012]
25 **Boonen A**, Stockbrügger RW, van der Linden S. Pericarditis after therapy with interferon-alpha for chronic hepatitis C. *Clin Rheumatol* 1999; **18**: 177-179 [PMID: 10357129 DOI: 10.1007/s100670050081]
26 **Conlon KC**, Urba WJ, Smith JW 2nd, Steis RG, Longo DL, Clark JW. Exacerbation of symptoms of autoimmune disease in patients receiving alpha-interferon therapy. *Cancer* 1990; **65**: 2237-2242 [PMID: 2346907 DOI: 10.1002/1097-0142(19900515)65:10<2237::aid-cncr2820651013>3.0.co;2-5]
27 **Crow MK**. Type I interferon in the pathogenesis of lupus. *J Immunol* 2014; **192**: 5459-5468 [PMID: 24907379 DOI: 10.4049/jimmunol.1002795]
28 **Baechler EC**, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A* 2003; **100**: 2610-2615 [PMID: 12604793 DOI: 10.1073/pnas.0337679100]
29 **Graninger WB**, Hassfeld W, Pesau BB, Machold KP, Zielinski CC, Smolen JS. Induction of systemic lupus erythematosus by interferon-gamma in a patient with rheumatoid arthritis. *J Rheumatol* 1991; **18**: 1621-1622 [PMID: 1765991 DOI: 10.1002/art.1780341023]
30 **Cantaert T**, Baeten D, Tak PP, van Baarsen LG. Type I IFN and TNFα cross-regulation in immune-mediated inflammatory disease: basic concepts and clinical relevance. *Arthritis Res Ther* 2010; **12**: 219 [PMID: 21062511 DOI: 10.1186/ar3150]
31 **Coroneos E**, Petrusevska G, Varghese F, Truong LD. Focal segmental glomerulosclerosis with acute renal failure associated with alpha-interferon therapy. *Am J Kidney Dis* 1996; **28**: 888-892 [PMID: 8957041 DOI: 10.1016/S0272-6386(96)90389-3]
32 **Bremer CT**, Lastrapes A, Alper AB, Mudad R. Interferon-alpha-induced focal segmental glomerulosclerosis in chronic myelogenous leukemia: a case report and review of the literature. *Am J Clin Oncol* 2003; **26**: 262-264 [PMID: 12796597 DOI: 10.1097/01.COC.0000020649.11411.2B]
33 **Markowitz GS**, Nasr SH, Stokes MB, D'Agati VD. Treatment with IFN-{alpha}, -{beta}, or -{gamma} is associated with collapsing focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 2010; **5**: 607-615 [PMID: 20203164 DOI: 10.2215/CJN.07311009]
34 **Migliorini A**, Angelotti ML, Mulay SR, Kulkarni OO, Demleitner J, Dietrich A, Sagrinati C, Ballerini L, Peired A, Shankland SJ, Liapis H, Romagnani P, Anders HJ. The antiviral cytokines IFN-α and IFN-β modulate parietal epithelial cells and promote podocyte loss: implications for IFN toxicity, viral glomerulonephritis, and glomerular regeneration. *Am J Pathol* 2013; **183**: 431-440 [PMID: 23747509 DOI: 10.1016/j.ajpath.2013.04.017]
35 **Dizer U**, Beker CM, Yavuz I, Ortatatli M, Ozguven V, Pahsa A. Minimal change disease in a patient receiving IFN-alpha therapy for chronic hepatitis C virus infection. *J Interferon Cytokine Res* 2003; **23**: 51-54 [PMID: 12639299 DOI: 10.1089/10799900360520450]
36 **Rettmar K**, Kienast J, van de Loo J. Minimal change glomerulonephritis with reversible proteinuria during interferon alpha 2a therapy for chronic myeloid leukemia. *Am J Hematol* 1995; **49**: 355-356 [PMID: 7639284 DOI: 10.1002/ajh.2830490417]
37 **Gordon A**, Menahem S, Mitchell J, Jenkins P, Dowling J, Roberts SK. Combination pegylated interferon and ribavirin therapy precipitating acute renal failure and exacerbating IgA nephropathy. *Nephrol Dial Transplant* 2004; **19**: 2155 [PMID: 15252182 DOI: 10.1093/ndt/gfh336]
38 **Kimmel PL**, Abraham AA, Phillips TM. Membranoproliferative glomerulonephritis in a patient treated with interferon-alpha for human immunodeficiency virus infection. *Am J Kidney Dis* 1994; **24**: 858-863 [PMID: 7977330 DOI: 10.1016/S0272-6386(12)80682-2]
39 **Cardineau E**, Le Goff C, Henri P, Reman O, Lobbedez T, Hurault de Ligny B, Leporrier M, Ryckelynck JP. [Nephropathies caused by interferon alpha: apropos of 2 cases]. *Rev Med Interne* 1995; **16**: 691-695 [PMID: 7481158 DOI: 10.1016/0248-8663(96)80773-9]
40 **Tsai MS**, Chen JH, Fang YW, Yang AH, Chang CH. Membranous nephropathy induced by pegylated interferon alpha-2a therapy for chronic viral hepatitis B. *Clin Nephrol* 2012; **77**: 496-500 [PMID: 22595393 DOI: 10.5414/cn106903]
41 **Auty A**, Saleh A. Nephrotic syndrome in a multiple sclerosis patient treated with interferon beta 1a. *Can J Neurol Sci* 2005; **32**: 366-368 [PMID: 16225183 DOI: 10.1017/S0317167100004303]
42 **Nishimura S**, Miura H, Yamada H, Shinoda T, Kitamura S, Miura Y. Acute onset of nephrotic syndrome during interferon-alpha retreatment for chronic active hepatitis C. *J Gastroenterol* 2002; **37**: 854-858 [PMID: 12424571 DOI: 10.1007/s005350200141]
43 **Miranda-Guardiola F**, Fernández-Llama P, Badia JR, Botey A, Estruch R, Darnell A, Rozman C, Revert L. Acute renal failure associated with alpha-interferon therapy for chronic hepatitis B. *Nephrol Dial Transplant* 1995; **10**: 1441-1443 [PMID: 8538940 DOI: 10.1038/ki.1995.44]
44 **Ferri C**, Giuggioli D, Colaci M. Renal Manifestations of Hepatitis C Virus. *Clin Liver Dis* 2017; **21**: 487-497 [PMID: 28689588 DOI: 10.1016/j.cld.2017.03.005]
45 **Ohta S**, Yokoyama H, Wada T, Sakai N, Shimizu M, Kato T, Furuichi K, Segawa C, Hisada Y, Kobayashi K. Exacerbation of glomerulonephritis in subjects with chronic hepatitis C virus infection after interferon therapy. *Am J Kidney Dis* 1999; **33**: 1040-1048 [PMID: 10352191 DOI: 10.1016/S0272-6386(99)70140-X]
46 **Fabrizi F**, Aghemo A, Messa P. Hepatitis C treatment in patients with kidney disease. *Kidney Int* 2013; **84**: 874-879 [PMID: 23823603 DOI: 10.1038/ki.2013.264]

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