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EDITORIAL

Is there still a role for sucralfate in the treatment of gastritis?

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Abstract

The endoscopic diagnosis of gastritis is usually made when a patient develops symptoms and undergoes an upper gastrointestinal endoscopy. There are often obvious aetiological causes such as smoking, alcohol *Helicobacter pylori* infection or drug treatment. Lifestyle changes can sometimes improve symptoms but often patients will be treated with a proton pump inhibitor. The stomach mucosa produces a protective mucous to prevent damage cause by gastric acid and exogenous agents can disrupt this layer. Repair of this protective layer can be enhanced by reduction in gastric acid secretion using H2 receptor antagonist or proton pump inhibitors or by cytoprotective drugs such as misoprostol, sucralfate, aluminium ions or bismuth subsalts. Sucralfate is a complex polymer which at a low pH changes its chemical configuration and binds to serum protein to form a protective layer protecting the mucosa against further injury. Cytoprotective drugs were the first line treatment for peptic disease including gastritis for many years but since the launch of cimetidine in 1976 and the subsequent launch of omeprazole in 1988, their use has slowly declined. First line treatment for patients with symptomatic gastritis after removal of potential causative factors is likely to be a proton pump inhibitor in 2019. This is despite the fact that there is some evidence that sucralfate is superior than a H2 receptor antagonist in the endoscopic healing rates in patients with gastritis. The logical treatment choice in patients with resistance symptoms is a combination of a proton pump inhibitor and sucralfate but evidence is lacking. Until such evidence is available In the meantime, we would suggest that there is a role for sucralfate in the treatment of intransigent gastritis and that mucosal protection should be considered even ahead of acid suppression given its favourable safety and toxicity profile.

Key words: Sucralfate; Enoscopy; Gastritis; Treatment; Anti-acid

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Core tip: An endoscopic diagnosis of gastritis is commonly made. When patients have

significant symptoms associated with this finding and no other explanation can be found for their symptoms the first line treatment tends to be with a proton pump inhibitor. The combination of a proton pump inhibitor and sucralfate can however, be useful in the treatment of these patients when conventional treatment has failed and symptoms are severe.

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Gastritis is a common endoscopic diagnosis and can range from asymptomatic to severely symptomatic and be acute or chronic. Experts have long argued whether the endoscopic diagnosis of gastritis is in fact a gastropathy rather than a gastritis but in the present of an endoscopic diagnosis and symptoms treatment is usually offered. If we accept the endoscopic diagnosis of gastritis or gastropathy can cause symptoms then gastritis (as we will term in from here) is an inflammation, irritation or erosion of the gastric mucosa and can have a number of aetiological factors that include alcohol, smoking, various medications, *helicobacter pylori*, bile reflux or bacterial/viral infections. This condition should be differentiated from non-ulcer dyspepsia which has been extensively studied and is not associated with any endoscopic abnormality[1].

Management of gastritis can sometimes be difficult as it can be unresponsive to conventional therapy. Where there is an obvious aetiology for the gastritis removal of the cause is the first line option such as stopping smoking and reducing alcohol intake. This becomes more difficult when the cause is medication related and the medication in question is important to either quality or quantity of life. Under these circumstances, it usually comes down to a balance of risks. Treatment of *helicobacter pylori* infection where present often improves symptoms and there might be dietary changes that can be made to improve symptoms. Once these options have been, exhausted conventional medical treatment will be directed towards a reduction in acid secretion from the gastric mucosa, mucosal barrier protection or where gastritis is believed to be alkaline, gastric pro-kinetic agents.

The symptoms associated with gastritis are non-specific and it is therefore important to exclude any other cause for the patient's symptoms before assuming that they are related to gastritis. Symptoms include epigastric discomfort, nausea and early satiety. Patients will usually require a minimum of an endoscopy, *helicobacter pylori* test and an ultrasound scan. A careful drug, surgical and lifestyle history is necessary.

The gastric mucosa is protected by a layer of water-insoluble mucus gel that is approximately 180 micron thick[2]. This adherent mucus is the first line in mucosal defence against gastric acid in the lumen. Exogenous agents such as alcohol and certain drugs can disrupt the gel layer. The disruption of this layer is in part responsible for exposing the gastric mucosa to either acid or alkali and this can lead to gastritis. Repair of the gastric mucosal barrier can be facilitated by either anti-secretory agents such as H2 receptor antagonists or proton pump inhibitors or by cytoprotective drugs such as misoprostol, sucralfate, aluminium ions or bismuth subsalts.

Sucralfate is a complex polymer of sucrose with multiple substitutions of sulphate and aluminium salts. At a low pH it changes its chemical configuration, which allows it to bind to serum protein to form a protective layer over ulcerated areas. This protects the mucosa against further injury. Sucralfate also stimulates the synthesis and release of prostaglandins, epidermal growth factor and nitric oxide as well as improving gastric mucosal blood flow, bicarbonate secretion and mucus production[3]. Sucralfate is not absorbed systemically and therefore has a good safety and toxicity profile.

Prior to the introduction of H2 receptor antagonist and the subsequent introduction of proton pump inhibitors mucosal cytoprotective drugs were the first line treatment for peptic disease including gastritis. Since the launch of cimetidine in 1976 and the subsequent launch of omeprazole in 1988, the use of cytoprotective agents in the treatment of dyspepsia has slowly declined. First line treatment for patients with symptomatic gastritis after removal of potential causative factors is likely to be a proton pump inhibitor in 2019. This is despite the fact that there is some evidence that sucralfate is superior than a H2 receptor antagonist in the endoscopic healing rates in

patients with gastritis[4,5]. There is little evidence of superiority of proton pump inhibitors over sucralfate in the treatment of gastritis although the only evidence appears to be in post-cholecystectomy biliary gastritis[6]. There is no evidence of benefit from sucralfate alone or in combination in non-ulcer dyspepsia[1].

The logical treatment strategy in patients with symptomatic gastritis is the combination of acid suppression and mucosal protection. There is little or no literature that addresses this combination in any peptic disorder[7] and specifically the combination of sucralfate and a proton pump inhibitor does not seem to have been assessed in the treatment of resistant gastritis.

In our own practice, we have a selective group of patients, who have been extensively investigated to exclude other causes of their symptoms and who have symptoms resistant to conventional acid suppression. We have found anecdotal evidence of symptom relief with the combination of a proton pump inhibitor and sucralfate or in those with strong evidence of bile reflux and therefore alkaline gastritis from sucralfate alone. Our advice is always to take the proton pump inhibitor before the sucralfate and wait for an hour so that absorption is not affected. Our only concern is a lack of an evidence base to support this regime.

As this type of patient is common in both general practice and secondary care it would seem logical to design and conduct a randomised controlled trial to assess whether this approach is supported by scientific data.

In the meantime, we would suggest that there is a role for sucralfate in the treatment of intransigent gastritis and that mucosal protection should be considered even ahead of acid suppression given its favourable safety and toxicity profile[8].

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SYSTEMATIC REVIEWS

L-carnitine supplementation in non-alcoholic fatty liver disease: A systematic review and meta-analysis

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) dominates the landscape of modern hepatology. Affecting 25% of the general population, there is critical unmet need to identify broadly available, safe and cost-effective treatments. Cumulative evidence in animal and human models suggests that intrahepatic and skeletal muscle fatty acid oxidation is impaired in NAFLD, such that lipid accretion is not matched by efficient utilisation. L-carnitine is a crucial mediator of fatty acid metabolism *in vivo*, promoting mitochondrial lipid β-oxidation and enhancing tissue metabolic flexibility. These physiological properties have generated research interest in L-carnitine as a potentially effective adjunctive therapy in NAFLD.

AIM

To systematically review randomised trials reporting effects of dietary L-carnitine supplementation on liver biochemistry, liver fat and insulin sensitivity in NAFLD.

METHODS

Search strategies, eligibility criteria and analytic methods were specified *a priori* (PROSPERO reference: CRD42018107063). Ovid MEDLINE, Ovid EMBASE, PubMed, Web of Science and the Cochrane Library were searched from their inception until April 2019. Outcome measures included serum concentrations of alanine and aspartate aminotransferase (ALT and AST), liver fat and insulin

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sensitivity assessed by the homeostasis model of insulin resistance (HOMA-IR). A random effects meta-analysis was performed for, ALT, AST and HOMA-IR measures separately. Between-study heterogeneity was measured using I^2 statistics.

RESULTS

Five eligible randomised trials were included in the qualitative and quantitative synthesis (n = 338). All of the 5 included trials assessed the effect of L-carnitine on serum ALT, identified from Italy, South Korea and Iran. Weighted mean difference (WMD) for ALT between L-carnitine and control groups after intervention was -25.34 IU/L [95%CI: -41.74-(-8.94); P = 0.002]. WMD for AST between L-carnitine and control groups was -13.68 IU/L (95%CI: -28.26-0.89; P = 0.066). In three studies (n = 204), HOMA-IR was evaluated. WMD for HOMA-IR between L-carnitine and control groups was -0.74 units [95%CI: -1.02-(-0.46); P < 0.001]. Two studies using validated outcome measures reported a significant reduction in liver fat in L-carnitine *vs* control groups post-intervention (P < 0.001).

CONCLUSION

Pooled results indicate that L-carnitine supplementation attenuates ALT, liver fat and insulin resistance in NAFLD cohorts, confirming a beneficial effect of L-carnitine for a highly prevalent condition with a growing economic burden.

Key words: L-Carnitine; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis; Liver function; Insulin resistance; Meta-analysis; Systematic Review

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Core Tip: Non-alcoholic fatty liver disease (NAFLD) presents a major public health challenge. As a leading cause of abnormal liver chemistry, rising in prevalence together with obesity and insulin resistance, there is critical unmet need to identify cost-effective, population-based treatment. We synthesised evidence from randomised trials published to date evaluating the effect of dietary L-carnitine supplementation on transaminases, liver fat and insulin resistance in NAFLD. We demonstrate a significant reduction in serum alanine aminotransferase, homeostasis model of insulin resistance and liver fat with dietary L-carnitine supplementation. L-carnitine could therefore present a novel therapeutic tool for NAFLD and its metabolic associations.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has rapidly emerged as a leading cause of chronic liver disease and liver transplantation worldwide[1]. The global prevalence of NAFLD is estimated to be 25.24%[2], rising to 66% and 90% in those with type 2 diabetes and obesity, respectively[3]. Although progression to non-alcoholic steatohepatitis (NASH) is limited to approximately 30% of individuals with NAFLD, the high population prevalence of NAFLD heralds a looming socioeconomic burden due to the consequences of its progression, including end-stage liver disease[4]. In EU4 countries alone (France, Germany, Italy, United Kingdom), the annual cost associated with NAFLD is estimated to be €35 billion[5]. Diet and lifestyle modification remain current standard of care, and there is no specifically licensed disease-modifying therapy available.

L-Carnitine is a naturally occurring water-soluble quaternary amine which acts as a crucial mediator of fatty acid metabolism *in vivo*. The role of carnitine as a key regulator of intracellular bioenergetics has gained traction in the search for broadly applicable treatments for metabolic disorders, including obesity and type 2 diabetes.

The ability of L-carnitine to regulate muscle mitochondrial fuel selection, through promoting both lipid oxidation and non-oxidative glucose disposal, renders it an attractive target for therapeutic intervention in the context of insulin resistance[6]. Cumulative evidence in both animal and human models suggests that intrahepatic and skeletal muscle fatty acid transport and oxidation is impaired in NAFLD and insulin resistance, such that excessive lipid accretion is not matched by efficient utilisation[7,8]. Thus, the effect of carnitine supplementation in the context of NAFLD specifically has been the focus of recent interest. This review aims to critically and systematically evaluate all human randomised trials investigating the effect of carnitine on liver fat and/or metabolic parameters in NAFLD.

MATERIALS AND METHODS

Search strategies, eligibility criteria and analytic methods were specified *a priori* in the study protocol, which was registered with the PROSPERO database (CRD42018 107063).

Search strategy

We performed a systematic literature search for randomised trials reporting the effects of dietary L-carnitine supplementation on liver biochemistry and liver fat in adult individuals with NAFLD and NASH.

The databases searched were PubMed, Ovid EMBASE, Ovid MEDLINE, Web of Science Core Collection and the Cochrane Library. The full search strategy used in Ovid MEDLINE is provided in Appendix 1. Databases were searched from their inception until April 2019. No language restrictions were used. For each database, a comprehensive list of alternative terms for NAFLD were combined with alternative terms for L-carnitine, using the Boolean operator AND. Reference lists of studies ultimately selected for inclusion were searched to identify any other relevant research.

Diagnostic criteria for NAFLD varied significantly between studies, but eligible studies included adult individuals diagnosed with NAFLD on the basis of validated histological, imaging or biochemical tests, including the following, and where other causes of hepatic steatosis had been excluded: (1) Liver histology; (2) Magnetic Resonance Imaging with proton density fat fraction (MRI-PDFF) or proton magnetic resonance spectroscopy (^{1}H-MRS); (3) Computed Tomography (CT); (4) Ultrasound and (5) Serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in conjunction with impaired glucose tolerance and in the absence of documented alcohol excess.

Primary outcome measures included change in serum concentrations of ALT, AST and liver fat (as assessed either by liver biopsy, cross-sectional imaging or ultrasound). Secondary outcome measures included changes in insulin sensitivity parameters (as assessed by the HOMA-IR) and, where available, markers of inflammation and oxidative stress.

Study selection was performed independently by two separate reviewers (PT and JC) with any disagreements revolved by a third researcher (GPA). Titles and abstracts of returned searches were evaluated against eligibility criteria. Those meeting eligibility criteria based on title and abstract were subsequently read in full. Wherever journal articles were found to contain insufficient information for critical analysis, attempts were made to contact the authors directly for clarification of missing details.

Eligibility criteria

Eligible published studies included human randomised trials evaluating the effect of carnitine supplementation on liver fat, liver enzymes, glucose and markers of inflammation or oxidative stress in adult individuals with NAFLD. Only full reports were considered as eligible for inclusion on the basis that they provided sufficient data to permit critical analysis. Studies were considered eligible for inclusion if they: (1) Were randomised in design; (2) Evaluated L-carnitine versus placebo, L-carnitine plus another intervention versus that intervention alone, or L-carnitine versus no intervention; (3) Included a patient population diagnosed with NAFLD and/or NASH based on validated histological, radiological or biochemical parameters as listed above; and (4) Included only subjects aged 18 years or above. Studies were excluded if they: (1) Were non-randomised in design *e.g.*, case reports, reviews or observational studies; (2) Included patients with another cause of hepatic steatosis *e.g.*, alcohol, genetic or viral liver disease; (3) Were animal studies; and (4) Did not evaluate outcomes of interest as detailed above.

There were no restrictions based on dosage, formulation or frequency of administration. Interventions in the control group included active placebo supplementation, hypocaloric diet and metformin therapy. Trials evaluating L-

carnitine supplementation together with other interventions, in the absence of a control group consisting of the other interventions alone, were excluded. No specific treatment duration was specified for inclusion in this review.

Data extraction and quality assessment

Data extracted from individual studies included (1) participant demographics; (2) intervention type and dose; (3) method of NAFLD diagnosis; (4) outcome measurements of liver fat, liver enzymes, glycometabolic profile and markers of inflammation and oxidative stress; (5) documentation as to whether informed consent was gained; (6) methods of randomisation; (7) allocation concealment; (8) participant and staff blinding; (9) blinding of outcome assessment; (10) presence of incomplete outcome data; and (11) evidence of any selective reporting. The Cochrane risk of bias tool[9] was then used to systematically appraise each included study in terms of methodological quality and validity according to the criteria of the Cochrane guidelines.

Statistical analysis

A random effects meta-analysis was performed for ALT, AST and HOMA-IR measures separately. Weighted mean difference (WMD) with 95%CI were calculated. Between-study heterogeneity was measured using I^2 statistics, with $I^2 > 50\%$ indicating significant heterogeneity. The analysis was performed using Stata software version 11.0 Stata (Version 11.2, StataCorp, College Station, Texas). Resultswere summarised using Forest plots.

RESULTS

Search results

Figure 1 depicts the PRISMA flowchart process of identification and selection of eligible studies for inclusion in the qualitative and quantitative syntheses. Primary database searches yielded 883 citations. After de-duplication, 692 remaining citations were screened for eligibility by reading titles and abstracts. Of these remaining studies, 675 were excluded. Of the remaining 17 citations, full text articles retrieved were read in full to determine eligibility. Twelve studies were subsequently excluded.

Table 1 summarises study characteristics for the five randomised trials ultimately included in the qualitative and quantitative synthesis[10-14]. In total, these trials comprised 338 patients (234 men, 104 women). In 3 trials, non-diabetic patients with NAFLD were recruited and the other 2 trials recruited individuals with type 2 diabetes mellitus and NAFLD.

Quality of included studies

Methodological quality was assessed using criteria set out in the Cochrane Handbook for Systematic Reviews (Figure 2). All trials described randomising participants to L-carnitine and control arms. However, only two of the five trials[10,11] reported the methods used to generate random allocation sequence (*i.e.*, computer generated tool) to a standard sufficient enough to be judged as having a low risk of bias. In the other 3 trials, no information was provided regarding methods of blinding and insufficient information was provided to enable informed decision-making regarding adequacy of randomisation. One trial reported randomising participants by using random numbers allocated to consecutive patients; this was considered to confer a high risk of selection bias[12].

Regarding allocation concealment, only two studies[10,11] reported efforts to conceal allocation sequences from personnel as well as patients. In two of the studies, genuine blinding was considered impossible due to lack of a placebo arm[13,14]. In the remaining two trials, insufficient information was given to determine whether a robust allocation concealment process was undertaken. Three of the five studies accounted for incomplete outcome data and clearly explained any loss to follow up and exclusions[10,11,13]. The other two studies lacked explanations for trial exclusion.

Selective reporting was considered to be present in two out of the five included studies. Alavinejad *et al*[12] (2016) with sonographic grade of liver fat as an outcome measure in their study, noted that "follow up ultrasonography did not show any significant change in comparison with baseline reports". However no baseline or post-intervention values were reported. Somi *et al*[14] (2014) reported changes from baseline in sonographically-determined grade of NAFLD in patients in the L-carnitine arm of the study, with nine of the L-carnitine treated patients having no evidence of NAFLD on post-intervention ultrasound. However it was unclear how many patients had progressed or regressed in steatosis grade.

In three out of the five trials, funding sources and conflicts of interests were

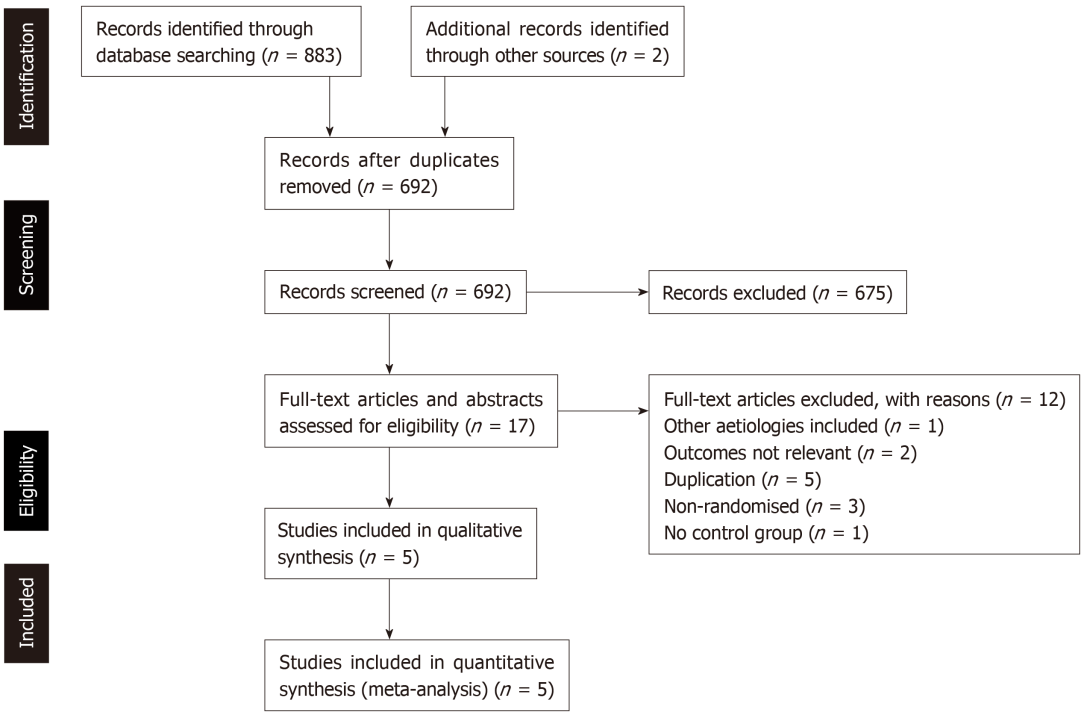


Figure 1 PRISMA flow diagram.

explicitly stated, whereas in the other two trials no mention was made of either[12,14]. Efficacy, safety and compliance measures were reported in 3 studies but no description of methods to evaluate compliance was made in the other two studies and Somi *et al*[12,14] (2014) did not report any safety visits for subjects during 24 wk of supplementation. Statistical analyses were reported in all five trials.

Outcomes

Estimates were made on the effect of L-carnitine on outcomes including liver transaminases, the HOMA-IR and liver fat (where measured).

ALT: All of the five included trials were for meta-analysis of ALT measures after intervention (Figure 3). The WMD for ALT between the L-carnitine groups and the control groups after intervention was -25.34 IU/L [95%CI: -41.74-(-8.94), P = 0.002] (Figure 3). The I^2 was 95.8%, indicating statistically significant heterogeneity between the studies.

AST: All five trials were included for meta-analysis of AST measures after intervention. The WMD for AST between the L-carnitine group and the control group after intervention was -13.68 IU/L (95%CI: -28.26-0.89) (P = 0.066) (Figure 4). The I^2 was 93.4%, indicating high statistical heterogeneity between the studies.

Liver fat: Four of the included studies evaluated hepatic steatosis at baseline and post-intervention. Outcome measures differed between the studies, precluding the possibility of a quantitative synthesis of results. In two studies, ultrasonography was used to grade liver fat at baseline and post-intervention[12,14]. In another study, CT imaging was used[11] and in the fourth study, liver fat was evaluated histologically[10].

Malaguarnera *et al*[10] (2010) assessed liver fat using paired biopsies; the group reported a significant reduction in steatosis in the group randomised to L-carnitine compared to placebo (1.68 ± 0.76 *vs* 0.94 ± 0.88; P < 0.001). However, within-group analysis also demonstrated that steatosis reduction was significant in the placebo group (hypocaloric diet + placebo) compared to baseline values (P < 0.001). In the same study, other histological features of NASH were shown to be significantly attenuated following 24 wk of L-carnitine therapy compared to placebo, including parenchymal inflammation (P < 0.001), hepatocellular injury (P < 0.05) and fibrosis (P < 0.05). However, it is again worth noting that within-group comparisons with baseline values also determined a significant reduction in these parameters following placebo supplementation.

Another study used hepatic CT with liver attenuation index (LAI) to evaluate steatosis before and after the study[11]. Authors reported that the patient group

Table 1 Published studies to date evaluating the effect of L-carnitine supplementation on liver fat and/or biochemistry in non-alocholic fatty liver disease

Ref.	Study population (diagnosis); comorbidities	Sample size (M/F) Control/ Carnitine	Age (yr)	BMI	Duration (wk)	Intervention (dose)	Outcome Measures	Control	Results
Malaguarnera *et al*[10]	Biopsy-proven NASH without diabetes	74 (40/34) 38 (CTRL) 36 (CAR)	47.8 ± 5.8 (CTRL) 47.9 ± 5.4 (CAR)	26.5 ± 3.8 (CTRL) 26.6 ± 3.7 (CAR)	24	L-carnitine (1000 mg BD) plus hypocaloric (1600 cal) diet	Primary: Improvement in histological features of NASH Other: ALT, AST, lipid profile, GLC, HOMA-IR, CRP, TNFα	Placebo plus Hypocaloric (1600 cal) diet	Primary Outcome: ↓ NASH activity score[1] (9.42-3.19), ↓ALT[1] ↓AST[1], ↓GGT[1] ↓TC[1], ↓LDL[1], ↑HDL[1], ↓GLC[1], ↓HOMA-IR[1], ↓CRP[1], ↓TNFα[1]
Bae *et al*[11]	NAFLD with type 2 diabetes	78 (54/24) 39 (CTRL) 39 (CAR)	52 ± 9.4 (CTRL) 50.6 ± 9.3 (CAR)	26.7 ± 3.7 (CTRL) 28.2 ± 2.6 (CAR)	12	Carnitine orotate complex (824 mg TDS)	Primary: Change in ALT Other: Liver attenuation index (CT)	Placebo	Primary outcome: ↓ALT (89.7% *vs* 17.9%)[1] Other: ↓Liver attenuation index[1] (0.74 ± 8.05 *vs* 6.21 ± 8.96)[1], ↓HbA1c, ↓GLC, ↓HOMA-IR[2]
Alavinejad *et al*[12]	NAFLD (ultrasound+ raised ALT) with T2DM	54 (38/16) 26 (CTRL) 28 (CAR)	59 ± 9 (CTRL) 60 ± 5 (CAR)	29.5 ± 3.6 (CTRL) 28.6 ± 4.6 (CAR)	12	L-carnitine (750 mg TDS)	Primary: AST, ALT Other: TG, GLC, HbA1c	Placebo	Primary outcome: ↓ALT[1], ↓AST[1] Other: ↓TG[2], ↓GLC[2], ↓HbA1c[2]
Hong *et al*[13]	NAFLD (plasma ALT 40-250) and impaired glucose tolerance (HbA1C ≥ 6.0%)	52 (36/16) 26 (CTRL) 26 (CAR)	52.0 ± 9.6 (CTRL) 51.5 ± 9.4 (CAR)	27.0 ± 3.1 (CTRL) 27.2 ± 2.6 (CAR)	12	Carnitine-orotate complex (300 mg TDS) + metformin	Primary: change from baseline ALT Other: GLC, HbA1c, m t DNA copy number, urine 8-OHdG	Metformin alone	Primary outcome: ↓ ALT[1] Other: ↓HbA1c[2], ↑ plasma mtDNA copy number[1], ↓GLC[2], ↓urine 8-OHdG
Somi *et al*[14]	NAFLD (ultrasound + plasma ALT ≥ 40)	80 (66/14)	40.7 ± 8	29.4 ± 3.9	24	L-carnitine (250 mg BD)	Primary: ALT, AST Other: Sonographic change in liver fat, BMI	No treatment	Primary outcome: ↓ALT[1], ↓AST[1] Other: ↓ liver fat on USS

NASH: Nonalcoholic steatohepatitis; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GLC: Glucose; HOMA-IR: Homeostasis model of insulin resistance; CRP: C-reactive protein; TNFα: Tumour Necrosis Factor alpha; HbA1C: Haemoglobin A1C; T2DM: Type 2 diabetes mellitus; TG: Triglyceride; mt DNA: Mitochondrial DNA; oHdg: 8-hydroxydeoxyguanosine.
[1]Denotes statistically significant results.
[2]Denotes non-statistically significant results.

receiving L-carnitine complex supplementation had a mean increase in LAI values of 6.21 ± 8.96 Hounsfield Units (HU) ($P < 0.001$), indicating significant reduction in liver fat, whereas the placebo group showed no significant change (LAI increased by 0.74 ± 8.05 HU, $P = 0.582$). The changes in LAI were found to correlate inversely with changes in ALT.

Alavinejad *et al*[12] (2016) reported no significant difference between baseline and post-intervention sonographic liver fat in either the L-carnitine or placebo groups in

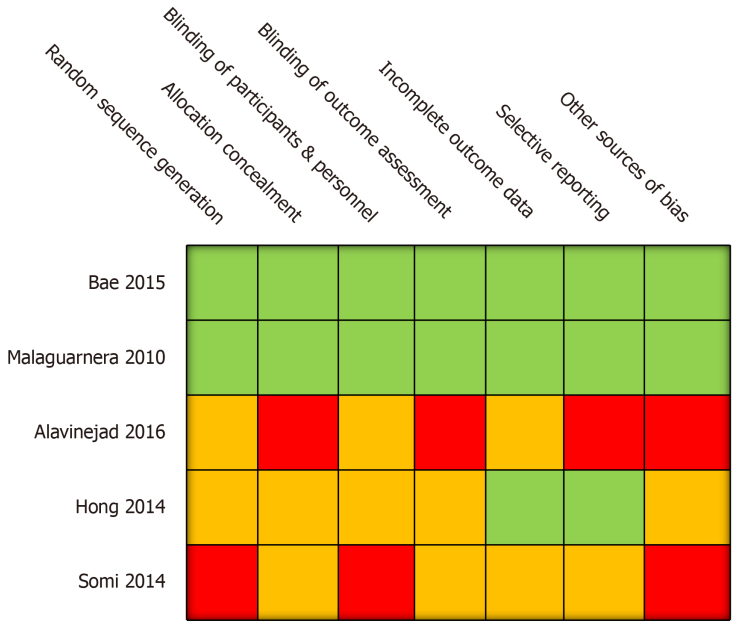


Figure 2 Methodological quality assessment according to the Cochrane risk of bias tool.

their study. However, absolute values were not provided in the published article. Finally, Somi *et al*[14] (2014) reported a significant reduction in patients with sonographic grade 2 liver fat in the placebo but not the L-carnitine groups. In the L-carnitine group, 9 patients with sonographic evidence of fatty liver at baseline had resolution of fatty liver disease on post-intervention ultrasonography, suggesting a beneficial effect of L-carnitine on liver fat; however, this was reported to be non-significant. Thus, two high quality RCTs utilising histology and cross-sectional imaging for quantifying liver fat reported significant outcomes following L-carnitine supplementation, whereas the two trials utilising ultrasonography reported no significant difference in liver fat with L-carnitine.

HOMA-IR and glycometabolic profile: Three papers were included for meta-analysis of HOMA-IR measures after intervention. The WMD for HOMA-IR between the L-carnitine and control groups after intervention was -0.74 units [95%CI: -1.02-(-0.46)] ($P < 0.001$) (Figure 5). The I^2 was 0%, indicating statistical homogeneity between the studies.

Adverse events: Adverse events (AE) were reported in 3 studies included in this review, including mild headache, musculoskeletal pain and gastrointestinal disturbance[10,11,13]. All AE rates were lower in the intervention versus placebo groups and no serious adverse events were reported.

Inflammation and oxidative stress: Malaguarnera *et al*[10] (2010) reported a significant reduction in hepatocellular injury ($P < 0.05$), parenchymal inflammation ($P < 0.001$), plasma C-reactive protein (CRP) and tumour necrosis factor alpha (TNFα) ($P < 0.001$) in L-carnitine treated patients compared to placebo. Hong *et al*[13] (2014) evaluated inflammation and oxidative stress at baseline and post-intervention in using high-sensitivity CRP (hs-CRP) and urine 8-hydroxy-2′-deoxyguanosine (8OHdG), respectively and report significant reduction in 8OHdG in the group treated with L-carnitine ($P = 0.034$).

DISCUSSION

Summary of evidence

To our knowledge, this is the first reported systematic review to evaluate the effect of dietary L-carnitine supplementation on liver fat, markers of liver injury and insulin resistance profiles in NAFLD populations. Pooled results from five randomised trials suggest that L-carnitine supplementation is associated with significant attenuation of liver fat, and reduction in serum ALT levels, the most commonly used surrogate biomarker of hepatocellular injury. A reduction in AST following L-carnitine therapy was also seen, though this did not reach statistical significance. Our results further

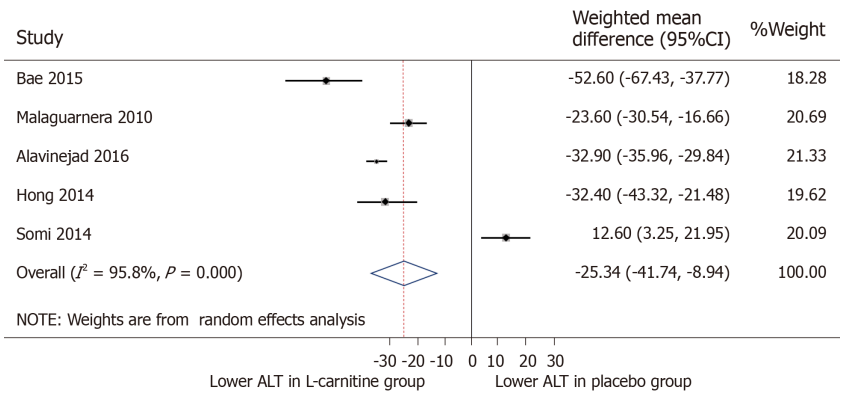


Figure 3 Meta-analysis of alanine aminotransferase measures after intervention.

suggest that L-carnitine can improve insulin sensitivity in NAFLD cohorts, as measured indirectly using the HOMA-IR index. These outcomes are potentially important to clinical practice, as they confirm a beneficial effect of a broadly available nutrient.

Strengths and limitations

This review comprised a robust and comprehensive database search with no language restrictions. The search strategy was implemented by two reviewers separately, and both authors agreed on papers selected for inclusion as well as reasons for exclusion. Included studies were read and assessed for bias independently by each reviewer; any disagreements in the risk of bias tool were referred to a third reviewer for final arbitration. No publication bias was found to be present for selected outcomes entered into the meta-analysis.

There were several limitations associated with this review. Firstly, despite an extensive literature search, only five randomised studies were available evaluating L-carnitine on liver markers in NAFLD. Of these, only four evaluated change in liver fat as an outcome measure. Despite another trial being published, we were unable to obtain the full text after attempts to contact the authors of the paper.

Secondly, poor methodological quality was inherent in three of the included studies. For example, while all studies claimed randomisation, only Malaguarnera *et al*[10] (2010) and Bae *et al*[11] (2015) described robust methods of random sequence generation. Lack of placebo control use in the studies conducted by Somi *et al*[13] (2014) and Hong *et al*[14] (2014) further reduced methodological quality and reliability of reported results. Double-blind trial design is an important tool in minimising bias and maximising reliability of research outcomes. In two of the included studies, there was insufficient evidence to judge that robust blinding of participants and personnel occurred. In the study conducted by Somi *et al*[14] (2014) no mention of blinding was made. This led us to judge these studies as having as high risk of bias and thus further reduced reliability of reported results.

There was heterogeneity of the trials with respect to duration, type of active comparator drug and background therapy, as well as carnitine formulation and dose. Two studies used carnitine in complex with orotic acid (carnitine-orotate); thus establishing a true effect of L-carnitine alone on outcomes measures was not possible. Doses varied widely, from 500 mg daily to 2.25 grams daily, resulting in differential exposure to active L-carnitine among patients in each individual study. The NAFLD patient populations studied were also heterogenous, including patients both with and without diabetes, patients with different stages of NAFLD and geographical differences in populations. However, all 5 studies reported reductions in ALT, suggesting a consistent beneficial effect of L-carnitine on hepatocellular injury across populations.

As a marker of treatment response, ALT appears to be reliable, and has been closely correlated to objectively measured reductions in liver fat using histological and imaging methods in NAFLD post-intervention[15,16]. In phase 2 RCTs, ALT continues to be used as a surrogate marker of disease activity in addition to estimation of fat[17]. The overall significant reduction in ALT following intervention in the included studies suggests a global reduction in hepatocellular injury in patients treated with L-carnitine. Indeed, Malaguarnera *et al*[10] (2010) demonstrated an improvement in all histological parameters in their biopsied NAFLD cohort following L-carnitine therapy, including steatosis, inflammatory activity and fibrosis. Steatosis reduction was confirmed using CT with liver attenuation index by Bae *et al*[11] (2015).

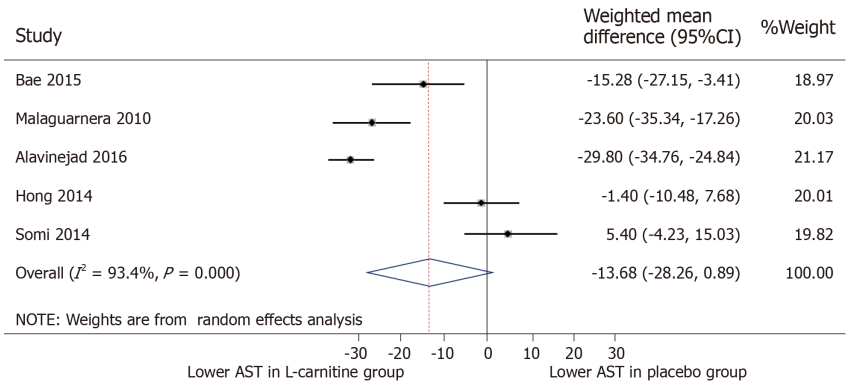


Figure 4 Meta-analysis of aspartate aminotransferase after intervention.

With respect to translation into clinical practice, none of the 5 trials evaluated plasma levels of Trimethylamine-N-Oxide, a metabolite of L-carnitine associated with increased risk of atherosclerosis. In a population already at high risk for cardiovascular outcomes, the safety profile of L-carnitine supplementation would require rigorous assessment in this context. However, the evidence base for a direct adverse link between L-carnitine supplementation and cardiovascular events is to our knowledge limited. On the contrary, a meta-analysis of 13 placebo-controlled trials including 3629 patients evaluated the clinical impact of L-carnitine supplementation in patients with ischaemic heart disease and concluded that carnitine administration was associated with clinical benefit, including reduced mortality and reduction in onset of cardiac arrhythmias and angina[18]. A recently published study further concluded that although 24-wk of L-carnitine supplementation increased plasma Trimethylamine-N-Oxide concentrations, no changes in lipid profile or other serum biomarkers of atherosclerosis were seen[19].

The evidence collated from studies included in this review forms a compelling argument for further robust, randomised trial data evaluating mechanisms of action of L-carnitine on liver and muscle tissue in a NAFLD phenotype, as well as its effect on validated outcome measures such as liver histology, magnetic resonance imaging and spectroscopy. Further, effects of L-carnitine supplementation on metabolic outcomes in NAFLD require specific attention, for example through utilising gold-standard measures of liver-specific and whole-body insulin sensitivity such as the euglycaemic hyperinsulinaemic clamp technique. As a naturally occurring, broadly applicable, safe and cost-effective agent, L-carnitine could overcome traditional barriers to translation to become routinely available for patients in clinical practice as an adjunctive treatment for NAFLD.

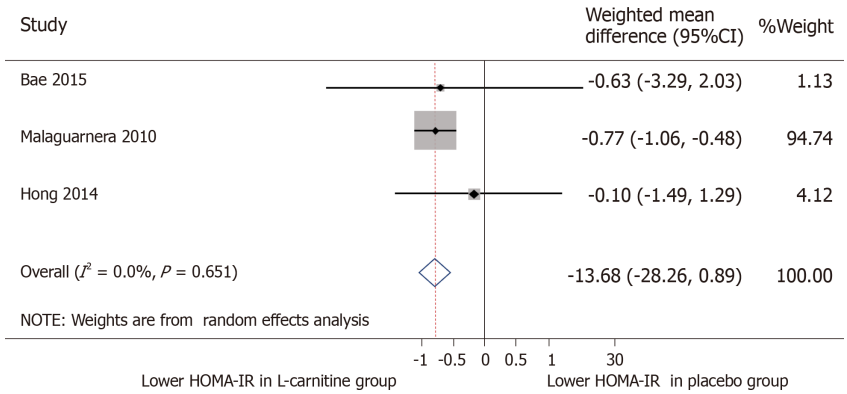


Figure 5 Meta-analysis of homeostasis model of insulin resistance measures after intervention.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of liver disease worldwide, affecting approximately 25% of the general population. To date, there are no licensed disease-modifying treatments to attenuate global burden of NAFLD. A growing body of evidence suggests that NAFLD is characterised at a cellular level by impaired mitochondrial fat oxidation. L-carnitine, a naturally occurring nutrient, is a key mediator of mitochondrial fuel selection and promotes lipid oxidation. In this article, we synthesise available evidence of a role for L-carnitine supplementation in the treatment of NAFLD.

Research motivation

L-carnitine has gained traction in recent years as a potential tool for the treatment of metabolic disorders including type 2 diabetes and heart disease. At the nexus of glucose and lipid metabolism, L-carnitine promotes mitochondrial lipid oxidation and enhances tissue metabolic flexibility. It may confer protective effects in NAFLD through these mechanisms. There is a critical unmet need for broadly applicable, population based treatment in NAFLD, which inspired this narrative and quantitative synthesis.

Research objectives

In this study, we aimed to systematically review randomised trials reporting effects of dietary L-carnitine supplementation on liver biochemistry, liver fat and insulin sensitivity in NAFLD.

Research methods

Ovid MEDLINE, Ovid Embase, PubMed, Web of Science and the Cochrane Library were searched from their inception until April 2019. Outcome measures included serum concentrations of alanine and aspartate aminotransferase (ALT and AST), liver fat and insulin sensitivity assessed by the homeostasis model of insulin resistance (HOMA-IR). A random effects meta-analysis was performed for, ALT, AST and HOMA-IR measures separately. Between-study heterogeneity was measured using I^2 statistics. A protocol for the systematic review was published a priori in the PROSPERO database (Reference: CRD42018107063).

Research results

Results from the synthesised evidence suggest that L-carnitine is associated with a significant reduction in serum alanine aminotransferase (ALT), the most commonly used biomarker of hepatocellular injury. In two robust, high-quality randomised trials, L-carnitine supplementation reduced liver fat significantly. Further, in subgroup analysis of studies assessing insulin resistance, L-carnitine supplementation was associated with a significant reduction in the HOMA-IR.

Research conclusions

We present an argument for further robust, randomised trial data evaluating mechanisms of action of L-carnitine on liver and muscle tissue in NAFLD populations. Currently availabile evidence suggests that as a naturally occurring, broadly applicable and cost-effective agent, L-carnitine could be an effective tool for patients in clinical practice as an adjunctive treatment for NAFLD. However, we emphasise that further research using robust and validated endpoints is required to consolidate existing evidence of benefit.

Research perspectives

Micronutrient supplementation presents a novel and exciting avenue for the treatment of population-level diseases, including NAFLD. A broader impact of L-carnitine on metabolic health (including improved insulin sensitivity) could have implications beyond NAFLD alone and its effect in other metabolically challenged populations deserves attention. Ultimately, further well-conducted prospective, randomised data will be required to translate a speculative

benefit of L-carnitine in NAFLD into the clinical sphere.

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META-ANALYSIS

Gender prevalence of cardiovascular diseases in the geriatric population of India: A meta-analysis using R

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Author contributions: Nanda H and Shivgotra VK both designed the study, the literature was reviewed by the first author, then by the second author; data collection, data analysis and writing of the paper was performed by Nanda H under the supervision of Shivgotra VK.

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Abstract

BACKGROUND

Population ageing is an important challenge for developed as well as developing countries due to the downward trends in mortality rates. The elderly population is increasing worldwide. Cardiovascular diseases (CVDs) are one of the most common diseases in the geriatric population. These diseases involve the heart or blood vessels and include hypertension, rheumatic heart disease, heart failure, and heart attack. An estimated 17.7 million people in India will die from CVDs representing 31% of all global deaths.

AIM

To perform a systematic review and meta-analysis of the gender prevalence of CVDs in the geriatric population of India.

METHODS

In the present study, we searched databases such as Google Scholar, PubMed and MEDLINE from the year 2003 to 2019 to identify the prevalence of CVDs in the Indian geriatric population. A meta-analysis was conducted using the statistical software R version 3.4.3 and the random effect model was used to determine the pooled estimate of the prevalence of CVDs in the geriatric population of India along with the 95% confidence interval rather than using the fixed effect model. The random effect model takes into consideration the heterogeneity across the various studies.

RESULTS

The prevalence of CVDs in the Indian geriatric population was determined in 6586 male subjects from 32 studies and 8164 female subjects from 32 studies, respectively. The overall prevalence of CVDs in the Indian geriatric population was 36.6% (95%CI: 31.9%-41.3%). In addition, calculation of the various heterogeneity statistics (Cochran's Q = 3836.85, I^2 = 98.6%, P < 0.0001) indicated heterogeneity in the prevalence of CVDs in the elderly Indian population in these studies. The prevalence of CVDs in elderly males was 38.0% (95%CI: 33.0%-43.0%) and the prevalence of CVDs in elderly females was 40.9% (95%CI: 35.5%-

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46.2%).

CONCLUSION
The results indicate that the prevalence of CVDs in the female geriatric population was relatively higher than that in the male geriatric population. Policy makers must take immediate steps to prevent CVDs and improve geriatric health care services in India.

Key words: Ageing; Cardiovascular diseases; Geriatric; Systematic review; Meta-analysis; Prevalence

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Core tip: A systematic review and meta-analysis of the prevalence of cardiovascular diseases in the geriatric population in India from the year 2003 to 2019 revealed that there was a higher prevalence of cardiovascular diseases in the female geriatric population (40.9%) as compared with the male geriatric population (38.0%). In addition, the measures of consistency such as I^2 and Cochran's Q suggested heterogeneity between the studies. These findings indicate that health care professionals should take immediate steps to improve geriatric health care services in India.

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INTRODUCTION

Ageing is a universal phenomenon associated with deteriorating health status. It is said that nobody grows old merely by living a certain number of years. With the passage of time, certain changes take place in an organism leading to morbidity, disability and even death. Populations around the world are growing old at a high rate with increasing life expectancy. The challenge for health care in the coming years is to ensure quality of life in the geriatric population[1]. The elderly consist of people surpassing the average life span of humans. The contribution of the elderly population to demographic figures is increasing day by day. Increasing problems in health care, psychological, personal and socio-economic factors are associated with the elderly[2]. The growing healthy ageing population is a source of both joy and worry. Joy as people are living longer and healthier lives, and worry about how to respond to a future with a larger older population and their demands and needs[3].

According to the World Health Organization (WHO) Report on Ageing and Health 2018, persons aged 60 years and older are considered elderly. It is suggested that between 2015 and 2050, the proportion of the world's elderly population will nearly double from 12% to 22%, respectively. By 2050, the world's population aged 60 years and older is expected to total 2 billion, up from 900 million in 2015[4]. According to the population census 2011, there are nearly 104 million elderly persons in India which constitutes approximately 8% of the total world population. This share of the population is expected to increase from 8% to nearly 12.6% in 2025 and to 19% in 2050[5].

The geriatric population is vulnerable to long-term diseases such as cardiovascular diseases (CVDs), cancer, diabetes, musculoskeletal disorders, depression, arthritis, kidney problems, *etc*. Due to the increase in life expectancy and modification of lifestyle, CVDs are emerging as one of the major problems in elderly people in India. CVDs generally refer to disorders of the heart and blood vessels and include hypertension, heart attack, cerebrovascular diseases or stroke, peripheral heart disease, rheumatic heart disease, congenital heart disease and cardiomyopathies[6]. The symptoms of heart diseases may vary with respect to disease type. Most CVDs can be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol, *etc*. CVDs are a major cause of mortality all over the world as well as in India. According to the WHO Report, an estimated 17.9 million people will die every year from CVDs representing

31% of all deaths worldwide and over 23 million people will die from CVDs by 2030[7]. Our study aims to provide a pooled estimate of the gender prevalence of CVDs in the geriatric population of India.

MATERIALS AND METHODS

Literature search

The search strategy, selection of publications and reporting of results for the review were conducted in accordance to the PRISMA guidelines. All published studies related to the prevalence of CVDs in India over the last 15 years (*i.e*., from the year 2003 to year 2018) were obtained from MEDLINE/PubMed, Google Scholar and from non-electronic materials such as journals, theses, *etc*. Internet searches using a combination of keywords such as prevalence, CVDs, hypertension, stroke, elderly, morbidity, *etc*. were used to identify prevalence studies of CVDs in the Indian geriatric population. The limitations included were: English for the language category and geriatric humans for the study category.

Selection of studies

All identified research papers were independently reviewed by the reviewers. Disagreements between the reviewers were resolved by considering the facts mentioned in the PRISMA checklist.

Inclusion criteria

Studies that m*et a*ll the following criteria were included in the present analysis: (1) The studies in the mentioned databases with full text, despite the language of the original text; (2) The studies were conducted in the Indian population and were prevalence studies conducted after the year 2002; (3) The study design was cross-sectional; and (4) The age group included in the study was 60 years and older.

Exclusion criteria

Studies which did not satisfy the above criteria were not included in our analysis. We also excluded studies in which age-specific prevalence was not reported. Some studies in which calculated proportions acted as outliers were also excluded from the study to ensure normal distribution of the data.

Data extraction and methodological assessment

Data extraction was performed by the first reviewer and confirmed by the second reviewer, and the whole process was discussed by both reviewers. The eligible studies were further reviewed and information on the author, year of publication, gender, location, total number of subjects, number of cases, type of study, *etc*. from each included study was extracted and the data were summarized in the form of a table.

Statistical analysis

The meta-analysis was used to combine the results of studies using the statistical software R version 3.4.3. The pooled estimate of prevalence of CVDs in the Indian geriatric population was provided by the random effect model. The random effect model assumes that observed estimates of treatment effect can vary across studies because of real differences in the treatment effect in each study as well as sampling variability (chance). Variation (heterogeneity) across studies must also be considered. The random effect meta-analysis allows for heterogeneity by assuming that underlying effects follow a normal distribution. Cochran's Q and I^2 were used to incorporate heterogeneity in the studies and 95% confidence intervals (CIs) are reported in the analysis. Forest plots and funnel plots were used for graphical representation of the meta-analysis. The squares in the forest plot represent the effect estimates of individual studies with their 95%CI of the prevalence of CVDs with the size of squares proportional to the weights assigned to each study in the meta-analysis. The diamond represents the overall result and 95%CI of the random effect meta-analysis. The funnel plots were used to investigate publication bias in the study. The inverted funnel shows that there is no publication bias, whereas the asymmetric funnel plot may suggest publication bias. The dots in the funnel plot represent the studies involved in the meta-analysis. Generally, the studies with larger power are placed towards the top, whereas the lower power studies are placed at the bottom.

RESULTS

Literature review

Figure 1 summarizes the search strategy carried out. Our search strategy identified approximately 720 articles. Of these, 390 duplicate articles were excluded. We also excluded 245 articles after screening the title and abstract. The full text of the remaining 85 articles was screened and 50 articles were further excluded due to lack of inclusion criteria and 35 articles were finally included in the study.

Study characteristics

We included 35 studies of CVDs in the Indian geriatric population in this meta-analysis. The included articles consisted of 32 studies based on male and female geriatric populations in India. Table 1 shows the characteristics of each study in our analysis after applying the exclusion criteria.

Meta-analysis

Meta-analysis of the prevalence of CVDs in the Indian geriatric population involved 6586 male subjects from 32 studies. The pooled estimate of prevalence of CVDs in the male geriatric population using the random effect model was 38.0% (95%CI: 33.0%-43.0%) (Table 2). Significant heterogeneity (I^2 = 95.0% and Cochran's Q = 621.09, P < 0.0001) among the 32 studies was found (Table 3). The forest plot (Figure 2) and the funnel plot (Figure 3) represent the proportion of subjects affected by CVDs in each study and the pooled estimate of the prevalence of CVDs in the male geriatric population, respectively.

Meta-analysis of the prevalence of CVDs in the female geriatric population included 8164 subjects from 32 studies. The pooled estimate of prevalence of CVDs in the female geriatric population using the random effect model was 40.9% (95%CI: 35.5%-46.2%) (Table 4). Significant heterogeneity (I^2 = 96.4% and Cochran's Q = 873.0, P < 0.0001) among the 32 studies was found (Table 5). The forest plot (Figure 4) and funnel plot (Figure 5) represent the proportion of subjects affected due to CVDs in each study and the pooled estimate of prevalence of CVDs in the female geriatric population, respectively.

DISCUSSION

CVD in the geriatric population is emerging as a major problem in India. In the present analysis, we attempted to provide an estimate of the pooled prevalence of CVDs among the Indian geriatric population along with the 95%CI. The estimated overall prevalence of CVDs in the Indian geriatric population was 36.6% (95%CI: 31.9%-41.3%).

The estimated prevalence of CVDs in the male geriatric population was 38.0% (95%CI: 33.0%-43.0%) which was less than the prevalence (46.9%) reported by Naushad *et al*[8] in Raipur, Chhattisgarh, India. The highest prevalence (64.2%) of CVDs in the male Indian geriatric population was observed by Hazarika *et al*[9]. Similar findings were reported by Lena *et al*[31] and Barman *et al*[3] in their respective studies. Rapid urbanization, lifestyle changes, dietary changes and increased life expectancy were factors attributed to this rising trend. The lowest prevalence (10.2%) of CVDs in the male Indian geriatric population was observed by Bardhan *et al*[10]. Chandrashekhar *et al*[1] also reported similar findings in their study in which the prevalence of CVDs in the geriatric population was 19.3%.

The estimated overall prevalence of CVDs in the female geriatric Indian population was 40.9% (95%CI: 35.5%-46.2%) which is higher than the prevalence (35.7%) reported by Shubha *et al*[11]. The highest prevalence (62.9%) of CVDs in the female Indian geriatric population was observed by Hazarika *et al*[9] Bharati *et al*[16], Joshi *et al*[25], Naushad *et al*[8] and Alam *et al*[12] who reported a similar high prevalence of CVDs in female subjects. The lowest prevalence (9.6%) was observed by Bardhan *et al*[10]. Studies by Banjare *et al*[14] and Shubha *et al*[11] also showed a similar pattern of low prevalence of CVDs in elderly female subjects.

The current statistics for CVDs show that almost 80% of premature heart attacks and strokes are preventable. The government should promote awareness of these diseases through mass media and other means and provide information on diet and physical health.

Conclusion

The present systematic review and meta-analysis demonstrated that the prevalence of CVDs in the male and female geriatric population in India was estimated to be 38.0% and 40.9%, respectively. These considerable prevalence rates require supportive interventions for the prevention and early diagnosis of CVDs in the geriatric population. It is imperative to provide geriatric health services at primary health

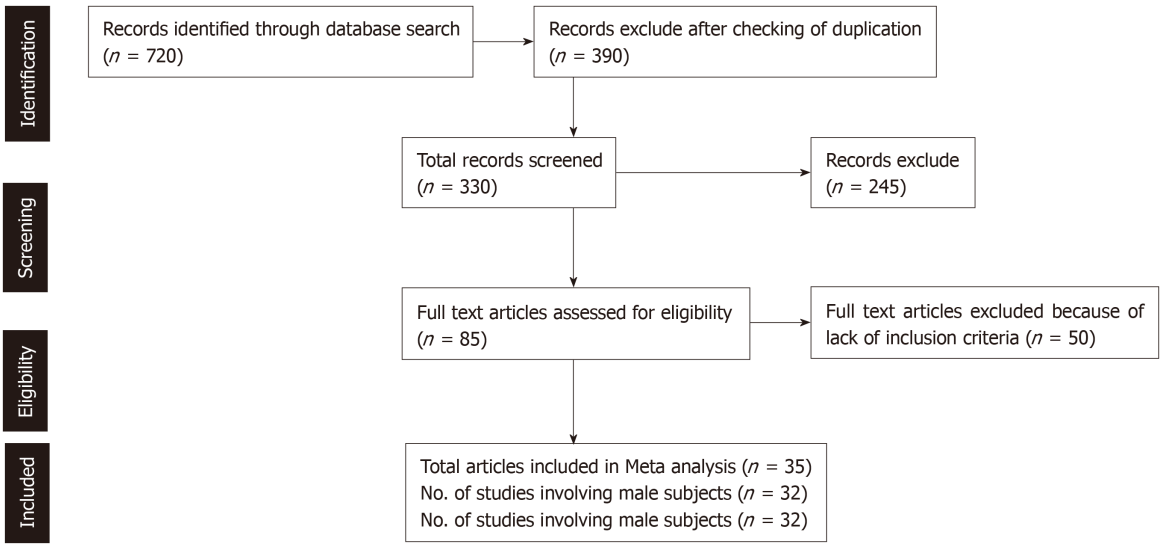


Figure 1 Flowchart showing the selection process of relevant studies.

centers in addition to increasing the awareness among elderly people of health, diseases and health care facilities in order to improve their quality of life.

Limitations

Given the limited amount of data available on the prevalence of CVDs in the geriatric population in India; studies conducted in subjects with different bio-social characteristics have been included in the meta-analysis. Also, the pooled estimate of prevalence of CVDs in the Indian geriatric population does not provide an overview of the problem of CVDs.

Implications for future research

The prevalence of CVDs in the Indian geriatric population presents a formidable challenge to the Indian health system. In future, researchers should identify the location- and age-specific pooled estimate of CVDs in the geriatric population of India. Other areas of research should include determination of the pooled prevalence of other morbidities such as diabetes, arthritis, *etc*. in the geriatric population.

Table 1 The characteristics of each study

Ref.	Study period	Study design	State	Sampling method	Age group	Sample size
Joshi *et al*[25]	2001	Cross-sectional	Haryana	Cluster sampling	≥ 60	200
Hazarika *et al*[9]	2002	Cross-sectional	Assam	Simple random sampling	≥ 60	888
Lena *et al*[31]	2003	Cross-sectional	Karnataka		≥ 60	213
Prakash *et al*[2]	2003	Cross-sectional	Rajasthan		≥ 60	300
Kishore *et al*[29]	2004	Cross-sectional	Uttarakhand		≥ 60	285
Khanam *et al*[28]	2004	Cross-sectional	Bangladesh	Simple random sampling	≥ 60	452
Bhatia *et al*[17]	2006	Cross-sectional	Haryana	Stratified random sampling	≥ 65	361
Chandrashekhar *et al*[1]	2008	Cross-sectional	Karnataka	Systematic random sampling	≥ 60	370
Bharati *et al*[16]	2008	Cross-sectional	Tamil Nadu	Simple random sampling	≥ 60	225
Bhatt *et al*[18]	2008	Cross-sectional	Gujarat	Simple random sampling	≥ 60	218
Jacob *et al*[23]	2010	Cross-sectional	Kerala	Cluster sampling	≥ 60	403
Sharma *et al*[36]	2010	Cross-sectional	Himachal Pradesh	Multistage simple random sampling	≥ 60	400
Ghosh *et al*[20]	2011	Cross-sectional	Bihar		≥ 60	431
Kamble *et al*[26]	2011	Cross-sectional	Maharashtra	Systematic random sampling	≥ 60	494
Qadri *et al*[33]	2011	Cross-sectional	Haryana	Simple random sampling	≥ 60	660
Banjare *et al*[14]	2012	Cross-sectional	Odisha	PPS, Systematic sampling	≥ 60	310
Dutta *et al*[19]	2012	Cross-sectional	Assam	Multistage sampling	≥ 60	370
Banjare *et al*[13]	2012	Cross-sectional	Odisha	Multistage simple random sampling	≥ 60	310
Barman *et al*[3]	2013	Cross-sectional	Bihar		≥ 60	160
Shubha *et al*[11]	2013	Cross-sectional	Karnataka	Simple random sampling	≥ 60	180
Karanth *et al*[27]	2013	Cross-sectional	Karnataka		≥ 60	500
Alam *et al*[12]	2014	Cross-sectional	Chhattisgarh	Multistage simple random sampling	≥ 60	640
Bardhan *et al*[10]	2014	Cross-sectional	Uttar Pradesh	Simple random sampling	≥ 60	980
Bartwal *et al*[15]	2014	Cross-sectional	Uttarakhand	Systematic sampling	≥ 60	440
Gupta *et al*[21]	2014	Cross-sectional	Punjab		≥ 60	534
Kumar *et al*[30]	2014	Cross-sectional	Uttar Pradesh	Stratified random sampling	≥ 60	402
Naushad *et al*[8]	2014	Cross-sectional	Chhattisgarh	Multistage simple random sampling	≥ 60	640
Jain *et al*[24]	2014	Cross-sectional	Maharashtra	PPS	≥ 60	600
Noor *et al*[32]	2014	Cross-sectional	Odisha	Simple random sampling	≥ 60	224
Venkateshkrishna *et al*[37]	2015	Cross-sectional	Karnataka		≥ 60	1452
Sahu *et al*[35]	2017	Cross-sectional	Uttar Pradesh		≥ 60	231
Reddy *et al*[34]	2017	Cross-sectional	Telangana	Systematic random sampling	≥ 60	1265
Gupta *et al*[22]	2018	Cross-sectional	Haryana	Simple random sampling	≥ 60	300

Table 2 Prevalence of cardiovascular diseases in the male Indian geriatric population

Ref.	*n*	Cases	Proportion	95%CI	W (Random) (%)
Joshi *et al*[25]	98	41	0.4184	[0.3195-0.5223]	3.0
Hazarika *et al*[9]	500	321	0.6420	[0.5982-0.6841]	3.3
Prakash *et al*[2]	190	84	0.4421	[0.3703-0.5158]	3.2
Kishore *et al*[29]	177	70	0.3955	[0.3229-0.4716]	3.1
Bhatia *et al*[17]	152	53	0.3487	[0.2733-0.4301]	3.1
Chandrashekhar *et al*[1]	181	35	0.1934	[0.1385-0.2585]	3.2
Lena *et al*[31]	92	53	0.5761	[0.4686-0.6785]	3.0
Bhatt *et al*[18]	74	19	0.2568	[0.1622-0.3716]	3.0
Bharati *et al*[16]	92	34	0.3696	[0.2712-0.4766]	3.0
Khanam *et al*[28]	204	74	0.3627	[0.2968-0.4328]	3.2
Kamble *et al*[26]	232	50	0.2155	[0.1644-0.2741]	3.2
Sharma *et al*[36]	196	64	0.3265	[0.2614-0.3970]	3.2
Qadri *et al*[33]	336	135	0.4018	[0.3489-0.4564]	3.2
Barman *et al*[3]	88	47	0.5341	[0.4246-0.6412]	2.9

Banjare *et al*[13]	153	40	0.2614	[0.1938-0.3385]	3.2
Noor *et al*[32]	132	62	0.4697	[0.3823-0.5585]	3.1
Ghosh *et al*[20]	196	96	0.4898	[0.4179-0.5620]	3.2
Kumar *et al*[30]	190	66	0.3474	[0.2799-0.4197]	3.2
Bardhan *et al*[10]	293	30	0.1024	[0.0702-0.1429]	3.3
Dutta *et al*[19]	162	57	0.3519	[0.2786-0.4307]	3.1
Banjare *et al*[14]	153	40	0.2614	[0.1938-0.3385]	3.2
Shubha *et al*[11]	42	15	0.3571	[0.2155-0.5197]	2.6
Alam *et al*[12]	267	113	0.4232	[0.3632-0.4849]	3.2
Gupta *et al*[21]	244	94	0.3852	[0.3239-0.4495]	3.2
Bartwal *et al*[15]	187	77	0.4118	[0.3405-0.4859]	3.2
Naushad *et al*[8]	241	113	0.4689	[0.4045-0.5340]	3.2
Jain *et al*[24]	262	133	0.5076	[0.4454-0.5697]	3.2
Venkateshkrishna *et al*[37]	530	145	0.2736	[0.2360-0.3137]	3.3
Sahu *et al*[35]	61	15	0.2459	[0.1446-0.3729]	2.9
Jacob *et al*[23]	135	77	0.5704	[0.4824-0.6552]	3.1
Reddy *et al*[34]	594	191	0.3215	[0.2841-0.3608]	3.3
Gupta *et al*[22]	132	59	0.4470	[0.3604-0.5359]	3.1

Table 3 Tests of heterogeneity among studies of the male Indian geriatric population

Test of heterogeneity	Prevalence	LCI 95%	HCI 95%
Pooled Statistics	0.38	0.33	0.43
Heterogeneity	4.48	4.02	4.98
I-squared	95.0	93.8	96.0
Cochran's Q	621.09		
χ^2, *P* value	0.0001		
τ^2	0.0197		

Table 4 Prevalence of cardiovascular diseases in the female Indian geriatric population

Study name	N	Cases	Proportion	95%CI	%W (Random)
Joshi *et al*[25]	102	57	0.5588	[0.4571-0.6571]	3.0
Hazarika *et al*[9]	388	244	0.6289	[0.5787-0.6771]	3.2
Prakash *et al*[2]	110	60	0.5455	[0.4477-0.6407]	3.0
Kishore *et al*[29]	108	48	0.4444	[0.3488-0.5432]	3.0
Bhatia *et al*[17]	209	97	0.4641	[0.3950-0.5342]	3.1
Chandrashekhar *et al*[1]	189	30	0.1587	[0.1097-0.2188]	3.2
Lena *et al*[31]	121	73	0.6033	[0.5104-0.6911]	3.0
Bhatt *et al*[18]	144	56	0.3889	[0.3088-0.4736]	3.1
Bharati *et al*[16]	122	68	0.5574	[0.4647-0.6472]	3.0
Khanam *et al*[28]	248	101	0.4073	[0.3455-0.4712]	3.2
Kamble *et al*[26]	262	69	0.2634	[0.2111-0.3211]	3.2
Sharma *et al*[36]	204	98	0.4804	[0.4101-0.5513]	3.1
Qadri *et al*[33]	324	159	0.4907	[0.4351-0.5466]	3.2
Barman *et al*[3]	72	34	0.4722	[0.3533-0.5935]	2.9

Noor *et al*[32]	92	46	0.5000	[0.3939-0.6061]	2.9
Ghosh *et al*[20]	235	82	0.3489	[0.2881-0.4136]	3.2
Kumar *et al*[30]	212	71	0.3349	[0.2717-0.4028]	3.1
Bardhan *et al*[10]	197	19	0.0964	[0.0591-0.1465]	3.2
Dutta *et al*[19]	208	77	0.3702	[0.3044-0.4397]	3.1
Banjare *et al*[14]	157	20	0.1274	[0.0796-0.1899]	3.2
Shubha *et al*[11]	138	19	0.1377	[0.0850-0.2066]	3.2
Alam *et al*[12]	373	207	0.5550	[0.5029-0.6061]	3.2
Karanth *et al*[27]	500	130	0.2600	[0.2221-0.3008]	3.2
Gupta *et al*[21]	290	158	0.5448	[0.4856-0.6031]	3.2
Bartwal *et al*[15]	253	99	0.3913	[0.3308-0.4544]	3.2
Naushad *et al*[8]	369	207	0.5610	[0.5087-0.6123]	3.2
Jain *et al*[24]	338	144	0.4260	[0.3727-0.4807]	3.2
Venkateshkrishna *et al*[37]	922	402	0.4360	[0.4037-0.4687]	3.2
Sahu *et al*[35]	170	52	0.3059	[0.2376-0.3811]	3.1
Jacob *et al*[23]	268	124	0.4627	[0.4018-0.5244]	3.2
Reddy *et al*[34]	671	193	0.2876	[0.2536-0.3235]	3.2
Gupta *et al*[22]	168	88	0.5238	[0.4455-0.6013]	3.1

Table 5 Tests of heterogeneity among studies of the female Indian geriatric population

Tests of heterogeneity	Prevalence	LCI 95%	HCI 95%
Pooled Statistics	0.409	0.355	0.462
Heterogeneity	5.31	4.82	5.84
I-squared	96.4	95.7	97.1
Cochran's Q	873.0		
χ^2, *P* value	0.0001		
τ^2	0.0229		

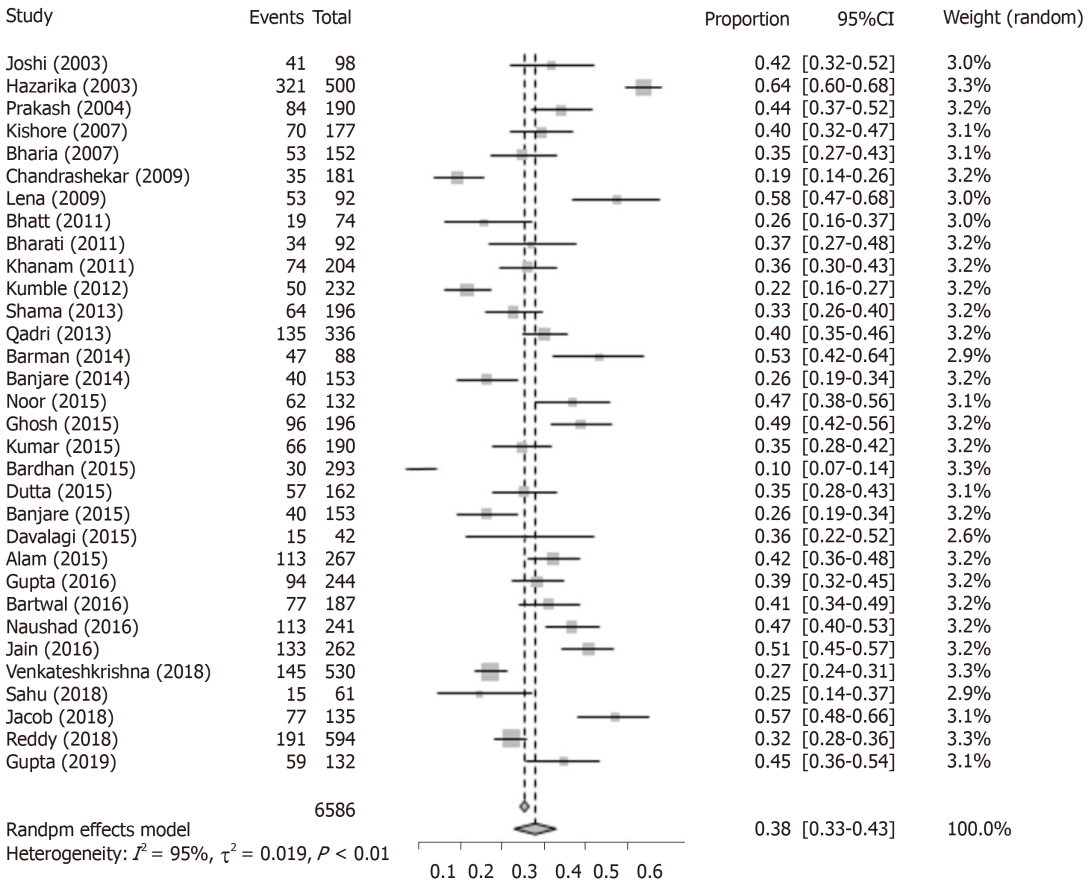


Figure 2 Forest plot of the meta-analysis of cardiovascular disease prevalence in the male Indian geriatric population.

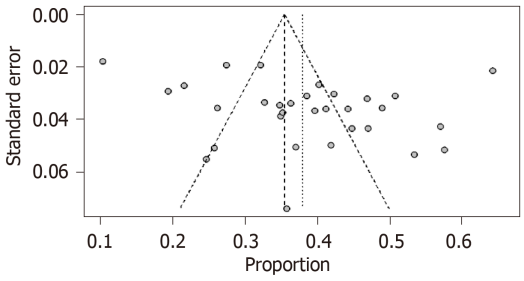


Figure 3 Funnel plot showing publication bias in the meta-analysis of the prevalence of cardiovascular diseases in the male Indian geriatric population.

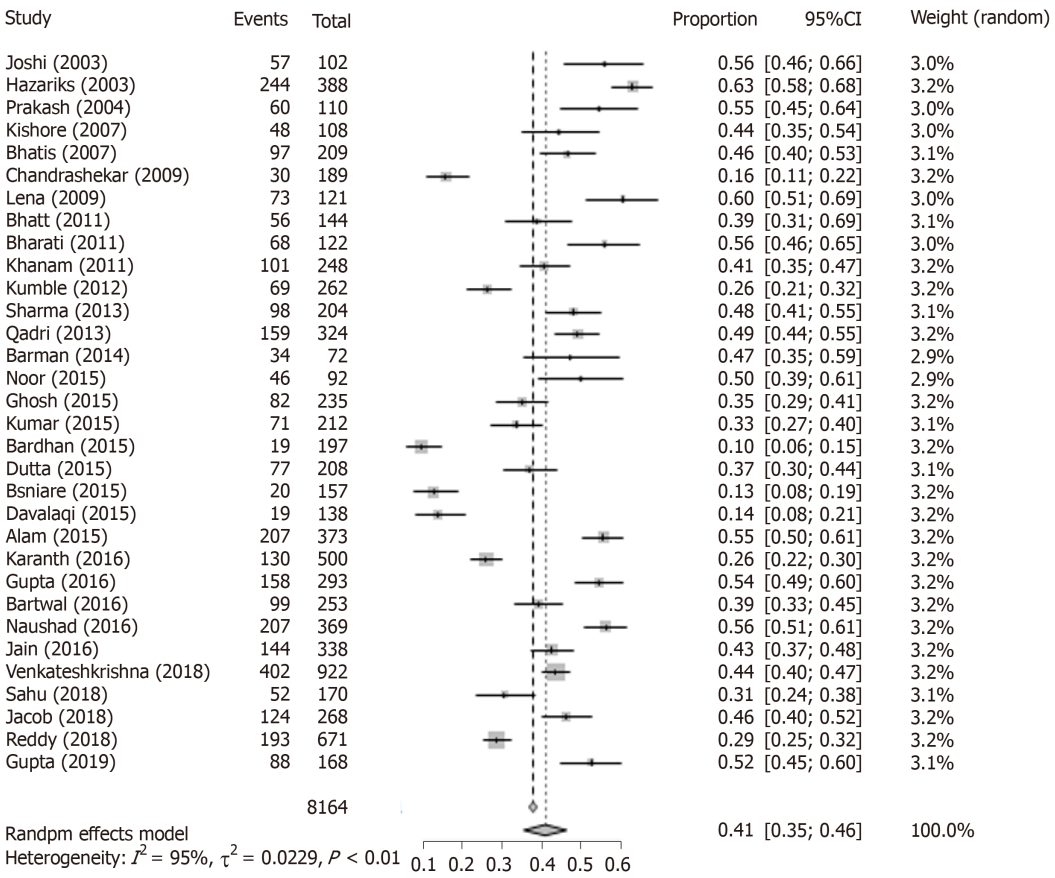


Figure 4 Forest plot of the meta-analysis of cardiovascular disease prevalence in the female Indian geriatric population.

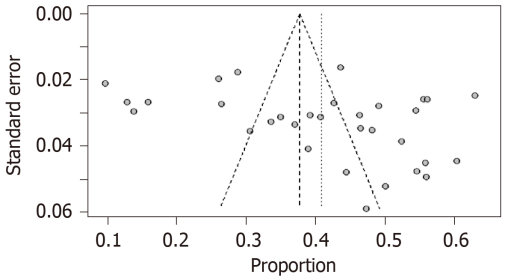


Figure 5 Funnel plot showing publication bias in the meta-analysis of prevalence of cardiovascular diseases in the female Indian geriatric population.

ARTICLE HIGHLIGHTS

Research background

India is in the phase of demographic transition. From the 2011 census, there are 104 million elderly persons in India as compared with 57 million elderly persons in 1991. The elderly are exposed to several morbidities such as cardiovascular diseases (CVDs), diabetes, hypertension, *etc*. CVDs have become the leading cause of mortality in India. An estimated 17.7 million people in India will die from CVDs representing 31% of all global deaths. Therefore, a precise estimate of the prevalence of CVDs in elderly males and females in India is required to assess the magnitude of the problem which needs to be addressed.

Research motivation

Very few studies are available on the prevalence of CVDs in India. The gender pooled estimate of CVDs from various studies conducted in different regions of the country can aid the development of preventive strategies. The difference in the prevalence of CVDs among male and female geriatric subjects was studied to provide an insight into the type of preventive and promotional services required.

Research objectives

The main objective of the study was to provide the gender pooled estimate of the prevalence of CVDs in the geriatric population of India. The large increase in the prevalence of CVDs in the Indian geriatric population has prompted health managers to take immediate steps for the prevention and early detection of these diseases. In addition, researchers are attempting to identify the location- and age-specific pooled estimate of the prevalence of CVDs. The main application of this meta-analysis is to consolidate the available data to determine the burden of CVDs in India.

Research methods

Secondary data related to the prevalence of CVDs in the geriatric population were collected from published research papers and then analyzed using meta-analysis in R software. A meta-analysis integrates the quantitative findings from separate studies and provides a numerical estimate of the overall effect of interest.

Research results

The overall prevalence of CVDs in the Indian geriatric population was estimated to be 36.6% (95%CI: 31.9%-41.3%). The prevalence of CVDs in the male geriatric population was 38.0%, whereas the prevalence of CVDs in the elderly female population was 40.9%.

Research conclusions

The pooled prevalence of CVDs in the female Indian geriatric population was greater than the pooled prevalence of CVDs in the male Indian population. These findings will help policy makers to take immediate steps to provide geriatric health care services in India. It is possible that an insight into the magnitude of the problem of CVDs can help to shape preventive programs for CVDs.

Research perspective

Future studies should be conducted to determine the pooled estimate of CVDs in the geriatric population in both rural and urban areas, and in different age-groups. Subgroup analysis in meta-regression can be used to calculate these values.

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META-ANALYSIS

Long-term efficacy of capecitabine plus oxaliplatin chemotherapy on stage III colon cancer: A meta-analysis

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Abstract

BACKGROUND

Many clinical studies for the long-term survival or efficacy of capecitabine plus oxaliplatin (XELOX) in colon cancer have already been studied, but its clinical benefit is controversial.

AIM

To evaluate the long-term efficacy of XELOX regimen in comparison with other adjuvant chemotherapy protocols in colon cancer.

METHODS

By searching the PubMed, EMBASE and Cochrane databases, a total of 12 randomized controlled trials involving 6698 stage III colon cancer cases (XELOX protocol: n = 3298 cases; other adjuvant chemotherapy protocol: n = 3268 cases) were included. The parameter outcomes included the overall survival and the disease-free survival. The quality control of selected literature was based on the Jadad scale and the GRADE system.

RESULTS

In comparison to other adjuvant chemotherapy regimen, XELOX regimen showed a better overall survival (odds ratio = 1.29, 95% confidence interval: 1.15-1.44, $P < 0.0001$) and a better disease-free survival (odds ratio = 1.32, 95% confidence interval: 1.18-1.46, $P < 0.0001$) for colon cancer patients, suggesting the

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XELOX regimen can be a good option for postoperative treatment of stage III colon cancer.

CONCLUSION

The XELOX regimen can be a preferred option for adjuvant treatment of stage III colon cancer after surgery.

Key words: Capecitabine plus oxaliplatin chemotherapy; Colon cancer; Meta-analysis; Long-term effect

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Core tip: Many clinical studies for the long-term survival of patients or the efficacy of capecitabine plus oxaliplatin (XELOX) in colon cancer have already been studied, but its clinical benefit is controversial. The long-term efficacy of the XELOX regimen in comparison with other adjuvant chemotherapy protocols in colon cancer was evaluated. By searching the PubMed, EMBASE and Cochrane databases, a total of 12 randomized controlled trials involving 6698 stage III colon cancer cases were included. Our findings showed that XELOX regimen had a better overall survival and a better disease-free survival. The XELOX regimen can be a preferred option for adjuvant treatment of stage III colon cancer after surgery.

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INTRODUCTION

Colorectal cancer (CRC) accounts for 9% of all cancers worldwide. It is the second most common cancer in women and the third most frequent cancer in men. More than 70% of the deaths associated with CRC are caused by metastasis to the liver. Although surgery may be potentially curable, less than 25% of cases can be managed with a recurrence rate of up to 70%[1]. The purpose of colon cancer treatment is to cure locally and to prevent metastasis and recurrence. Therefore, in the local excision of colon cancer at the same time, the treatment should be according to individual condition, and chemotherapy is an important method that is based on the patient's condition, surgical situation and clinical stage of appropriate postoperative adjuvant chemotherapy.

Many clinical studies for the long-term survival benefit or efficacy of capecitabine plus oxaliplatin (XELOX) in colon cancer have already been studied[2-4], but its clinical benefit is controversial[5]. Since the 1990s, the introduction of irinotecan or oxaliplatin has extended the spectrum of therapeutic options. The combination of oxaliplatin or irinotecan with 5-fluorouracil (5-FU) plus leucovorin (LV or FA) has been considered the standard regimen for first-line treatment of metastatic CRC. However, this is an inconvenient therapeutic option due to the requirement for continuous vascular infusion of 5-FU. A retrospective study on XELOX plus bevacizumab *vs* LV plus 5-FU plus irinotecan (also known as FOLFIRI) plus bevacizumab treatment for metastatic colon cancer reported that XELOX plus bevacizumab was more effective in response rate and overall survival (OS) compared with LV plus 5-FU plus irinotecan plus bevacizumab[6].

Capecitabine is an orally administered fluoropyrimidine that was rationally designed to generate 5-FU preferentially at the tumor site. Capecitabine demonstrated a safety profile superior to that of 5-FU/LV, with a significantly lower incidence of diarrhea, stomatitis, nausea, alopecia and grade 3/4 neutropenia. Also, oral administration of capecitabine simplifies chemotherapy and provides convenient outpatient therapy. Because capecitabine has been adopted as a substitute for infused 5-FU/LV to overcome the inconvenience of 5-FU, subsequent data have found XELOX (also known as CAPOX) to be a comparable therapeutic regimen to infused 5-FU/LV plus oxaliplatin (known as FOLFOX-4 or FUOX). In the Loree *et al*[7] study,

XELOX and FOLFOX were compared in the treatment of colon cancer. The results showed that XELOX may be associated with improved disease-free survival (DFS) despite greater toxicities and reduced adjuvant chemotherapy duration to 3 mo. In a safety analysis of adjuvant chemotherapy for stage III colon cancer after radical resection of stage III colon cancer, mFOLFOX6/XELOX regimens are acceptable[8].

Randomized phase III trials demonstrated that outcomes using first-line XELOX are comparable with those achieved using continuous infusion of 5-FU and FOLFOX. There are many chemotherapy options for advanced CRC, and the long-term benefit is uncertain. Combining XELOX is advantageous for the reasons as follows: Synergistic effects, no overlapping toxicities, easy to administer and outpatient management[9-12]. XELOX has been studied extensively in rectal cancer where the standard therapy is XELOX plus radiation therapy. To determine the efficacy of XELOX in colon cancer, the long-term efficacy of capecitabine combined with oxaliplatin (XELOX regimen) in comparison with other adjuvant chemotherapy protocols was evaluated.

MATERIALS AND METHODS

This meta-analysis is in terms of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 declaration.

Search strategy

Two researchers independently retrieved randomized controlled trials (RCT) articles involved in oxaliplatin combined with capecitabine in CRC published in PubMed, EMBASE, Cochrane, web of science, clinical trial and China National Knowledge Infrastructure databases from 1991 to August 2017. The retrieval languages were Chinese and English. The retrieval was performed using the following keywords. English search terms for: (PubMed): Search (((Colonic Neoplasms [MeSH Terms]) OR ((((((((((Colonic Neoplasm [Title/Abstract]) OR Colon Neoplasm* [Title/Abstract]) OR Neoplasm*, Colonic) OR Neoplasm*, Colon [Title/Abstract]) OR Cancer of Colon [Title/Abstract]) OR Cancer of the Colon [Title/Abstract]) OR Colonic Cancer* [Title/Abstract]) OR Cancer*, Colonic [Title/Abstract]) OR Colon Cancer* [Title/Abstract]) OR Cancer*, Colon [Title/Abstract])))) AND ((((((((((ECX) [Title/Abstract] OR XELOX) [Title/Abstract] OR Xeloda) [Title/Abstract] OR Capecitabine [Title/Abstract])) ORN (4) - pentyloxycarbonyl – 5′ - deoxy- 5-fluorocytidine [Title/Abstract]) OR Capecitabine)) OR Capecitabine [MeSH Terms]) AND (((oxaliplatin [MeSH Terms]) OR oxaliplatin [Title/Abstract]) OR ((((((((((((((((1, 2 - diamminocyclohexane (trans-1) oxolatoplatinum (II) [Title/Abstract]) OR oxalato-(1,2-cyclohexanediamine) platinum II [Title/Abstract]) OR L-OHPcpd [Title/Abstract]) OR oxaliplatine [Title/Abstract]) OR1,2-diaminocyclohexane platinum oxalate [Title/Abstract]) OR platinum (II)-1,2-cyclohexanediamine oxalate [Title/Abstract]) OR cis-oxalato-(trans-l)-1,2-diaminocyclohexane - platinum (II) [Title/Abstract]) ORoxaliplatin, (SP-4-3-(cis))-isomer [Title/Abstract]) OR oxaliplatin, (SP-4-2-(1R-trans)) - isomer [Title/Abstract]) OR oxaliplatin, (SP - 4 - 2 - (1S-trans)) - isomer [Title/Abstract]) OR ACT-078 [Title/Abstract]) OR ACT-078 [Title/Abstract]) OR Eloxatine [Title/Abstract]) OR Sanofi Synthelabo brand of oxaliplatin [Title/Abstract]) OR Sanofi brand of oxaliplatin [Title/Abstract]) OR Eloxatin [Title/Abstract]))))). Chinese search terms for: Capecitabine, oxaliplatin, XELOX, colon cancer.

Selection criteria

Literature was retrieved and screened in accordance with the PRISMA guidelines. Two reviewers independently screened literature and abstracts based on predefined inclusion and exclusion criteria and screened the full text if necessary. In the literature that met the inclusion criteria, two reviewers used a unified data extraction table to independently extract data. Disagreements were resolved through consultation or by a third researcher.

Inclusion criteria

The inclusion criteria was: (1) Experimental design for RCT; (2) The research object for the pathological diagnosis of patients with CRC; (3) The experimental group intervention for capecitabine combined with oxaliplatin, the control group for other chemotherapeutic drugs; (4) Observation results for patients with long-term efficacy: OS and DFS; (5) If the study included many cases, then only select the required part; and (6) The selected patients were stage III colon cancer patients and had undergone surgery without neoadjuvant chemotherapy.

Exclusion criteria

Studies were excluded if: (1) Non-RCT; (2) Subjects included rectal cancer; (3) DFS or OS was not compared with two chemotherapy regimens in the same trial; (4) No specific data were provided; and/or (5) Repeated publication.

Data extraction

Data for each article was extracted, including the first author and title of the RCT, sample size, follow-up time, publication time, medication regimen, DFS and OS.

Methodological quality and statistical analysis of the RCTs were evaluated with the following criteria. The offset assessment of a single study was evaluated by two independent researchers. Any disagreements were evaluated by a third researcher. Quality evaluation mainly included random sequence generation, randomization concealment, blindness, withdrawal and withdrawal, and the Jadad scale was used to evaluate the score. We defined 1-3 points as low quality and 4-7 points as high quality. At the same time, the meta-analysis software Review Manager Version 5.3 recommended by the Cochrane library was used to test the heterogeneity and calculate the combination of odds ratio (OR) value and 95% confidence interval (CI).

Statistical detection of P values less than 0.05 were considered statistically significant. The heterogeneity of the study was determined by the t test and the I^2 test. The unified random effect model was used for consolidation. $I^2 < 50\%$ were considered as no statistical heterogeneity among the studies, and a random effect model was used to merge effects. Conversely, the random effect model was used to combine the effects. The output combined the OR value and the 95%CI and tests the merging statistic. The Z test was used. The test level was $\alpha = 0.05$.

Sensitivity analysis

Sensitivity analysis included a sensitivity analysis performed in subgroup analyses. If a document was excluded and the impact on the overall outcome was greater, then the literature was reread and the quality was evaluated. Then it was determined whether it was eventually incorporated.

Methodological quality

The literature was included and the GRADE system was used to assess methodological quality. In order to thoroughly reveal the source of heterogeneity, we also conducted meta regression and subgroup analysis. In addition, the funnel plot and application of shear reinforcing method (if the funnel plot was asymmetric or incomplete, then there was publication bias, and shear reinforcing method was applied; symmetry indicates that the publication bias is less likely without using the shear reinforcing method). Begg's funnel plots and Egger's tests were conducted to assess potential publication bias. Data analysis was performed using Stata 12.0 edition.

RESULTS

After searching the database, 360 documents were selected. Eighteen repetitive documents were excluded. After reading the title and abstract, 285 papers were excluded from the study of capecitabine combined with oxaliplatin compared with other drugs that affect colon cancer. After reading the full text, 41 articles were excluded because they did not involve relevant outcome indicators. Two articles were excluded because there were no relevant data, and two articles in the control group did not meet the inclusion criteria. Finally, 12 articles met the requirements[13-24] (Figure 1).

Data and quality evaluation were included in a clinical controlled trial (Table 1).

Statistical analysis

OS: The meta-analysis obtained OS data from 11 articles. Finally, ten papers were included. The combined analysis showed that the XELOX group had longer OS compared with other chemotherapy groups (Figure 2A). The study has statistical significance (OR = 1.29, 95%CI: 1.15-1.44, $P < 0.0001$). There was no heterogeneity among the study sites ($P = 0.46$, $I^2 = 0\%$).

Results of the OS subgroup analysis (subgroup analysis of different follow-up visits) (Figure 3): Annual survival was statistically significant: (OR = 1.70, 95%CI: 1.13-2.56, $P = 0.01$). There was no heterogeneity in the study ($P = 0.66$, $I^2 = 0\%$). The 2-year survival rate was statistically significant: (OR = 3.30, 95%CI: 1.37-7.97, $P = 0.01$). There was no heterogeneity in the study ($P = 0.99$, $I^2 = 0\%$). The 3-year survival rate was not statistically significant: (OR = 0.97, 95%CI: 0.56-1.69, $P = 0.93$). There was no heterogeneity in the study ($P = 0.93$, $I^2 = 0\%$). The 7-year survival rate was statistically

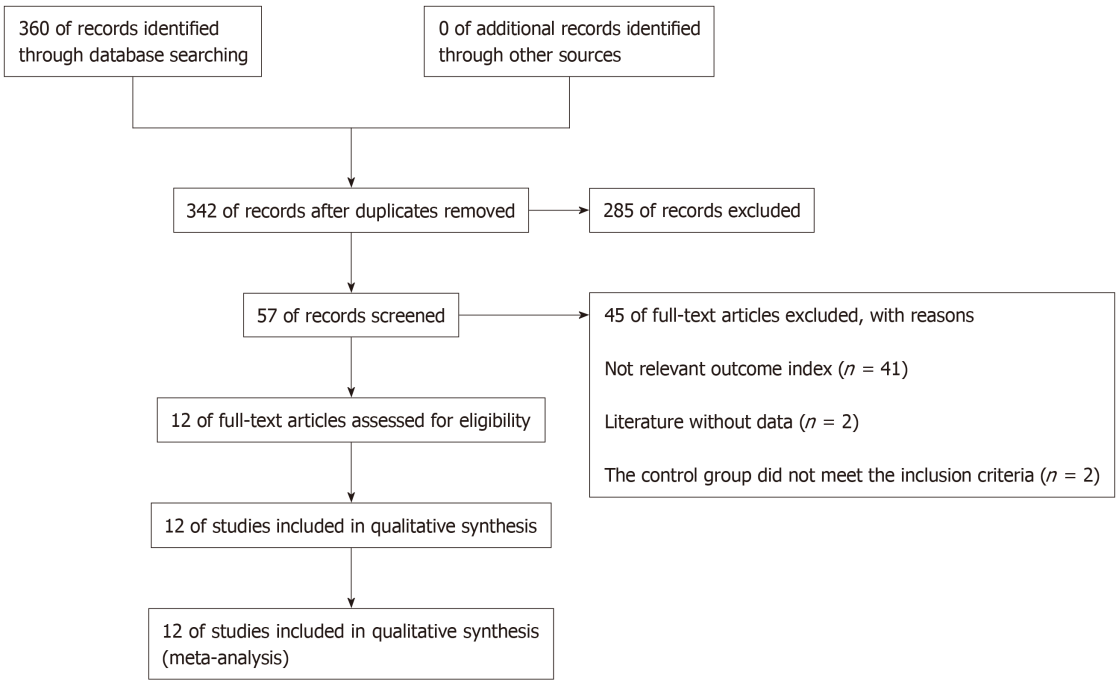


Figure 1 Flow chart of the 12 articles that met the inclusion criteria.

significant (OR = 1.30, 95%CI: 1.13-1.50, P = 0.0003). There was no heterogeneity in the study (P = 0.75, I^2 = 0%).

Results of the OS subgroup analysis (XELOX *vs* other chemotherapy groups) (Figure 4A): XELOX *vs* FU/FA: The study was statistically significant: (OR = 1.24, 95%CI: 1.07-1.44, P = 0.005). There was no heterogeneity in the study (P = 0.73, I^2 = 0%). XELOX *vs* FOLFOX: The study was statistically significant: (OR = 1.29, 95%CI: 1.07-1.55, P = 0.008). There was no heterogeneity in the study (P = 0.32, I^2 = 0%). XELOX *vs* capecitabine: The study was statistically significant: (OR = 1.28, 95%CI: 1.14-1.44, P = 0.008). There was no heterogeneity in the study (P = 0.99, I^2 = 0%).

DFS: In this meta-analysis, DFS data were obtained in five articles, and four articles were finally included. The combined analysis showed that the XELOX group had longer DFS compared with other chemotherapy groups (Figure 2B). The study had significant statistical significance (OR = 1.32, 95%CI: 1.18-1.46, P < 0.0001). There was no heterogeneity in the study (P = 0.68, I^2 = 0%).

Results of the DFS subgroup analysis (XELOX and other chemotherapy drug groups (Figure 4B): XELOX *vs* FU/FA: DFS had significant statistical significance: (OR = 1.34, 95%CI: 1.17-1.53, P < 0.0001). There was no heterogeneity among the study sites (P = 0.94, I^2 = 0%). XELOX *vs* FOLFOX: DFS had statistical significance: (OR = 1.28, 95%CI: 1.08-1.53, P = 0.004). There was low heterogeneity among the studies (P = 0.24, I^2 = 27%).

Process of deflection analysis

Included 11 articles from OS meta-analysis: There was statistical significance (P = 0.004) with moderate heterogeneity in this meta-analysis (P = 0.007, I^2 = 59%) **(**Figure 5A**)**.

Articles were included in the DFS meta-analysis: There was statistical significance (OR = 1.24, 95%CI: 1.04-1.46, P = 0.01) with moderate heterogeneity in this meta-analysis (P = 0.09, I^2 = 51%) **(**Figure 5B**)**. In its subgroup analysis, there was statistical significance (P = 0.02) with high heterogeneity in this meta-analysis (P = 0.02, I^2 = 73%) (Figure 5C).

The sources of heterogeneity

Sensibility analysis 1: There was heterogeneity in the article published by Zhang *et al*[15] (Figure 2A). After evaluating the article, the quality was low and samples were excluded. After exclusion (OR = 1.29, 95%CI: 1.15-1.44, P < 0.0001), there was no heterogeneity among the studies (P = 0.46, I^2 = 0%).

Sensibility analysis 2: The sensitivity analysis (Figure 2B) showed heterogeneity in

Table 1 Data and quality evaluation of clinical controlled trials

Study	Year	T event	T no event	T total	C event	C no event	C total	Outcome	The Jadad score
Haller *et al*[14]	2011	643	295	938	573	353	926	DFS	7
		741	197	938	701	225	926	OS	
Kubicka *et al*[16]	2016	541	320	861	482	379	861	DFS	7
		619	242	861	575	286	861	OS	
Pectasides *et al*[20]	2014	169	44	213	160	41	201	DFS	6
		185	28	213	175	26	201	OS	
Schmoll *et al*[22]	2012	594	350	944	527	415	942	DFS	7
		689	255	944	631	311	942	OS	
Diao *et al*[13]	2008	47	24	71	69	18	87	DFS	2
Xun *et al*[21]	2016	9	21	30	3	25	28	OS	3
Song *et al*[23]	2016	63	10	73	49	6	55	OS	3
Lei *et al*[17]	2016	5	16	21	2	19	21	OS	4
Li *et al*[18]	2012	43	17	60	32	28	60	OS	3
Lian *et al*[19]	2016	2	54	56	2	48	50	OS	4
Zhang *et al*[15]	2011	20	5	25	3	22	25	OS	2
Wang *et al*[24]	2011	8	22	30	3	27	30	OS	3

OS: Overall survival; DFS: Disease-free survival.

the article from Diao *et al*[13]. After evaluating the article, the quality was low and samples were excluded. After exclusion (OR = 1.32, 95%CI: 1.18-1.46, $P < 0.0001$), there was no heterogeneity among the studies ($P = 0.68$, $I^2 = 0\%$). After completely excluding Diao *et al*[13], the study remained statistically significant ($P = 0.004$), and the heterogeneity of the study was greatly reduced ($P = 0.24$, $I^2 = 27\%$) (Figure 4B).

Bias detection of OS: The funnel plot was conducted to evaluate the publication bias. However, we did not observe clear asymmetry suggesting the results from this study are reliable (data not shown).

DISCUSSION

Li *et al*[18] reported that in elderly CRC patients (age above 70-years-old), a reduction in chemotherapy dose did not decrease the DFS with a benefit of less mortality. But, elderly patients receiving 50% of planned cycles had shorter DFS and higher CRC mortality than elderly patients receiving the full planned cycles[25]. Therefore, a reduced dose but full cycles should be considered for elderly CRC patients. Our results are consistent with Kim *et al*[26] who found that OS was better in patients receiving at least 75% of expected cycles, but a dose reduction did not affect OS. These results suggest that a primary dose reduction in elderly patients may reduce the side effects of chemotherapeutics and help them finish the full planned cycles. The choice between mFOLFOX6 and XELOX should be discussed based on the gene subtypes of colon cancer[27-36].

It is well known that the prognosis of CRC patients has significant association with gene mutations. It is generally accepted that dMMR confers favorable prognosis in patients with resected colon cancer[10,11]. Sinicrope *et al*[12,37] found that *KRAS* mutations were associated with adverse prognosis specifically in pMMR tumors, while Blons *et al*[38] showed that *KRAS* mutations conferred shorter DFS in patients with left colon primaries.

Patients with poor compliance may affect the accuracy of the results[39]. Cancer patients are generally expected to have higher adherence to treatment than other patients because they are highly motivated by the gravity of their disease[40,41]. However, studies have shown cancer patients to have similar adherence rates to patients with other diseases[42-45]. Treatment duration plays a role in adherence to the regimen: when medication is continued over a longer period of time, patients become less adherent[46].

For oral cytotoxic agents, which require close monitoring of side effects and regular patient visits. There is no gold-standard measurement, and all methods have limitations[42,47]. Previous studies of oral cytotoxic chemotherapeutic drugs have

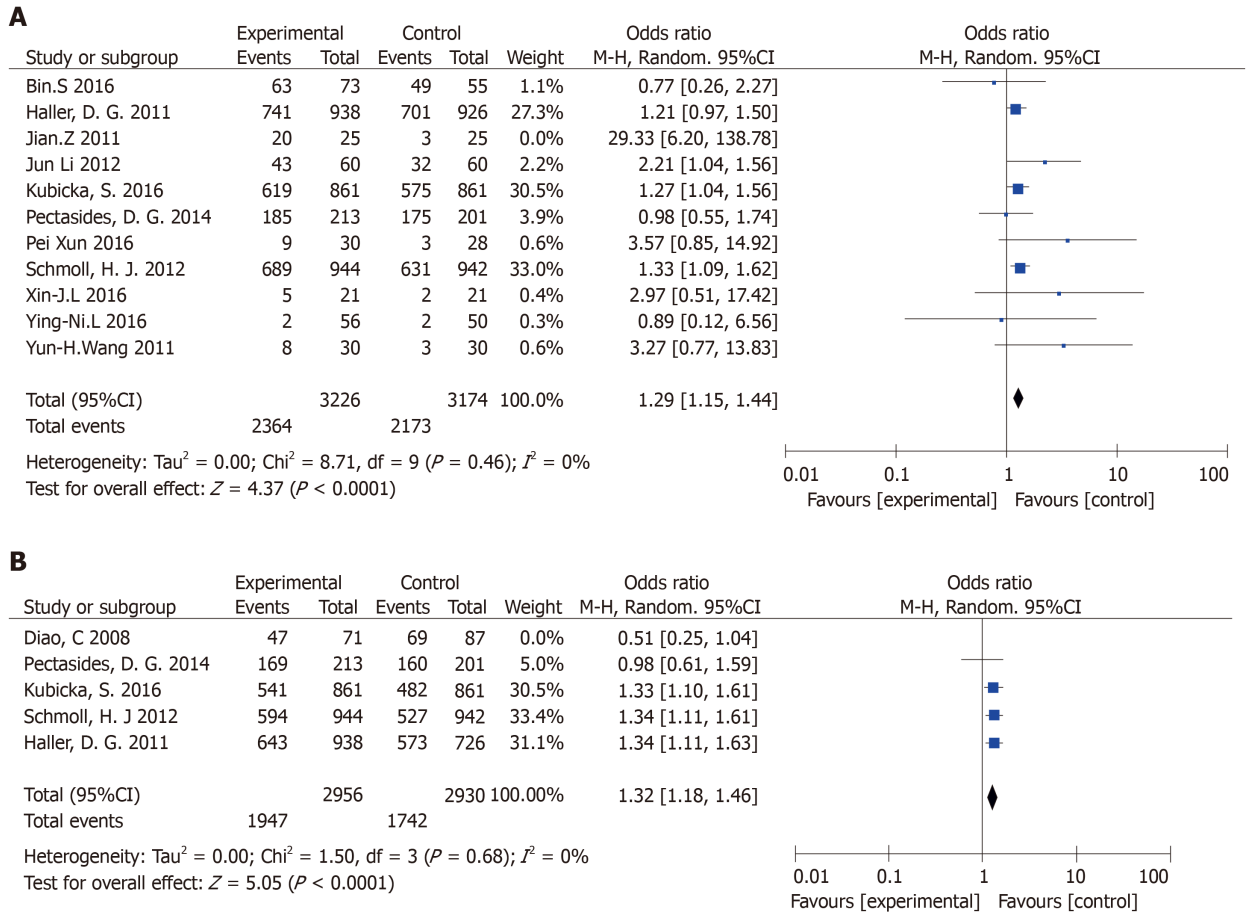


Figure 2 The combined analysis. A: The capecitabine plus oxaliplatin (XELOX) group had longer overall survival compared with other chemotherapy groups; B: The XELOX group had longer disease-free survival compared with other chemotherapy groups.

mainly used self-reported questionnaires[48], which tend to overestimate adherence because patients are inclined to over-report to please their doctors.

The side effects of commonly used adjuvant chemotherapy regimens FOLFOX is more serious[49]. The annoying neurotoxicity side effect of FOLFOX appears at the 8-10th cycle of administration. This time period is the critical time to gain or lose survival benefits. Although treatment series of fewer cycles showed some potential to ameliorate this neurotoxicity[50,51], recent studies failed to show any convincing benefit[52-55], even on a molecular basis[56]. It is still a challenge to be solved. Any "wait and go" policy to reduce side effects needs to be evaluated in a larger cohort of patients[57].

To our knowledge, many studies have indicated that monocyte count is associated with poor survival in patients with many types of cancer, but the potential mechanisms remain unknown[58]. Low monocyte to lymphocyte ratio (MLR) level may help improve the demographic and clinicopathological characteristics[59]. We found that low MLR, low monocyte and high lymphocyte were all associated with better prognosis in advanced gastric cancer patients. Meanwhile, our study indicated that low level MLR and low level neutrophil or high level lymphocyte correlated with better median DFS and OS for all patients. The 5-year DFS and OS rates of patients with low level MLR were higher than those with high level MLR[58,60-67]. We speculate that there may be similar mechanisms in CRC.

Nowadays, tumor molecular pathology assessment serves as a regular part of clinical practice. Treatment effect is unlikely uniform across different molecular subtypes. Molecular pathological epidemiology is an integrative science to determine the molecular pathology in relation to clinical features and outcome in patients and populations. Molecular pathological epidemiology will be a future direction for personal treatment[68,69].

In this study, some of the non-English articles were not included because of the low assessed quality. Secondly, a limited number of RCT trials and small number of included patients may limit the conclusion of this study. Finally, a diversity in genetics, tumor staging and XELOX dose may also influence the results. Therefore,

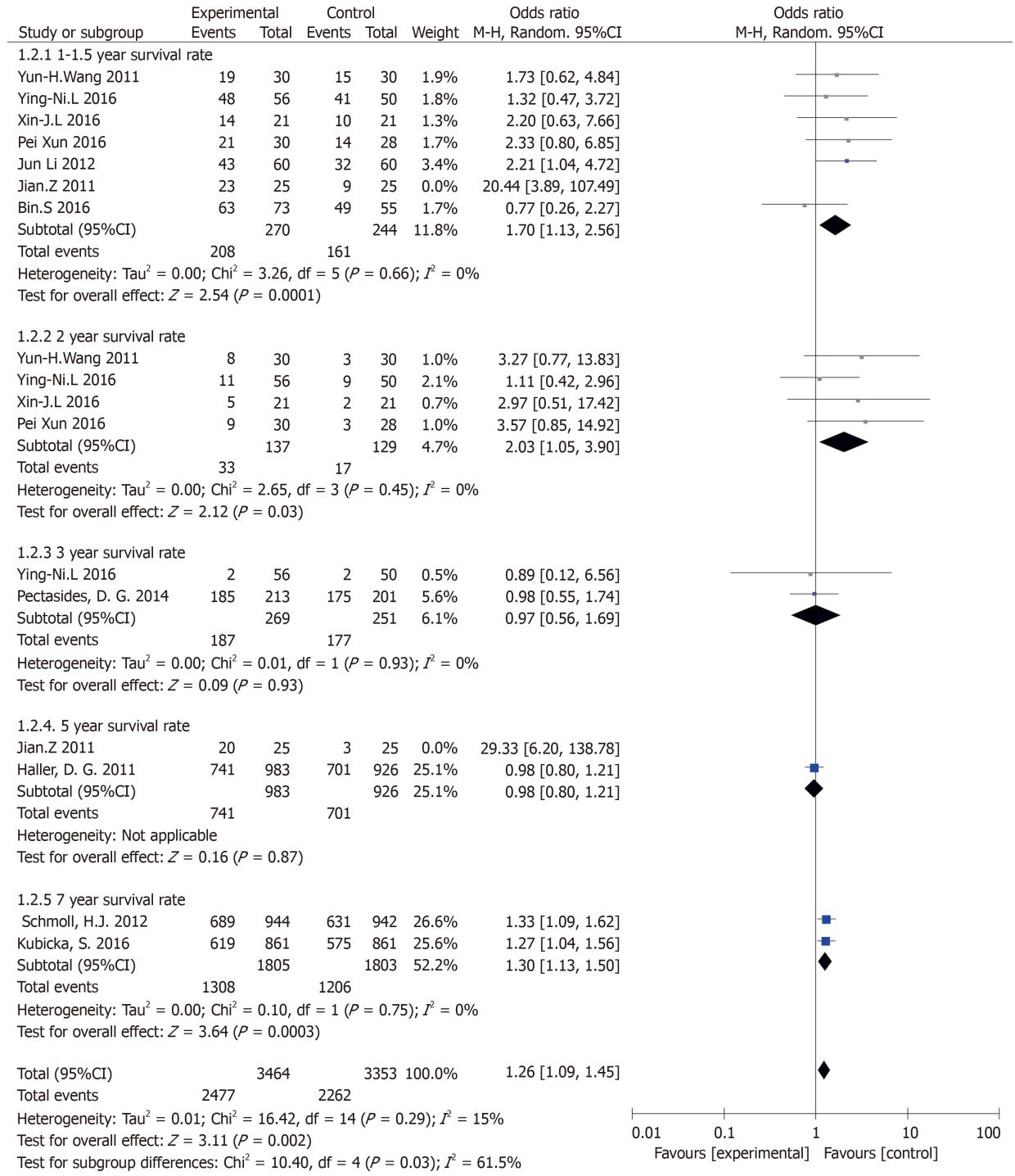


Figure 3 Results of the overall survival subgroup analysis (subgroup analysis of different follow-up visits).

our conclusion needs to be further validated by a large RCT trial in the future.

In conclusion, the XELOX regimen is recommended for stage III colon cancer after surgery.

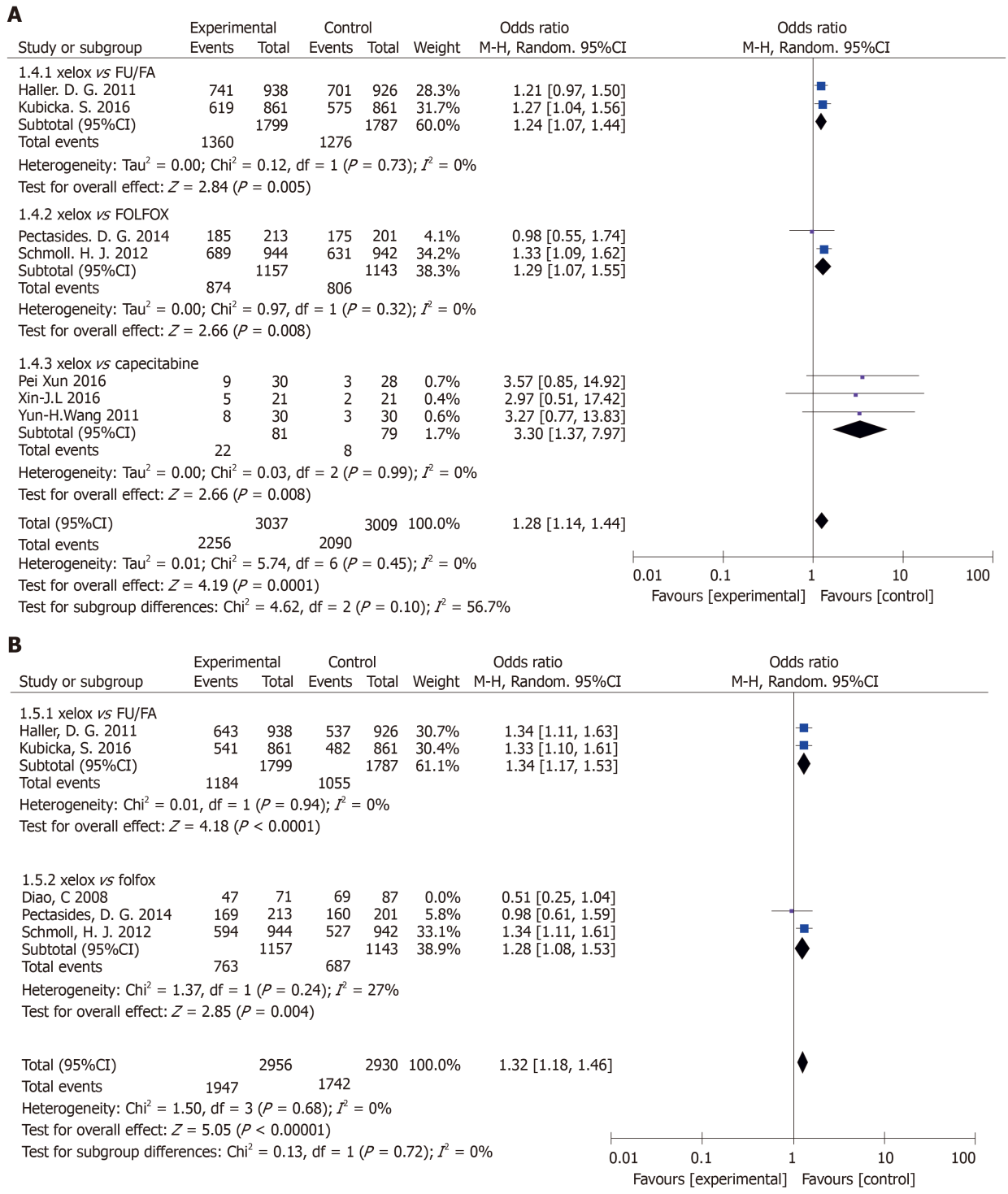
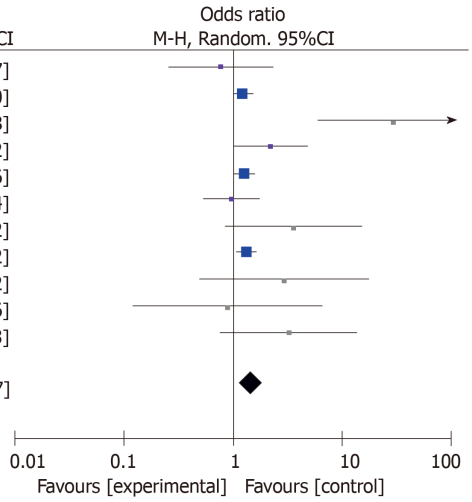


Figure 4 Subgroup analysis of overall survival and disease-free survival [capecitabine plus oxaliplatin (XELOX) *vs* other chemotherapy groups]. A: The capecitabine plus oxaliplatin (XELOX) group had longer overall survival compared with other chemotherapy groups; B: The XELOX group had longer disease-free survival compared with other chemotherapy groups.

A

Study or subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds ratio M-H, Random. 95%CI
Bin.S 2016	63	73	49	55	4.5%	0.77 [0.26, 2.27]
Haller, D. G. 2011	741	938	701	926	21.5%	1.21 [0.97, 1.50]
Jian.Z 2011	20	25	3	25	2.4%	29.33 [6.20, 138.78]
Jun Li 2012	43	60	32	60	7.8%	2.21 [1.04, 4.72]
Kubicka, S. 2016	619	861	575	861	21.8%	1.27 [1.04, 1.56]
Pectasides, D. G. 2014	185	213	175	201	11.1%	0.98 [0.55, 1.74]
Pei Xun 2016	9	30	3	28	2.8%	3.57 [0.85, 14.92]
Schmoll, H. J. 2012	689	944	631	942	22.1%	1.33 [1.09, 1.62]
Xin-J. L. 2016	5	21	2	21	1.9%	2.97 [0.51, 17.42]
Ying-Ni. L 2016	2	56	2	50	1.5%	0.89 [0.12, 6.56]
Yun-H. Wang 2011	8	30	3	50	2.7%	3.27 [0.77, 13.83]
Total (95%CI)		3251		3199	100.0%	1.45 [1.13, 1.87]
Total events	2384		2176			

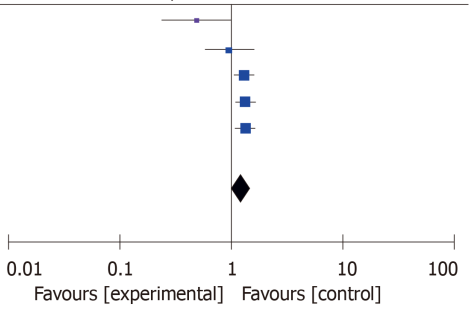
Heterogeneity: $Tau^2 = 0.07$; $Chi^2 = 24.20$, df = 10 ($P = 0.007$); $I^2 = 59\%$
Test for overall effect: $Z = 2.88$ ($P = 0.004$)



B

Study or subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds ratio M-H, Random. 95%CI
Diao, C 2008	47	71	69	87	4.9%	0.51 [0.25, 1.04]
Pectasides D. G. 2014	169	213	160	201	9.7%	0.98 [0.61, 1.59]
Kubicka, S. 2016	541	861	482	861	28.1%	1.33 [1.10, 1.61]
Schmoll, H. J. 2012	594	944	527	942	29.0%	1.34 [1.11, 1.61]
Haller, D. G. 2011	643	938	573	926	28.2%	1.34 [1.11, 1.63]
Total (95%CI)		3027		3017	100.0%	1.24 [1.04, 1.46]
Total events	1994		1811			

Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 8.09$, df = 4 ($P = 0.09$); $I^2 = 51\%$
Test for overall effect: $Z = 2.47$ ($P = 0.01$)



C

Study or subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds ratio M-H, Random. 95%CI
1.5.1 xelox *vs* FUFA						
Diao, C 2008	47	71	65	98	2.9%	0.99 [0.52, 1.90]
Haller, D. G. 2011	643	938	573	926	28.8%	1.34 [1.11, 1.63]
Kubicka, S. 2016	541	861	482	861	28.5%	1.33 [1.10, 1.61]
Subtotal (95%CI)		1870		1885	60.2%	1.32 [1.16, 1.51]
Total events	1231		1120			

Heterogeneity: $Chi^2 = 0.78$, df = 2 ($P = 0.68$); $I^2 = 0\%$
Test for overall effect: $Z = 4.09$ ($P < 0.0001$)

1.5.1 xelox *vs* FUFA						
Diao, C 2008	47	71	69	87	3.3%	0.51 [0.25, 1.04]
Pectasides D. G. 2014	169	213	160	201	5.4%	0.98 [0.61, 1.59]
Schmoll, H. J. 2012	594	944	527	942	31.1%	1.34 [1.11, 1.61]
Subtotal (95%CI)		1228		1230	39.8%	1.22 [1.03, 1.44]
Total events	810		756			

Heterogeneity: $Chi^2 = 7.42$, df = 2 ($P = 0.02$); $I^2 = 73\%$
Test for overall effect: $Z = 2.33$ ($P = 0.02$)

Total (95%CI)		3098		3115	100.0%	1.28 [1.15, 1.42]
Total events	2041		1876			

Heterogeneity: $Chi^2 = 8.69$, df = 5 ($P = 0.12$); $I^2 = 42\%$
Test for overall effect: $Z = 4.65$ ($P < 0.00001$)
Test for subgroup differences: $Chi^2 = 1.52$, df = 1 ($P = 0.47$); $I^2 = 0\%$

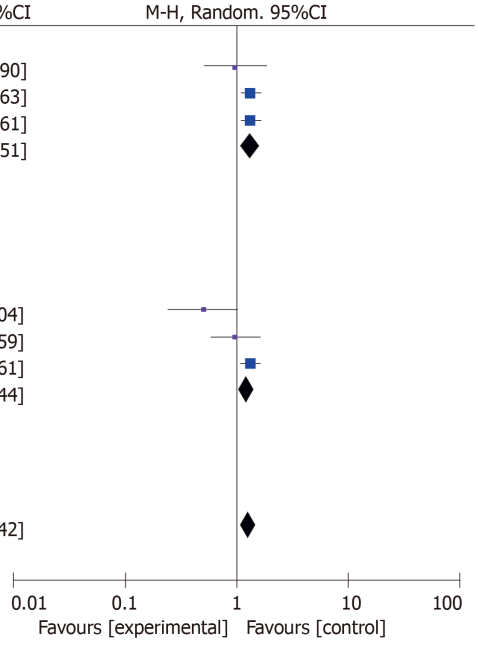


Figure 5 Process of deflection analysis. A: Overall survival (The capecitabine plus oxaliplatin (XELOX) group *vs* other chemotherapy groups); B: Disease-free survival (The XELOX group vs other chemotherapy groups); C: Subgroup analysis of disease-free survival (The XELOX group vs other chemotherapy groups).

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) accounts for 9% of all cancers in the world. In the last decade, it is the third most common malignant tumor in Europe and the United States. There is an urgent need to establish an effective standard treatment for CRC. In addition, more than 70% of CRC-related deaths are associated with the liver metastasis. A recurrence rate and poor overall survival make CRC a serious public health problem.

Research motivation

The aim of treatment for CRC is to cure locally and prevent metastasis and recurrence. Generally, comprehensive treatment is the focus of CRC, and chemotherapy is one of the important treatment methods. Reasonable and effective chemotherapy can prolong the life span and improve the quality of life of patients. Therefore, local resection of colon cancer should be combined with individual treatment. For patients with CRC, the choice of chemotherapy is very important for their prognosis. In patients with CRC, the purpose of adjuvant chemotherapy is to eliminate the occult micrometastasis during surgery, so as to improve the overall survival.

Research objectives

The purpose of this study was to explore the efficacy of capecitabine plus oxaliplatin (XELOX) regimen over other chemotherapy regimens, specifically XELOX *vs* 5-fluorouracil plus leucovorin, XELOX *vs* 5-fluorouracil plus leucovorin plus oxaliplatin, XELOX *vs* capecitabine and XELOX *vs* oxaliplatin plus 5-fluorouracil.

Research methods

By searching the PubMed, EMBASE and Cochrane databases, a total of 12 randomized controlled trials involving 6698 stage III colon cancer cases (XELOX protocol: n = 3298 cases; other adjuvant chemotherapy protocol: n = 3268 cases) were included. The parameter outcomes included the overall survival and the disease-free survival. The quality control of selected literature was based on the Jadad scale and the GRADE system.

Research results

In comparison to other adjuvant chemotherapy regimen, the XELOX regimen showed a better overall survival and a better disease-free survival for colon cancer patients.

Research conclusions

In clinical application, XELOX and 5-fluorouracil plus leucovorin plus oxaliplatin showed similar efficacy, but different types of patients may have different benefits from treatment. According to our data, in comparison to other adjuvant chemotherapy regimen, XELOX regimen showed a better overall survival and a better disease-free survival for colon cancer patients, suggesting the XELOX regimen can be a good option for postoperative treatment of stage III colon cancer.

Research perspectives

The XELOX regimen is recommended for stage III colon cancer after surgery. In addition, our conclusion needs to be further validated by a large RCT trial in future.

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OPINION REVIEW

European Specialty Examination in Gastroenterology and Hepatology examination — improving education in gastroenterology and hepatology

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Abstract

The Federation of Royal Colleges, the British Society of Gastroenterology (BSG) and the European Section and Board of Gastroenterology and Hepatology developed the European Specialty Examination in Gastroenterology and Hepatology (ESEGH) from the United Kingdom Specialty Certificate Examination, which was the original examination. Since 2018 the Specialty Certificate Examination and the ESEGH were combined into a single exam, identical across Europe and the rest of the world. The ESEGH is mandated in 4 countries (United Kingdom, Switzerland, The Netherlands and Malta) and the number of entries increased from 50 in 2014 to 490 in 2019. Candidates from countries were the ESEGH is not mandated are sitting the Exam, showing us they realize the enormous interest of holding a certificate for knowledge in their Curriculum. We also have an increasing number of candidates from countries

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outside Europe.

Key words: Examination; Specialty; Education; Gastroenterology; Hepatology; Curriculum

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Core tip: The examination is one of the largest Physician Specialty Examination in Europe, and is a four-way cooperation between Union of European Medical Specialties, The European Board of Gastroenterology and Hepatology, the British Society of Gastroenterology and the Federation of Royal Colleges of the United Kingdom. The United Kingdom GMC formally approved the European Examination in January 2018. The European Specialty Examination in Gastroenterology and Hepatology is mandated in 4 European countries and the increasing number of candidates in countries were not mandated is a measure of success.

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INTRODUCTION

The European Specialty Examination in Gastroenterology and Hepatology (ESEGH) Examination was first introduced in 2014, as a pilot programme based on the United Kingdom Specialty Certificate Examination (SCE) that was established in 2007. The ESEGH was developed from the United Kingdom SCE as the original examination. In the first sitting there were 50 entries (32 from Europe) for the European exam. In 2018 the SCE and ESEGH were combined into a single exam which was identical across Europe, the United Kingdom and the rest of the world, and in 2019 there were 490 entries (156 from Europe) (Figure 1).

This substantial increase in entries is due to the examination being mandated for trainees in several European countries and a significant increase in International candidates. The examination is one of the largest Physician Specialty Examination in Europe, and is a four-way cooperation between Union of European Medical Specialties (UEMS), The European Board of Gastroenterology and Hepatology, the British Society of Gastroenterology and the Federation of Royal Colleges of the United Kingdom. The United Kingdom GMC formally approved the European Examination in January 2018.

This paper describes how the quality and the governance of the examination are managed and discusses plans for future improvements.

EXAMINATION FORMAT

The ESEGH is a computer-based, multiple-choice single best answer test divided into two papers. Candidates are allowed three hours to answer each paper, each comprising 100 items. Each question presents a clinical scenario, with the results of some investigations and perhaps an image or scan, and tests medical knowledge and competency in diagnosis, investigation, management and prognosis. Candidates are asked to choose the best answer from five options. The test is taken on one day annually at independently operated assessment centres. These centres run in most countries.

ELIGIBILITY REQUIREMENTS

There are no entry requirements for the examination.

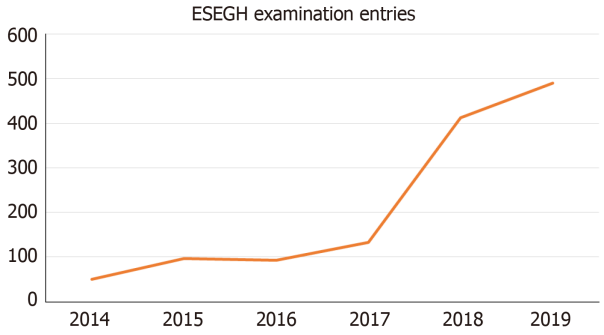


Figure 1 Annual entries to the European Specialty Examination in Gastroenterology and Hepatology. ESEGH: European Specialty Examination in Gastroenterology and Hepatology.

European Union (EU) candidates are encouraged to take the examination towards the end of their specialist training.

CURRICULUM AND KNOWLEDGE RESOURCES

Curriculum — The Blue Book - https://www.eubogh.org/blue-book/

The Blue Book, 2017, approved by the UEMS, defines the curriculum of Gastroenterology and Hepatology training for UEMS–affiliated countries. The curriculum, revised on a five yearly cycle, has a writing group composed of European Section and Board of Gastroenterology and Hepatology Examination (ESBGH) delegates and representatives from the United European Gastroenterology (UEG), European Association for the Study of the Liver and the European Junior Doctors committee.

Knowledge resources

Those recommended can be found in the website www.eubogh.org.

HOW TO PREPARE FOR THE EXAMINATION

Candidates are encouraged to plan their study in advance. As the field of gastroenterology and hepatology is rather vast, the subject material to be covered can be quite large. For a typical full-time trainee, it is recommended to start 3-4 mo prior to the exam.

In order to help guide their studies, candidates are encouraged to explore both the ESBGH and the Membership of the Royal Colleges of Physicians websites as they host invaluable information pertaining to the training curriculum (Blue Book) as well as resources, statistics and advice.

There are many possible resources available, from books to guidelines to journals to online websites. Overall, guidelines are possibly the best source of information as they are up to date with newly published data. When choosing which guidelines to study, it is recommended to pick those that are internationally recognized since the examination is European and not affiliated to a specific country. The major European societies, (such as UEG, European Association for the Study of the Liver, and BSG), have excellent material that is invaluable for learning. These include short videos, previous presentations and online classroom courses. Candidates are also encouraged to find resources that contain radiology, pathology or histology images and to familiarize themselves with their interpretation. The Post-Graduate Teaching Programme organized by UEG has a three year rolling cycle covering all major topics in the Blue Book.

It is particularly important that candidates are familiar with the subject areas of Nutrition, Functional Bowel Disorders and Infectious Diseases affecting the gastrointestinal tract and liver, as these subjects are not universally covered in all European National Curricula.

The exam is in the format of "Single Best Answer" multiple choice questions (MCQs). As there is a technique to answering the questions, which involves focusing on the important points in the stem and reading the question well, candidates should try to practice as many MCQs as possible. Mock MCQs can be found in dedicated books, various websites that contain question banks and the Membership of the Royal

Colleges of Physicians website. Practising MCQs will also give a good indication of the level of knowledge that is required and the level of English that is used.

The examination is computer based, held at a designated centre and hosted by Pearson VUE. Candidates can familiarize themselves with the navigation of the test using the following website: https://wsr.pearsonvue.com/demo/.

HOW ARE THE QUESTIONS WRITTEN?

The ESEGH is based on 200 single best answer questions, chosen to cover all the training curriculum of gastroenterology and hepatology, according to the European Training program, (The Blue Book). The numbers of questions in each area of gastroenterology and Hepatology is defined by the exam "blueprint" (Table 1).

The writing and choosing of these 200 questions is a multistep process involving three groups - the Question Writing Group, the Examining Board (EB) and the Standard Setting Group (SSG).

The members of these three groups are selected by the three examination partners. They are chosen according to their areas of expertise and are accredited members of the organizations who nominated them. Prior to working in the question writing and selection process, these experts undergo a mandatory training course arranged by the Federation of Royal Colleges.

The members of the Question Writing Group are tasked to submit 10-12 new questions each year, usually from their area of expertise. The format of these questions is strictly defined, in order to harmonize the writing style and question content. Negative questions, and questions where all, or none, of the options are correct, are not allowed.

The questions are edited at the Federation of Royal Colleges, following which there is an annual meeting, where all the questions are discussed by groups of question writers, to verify that they ask about areas of knowledge suitable for an examination whose aim is to certify that the passing candidate has achieved the knowledge necessary to fulfil the requirements of the ESBGH curriculum which is a Europe-wide description of the level expected of a specialist in Gastroenterology and Hepatology.

All questions are generated according to current international guidelines. Once the questions have been edited and accepted, they are stored in a question bank (which already includes around 2500 questions). If the questions are not accepted, they may go back to the author for revision or are simply rejected.

Following this process, the EB meets to decide which questions are selected for the next examination based on the examination blueprint. They review 70-80 questions (not of their authorship) and present them at the meeting, with a recommendation for accepting, review, send back for rewriting or rejection. Approximately 350 questions are reviewed at this meeting, in order to choose the final 200 for the exam.

This meeting is followed by the review of questions at the SSG, using the Angoff technique[1]. Here the group members evaluate the degree of difficulty of each question. Prior to the meeting each member rates all the questions regarding what percentage of just passing candidates should pass the specific question. At the SSG each question is discussed and the percentage re-evaluated. Again questions can be send back for rewriting or rejected. Within the group of examination there are between 30 and 40 question that have been used in previous exams for which data are available about their performance. These "anchor" questions are used to determine the performance of each cohort of candidates across different years.

The final group of 200 questions, will be compiled into 2 papers uploaded into the electronic delivery system, before a final review to ensure that they are correctly formatted and that the coded answers are correct, before being sat in the following year. After the examination has been sat, the "performance" of each question is reviewed in order to ensure that questions are fair. This final quality assurance step picks up questions where there is different clinical practise across countries, or where the evidence base or guidance has changed between development of the exam and the day it is taken. Such questions may be removed from the final mark. This usually occurs of 0-2 questions per year.

HOW IS THE PASS MARK DETERMINED?

The criterion-referenced pass mark

To produce a criterion-referenced pass mark, a mean score was calculated for each examiner. To calculate a final score for the paper, the mean of the examiners' mean scores was calculated. The process results in a pass mark that is usually 60% (+/- 2%)

Table 1 Examination blueprint

Candidates are tested on a wide range of common and important disorders as set out in the syllabus of the curriculum	
The composition of the paper is as follows:	
Topic	Number of questions[1]
Biliary tree	16
Gastrointestinal haemorrhage	10
Inflammatory bowel disease and colonic disorders	40
Liver disorders	40
Nutrition	10
Oesophageal disorders	16
Pancreatic disorders	16
Small intestinal disorders	20
Stomach and duodenal disorders	20
Other[2]	12
Total	200

[1]This should be taken as an indication of the likely number of questions – the actual number may vary slightly. The questions in each category are distributed across both papers.
[2]Other is made up of the following topics: (1) Mouth and salivary gland; (2) Endoscopy; (3) Gastrointestinal physiology; (4) Gastroenterology investigations; (5) Gastroenterology symptoms/signs; and (6) Anal disorders.

for each exam[2].

PERFORMANCE OF CANDIDATES – 2014-2019

The performance of all candidates is showed in Table 2. The performance of European candidates is presented in Figure 2. In Figure 3 we can see the results of 2 countries were the ESEGH is mandated and a third (Portugal) were trainees sat the ESEGH on a voluntary basis. In 2019 all trainees who finished their training that year sat the ESEGH.

COMMENT

The European Board Examination ran from 2014-2017, as a separate exam to the United Kingdom SCE, and the two merged into a single identical exam from 2018 onwards. Trainees have been mandated to take the examination in Switzerland since 2015, and the Netherlands since 2017. United Kingdom trainees, for whom the SCE had been the mandated exam since 2007, have been mandated to take the combined ESEGH since 2018. In Malta the ESEGH is also mandatory.

There have been rapid increases in the numbers of entries over the last two years, with a particular expansion of International candidates, notably from the Middle East and South Asia.

The examination fully complies with the CESMA criteria agreed by UEMS in 2015[3].

WHY SHOULD POTENTIAL CANDIDATES TAKE THIS EXAMINATION?

European postgraduate medical assessments will facilitate mobility of medical practitioners throughout the EU by assessing specialist knowledge at an agreed EU standard. Furthermore, European examinations will enable benchmarking of candidates against their European colleagues in other EU countries.

However, passing a European postgraduate medical assessment will not imply a licence to practise. It is only the National Certifying Authority (NCA) in each country that will be able to provide such a licence. Nevertheless, where full NCA recognition of a qualification applies, European postgraduate medical assessments may provide a means for the NCA to determine eligibility for a licence to practise.

As stated above, some countries have mandated passing of the examination, as a necessity to complete specialist training.

Table 2 Results (2014-2019)

Diet	Pass mark	Pass rate United Kinfdom trainees	Pass rate European centres	Pass rate all candidates
2019	59% (118/200)	78.7% (111/141)	71.2% (111/156)	59.2% (290/490)
2018[1]	60% (120/200)	83% (146/176)	73.9% (65/88)	67.3% (278/413)
2017	61.3% (122/199)	76.4% (110/144)	60.9% (81/133[1])	59.1% (146/247)
2016	60.8% (121/199)	82% (100/122)	68.1% (62/91[1])	65% (147/226)
2015	62% (124/200)	72.5% (87/120)	63.2% (60/95[1])	55.4% (128/231)
2014	62.24% (122/196)	76.2% (80/105)	64% (32/50[1])	53.5% (106/198)

The "total" number of candidates includes international doctor who take the examination outside of Europe and the United Kingdom.
[1]Candidates sitting in European centres are excluded from the total candidates in 2014-2017 data but included in 2018 when the Specialty Certificate Examination and European Section and Board of Gastroenterology and Hepatology Examination merged.

SUBJECT AREAS AND PERFORMANCE IN THE EXAMINATION

The main subject areas of the 200 questions have remained fairly constant since the examination's introduction and reflect the European Curriculum as described in the Blue Book, with approximately 40% of the questions covering liver or inflammatory bowel disease.

During the 6-year tenure of the examination, candidates have persistently performed less successfully on questions covering nutrition and gastrointestinal haemorrhage. Informal discussions with National Training Programme leads concerning these findings suggest that nutrition is not taught in some institutions, and trainees have less exposure to the acute care of patients with gastrointestinal bleeding than existed previously.

Pass rates of candidates taking the examination in European Centres have risen to over 70% in the 6-year tenure of the examination, with consistently higher rates in those countries where the examination is mandated and in countries where most of the eligible trainees sit the examination. It appears that structured training towards the examination leads to higher pass rates.

GENDER/AGE

Trainees in the younger age group, 30-34, have higher pass rates, presumably due to their being in active training programmes when sitting the examination. There are no gender differences in outcome of the examination.

THE FUTURE

Candidates in Europe and outside Europe are sitting the ESEGH in increasing numbers in countries where the ESEGH is not mandatory, as the examination is recognised as a benchmark of the knowledge of successful candidates.

The ESBGH hopes that more and more EU countries will mandate the examination for their trainees. Our cooperative work with National Associations and Scientific Societies supports the widespread adoption of the Blue Book as a reference European Curriculum for Gastroenterology and Hepatology and the next revision due in 2021, will integrate even further with the examination.

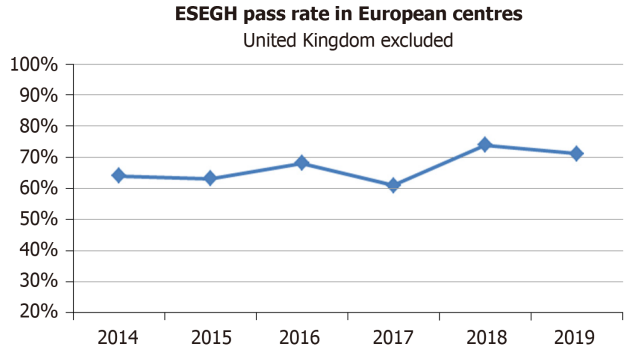


Figure 2 European Specialty Examination in Gastroenterology and Hepatology pass rate in European centres (United Kingdom excluded). ESEGH: European Specialty Examination in Gastroenterology and Hepatology.

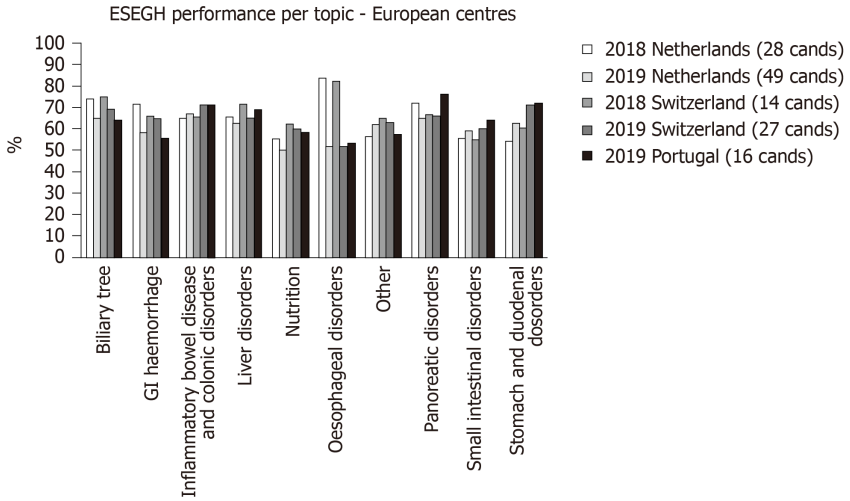


Figure 3 European Specialty Examination in Gastroenterology and Hepatology performance per topic in European centres (2018 and 2019). ESEGH: European Specialty Examination in Gastroenterology and Hepatology.

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OPINION REVIEW

Effectiveness and safety of sedation in gastrointestinal endoscopy: An opinion review

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Abstract

Although endoscopy is a less invasive procedure than surgery, patients can experience pain without sedation. Patients expect reduced pain during endoscopies from effective and safe sedatives. Midazolam and propofol are used for endoscopic sedation in many countries and regions. Midazolam is a widely available benzodiazepine, and many clinical trials have shown it to be an effective sedative. However, patients who are sedated with midazolam require rest in the recovery room due to its relatively long half-life, and an antagonist such as flumazenil may need to be administered in cases of deep or prolonged sedation. Propofol is a short-acting sedative with a short half-life and a quick recovery time. Therefore, the use of propofol has been increasing. However, propofol has a narrow margin of safety and often induces adverse effects such as respiratory depression. Also, propofol has no specific antagonist, and should be administered by an anesthesiologist or an endoscopist familiar with anesthesia. Remimazolam, which is a novel ultra-short-acting benzodiazepine, has recently gained attention. Remimazolam has a short half-life and an antagonist. Both effective and safe sedation is desired in accordance with the increasing need for sedative endoscopies. Therefore, in this review each sedative is summarized.

Key words: Gastrointestinal endoscopy; Conscious sedation; Propofol; Midazolam; Remimazolam; Benzodiazepine

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Core tip: The need for sedation during gastrointestinal endoscopies is ever increasing. Currently, benzodiazepines such as midazolam and the short acting propofol are the most commonly used sedatives for an endoscope. However, midazolam requires the patient to have an extended recovery period and in cases of a deep sedation an antagonist administered. Although short acting, propofol must be administered by an anesthesiologist due to its potential side effects and does not have an antagonist. Remimazolam is ultra-short acting and has both a short half-life and if required an

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antagonist. In this review we discuss the advantages and disadvantages of each sedative.

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INTRODUCTION

Recently, the advances in gastrointestinal endoscopy is remarkable. Gastrointestinal endoscopy has been applied to not only endoscopic diagnoses of gastrointestinal tract cancers including stomach, esophagus and colon, but also endoscopic treatment for gastrointestinal diseases such as endoscopic submucosal dissection and endoscopic retrograde cholangiopancreatography[1-4]. The need for endoscopic treatment for gastrointestinal disease has been gradually increasing, because this is a less invasive procedure than surgery. Sedation during endoscopic treatment is essential considering its time consumption. Although endoscopic examination is less invasive and less time-consuming than endoscopic treatment, patients often experience pain and discomfort without sedation. Conscious sedation is considered effective for gastrointestinal endoscopic examinations[5,6]. Both patient satisfaction and re-examination compliance are superior in those patients who received sedation compared to those who did not have sedation[7,8]. Only 65% of patients who underwent upper gastrointestinal endoscopy without sedation were willing to have a repeat examination. Effective sedation is not only important for pain reduction in patients, but also for endoscopists to facilitate a successful examination[9]. In elderly patients or patients with various comorbidities, the risk of adverse events associated with sedation is increased[10]. Sedation is required not only to increases the completion rates of endoscopic examinations and reduces pain in patients, but also to create a safer system.

CURRENT STATUS IN THE USAGE OF SEDATIVES

Although sedation during endoscopic treatment is essential, the use of conscious sedation, without tracheal intubation, during endoscopic examination varies greatly by country and region, depending on the endoscopic facilities or insurance systems. Sedation is used during 98% of upper gastrointestinal endoscopies and colonoscopies in the United States, and in 90% of these procedures in Canada[11-13]. While in Europe, the use of sedation during endoscopy was lower than in the Unites States and Canada. However, its rate is gradually increasing. Sedation during endoscopy is already standard practice in Italy[14]. In Greece, the rates of sedation are 64% in upper gastrointestinal endoscopies and 78% in colonoscopies[15]. In Germany, sedation is used in 74% of upper gastrointestinal endoscopies and 87% of colonoscopies[16]. In Japan, the rate of sedation during endoscopy has been increasing. Sedation is used during upper endoscopies in up to 75% of 544 institutions. Sedation will be increasingly required during endoscopic examinations in the future.

Midazolam, which has the shortest half-life out of all of the conventional benzodiazepines, is administered either alone or with opioids such as fentanyl or pethidine in many countries. Recently, the use of propofol has been increasing because endoscopists are satisfied with its rapid onset of action and quick recovery time.

MIDAZOLAM'S CHARACTERISTICS AND CLINICAL TRIALS

Midazolam, as well as other benzodiazepines, enhances the effect of gamma-aminobutyric acid, a suppressive neurotransmitter in the central nervous system, at its receptor. Therefore, hypnotic, sedative, anxiolytic, amnesic, anti-convulsive, and muscle-relaxing effects are achieved. The active duration of midazolam is the shortest (2-6 h) out of all the conventional benzodiazepines, which is metabolized *via* the liver. Severe adverse effects include respiratory depression, hypotension, and bradycardia

depending on the dosage. There is no vascular pain at the time of intravenous injection[17].

Various clinical trials have been conducted on the use of midazolam for endoscopic sedation. A randomized controlled trial that compared endoscopies using either midazolam or a placebo, showed that the midazolam group had higher rates of procedural success, patient satisfaction, and compliance with re-examination. There was no significant difference in the rate of severe hypoxemia between the two groups[7,18].

Benzodiazepines are sometimes used in combination with analgesics such as fentanyl and pethidine hydrochloride. A randomized controlled trial that compared endoscopies using a benzodiazepine alone and a benzodiazepine in combination with an analgesic, showed that there were no significant differences in the patient satisfaction or cardiorespiratory depression between the two groups, but a higher endoscopist satisfaction was achieved in the benzodiazepine combined with an analgesic group[19,20]. However, unpredicted deep sedation was reported to occur in the benzodiazepine combined with an analgesic group[21]. The combination of a benzodiazepine and an analgesic might be limited for elderly patients or those patients with comorbidities.

PROPOFOL CHARACTERISTICS AND CLINICAL TRIALS

Propofol is a short-acting intravenous anesthesia, which is a phenol derivative that easily crosses the blood-brain barrier due to its high fat solubility. Rapid induction and a short recovery time can be achieved with propofol compared with conventional Benzodiazepines. Propofol's sedative effect can be achieved within 30-60 s after administration. The half-life of propofol in the blood is 1-4 min. A 15%-30% reduction in systolic blood pressure as a cardiovascular side-effect of this drug occurred in 53% of patients following induction of anesthesia with propofol because of cardiovascular depressant and dilation of the peripheral vessels[22].

Various randomized controlled trials have been conducted to compare sedation using a benzodiazepine such as midazolam, and propofol[23-26]. Although patient satisfaction differed from report to report, the recovery time from sedation was shorter in the propofol groups than in the benzodiazepines groups in all reports. The recovery time with propofol was significantly shorter than with midazolam, and without the increase of cardiorespiratory adverse effects according to a meta-analysis[27-29].

Several studies have shown the efficacy and safety of propofol compared with the benzodiazepines, regardless of whether an anesthesiologist or gastroenterologist (non-anesthesiologist) administered the propofol sedation[30,31]. However, propofol has a narrow range between sedation and anesthesia, therefore sometimes adverse effects can be induced such as respiratory and cardiac depression. Furthermore, there is no specific antagonist for propofol[32,33]. The written information inside the packaging of propofol cautions that sedation with propofol should be inducted by an anesthesiologist or independent physician familiar with anesthesia for safety, and that patients should be carefully monitored until they have completely recovered. Although the American Gastroenterological Association proposed to remove this caution, this proposal was rejected by the Food and Drug Administration.

WHICH DRUG IS MORE EFFECTIVE FOR SADATION MIDAZOLAM OR PROPOFOL?

An endoscopic examination is a relatively short procedure and is usually conducted without admission to the hospital. Endoscopists must conduct many endoscopic examinations per day, while responding to the needs of patients to eliminate pain. Therefore, sedatives that are safe, have a rapid onset of action, rapid recovery, and have an antagonist are desired.

Due to Midazolam's half-life, recovery takes approximately 30 min-1 h after the endoscope. Patients can usually return home once they are able to walk unaccompanied. There are various medical costs associated with endoscopic sedation such as securing a large recovery space in the hospital and medical staff to watch over patients. Flumazenil acts as a midazolam antagonist and is sometimes used to reduce recovery time in patients who are either in a deep or prolonged sedation. However, re-sedation might occur after reversing sedation because the active duration of flumazenil (approximately 50 min) is shorter than that of Midazolam, and some active metabolites profoundly contribute to the sedative profile of midazolam[34,35].

However, appropriate monitoring and observation of those sedated with propofol is required because it has a narrow therapeutic range with the potential to cause cardiorespiratory depression. Although propofol acts *via* gamma-aminobutyric acid receptors, similar to the benzodiazepines, there is no antagonist for propofol unlike the benzodiazepines. Lack of an amnestic effect and pain during administration is a disadvantage of propofol sedation. Furthermore, at the time of writing propofol can only be administered by an anesthesiologist or independent physician familiar with anesthesia.

For the above reasons, propofol is currently only used in institutions where independent anesthesiologists or endoscopists can administer the drug and monitor the patient's sedation. In other institutions, midazolam is used as a sedative.

REMIMAZOLAM: A NEW SEDATIVE

Remimazolam is an ultra-short-acting intravenous novel benzodiazepine sedative, with a shorter half-life (approximately 40 min) compared with other conventional benzodiazepines. It can rapidly pass through the blood-brain barrier and provide a rapid effect because it is a fat-soluble drug. Remimazolam is rapidly metabolized by carboxylic acid elastase, which does not involve the liver enzyme CYP3A4, and shows organ-independent metabolism. Its organ-independent metabolism makes it less likely to impair liver and kidney function. Remimazolam's metabolites are pharmacologically inactive. Therefore, the adjustability of remimazolam seems to be superior to Midazolam.

Remimazolam is safer than propofol because it can be reversed with flumazenil to rapidly terminate sedation, similar to other benzodiazepines if necessary. Furthermore, remimazolam's half-life is as short as flumazenil's and therefore, there is low risk of re-sedation unlike other benzodiazepines.

In a phase IIa study conducted in the United States, the induction times from drug administration to sedation was 1.5-2.5 min in remimazolam (0.10-0.20 mg/kg) and 5 min in midazolam (0.075 mg/kg) in upper gastrointestinal endoscopies[36]. The time to recover from sedation (3 consecutive Modified Observer's Assessment of Alertness and Sedation scores of 5) was significantly shorter in remimazolam than that in midazolam (6.8-9.9 min *vs* 11.5 min, respectively). These results suggest that remimazolam had a faster onset of action and a faster recovery time after endoscopic examination/treatment compared with midazolam.

In a phase IIb study conducted in the United States, remimazolam (5.0 mg, 7.0 mg, and 8.0 mg) achieved a lower rate of additional administrations compared with Midazolam (2.5 mg) during colonoscopies[37].

Similarly in a phase III study conducted in the United States, remimazolam (5 mg) achieved a higher procedural completion rate without the requirement for additional fixed doses (5 doses in any 15-min interval), compared with placebo (5 doses in any 15-min interval) and midazolam (3 doses in any 12-min interval; aged < 60 years, 1.75 mg; aged ≥ 60 years, 1.0 mg) in outpatient colonoscopies[38].

All studies have suggested that remimazolam was as safe as Midazolam as a sedative for gastrointestinal endoscopy. Remimazolam, has the combined advantage of a short half-life, similar to propofol and an antagonist like midazolam. Furthermore, it can be managed by non-anesthesiologists. Therefore, remimazolam may increasingly be used as a sedative for gastrointestinal endoscopies.

The use of Remimazolam in clinical practice remains insufficient. New issues might arise after clinical administration. To the best of our knowledge, there are no studies comparing the clinical outcomes of remimazolam with propofol. There is a lack of clinical data on Remimazolam. Various additional clinical studies to improve the efficacy and safety of remimazolam as a sedative for endoscopic procedures is desired in the future. Preparation for clinical trial for insurance coverage are currently in progress in Japan.

CONCLUSION

Conscious sedation, without tracheal intubation, during endoscopy differs greatly depending on the country and region. Endoscopic examination using sedation should be safely completed without pain. Currently, benzodiazepine sedatives and propofol are the predominant drugs administered during endoscopic examinations. Propofol might be useful for patients in countries or regions with sufficient anesthesiologists. While a novel benzodiazepine sedative, remimazolam, could be a desired option in countries or regions without adequate numbers of anesthesiologists to attend

endoscopic procedures.

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REVIEW

Probiotics in inflammatory bowel disease: Does it work?

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Abstract

The number of patients with inflammatory bowel disease (IBD), a group of diseases mainly represented by Crohn's disease (CD) and ulcerative colitis (UC), has increased in recent decades. As a consequence, the number of people undergoing any drug treatment against these diseases has expanded. However, IBD conventional therapies present several limitations, which lead researchers to look for better alternatives to improve the quality of life of patients. Moreover, microbiome imbalance seems to play a crucial role in the pathogenesis of IBD, since important alterations in bacterial, viral, protist and fungal populations are observed in the gut microbiota of affected individuals. Given the importance of such life forms in that context, the use of probiotics becomes a plausible alternative for treating affected patients. Trials have been developed aiming the evaluation of probiotics potential to induce and to maintain remission in CD and UC. Regarding the tested microorganisms, various non-pathogenic bacteria and fungi have been assessed. However, consistent results have been obtained only with some of them, including *Escherichia coli* Nissle 1917, VSL#3, *Saccharomyces boulardii*, *Lactobacillus*, and *Bifidobacterium*. Therefore, this minireview aims to explore the role of microbiota in the genesis of such a disorder and to compile the most concrete data on probiotic-related efficiency in IBD treatment.

Key words: Inflammatory bowel disease; Probiotics; Crohn's disease; Ulcerative colitis

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Core tip: The clinical management of ulcerative colitis and Crohn's disease represent a major challenge in the gastroenterology field since conventional therapies present several limitations. Interestingly, changes in gut microbiota are linked to the development of these diseases. In this sense, the use of probiotics becomes a plausible alternative for treating affected individuals. Although several microorganisms have been tested for this purpose, satisfactory results have been obtained only with a portion of them. Therefore, this minireview aims to explore the role of microbiota in the pathogenesis of

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inflammatory bowel disease and to compile the most concrete data on probiotics efficiency in its treatment.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic diseases that significantly affects patients quality of life and is mainly represented by Crohn's disease (CD) and ulcerative colitis (UC)[1]. Although IBD pathophysiology is widely studied and intestinal microbiota seems to play a crucial role in this process, there are still several unclear points about that[2]. However, it is well known that the existence of positive first-degree relatives for these diseases, as well as environmental exposures including psychological stress, antimicrobial use, and dietary factors, are risk factors for IBD development[3-5].

More than 3.6 million people are estimated to be affected by IBD across the globe, though data scarcity from some regions hinders this calculation[6,7]. In addition, recent studies show that its prevalence has risen in recent decades, with an increase of 75% and 60% in the number of UC and CD patients, respectively, in North America and Europe over the last 20 years[7]. This data becomes even more important if we consider the significant negative impacts caused by these diseases in the quality of life of affected individuals, which include social, professional, sexual, self-esteem and functional prejudices[8,9].

Furthermore, current IBD therapy represents an important economic burden to health systems as it is considered one of the most expensive treatments in the gastroenterology field[10]. Besides that, conventional therapeutic options for IBD also present several limitations regarding the adverse effects associated with their use. Such negative points have motivated researchers to look for better alternatives aiming the clinical control of these diseases, and, in this sense, probiotics emerge as a new option, although there is still limited evidence supporting their use[1,11].

According to the World Health Organization, probiotics are "live organisms which when administered in adequate amounts confer a health benefit on the host"[12]. In this framework, the beneficial effects provided by these agents to IBD patients could arise from various mechanisms that potentially promote attenuation of bowel inflammatory activity, such as antimicrobial properties, immune modulation, and improvement of intestinal barrier integrity[13,14].

Various probiotics have been tested in IBD. However, satisfactory effects were observed only with a portion of them, including *Escherichia coli* Nissle 1917, VSL#3, *Saccharomyces boulardii*, *Lactobacillus*, and *Bifidobacterium*[15-18]. In this context, our study aims to review the main theories about the role of microbiota in IBD pathophysiology and to gather the most consistent results on probiotic-related effectiveness in the treatment of that condition.

NORMAL GUT MICROBIOTA

The current evidence show that the intestinal microbiota is influenced by various factors and can vary between individuals and even be contrasting in different gastrointestinal areas[19]. Although the complete elucidation of the gut microbiota composition is challenging, it is well established that Bacteroidetes and Firmicutes are its main constituents[20]. It is believed that there is a relationship of commensalism between most microorganisms of the gastrointestinal tract (GIT) and host. Whereas the first ones benefit from the nutrients found in GIT environment, the second one takes advantage from important functions performed by the microbes[21].

Among these functions, we highlight the metabolization of nutrients - such as carbohydrates, lipids, and K and B vitamins[22-26], the protection against pathobionts - producing acids, thickening the protective wall and inducing production of immunoglobulins[27], and the immunomodulation of the innate and adaptive systems[28]. Besides that, the relationship between gut microbiota and human health

has been widely discussed, not only in the gastroenterology field, but also when the elucidation of pathological manifestations outside GIT, such as allergic processes and neurodegenerative manifestations, is aimed[29,30].

GUT MICROBIOTA IN IBD

The role of gut microbiota in the pathogenesis of IBD has been extensively discussed. Although intestinal microbiota is mainly represented by bacteria, researches have also highlighted the importance of viruses, fungi, and protists in that process (Figure 1)[31-33]. Moreover, there is no consensus on whether the changes observed in the microbiota of IBD patients are causes or consequences of the disease.

Bacterial role

Besides Bacteroides and Firmicutes, Actinobacteria and Proteobacteria phyla make up the group of the most common bacteria in human gut[31,34-38]. However, nowadays, it is being questioned whether this is a pattern among all individuals or whether factors such as genetic susceptibility and inheritance factors can change this profile and facilitate the occurrence of IBD[39].

The decrease of Bacteroides and Firmicutes phyla, as well as the increase of Proteobacteria and Actinobacteria, stand out as the main alterations in the microbiota from feces and intestinal mucosa of affected individuals[40,41]. Furthermore, the abnormal presence of pathogenic microorganisms might also contribute to the above-mentioned imbalance and to IBD emergence, since *Mycobacterium avium paratuberculosis, Salmonella, Campylobacter*, and *Fusobacterium nucleatum* have been positively associated with IBD[42-45]. Adherent-invasive *E. coli* is also supposed to be implicated in IBD pathogenesis, and recent evidences show that its presence not only propitiates the occurrence of IBD but also seems to predispose relapses in affected patients[46]. In addition, studies have correlated IBD relapses to *Clostridium difficile* infection[47]. Ultimately, Enterobacteriaceae and *Streptococcus* might also play a role in dysbiosis and further pathogenesis of IBD[48,49], with positive experimental trials for this relation[50].

Few studies evaluated the protective role of gut microbiota against IBD. Presti *et al*[51] suggest a protective role of *Akkermansia muciniphila*, since IBD patients had a lower presence of this species when compared to control and irritable bowel syndrome groups. Moreover, decreased abundance of *Faecalibacterium prausnitzii* in IBD has also been reported[51,52]. It is important to be highlighted that there is also a difference in the composition of the microbiome of IBD patients when comparing active and quiescent phases of the disease[53]. In view of the foregoing, it is conclusive in this topic that not only one, nor a few, but many bacteria can be related to IBD manifestations.

Viral role

The gut virobiota, unlike the bacterial microbiota, is not well described, neither in healthy individuals nor in IBD patients. It is known that the human gut virome is composed of eukaryotic viruses (*e.g.*, herpesviruses, adenoviruses) and prokaryotic viruses (*e.g.*, Microviridae and Caudovirales). However, many of them are not yet described, since there is a lack of studies on this area[54,55]. A study from 2016 aimed to identify the components of healthy human gut virome, which were divided into three different groups: the core, the common and the unique. The first group contains viruses found in more than half of the analyzed individuals, the second one is composed of species shared by many of the individuals, and the last one includes those found in a limited number of individuals. Drawing attention to the first group, it was noticed that the 23 bacteriophages that composed it were significantly reduced in IBD patients, bringing up the discussion that these common bacteriophages could have an important role in the pathogenesis of UC and CD when reduced[56].

Furthermore, differences have been observed between the gut virobiota of CD and UC patients. An increase of virobiota abundance in UC patients–mainly of *Caudovirales* bacteriophages-was reported by Zuo *et al*[57], with a concomitant identification of decreased viral diversity. Among CD patients, Pérez-Brocal *et al*[58] also observed a dysbiosis in virobiota, with abundance of phages that infect *Clostridiales, Alteromonadales*, and *Clostridium*. It was also detected a high abundance of the Retroviridae family in individuals with IBD.

Fungal and protist microbiota role

Although mycome represents only 0.1% of the human gut microbiome, a study from 2017 demonstrated that it presents a significant variability between healthy and IBD positive individuals. In the latter, a higher presence of *Candida albicans* and a lower

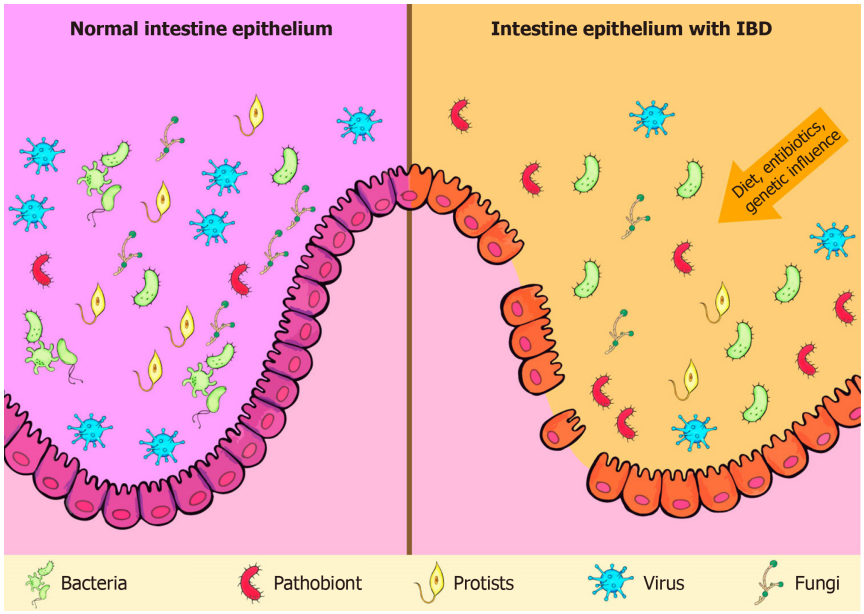


Figure 1 Comparison between normal intestinal microbiota and intestinal microbiota with inflammatory bowel disease. IBD: Inflammatory bowel disease.

presence of *Saccharomyces*, when compared to the control group, was described. Furthermore, the fungal diversity was reduced in the IBD[59]. Tests on animal subjects also corroborates this theory, as mice treated with antifungal drugs presented a higher incidence of acute and chronic colitis when compared to control groups[60].

Regarding the protist microbiota, it is described that the presence of such microorganisms can represent a protective factor against IBD. Comparing the protozoans found in the feces of healthy and IBD positive patients, the latter presented a reduced number of *Blastocystis*, suggesting that it might play a role in the balance of human healthy gut environment[61].

Microbiota, age and IBD

Concerning individual characteristics that may facilitate the occurrence of IBD, incidence peaks in certain ages (around 25 years old and close to 60 years old) evoke a discussion about what changes in these periods predispose the occurrence of the first episodes of CD and UC. Interestingly, these stages of life are moments in which the microbiome undergoes significant alterations. The first peak is marked by the host adaptation to new microorganisms in intestinal microbiota, while, in the second one, a global decrease in these life forms is observed in the human gut[62].

Immune response in IBD

In the last decades, the scientific community has increasingly investigated the role of host-microbial interactions in the human body immune regulation. Taking into consideration the gastrointestinal scenario, gut microbiome has been described as an integrating system that regulates the intestinal metabolism by means of environmental, genetic and immunological interactions. Therefore, it is expected that disturbances in such an important regulator can lead to complex diseases[63]. Indeed, the normal development of the immune system in the intestine have shown to be directly associated with adequate bacterial colonization during the early life and, in line with that, the result of a study indicated that germ-free mice present deficiencies in their immune functions[64,65]. It is also known that T and B immune cells from the intestinal mucosa play a crucial role in maintaining immune homeostasis, suppressing responses to non-pathogenic antigens and reinforcing the integrity of the intestinal mucosal barrier functions[66]. Among specific mechanisms through which bacteria influence immune response, it has already been observed that segmented filamentous bacteria induce the production of interleukin (IL)-17 and IL-22, which present a pro-inflammatory function[67]. Moreover, a series of 17 bacterial species have shown their potential to stimulate the expression of regulatory T cells and IL-10, which are associated with anti-inflammatory activity[68].

Regarding IBD, recent studies have described that it results from chronic intestinal inflammation which is due to a dysregulation in the expression of pro-inflammatory and anti-inflammatory molecules from the innate and adaptive responses of the

intestinal immune system[69]. As an example, a study that evaluated 66 children with early onset IBD found a loss of function in the genes that encode and regulate IL-10 and the IL-10 receptor in those patients, what leads to deficient anti-inflammatory function in the gut environment, favoring the appearance of intestinal diseases[70]. Furthermore, other studies indicate that there is probably an important increase in the expression of pro-inflammatory cytokines in IBD, such as IL-1, IL-6, IL-18, TNF, IL-12, and IL-23, by antigen presenting cells, neutrophils, monocytes, and macrophages[71]. Given the importance of those molecules in IBD, therapeutic alternatives targeting them have been tested. Among which, anti TNF-α agents stand out since they present satisfactory effectiveness in the treatment of ulcerative colitis, being included in the current guidelines for IBD treatment[72]. In summary, the literature has not yet completely understood the role of immunopathogenesis in IBD. In addition, most available data are from association studies and from researches that evaluate molecules expression in patients that already manifested IBD, what impairs the understanding of the immunological phenomenons that occur during the onset of the disease.

CONVENTIONAL IBD TREATMENT

Besides the significant negative impacts caused by the disease on the quality of life of patients, important economic impact is generated by IBD treatment, as it is considered one of the most expensive therapeutics in gastroenterology field[73]. Some guidelines have been published over the years to standardize and to guide IBD treatment[74,75]. The current consensus about this issue aim to improve the symptoms and quality of life of individuals, as well as to reduce the risk of complications and surgical interventions. Moreover, the immediate therapeutic target is the induction of clinical remission of the disease and, subsequently, its maintenance[76,77].

Mesalazine, corticosteroids, immunosuppressive drugs, and monoclonal antibodies targeting TNF-α are some of the IBD therapeutic options, which are arranged along with their main adverse effects in Table 1. Some drug classes are used in both CD and UC management, and the therapeutics of this last condition significantly varies according disease activity and extent[78-80]. Furthermore, new research is being conducted on the incorporation of new corticosteroids, biosimilars, TGF-beta, immunomodulators, anti-TNF agents, and even intestinal microbiota manipulation in the treatment of affected individuals[81].

It is well established that corticosteroid therapy with prednisone, methylprednisolone or budesonide is indicated in the induction of CD remission[82,83]. However, such therapies present important limitations due to their adverse effects, that include cosmetic effects such as acne and moon face, as well as other multisystemic repercussions associated with a prolonged therapy from which stand out posterior subcapsular cataract, osteoporosis, and a higher susceptibility to infections[77,84,85]. Moreover, the abstinence to these drugs is associated with acute adrenal insufficiency, arthralgia, increased intracranial pressure and pseudo-rheumatism syndrome[86]. Budesonide may have fewer side effects when compared to other corticosteroids, but its use is not recommended in severe CD or exacerbations[87].

Clinical trials with antibiotic therapy generally use ciprofloxacin, metronidazole, rifaximin, clarithromycin, and antituberculosis regimens combined or not with steroids or immunosuppressants[88]. Those therapies are often suitable for infectious complications, especially in perianal disease[89]. Adverse effects of the main antibiotics used include photosensitivity, tendinitis, tendon rupture, cartilage growth inhibition in fetuses and children, oral candidiasis, gastrointestinal disorders and may cause peripheral neuropathy[90-92].

Although used in UC, aminosalicylates were initially considered effective in the treatment of mild CD. However, current meta-analyzes have not observed action in preventing relapse with sulfasalazine and mesalazine[93]. Blood dyscrasias are more frequent in use of the first one, whereas nephrotoxicity and pancreatitis are more common adverse effects when the latter treatment is chosen[94,95].

In active CD, the use of anti-TNF therapeutic strategy is effective. In this sense, adalimumab, infliximab and certolizumab are used in both induction and maintenance protocols of CD and UC[77,96]. Infection represents the worst adverse effect on anti-TNF use and, if it occurs, its use shall be suspended due to the risk of septicemia development[97]. Therefore, any presentation of systemic symptoms suggestive of infection in patients under that therapy demand the exclusion of opportunistic infections[98].

Thiopurines are represented by azathioprine or mercaptopurine and they may be used as an adjunctive treatment[99]. The efficacy of this drug class in inflammatory

Table 1 Treatments used in inflammatory bowel disease and their side effects

Classes	Adverse effects	Ref.
Aminosalicylates	Mesalazine-nephrotoxicity and pancreatitis; sulfasalazine-blood dyscrasias	[10,11]
Antibiotics	Photosensitivity, tendonitis, tendon rupture, cartilage growth inhibition in fetuses and children oral candidiasis, gastrointestinal disorders, peripheral neuropathy	[14-16]
Corticosteroids	Acne, moon face and edema, sleep and mood disorders. Posterior subcapsular cataract, osteoporosis, myopathy, and susceptibility to infection. Acute adrenal insufficiency, arthralgia, increased intracranial pressure and pseudo-rheumatism syndrome	[7,20-22]
anti-TNF	Septicemia	[28]
Thiopurine	Hepatotoxicity, gastric intolerance and pancreatitis	[32]
Methotrexate	Nausea, vomiting and diarrhea	[34]

bowel disease is already evidenced by important studies and it is used for both induction and remission of CD[100]. Its main adverse reactions are hepatotoxicity, gastric intolerance and pancreatitis[101]. Another agent with an interesting immunosuppressive action is methotrexate, which can be also used in the scenarios the thiopurines are indicated[102]. Gastrointestinal changes represented by nausea, vomiting and diarrhea are its main adverse effects[103].

Facing the inconveniences associated with the above-mentioned side effects, IBD patients have gradually searched for alternative therapies[104]. Some potential therapies use plants, including *Cannabis sativa*, and their active ingredients. However, there is no robust evidence that prove their effectiveness in modifying the course of the disease[105]. Moreover, there is a higher prevalence of psychological disorders among IBD patients, such as stress, anxiety and depression[106,107]. These comorbidities calls attention for non-pharmacological therapies aiming the increase of patients' quality of life, including cognitive and behavioral therapy, hypnotherapy, psychodynamic therapy, meditation, yoga, acupuncture, and exercise, but all of them present a limited level of evidence[104]. In that context, probiotics still have many conflicting works, however, they emerge as a new perspective for the treatment of these diseases[108].

USE OF PROBIOTICS FOR IBD TREATMENT

Since microbiota plays a crucial role in IBD pathophysiology, efforts have been directed towards the evaluation of the effectiveness of microbial-based therapies for its management, among which the use of probiotics rises as a promising alternative[109]. It is important to be highlighted that fecal microbiota transplantation is also a possibility that have been tried in this scenario, but a recent meta-analysis that included 18 studies did not demonstrate a consistent effectiveness of that method[110]. Regarding probiotics, several studies have been developed in order to evaluate their potential in inducing and maintaining remission in both CD and UC[111-113]. Moreover, encouraging results have been obtained with the use of non-pathogenic bacteria and fungi in the treatment of these patients (Table 2)[15,114,115].

In 1997, the *Escherichia coli* Nissle 1917 (EcN) was tested in a double-blind trial in order to evaluate its efficacy in maintaining UC remission[16]. That study included 120 patients and observed an equivalence between this probiotic and mesalazine in preventing disease relapses, whose rates were 11.3% in mesalazine group and 16.0% in probiotics group, with a relapse-free time of 103 ± 4 d *vs* 106 ± 5 d, respectively. Since then, other studies on EcN efficacy were performed[116-118], and two meta-analyses reaffirmed the results found in the above-mentioned study[111,112]. The first of them included six trials, embracing 719 patients, and found that EcN induced remission in 61.6% of patients, while in mesalazine that rate was 69.5%[111]. The most recent one, in its turn, comprehended 10 studies, totaling 1049 patients, and observed a related ratio (RR) of 0.94 (95%CI: 0.8-1.03, P = 0.21) in remission rate and of 1.04 (95%CI: 0.82-1.31, P = 0.77) in relapse rate when EcN and Mesalazine groups were compared[114]. Moreover, a current practice position from European Crohn's and Colitis Organization (ECCO) consider that EcN may be effective in inducing and maintaining remission in UC[109].

Table 2 Clinical activity of probiotics in inflammatory bowel disease

Probiotic	Clinical activity in IBD	Ref.
Escherichia coli Nissle 1917	Induction and maintenance of UC remission	[16,57,58,62]
VSL#3	Induction and maintenance of UC remission; prevention of relapses in chronic pouchitis	[65,66,68,69]
Saccharomyces boulardii	Clinical remission of UC	[68-70]
Bifidobacterium longum	Objective improvements in UC parameters	[71]
Lactobacillus acidophilus La-5 + *Bifidobacterium* BB-12	Probable improvement of intestinal parameters in IBD	[72]

UC: Ulcerative colitis; CD: Crohn's disease; IBD: Inflammatory bowel disease.

Probiotics formulations containing multiple species with different combinations of microorganisms are also commonly applied[115]. The VSL#3 is a widely studied and commercialized combined preparation that contains eight strains of lactic acid-producing bacteria (*L. plantarum*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, *L. acidophilus*, *B. breve*, *B. longum*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus*)[17]. This formulation was firstly tested in 2000 for maintenance of clinical remission in patients with UC and chronic pouchitis in a double-blind placebo-controlled trial. That study included 40 patients during disease remission and its results pointed to the efficacy of this agent in preventing clinical relapses when compared to placebo[119]. Their results showed that only 15% of the patients who received the probiotic therapy presented relapses within 9 months, while all of the individuals from placebo group (100%) experienced such intercurrences ($P < 0.01$). After that, encouraging results were observed in the use of VSL#3 aiming the remission of acute mild-to-moderate UC[120-122]. Increased regulatory cytokines levels and reduced pro-inflammatory cytokines and toll-like receptors (TLRs) expression are supposed to be induced by this probiotic[123]. According to a new study that used a murine model, the inhibition of NF-κB and TNF-α expression by means of TLR4-NF-κB signal pathway might play an important role in such promising VSL#3 effects on UC[124]. Recently, a meta-analyses concluded that VSL#3 is effective in preventing pouchitis episodes and may have beneficial effects in inducting UC remission (RR = 1.67, 95%CI: 1.06-2.63, $P = 0.03$) and in avoiding UC relapses (RR = 0.29, 95%CI: 0.10-0.83, $P = 0.02$) when compared to placebo.

Besides the presence of *Lactobacillus* and *Bifidobacterium* in VSL#3 composition, these genera are also evaluated singly or in other combinations, being them the most clinically tested genera in IBD[114]. With regards to *Bifidobacterium*, a recent double-blind study including 195 patients found that *B. breve* strain Yakult fermented milk had no effect in maintaining remission in UC patients[18]. On the other hand, a randomized, placebo-controlled, double-blinded trial that included 56 patients demonstrated that *B. longum* 536 strain promoted a reduction in UC Disease Activity Index (UCDAI) after 8 wk of treatment (3.8 ± 0.4 at baseline *vs* 2.6 ± 0.4 at week 8, $P < 0.01$), while no significant improvement in UCDAI was observed among patients that received placebo (4.5 ± 0.5 at baseline *vs* 3.2 ± 0.6 at week 8, $P = 0.88$)[125]. Another trial with 305 IBD patients showed that *Lactobacillus* acidophilus La-5 associated with *Bifidobacterium* BB-12 probably improves intestinal parameters of affected individuals by means of increasing the prevalence of probiotic bacteria in intestine and colon[126].

The fungus *Saccharomyces boulardii*, a yeast that induces anti-inflammatory activity, has also been studied in IBD[15]. Some clinical trials observed satisfactory effects when using *S. boulardii* for the prevention of relapses in CD patients and in clinical remission of UC. A randomized non blinded study with 32 CD patients showed that the clinical relapses rates during six months in *S. boulardii* plus mesalazine group (6.25%) were lower than in those patients that used mesalazine alone (37.5%)[127], while the other one found improved bowel permeability among patients in whom this probiotic was added to baseline therapy[128]. Regarding UC, a pilot study found an improvement in Rachmilewitz clinical activity index among treated individuals[129]. However, these researches included small populations and were performed using distinct *S. boulardii* doses.

It is important to be highlighted that the number of randomized controlled trials that evaluate the efficacy of probiotics in IBD remains low. Besides that, the meta-analyses about this therapy modality present potential biases due to the reduced number of included studies[11,111,112]. Furthermore, the lack of standardization of the therapeutic protocols leads to probiotics administration in different doses and frequency in distinct studies. Moreover, a study showed that probiotics composed by identical microorganisms, when underwent to different manufacturing methods,

present distinct metabolic characteristics[130]. Complementarily, recent research showed that the effectiveness of a multispecies probiotic formulation depends on microbial metabolic properties, which affect its anti-inflammatory activity[131].

CONCLUSION

Considering the apparent pivotal role of microbiota in IBD genesis and the negative points observed in its conventional treatment, the application of microbial-based therapies seems to be a plausible alternative for affected patients with UC disease. To date, the use of probiotics seems to have no consistent benefit in treating DC. Although more evidence is needed in the evaluation of probiotics efficacy, promising results have been obtained in UC, mainly regarding *E. coli* Nissle 1917 and VSL#3. Lastly, standardizing therapeutic protocols and probiotics manufacturing methods could improve future studies, minimizing their potential biases.

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REVIEW

Pathological characterization of occult hepatitis B virus infection in hepatitis C virus-associated or non-alcoholic steatohepatitis-related hepatocellular carcinoma

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Abstract

Occult hepatitis B virus (HBV) infection, by definition, is a state in which infection with this virus does not manifest with the conventional diagnostic laboratory criteria reserved for the obvious form of HBV infection. As a result, occult HBV infection is commonly a surprise finding discovered accidently during the evaluation of other apparent liver diseases, such as hepatitis C virus (HCV) infection or non-alcoholic fatty liver disease and, more importantly, their evolution into life-threatening hepatocellular carcinoma. As infection with HCV and occult HBV is rarely considered when assessing these more obvious conditions, and in an attempt to offer a better understanding of this phenomenon, this study attempted to shed some light onto the uniqueness of occult HBV infection by addressing the natural history of HBV and HCV infections, as well as non-alcoholic fatty liver disease. This was carried out by taking into account the exclusive integration process undertaken by the HBV genome into infected host hepatocytes, with consideration given to conditions which afford reactivation of the occult infection and stress on the molecular mechanisms that underlie occult HBV infection. Finally, the clinical outcome of occult HBV infection and its relation to hepatocellular carcinoma is analyzed.

Key words: Occult hepatitis B virus; Hepatitis C virus; Non-alcoholic fatty liver disease; Hepatocellular carcinoma; Clinical outcome; Pathophysiology of occult hepatitis B virus

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Core tip: Occult hepatitis B infection is a common clinical situation among chronic liver diseases including hepatitis C virus infection and non-alcoholic fatty liver disease. It is masked, not routinely diagnosed by common laboratory tools, it has a different clinical impact and may increase the incidence of hepatocellular carcinoma in patients with these chronic liver diseases. This systematic review analyzes the data on this clinical situation and highlights different studies which have investigated this clinical entity.

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NATURAL HISTORY OF HEPATITIS B VIRUS, HEPATITIS C VIRUS AND NON-ALCOHOLIC FATTY LIVER DISEASE

Despite an increased understanding of the immunopathogenesis and virology of hepatitis B, the directional course of chronic hepatitis B (CHB) viral infection remains uncertain. Nevertheless, it has been established that the natural history of this disease is primarily dependent on the age at time of exposure to the infecting virus[1].

Chronicity is the hallmark of hepatitis B virus (HBV) infection acquired in infancy, with over 90% of cases developing CHB, while this same percentage of patients undergo resolution of the disease if the infection occurs in adulthood. Therefore, with consideration of the levels of hepatitis B e-antigen (HBeAg) and HBV DNA, in addition to alanine aminotransferase (ALT) values and extent of liver inflammation, the clinical practice guidelines for the management of HBV infection established by the EASL in 2017 have classified the natural history of chronic HBV infection into five phases[2] (Figure 1).

Phase I includes patients with HBeAg-positive chronic HBV infection. Previously coined as "immune tolerant", these patients are characterized as having detectable serum HBeAg associated with high HBV DNA levels, while ALT levels remain in the normal range (ULN, approximately 40 IU/L)[3]. Histologically, little or no necroinflammatory changes or fibrosis are detected in these cases. However, the presence of integration affiliated with the increased HBV DNA levels and clonal hepatocyte expansion suggest that the hepatocarcinogenic process may be initiated early in the course of this infection[3,4]. Phase 2 comprises HBeAg-positive CHB patients, formerly termed "immune reactive HBeAg positive". In addition to detectable serum HBeAg and elevated HBV DNA levels, these patients are distinguished by increased ALT levels associated with moderate to severe hepatic necroinflammation with increased evolution to the development of fibrosis. Patients in phase 3, who have antecedently been called "inactive carriers", are now known to have HBeAg-negative chronic HBV infection. Described by a lack of detectable serum HBeAg with absent or low (< 2000 IU/mL) levels of HBV DNA and normal ALT values, these patients have minimal liver necroinflammation and fibrosis.

Phase 4 of this categorization constitutes cases of HBeAg-negative CHB infection. These patients demonstrate absence of serum HBeAg in association with moderate to high levels of HBV DNA (> 2000 IU/mL). However, ALT levels in these subjects are elevated, the manner being either persistent or fluctuating, and hepatic necroinflammatory activity and fibrosis are evident. The final phase of the EASL classification, phase 5, is the hepatitis B surface antigen (HBsAg)-negative phase, which includes patients with negative serum HBsAg, regardless of the appearance of antibodies to HBsAg (anti-HBs), and the presence of detectable antibodies to hepatitis B core antigen (HBcAg) (anti-HBc). Known as "occult HBV infection", patients in this phase most commonly exhibit undetectable HBV DNA in serum and ALT levels within the normal range. However, histological examination often demonstrates the presence of hepatic HBV DNA in the form of covalently closed circular DNA (cccDNA). As a consequence, reactivation of HBV infection may occur in patients undergoing immunosuppressive therapy[3].

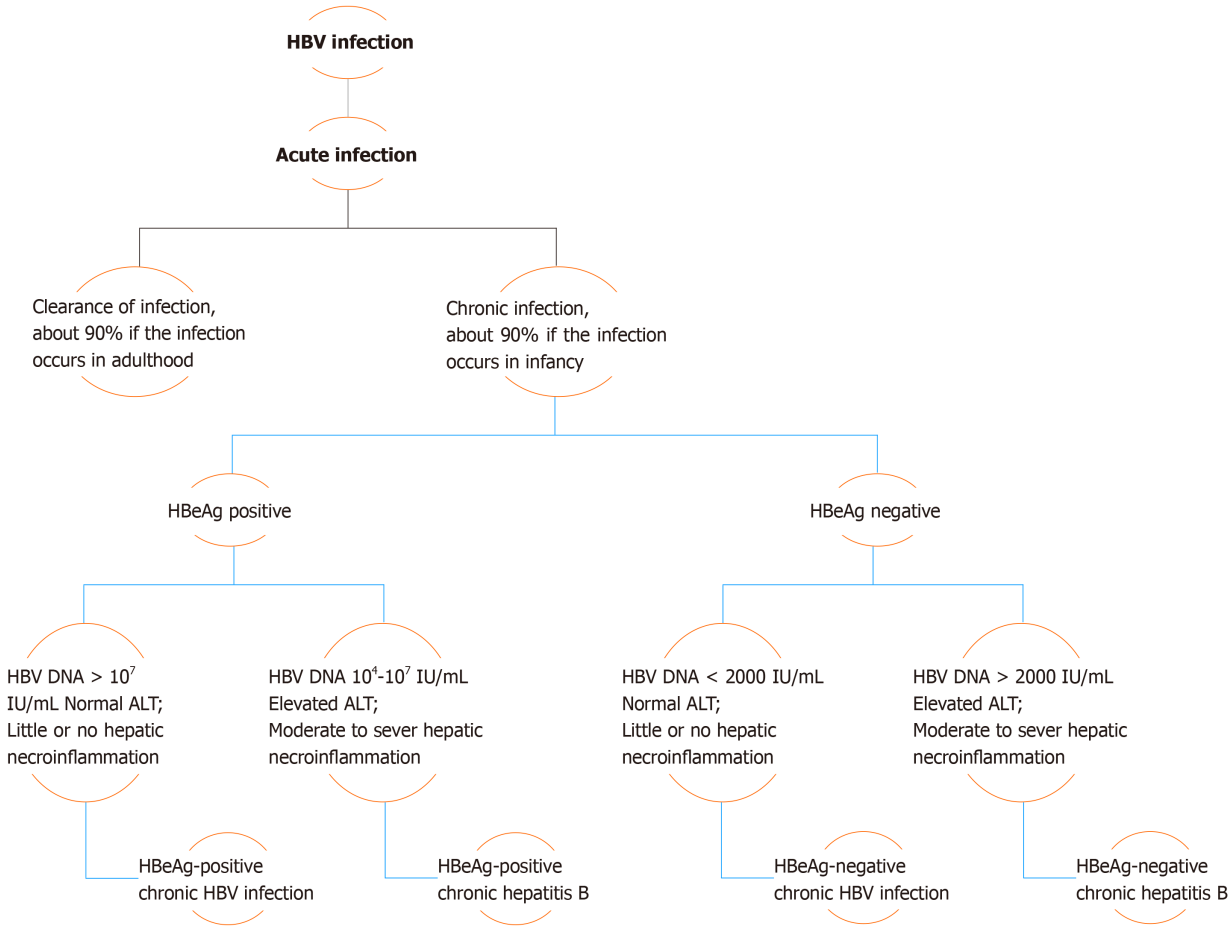


Figure 1 Natural history and assessment of patients with chronic hepatitis B virus infection. HBV: Hepatitis B virus.

Progression of hepatitis B infection to the stage of cirrhosis and the development of hepatocellular carcinoma (HCC) is of variable incidence and largely affected by immune response of the infected patient to the virus. Untreated CHB patients have a 5-year cumulative incidence of developing cirrhosis of approximately 8%-20%. Those who do progress to cirrhosis have a 5-year cumulative risk of advancing to hepatic decompensation of 20% and an annual risk of HCC of about 2%-5%[5].

As occult HBV infection can only be diagnosed during assessment and evaluation of other liver diseases, such as HCV infection or non-alcoholic fatty liver disease (NAFLD), the natural history of these diseases should be taken into consideration. HCV infection may either manifest mildly, as in acute cases, or present with more serious symptoms in either acute or chronic cases. Chronicity develops in about 80% of patients infected with HCV, and is predicted by several factors such as male sex, age over 25 years at initial infection, mild asymptomatic acute infection, intake of immunosuppressive therapy, and co-infection with human immunodeficiency virus (HIV). The intensity of chronic hepatitis is variable between chronically infected patients but, in general, 5%-15% of patients with chronic HCV infection advance to develop liver cirrhosis over a period of 20 years. Of these cirrhotic patients, 4%-9% will progress to liver failure, with a 2%-5% annual risk of developing HCC[6] (Figure 2).

Progression of liver disease identified as simple steatosis to more advanced steatohepatitis with transformation to fibrosis and development of cirrhosis forms the basis for what is collectively defined as NAFLD. While progressive liver disease is a highly unlikely consequence of simple steatosis, the development of non-alcoholic steatohepatitis (NASH) and associated fibrosis have both been shown to be associated with poor prognosis in these patients. This was demonstrated in a study of patients with paired biopsies and showed that 49% of subjects demonstrating features of NASH at baseline proceeded to develop advanced fibrosis in contrast to only 17% of those presenting with simple steatosis[7]. However, no difference in mortality was detected among patients with NAFLD in a large population study utilizing blood tests and ultrasonography for assessment of this condition[8]. However, compared with the general population, the overall mortality rate was found to be higher in

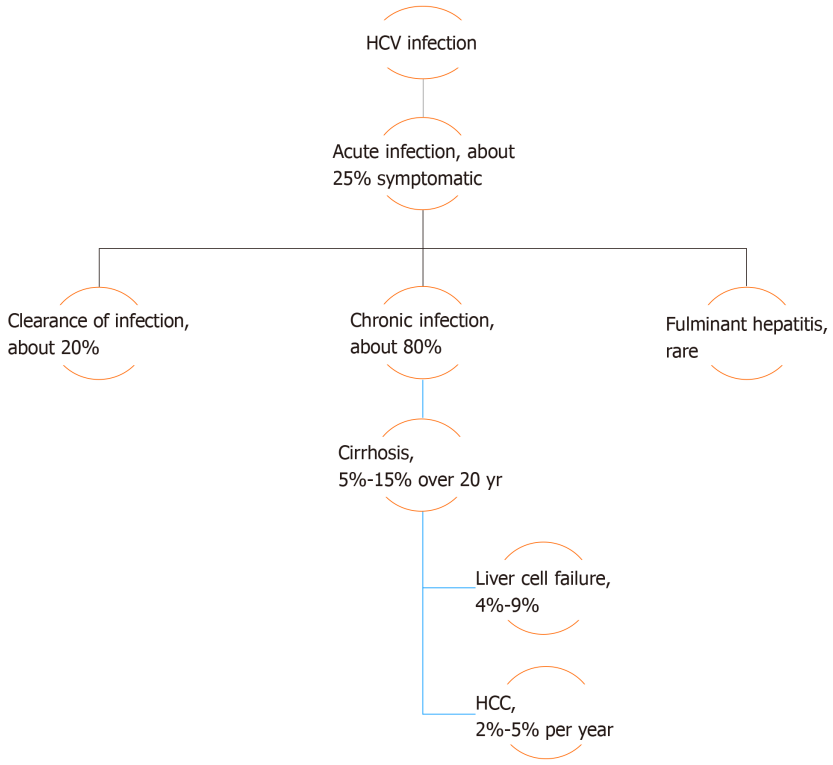


Figure 2 Natural history of hepatitis C virus infection. HCV: Hepatitis C virus.

NAFLD patients assessed primarily by radiological evaluation with ultrasound[9]. Nevertheless, upon scrutinizing the results of an overlapping cohort with 26.4 years as the mean period of follow-up, only patients diagnosed with late-stage fibrosis had an increased risk of all-cause mortality when compared to the reference population[10]. The risk of developing HCC is increased in patients afflicted with cirrhosis in the setting of NAFLD, with about 1%-2% of primary hepatic cancer appearing in patients also suffering from NAFLD[11].

INTEGRATION OF HBV GENOME INTO HEPATOCYTES

HBV is a member of the *Hepadnaviridae* family characterized by its incorporation of a partially double-stranded relaxed circular DNA of about 3.2-kb that comprises four open reading frames (ORF), each of which encodes for different virion proteins[12]. The *S* ORF encodes the large, middle, and small viral surface envelope glycoproteins collectively known as HBsAg, these being categorized into pre-S1, pre-S2 and S regions based on both structure and function. The *C* ORF encodes one of two proteins depending on the site of translation initiation, either the HBcAg or viral nucleocapsid, initiated from the core region of the viral genome, or the HBeAg initiated from the pre-core region. Viral polymerase, distinguished by reverse transcriptase activity, is encoded by the *P* ORF, while the small regulatory HBV X protein (HBxAg) is encoded by the *X* ORF[13,14].

Upon entry of an infectious virion into the host hepatocyte, possibly by way of the pre-S protein[15], the process of replication begins with the uncoating and release of the relaxed circular DNA (RC-DNA) followed by its transport to the nucleus of the cell where it is re-organized into the stable form of the viral genome known as the cccDNA. This cccDNA is used as a template for transcription of a group of RNAs utilizing RNA polymerase II enzyme, including pre-genomic RNA (pgRNA), pre-core RNA and sub-genomic HBV RNAs. PgRNA functions as a template for completion of HBV DNA synthesis and serves as the messenger RNA for polymerase and core protein[16], while sub-genomic HBV RNA acts as mRNA for translation of the different sized surface proteins of HBsAg as well as HBxAg protein. At the cytoplasm, produced nucleocapsids are gathered into a glycoprotein envelope in the endoplasmic reticulum forming mature virions to be secreted extracellularly[17].

Development of occult HBV infection, as well as HCC, may possibly be explained by the phenomenon of hepatitis B viral integration into the host genome[18], a process

that comprises the redistribution of HBV DNA sequences into host chromosomal DNA. This integration mechanism is often defective[19], and is associated with decreased virion production accompanied by undetectable HBV DNA and HBsAg in the serum of infected patients[7]. Integration is commonly seen in chronically infected HBV patients, particularly those with HBsAg-positive HCC[20], although it has also occasionally been seen in HBsAg-negative HCC cases. However, HBV viral integration has not been detected in patients co-infected with HCV[21-23].

Defects that have been associated with HBV DNA integration include loss of HBV core gene with consequent loss of core protein, leading to viral assembly at substandard levels with excessive aggregation of viral DNA in hepatocytes. This may possibly offer clarification for findings of detectable HBV DNA in the liver but not in serum of infected patients[18]. Excessive translation of the large protein of HBsAg causes impaired release of other forms of the surface protein, leading to their aggregation in the form of granules in the hepatocyte cytoplasm[24]. In addition, HBsAg expression is also affected by HBV DNA disruption and rearrangement when the downstream region of the *S* ORF is replaced with the pre-S1 promoter, leading to decreased S gene transcription associated with impaired secretion of S protein[18].

REACTIVATION OF OCCULT HBV INFECTION

Patients with occult HBV infection (OBI) show HBV DNA existing in two forms, either as episomal free cccDNA or integrated into DNA of the host hepatocyte. The state of OBI may be induced by a number of situations including the subsequent resolution of an acute HBV infection, the "a" determinant arising from mutation in the HBsAg gene, co-infection with HCV or HIV, or epigenetic changes in the cell. OBI is defined by the absence of detectable HBsAg in serum regardless of the status of the antibodies against HBcAg (anti-HBc)[25].

Therefore, cases of immune suppression are associated with cessation of the decreased viral replication and suppressed gene expression that commonly accompany OBI, resulting in reactivation of viral replication consequent to the deterioration of control by the immune system. This provides crucial, albeit indirect, evidence of the important function of immunological control in induction of the state of occult HBV infection. However, recent studies have reported that reactivation of OBI also occurs with histone deacetylase inhibitor use, thus providing confirmation of the role of epigenetic mechanisms in maintaining HBV activity in check with modification of the structure and dynamics of viral cccDNA mini-microsome, these also being projected as probable causes of reactivation of HBV[26].

Conditions associated with a high risk of reactivation of OBI include hematological malignancies, in addition to transplantation with hematopoietic stem cells and immunosuppressive therapies consisting of anti-CD20 monoclonal antibody (Rituximab), drugs included in the CHOP regimen as well as fludarabine. However, only a small number of patients undergoing these therapeutic interventions experience severe clinical manifestations following alteration of the HBV serological profile[27].

On the other hand, reactivation of OBI appears to occur infrequently in sufferers of rheumatologic diseases subjected to high doses of corticosteroids or biological agents for prolonged periods of time. In addition, liver cancer patients going through transarterial chemoembolization (TACE) and those with inflammatory bowel diseases treated with biologics rarely experience OBI reactivation, although patients undergoing chemotherapy for solid tumors have increasing shown HBV reactivation. As a result of these inconsistencies, consensus regarding this topic remains inconclusive, with the entire scope of risk remaining obscure[27]. However, the possibility of reactivation of HBV in patients with OBI undergoing direct acting antiviral therapy for the treatment of HCV has been raised in recent reports, although there appears to be negligible risk in these instances and no clinical or virological consequences[27].

MOLECULAR MECHANISMS UNDERLYING OCCULT HBV INFECTION

HBV genome mutations and deletions

A double looped structure comprising amino acids 124 to 147 is known as the "a" determinant of HBsAg. On account of its abundant cysteine residues, it is known to take part in disulfide bond formation and maintenance of this region[28]. Mutations in the "a" determinant propose a serious health burden in that they are undetectable by

certain commercial HBsAg assays as well as having the capability to infect both vaccinated and unvaccinated persons[29]. The first mutation in the "a" determinant of HBsAg to be reported was the sG145R mutation, which was described in a patient who was both actively and passively immunized[29], after which other mutations were later disclosed both inside and outside the "a" determinant[30,31]. In addition, this mutation most likely causes reinfection with HBV in patients following liver transplantation in spite of prophylaxis with hepatitis B immunoglobulin (HBIG), probably due to HBIG induced suppression of the secretion of HBsAg into serum without affecting replication of the HBV DNA[32].

Similar mutations characterized by being undetectable by some commercial HBsAg assays as well as infecting vaccinated subjects, are mutations with the added health complication of inducing lamivudine resistance in patients undergoing this treatment[29]. These include the Q563S mutation in HBV polymerase, surface gene mutation sS207R, the V539I mutation in the "C" domain of the polymerase, sS143L substitution in the "a" determinant of HBsAg, and M204I and L180M/M204I mutations of the polymerase gene[33,34].

Furthermore, deficient HBsAg expression associated with low replication is also seen with the G-to-A mutation of the surface gene at position 458. Due to the close proximity of this position to the 5′ splice site of the S gene mRNA, this mutation impedes S gene mRNA splicing through a co-/posttranscriptional mechanism with resultant defective export of S gene mRNA or dysregulated RNA folding[35]. Genotype D strains of HBV are the only ones that have evolved to accommodate the accumulation of substitutions thus advocating positive selection, as well as having an acceptor site at nucleotide 202. Splicing at this site as well as at the nucleotide 2986 donor site produced intracellular viral particles lacking the surface protein, this resulting in the accumulation of mutations subsequent to alleviation of coding restrictions[36].

Pre-S region deletion mutations are associated with eradication of HLA-restricted B-cell and T-cell epitopes, and are another form of mutation also accompanied by inadequate expression of the surface proteins of HBV[37,38]. Deletions and mutations in the pre-S gene result in modified expression of HBsAg as well as reduced HBeAg and HBV DNA levels in hepatocyte cell lines[39]. In addition, pre-S1 and pre-S2 region mutations were found to be associated with low secretion of HBsAg in cell culture systems[40].

CO-INFECTION OF HBV WITH OTHER VIRUSES

Contradictory data exists with regards to the clinical outcome of occult HBV co-infection with HCV. A significant association was noted between occult HBV infection and cirrhosis in patients infected with HCV in a study by Cacciola *et al*[41], in spite of the enhanced response to interferon therapy reported in these co-infected patients. A higher likelihood of developing HCC in patients co-infected with HCV and occult HBV has also been reported[42], although no correlation was found between the presence of infection with occult HBV and the severity of liver disease related to HCV[43]. Co-infection with HBV and HCV is associated with reduced levels of HBV replication accompanied by diminished expression of hepatic HBsAg[44,45], but patients infected with HBV alone demonstrated significantly higher rates of spontaneous HBsAg clearance[46].

Inhibition of HBV replication and HBV protein production by HCV occurs through a variety of underlying mechanisms, including co-localization of HBV and HCV genomes within the nucleus in which the inhibitory effect of HCV on HBV replication was demonstrated using a double fluorescent in situ hybridization technique[47], although this result could not be replicated in Huh-7 cells[48]. In addition, replication of HBV and expression of HBsAg can be inhibited by HCV core protein[49]. HBV core and polymerase proteins interact with the package signal (Σ) situated at the 5′ end of HBV pgRNA to start the process of encapsidation[50]. Direct interaction of HCV core protein with HBx protein causes obstruction of the core and polymerase binding to this package signal within the hepatocyte, thus preventing encapsidation of HBV with consequent inhibition of HBV gene expression[29]. Furthermore, HCV NS2 protein downregulates secretion of HBsAg and HBeAg accompanied by suppression of different cellular and viral promoters[51].

Another viral infection in which occult HBV infection is commonly seen is HIV, in which HBV DNA is also detected intermittently. As a result, recurrent sampling has been proposed for HBV DNA detection in HIV-positive patients, although this recommendation remains controversial[52].

APOLIPOPROTEIN B MRNA-EDITING ENZYME CATALYTIC POLYPEPTIDE

Apolipoprotein B mRNA-Editing Enzyme Catalytic Polypeptide (APOBEC) has been shown to play a role in cytidine deamination and is known to inhibit and edit replication of HIV[53]. Similarly, expression of APOBEC3G has been associated with a 50-fold reduction in HBV DNA[54]. Inhibition of HBV replication by APOBECs occurs in either a deamination-dependent or a deamination-independent manner.

APOBECs have been shown to edit up to 35% of the HBV genome present in the liver[55], including regions encoding for the surface proteins, the polymerase, and the HBx protein[56]. While little data exists demonstrating the non-cytolytic clearance of HBV by APOBEC proteins, this has steadily been improving over the past few years with the appearance of more studies[29].

On the other hand, deamination-independant inhibition can be mediated by enhanced nuclease susceptibility of the pre-genomic RNA associated with HBV core protein[57]. In addition, targeting of single-stranded or hybrid HBV DNA is another mechanism of inhibiting early stage HBV DNA[58]. Secretion of both HBsAg and HBeAg can also be inhibited by APOBEC3B, although the mechanism remains unknown[59].

HOST IMMUNE RESPONSES AND OCCULT HBV INFECTION

Occult hepatitis B virus infection may be caused by impedance of the immune response to HBV. Modulation of both replication of HBV and synthesis of HBV proteins are the outcome of a number of mechanisms related to the host immune response, including apoptosis, T-cell responses of both cytolytic and non-cytolytic nature, and polymorphisms of vitamin D receptor (VDR)[29]. Compared to patients with CHB, those with OBI demonstrated lower expression levels of the apoptotic factor Fas, which plays a role in the apoptosis of infected hepatocytes and removal of old hepatocytes[60]. Another potential mechanism for occult HBV is the non-cytolytic immune responses seen to be associated with indistinquishable HBsAg levels[61]. Similarly, VDR gene polymorphisms were associated with occult HBV infections and were characterized by variable levels of HBV DNA with HBeAg loss[62,63].

EPIGENETIC CHANGES

Occult HBV infection may be the result of modulated HBV replication and virion production due to control of transcriptional activity by methylation[29]. Cytosine methylation of CpG dinucleotides within CpG islands inside the gene promoters has been linked to silencing of genes[64]. Furthermore, methylated HBV cccDNA has been detected in liver tissues of infected humans, this being associated with a 90% diminution of HBsAg secretion in hepatocyte cell lines[38]. In addition, HBeAg-negative individuals demonstrated methylated cccDNA in a higher ratio to total cccDNA when compared to HBeAg-positive subjects[65].

In addition, enhanced replication of HBV in cell culture is associated with hyperacetylation of cccDNA-bound histones, a finding similarly demonstrated with histone deacetylase inhibitors resulting in acetylated histones bound to cccDNA leading to elevated HBV replication with high levels of HBV transcripts. Conversely, hypoacetylation of cccDNA-bound histones is linked to diminished viral loads associated with recruitment of histone deacetylase[66]. Remodelling of the HBV minichromosome with regulation of HBV replication is the culmination of histone phosphorylation and methylation[67].

GENOME INTEGRATION

Integration of HBV DNA sequences into the host genome is an established mechanism by which chronic HBV patients develop HCC[68]. During the integration process, interruption and rearrangement of genes into chromosomal DNA may result in either loss of serum HBsAg, reduced virus production, or the appearance of undetectable serum HBV DNA. Therefore, particularly in patients suffering from chronic HBV infection for several years, the fundamental mechanism underlying occult HBV is integration of HBV DNA[29].

Loss of HBV core gene during HBV DNA integration results in HBV core protein loss which is associated with minimal levels of viral assembly with aggregation of unencapsidated HBV DNA in the hepatocyte, this offering an explanation as to why HBV-related HCC is associated with undetectable serum HBV DNA although it may be easily detected in liver tissure[18].

IMMUNE COMPLEXES IN OCCULT HBV INFECTION

Immune complexes containing HBsAg have been demonstrated in the serum of patients with acute HBV infection, those with chronic HBV infection, as well as HBsAg carriers who are asymptomatic[69]. HBsAg may be become entrapped with anti-HBc, thus hindering detection of HBsAg by conventional serological assays. In addition, these HBsAg-containing immune complexes have been found in HBsAg-negative occult HBV infected patients with HCC[70]. In fact, about 40% of blood donors identified as HBsAg-negative but having positive anti-HBc were found to have detectable HBV DNA, most of these being shown as having HBV-containing immune complexes. These circulating immune complexes demonstrated an absence of nucleic acid changes that may cause changes in the major epitopes of HBsAg, thus providing further confirmation of the role of these immune complexes in OBI[71].

CLINICAL OUTCOME OF OBI (OCCULT HBV INFECTION) AND ITS RELATION TO HCC

Although the clinical characteristics of HCC developing in the setting of OBI has not been extensively studied, several attempts have been made to explore this point. A recent study by El-Maksoud *et al*[72], performed on 50 Egyptian patients with HCC who had undergone either resection or transplantation, found that over 30% of chronic hepatitis C patients with HCC had OBI. Although no significant association could be ascertained between OBI and clinical tumor characteristics such as size and number, BCLC staging or vascular invasion, findings showed that these patients were younger with histology demonstrating a more advanced grade of 3 or 4. In addition, most of these patients had serological positivity for HBcAg, a possible indicator for prediction of occult infections[72]. These findings have been supported by a number of studies[73-75]. Therefore, the complexity yet uniqueness of the state of OBI grant it an exceptional status that is worthy of consideration when investigating any liver disease.

CONCLUSION

As OBI does not demonstrate the diagnostic laboratory criteria of conventional HBV infection, it is often missed in patients with HCC developing in the settings of HCV infection or NAFLD. Addressing the natural history of these conditions, and the molecular mechanisms by which hepatitis B virus becomes occult then reactivated in circumstances of immunosuppression emphasizes the unique characteristics of this infection. Studies conducted on occult HBV in patients with HCC on top of HBV have collectively concluded that although no significant association could be determined between OBI and tumor characteristics, the presence of OBI in up to 30% of patients, these being mostly younger patients who also tested positive for HBcAg, does in fact warrant its consideration during the investigative workup of other liver diseases.

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REVIEW

Hypoxia and oxidative stress: The role of the anaerobic gut, the hepatic arterial buffer response and other defence mechanisms of the liver

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Abstract

The liver is considered a vital organ and is the hub for multiple chemical functions, such as intermediary metabolism and the detoxification of ingested toxins, which are essential for the preservation of life, hence, the origin or the word "liver". The liver has enormous, highly diversified catalytic potential. This enormous catalytic potential generates massive oxidative stress, which is important for the functions of the liver but is detrimental to the viability of the liver. The liver receives approximately 80% of its blood supply from the portal vein, which brings less saturated blood from the gastrointestinal tract. Hepatocytes operate in a relatively hypoxic microenvironment due to this portal inflow. The development of this hypoxic microenvironment of the liver is an important evolutionary adaptation for its detoxification function that is not recognized in the literature as a defence mechanism against the oxidative stress generated during the detoxification process. This review describes liver function in relation to its oxidative catalytic potential and the oxidative stress generated by it as well as the evolutionary defence mechanisms present in the liver against this oxidative stress to provide new insights into liver function.

Key words: Anaerobic gut lumen; Oxidative stress; Liver defences against oxidative stress; Hepatic arterial buffer response; Hypoxia; Liver function

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Core tip: This review presents a prototypic view of the role of hypoxia as a regulator and a defence mechanism against oxidative stress in the gastrointestinal tract, including in the liver. The functional significance of having an anaerobic gut lumen for the preservation of energy sources is described in the article. Furthermore, the significance of the hepatic arterial buffer response in the maintenance of the hypoxic microenvironment of hepatocytes to regulate the oxidative stress generated by the

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enormous catalytic potential of the liver and other hypoxia-mediated defences against this oxidative stress are described.

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INTRODUCTION

The gastrointestinal tract has evolved for the digestion of food and absorption of nutrients to provide energy and nutritional requirements for the body. In nature, food is scarce and in intermittent supply; however, the energy needs of the body for metabolic processes are continuous. Evolutionary mechanisms were needed to store energy and nutrients during times of surplus (feeding episodes) for utilization during episodes of scarcity (episodes of fasting). In the never-ending quest for food, an innumerable number of toxic xenobiotic molecules occurring in nature can enter the body with food. These toxins are generated as by-products of chemical processes for the synthesis of food or are present as contaminants or are generated in the putrification of food and pose a risk to the health and the life of the animal.

The liver, which develops from the liver diverticulum, has evolved as the main storage organ for energy substrates and as the main detoxifying organ for ingested toxic xenobiotics. The liver has enormous catalytic potential due to the enzymes present within hepatocytes, which generate large amounts of oxidative stress that is injurious to hepatocytes.

Similarly, oxidative stress is generated within the intestinal lumen due to the oxidative enzymes of intestinal bacteria, which could oxidize and therefore lead to the breakdown of energy substrates, such as glucose, resulting from the digestion of complex carbohydrates.

Due to the unique arrangements of the blood supply of the intestines and the liver, the intestinal lumen and hepatocytes operate in a relatively hypoxic microenvironment. This hypoxic microenvironment is an important defence mechanism against oxidative stress at these sites, as will be elaborated in this review.

Challenge of storing energy

Energy sources (mainly complex carbohydrates and lipids) are digested and absorbed mainly in the small intestine. Complex carbohydrates are enzymatically broken down, mainly to glucose, in the small intestine, absorbed into the portal vein and transported to the liver for storage.

With regard to the enzymatically catalysed oxidation of glucose, two main substrates for the reaction are oxygen and glucose, whereas the end products can differ depending on whether it is a complete or partial oxidation. As per the chemical kinetics of any chemical reaction, the rate-limiting factor for the oxidation of glucose would be the concentration of the substrate with the lowest concentration of the two substrates if all other factors that affect the rate of the reaction are constant. On the other hand, if concentrations of both substrates are high in a catalytic environment of enzymes, that would accelerate the rate of the reaction, leading to the consumption of both substrates.

Hence, according to Michaelis-Menten kinetics for oxidative phosphorylation (occurring intracellularly) and other oxidative reactions of glucose mediated by bacterial oxidative enzymes (occurring in the lumen of the gut), the oxidation of glucose is stimulated in a catalytic environment of enzymes in the presence of high glucose concentrations if the oxygen tension of the milieu is also high[1,2]. This can pose a challenge for the preservation of glucose in the intestines themselves if the oxygen tension of the intestinal lumen is high, as in the intestinal lumen, a high concentration of glucose is achieved by the digestion of carbohydrates and by bacteria that possess enzymes that can oxidize glucose, leading to the breakdown of glucose even before it reaches the liver for storage as glycogen.

Although, for practical purposes, the small intestine is traditionally considered "sterile", it contains up to 10^4 microorganisms/mL of intestinal secretions[3]. Therefore, evolutionary adaptations are needed to prevent the oxidation of glucose within the

intestines. The evolutionary adaptation in the intestines to overcome this challenge was to make the lumen deeply hypoxic to the extent that the oxidation of energy substrates such as glucose are inhibited due to the lack of oxygen. The degree of hypoxia achieved in the intestinal lumen is so severe that the intestinal lumen is traditionally considered "anaerobic", and only facultative anaerobes and obligate anaerobes colonize it[4]. Friedman *et al*[5] attributed the colonization of intestinal lumen by anaerobic bacteria to the consumption of luminal oxygen by facultative anaerobes and to other chemical reactions that utilize oxygen, which convert the lumen into a deeply hypoxic environment. These microorganisms consume oxygen that diffuses into the gut lumen from mucosal capillaries. The action of these microbes is prominent only in the colon, contributing substantially to deep hypoxia of the colon only, where the size of the biomass is considerably larger[5].

Friedman *et al*[5] considered chemical reactions consuming oxygen (*e.g.*, oxidative reactions such as lipid peroxidation, seen in germ-free mice) as one of the important mechanisms contributing to the generation of the anaerobic intestinal lumen, especially in the proximal small intestine. Although they considered chemical reactions consuming oxygen (*e.g.*, lipid peroxidation) as an important mechanism contributing to the anaerobic gut lumen in the small intestine, these oxidative reactions can also be viewed as undesirable yet unavoidable reactions due to oxidative stress resulting in the breakdown of energy substrates. This undesirable lipid peroxidation in the small intestine has been minimized by another evolutionary adaptation to make the lumen of the intestine as hypoxic as possible, as described below.

In the small intestine, where the energy substrate concentrations are highest, the anaerobic (more strictly, deeply hypoxic) gut lumen is achieved by a remarkable evolutionary adaptation in the microvasculature, preventing the diffusion of oxygen into the gut lumen. In small intestinal villi, there are hairpin loop arrangements between arterioles and venules with multiple cross connections between them. Within the villi, afferent and efferent vessels are separated by a distance of only 20 μm[6,7]. This arrangement allows for a countercurrent shunt in which oxygen carried into the villus is able to diffuse to the venule without being transported through the vascular circuit bound to red blood cells, thus reducing the oxygen content of blood delivered to the villous tip[7,8] (Figure 1).

These adaptations help to maintain the lumen of the small and large intestine in a deeply hypoxic state, supporting the colonization of the small and large intestine with anaerobic bacteria and discouraging the growth of aerobic bacteria. Anaerobic fermenters of the gut oxidize glucose and other simple sugars to lactate, which can be salvaged in the liver with the help of the Cori cycle to re-synthesize glucose[9]. If the luminal environment of the gut was conducive to aerobic bacteria that possess cytochromes and other oxidizing enzymes, they would oxidize glucose to CO_2 and water, leading to complete wastage of energy substrates.

There is a similar challenge in preventing the oxidation of these energy substrates by the action of oxidative enzymes present in high amounts in hepatocytes once they reach the liver *via* the portal vein. In this catalytic environment, during the fed state, glucose is oxidized to pyruvate, then is fluxed into mitochondria along the concentration gradient and is oxidized at a rapid rate if the oxygen concentration in this milieu is also high. However, if the oxygen concentration (tension) of this environment is maintained at low levels, oxidative phosphorylation is inhibited due to hypoxia, and pyruvate will be reconverted to glucose in the cytoplasm. This glucose can then be stored as glycogen once the adenosine triphosphate (ATP), guanosine triphosphate and uridine triphosphate levels become sufficiently elevated as a result of anaerobic metabolism of glucose.

Multiple lines of evidence for the metabolic stress placed upon the liver for the oxidation of energy substrates exist. The liver is the largest contributor to the basal metabolic rate of 27% in the adult and is one of the four high-metabolic-rate organs[10,11]. The contribution of the liver to basal thermogenesis and, more importantly, the contribution of the liver to diet-induced thermogenesis can be considered additional evidence for the stress placed upon the liver for the oxidation of energy substrates due to its enormous catalytic reserve[12-15]. It has been shown that diet-induced thermogenesis is more prominent with meals containing medium-chain fatty acids that are absorbed into the portal vein than with meals containing long-chain fatty acids that are absorbed into the lacteals and delivered *via* the intestinal lymphatics to the systemic circulation, bypassing oxidative metabolism in the liver[16].

Challenge of protecting the body from the toxic xenobiotics present in food

During digestion, the gastrointestinal tract is exposed to an innumerable number of naturally occurring toxic xenobiotics in food, of which some are lipid soluble and penetrate biological membranes with ease. The body is confronted with the challenge

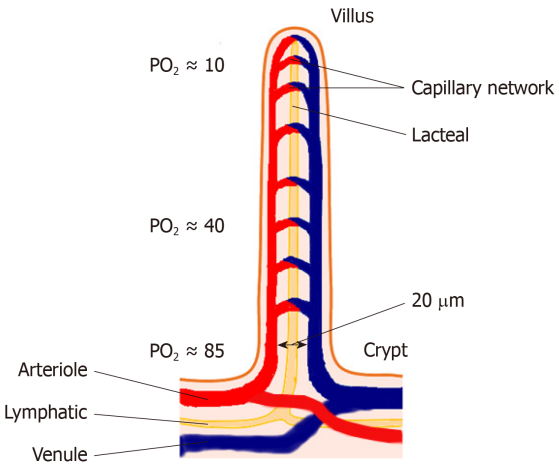


Figure 1 Schematic diagram of countercurrent blood flow within the small intestinal villus allowing diffusion of O_2 from the arteriole to the venule, creating a luminal hypoxia from the crypt to the villous tip. PO_2 is shown in mmHg.

of protecting the organ systems, especially the central nervous system, from these toxic molecules through detoxification and excretion mechanisms. The liver, developing from the liver diverticulum of the embryonic gastrointestinal tract, which also drains the blood supply of the gastrointestinal tract *via* the portal vein, has evolved as the organ for the detoxification of these substances prior to their release into the systemic circulation. The liver accomplishes this task mainly by the oxidative metabolism of these toxic substances within hepatocytes and converting them to polar, water-soluble substances (to which cell membranes are relatively impermeable). These toxic metabolites are either excreted in urine or conjugated with glucuronic acid and excreted back into the gastrointestinal tract with bile to be excreted with faeces (*e.g.*, the excretion of haem in food as bilirubin diglucuronide).

The detoxification process of most toxic substances by the P450 enzyme system within the hepatocyte is achieved by binding these toxic molecules to P450 enzymes and subjecting them to controlled oxidation using oxygen, leading to less toxic end products[17,18]. However, during this oxidative process, highly reactive free radicals and oxygen species are formed inside hepatocytes, which can react and damage the cytochrome P450 system itself as well as other cellular proteins, cell membrane constituents (lipid peroxidation) and genetic material, including DNA, leading to hepatocellular damage and necrosis. It has been shown that hepatocytes experience increased oxidative stress and suffer more cellular injury during the oxidative metabolism of toxic substances such as acetaminophen if the hepatocytes are exposed to higher oxygen tensions during the oxidative process[19].

Indirect evidence for the generation of increased amounts of reactive oxygen species and oxidative stress that damage the cytochrome P450 enzymes and other cellular elements comes from a study by Suleiman *et al*[20], where they showed reduced rat hepatocyte viability, gradual loss of cytochrome P450 and the loss of cellular respiration when hepatocytes are cultured under higher oxygen tensions.

It is well established that the drug-induced hepatotoxicity of halothane is caused by the formation of highly reactive trifluoroacetyl metabolites of halothane during oxidative metabolism by liver microsomal P450 enzymes[21]. These trifluoroacetyl metabolites then react with hepatocyte proteins to form antigens, triggering immunological damage to hepatocytes. It has been shown that the formation of these trifluoroacetyl metabolites is increased by increasing oxygen tensions[21]. This means that reactive metabolite and reactive oxygen species production is increased when the oxygen tension of the hepatocyte milieu is increased during the detoxification of xenobiotics.

To minimize this damage, the cytochrome P450 system of the liver has evolved to operate under highly controlled conditions where oxygen tension of the reactive environment is kept to a minimum, minimizing the generation of free radical and toxic intermediates while providing sufficient oxygen to produce adequate ATP to power the cellular processes. If the cytochrome P450 system is exposed to high oxygen tensions during this process, large quantities of highly reactive toxic intermediates and oxygen species are produced, leading to increased hepatocellular damage and sometimes fulminant hepatic failure.

DEFENCE MECHANISMS OF THE LIVER AGAINST OXIDATIVE STRESS

There are multiple protective mechanisms in the liver to circumvent oxidative stress. The role of the intracellular antioxidant glutathione in combating oxidative stress is extensively described in the literature and will not be discussed here[22].

Some of the most important protective mechanisms to circumvent oxidative stress in the liver, undescribed in the form conceptualized in this article until now, are the evolutionary adaptations to maintain a relatively hypoxic microenvironment within hepatic sinusoids to achieve a low oxygen tension inside hepatocytes.

Hepatic arterial buffer response as a defence against oxidative stress

The liver receives 75%-80% of its blood supply from the portal vein, which drains the partially deoxygenated venous blood from the spleen, stomach, small and large intestine, gallbladder and pancreas, while the hepatic artery accounts for the remaining 20%-25%, supplying the liver with well-oxygenated blood[23]. Branches of the portal vein and hepatic artery supply the hepatic sinusoids, in which the mixed blood flows towards the hepatic venules at the centre of the hepatic lobules. These hepatic sinusoids are lined on their external surface by plates of hepatocytes, which modify the composition of the blood flowing in the sinusoids.

The functional unit of the liver is a matter of debate, and the concepts of the "hepatic acinus" and the "primary lobule", which are based on the blood supply and share some features in common, have gained wide acceptance within the scientific community[24,25]. The concept of the hepatic acinus by Rappaport in particular has gained popularity since it allows for the explanation of lesions such as bridging necrosis and fibrosis[26-28]. Each acinus is at the end of a vascular stalk containing terminal branches of portal veins, hepatic arteries, and bile ducts. Blood flows from the centre of this functional unit through the hepatic sinusoids to the hepatic venules at the periphery of the acinus, and the blood flow in the hepatic sinusoids is classically described as periportal-to-perivenular blood flow.

This gives rise to a functional zonation within the hepatic acinus where the central portion of the acinus closest to the mixed inflow of blood from the portal vein and hepatic artery (zone 1) receives the blood with the highest oxygen concentration, the intermediate zone (zone 2) receives less oxygenated blood, and the peripheral zone (zone 3) closest to the hepatic venules receives the least oxygenated blood[29] (Figure 2).

The mixed inflow of blood to the acinus from the branches of the portal vein and the hepatic arterioles is regulated in a manner where there is an inverse relationship between the hepatic arterial and the portal venous blood flow. The regulation of hepatic arterial blood flow inversely with portal venous blood flow is called the "hepatic arterial buffer response" (HABR) and is mediated by adenosine[23]. According to the currently accepted hypothesis for the adenosine-mediated mechanism of the HABR proposed by Lautt *et al*[30], adenosine is produced by hepatic metabolism in an oxygen-independent manner and is released at a constant rate into fluid in the space of Mall that surrounds the hepatic resistance vessels and portal venules. The concentration of adenosine is regulated by washout into the portal vein and the hepatic artery. If portal venous blood flow is reduced, less adenosine is washed away from the space of Mall, leading to an increase in adenosine levels, producing dilation of the hepatic artery with a subsequent increase in hepatic arterial inflow.

Although the existence of the HABR and the "adenosine washout hypothesis" are widely accepted, the main deficiency of Lautt's theory is the inability to explain the functional significance of the HABR. This deficiency is connected to the source of adenosine for the HABR in Lautt's theorem. According to Lautt's hypothesis, adenosine for the HABR is derived from the demethylation of S-adenosylhomocysteine, a reaction that is oxygen independent. According to Lautt, adenosine, which is produced in huge amounts by hepatocytes in response to hypoxia, is secreted into hepatic sinusoids, where the blood flows away from the hepatic resistance vessels; therefore, the hepatic resistance vessels are not exposed to or influenced by adenosine produced in this manner. Nevertheless, in his "1995 Ciba-Geigy Award Lecture", Lautt admitted that the pathway of adenosine production is an unresolved aspect of his hypothesis, and in this lecture, he proposed the demethylation of S-adenosylhomocysteine as the source of adenosine for the HABR[31]. This proposal has gained wide acceptance as the source of adenosine for the HABR since then.

Significance of adenosine washout

Other researchers have subsequently shown the existence of hypoxia/anoxia-induced adenosine secretion by hepatocytes as an important mechanism of hepatic arterial

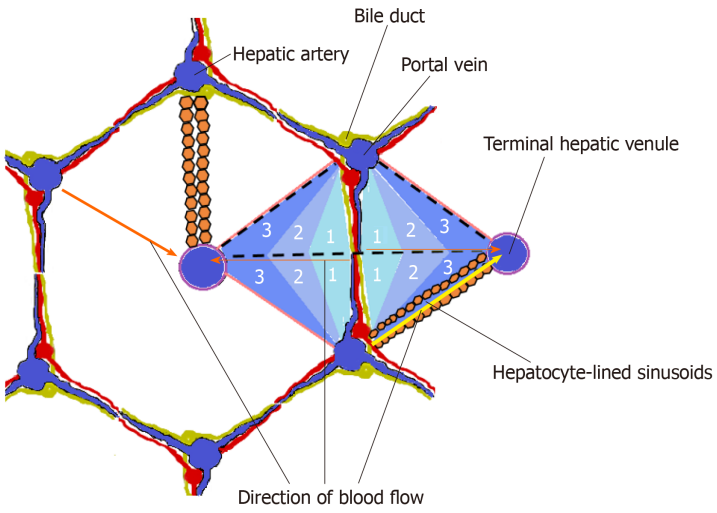


Figure 2 Schematic representation of a hepatic acinus within a conventional hepatic lobule. The demarcation of the hepatic acinus is shown by the dashed lines. Arrows indicate the direction of blood flow within hepatic sinusoids in a periportal-to-perivenous direction, giving rise to zones 1, 2 and 3 (numbered) within the acinus. Note that all three zones are represented in a single sinusoid.

vasodilatation during periods of increased hepatic hypoxia associated with portal flow reduction[32-36]. Kurbel *et al*[36] described a model of dual circulation in hepatic acini, with hypoxia-regulated adenosine secretion that can drive the HABR, but this model has not reached wider acceptance despite its ability to support the functional significance of the HABR.

There is no rationale to assume that autoregulatory arteriolar dilatation due to hypoxia-mediated adenosine production seen in other organs like the heart will not occur only in the liver. Adenosine production can occur by the breakdown of adenine nucleotides or cyclic adenosine monophosphate, and these sources are directly linked to the energy status of the cells[31]. In turn, the energy status of the cells is dependent on the oxidative phosphorylation of energy substrates such as glucose, which relies on an adequate oxygen supply.

The production of adenosine from adenine nucleotides, which occurs through multiple enzymatic steps, can be summarized as shown in Figure 3, which is in dynamic equilibrium.

This equilibrium is shifted towards ATP production under less hypoxic conditions that support oxidative phosphorylation and towards ATP depletion/adenosine production under critically hypoxic conditions. Thus, if the hypoxic microenvironment of the hepatocytes becomes critically hypoxic to the extent that it compromises oxidative phosphorylation with ATP depletion, adenosine production will be increased, producing hepatic arterial vasodilatation, with the correction of critical hypoxia. In other words, the characteristics of hepatic blood supply allow hepatocytes to operate under low oxygen tensions, which facilitates the controlled oxidation of toxic substances, minimizing the generation of harmful reactive intermediates and oxygen free radicals. This also minimizes the uncontrolled utilization of energy substrates, thus favouring the synthesis of storage polymers.

Enzymatic zonation as a defence against oxidative stress

Another protective mechanism of the liver against oxidative stress is the enzymatic zonation of different hepatocyte zones within the hepatic acinus, according to blood flow-dependent oxygen tensions, leading to metabolic zonation inside the hepatic acinus[37-39]. Thus, metabolic processes requiring higher oxygen tensions/high CO_2 levels/high amounts of ATP, but that do not generate reactive intermediates and free radicals (*e.g.*, oxidative energy metabolism, gluconeogenesis, urea synthesis, conversion of pyruvate to glycogen with the consumption of 6 ATP molecules), occur in the periportal area, which receives oxygen-rich blood. In contrast, reactions that require a lower oxygen tension for controlled oxidation occur in the perivenous zone, where the oxygen tension is low. Examples of such reactions include the controlled oxidation of glucose to glucuronic acid and glucuronidation, reductive reactions such as glycogen synthesis *via* UDP glucose, glutamine formation and finally reactions that require the prevention of the uncontrolled oxidation of certain substrates to minimize the generation of reactive intermediates and oxygen free radicals (*e.g.*, xenobiotic metabolism *via* the microsomal cytochrome P450 system). This is achieved by the

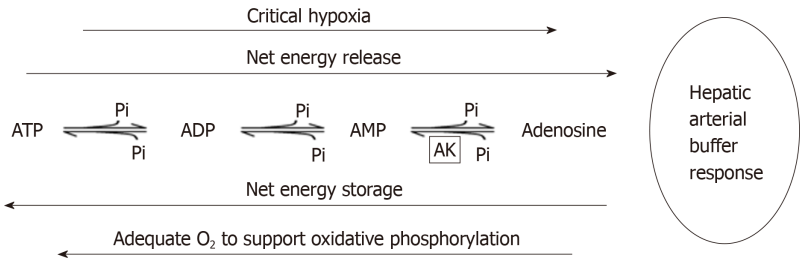


Figure 3 Production of adenosine from adenine nucleotides. ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate; Pi: Inorganic phosphate; AK: Adenosine kinase.

differential expression of enzymes among periportal and perivenous zones (called enzymatic zonation) and is probably achieved by an oxygen-mediated/dependent mechanism for gene expression in hepatocytes[40].

Perivenous (zone 3) zonation of the cytochrome P450 system confers a survival advantage by minimizing the generation of reactive intermediates and oxygen species during xenobiotic metabolism, thus minimizing hepatocellular damage. These perivenous hepatocytes may completely clear the blood of xenobiotic toxins during their first pass through the liver when the dose ingested is low. This advantage is not present when xenobiotic toxins are ingested in large doses or enter the body *via* the parenteral route or *via* inhalation (*e.g.*, halothane).

This enzymatic zonation according to the oxygen gradient within the hepatic acinus also leads to the manifestation of zone-specific patterns of liver disease. For instance, most xenobiotics (including the "cultural poison" ethanol) that are metabolized by the microsomal cytochrome P450 system, which is preferentially expressed in the perivenous area (zone 3), give rise to hepatocellular damage and necrosis in the perivenous area due to the generation of reactive intermediates and oxidative stress[41]. Acetaminophen overdose also causes zone-3 necrosis when the highly reactive oxidative metabolite of acetaminophen, N-Acetyl-p-benzoquinone-imine (NAPQI), exhausts the capacity of the glutathione scavenger system to clear NAPQI, leading to covalent binding of NAPQI to cellular proteins[42]. It is interesting to note that although N-acetylcysteine and methionine are successful in replenishing hepatocellular glutathione, most of the other antioxidants may not be able to penetrate into hepatocytes to scavenge oxygen free radicals and reactive intermediates. Halothane hepatotoxicity also causes zone 3 necrosis due to its metabolism by the microsomal cytochrome P450 system expressed preferentially by perivenous hepatocytes[43]. It has been shown that microsomal cytochrome P450 inducers such as isoniazid, phenytoin, phenobarbital and alcohol can augment hepatocellular damage due to oxygen free radicals and reactive intermediates.

Most of the aetiologies that produce cirrhosis, such as alcohol, haemochromosis, Wilson disease and non-alcoholic steatohepatitis, lead to hepatocellular damage through disturbances they cause to the oxidative catalytic potential, and when the oxidative stress is overwhelming, many of these diseases show Fenton or Fenton-like reactions within the hepatocytes with the production of highly toxic hydroxyl free radicals, further perpetuating the liver damage[44-46].

Regenerative capacity of the liver as a defence mechanism against oxidative stress

Of all the vital organs in the body, the liver has the most remarkable regenerative capacity. It can regenerate to its original size after a 70% hepatectomy within approximately two weeks while preserving all liver functions and requires no more than two cycles of hepatocyte replication[47]. More importantly, the liver can regenerate fully after massive hepatocyte necrosis, as occurs in toxin-mediated necrosis, such as acetaminophen hepatotoxicity or ischaemic necrosis[47,48]. This is particularly important as reactive intermediates and oxygen free radicals can bind to genetic material within hepatocytes and cause apoptosis or hepatocellular necrosis.

The remarkable regenerative capacity of the liver, which differs from other organs of the body, is a property of the liver due to its hypoxic microenvironment. Although critical hypoxia leads to cell cycle arrest in most tissues, a lesser degree of hypoxia can also stimulate cellular proliferation in certain tissues and cells, such as embryonic tissues and cancer cells, as well as in hepatocytes, and this replication is orchestrated by signalling molecules, such as hypoxia inducible factors[49-52].

Hepatocytes can regenerate from a variety of precursors, namely, by the replication of existing hepatocytes, by the differentiation of oval cells (cells originating in the terminal branches of the bile ductular system and the canals of Hering) or from bone marrow cells under different circumstances with different triggering stimuli and

different signalling molecules mediating such replication[47]. Wang *et al*[53] recently identified a population of proliferating and self-renewing hepatocytes in centrilobular zone 3 adjacent to the central vein of the lobule in mice in the absence of any liver injury. These cells were shown to proliferate twice as fast as the other hepatocytes and replace more than one-third of the mouse liver lobule around the central vein during homeostatic renewal in approximately one year. On the other hand, there is another group of hepatocytes, called hybrid hepatocytes, in zone 1, near the portal triad, which undergo extensive proliferation and replenish liver mass after chronic hepatocyte-depleting injuries[48]. Therefore, it can be concluded that different hepatocytes harbour different proliferative capacities. This seems to carry functional significance with regard to different triggers that invoke different patterns of hepatocellular damage and necrosis and for the restoration of hepatic mass and architecture or for the development of nodular regeneration (cirrhosis).

INSIGHTS INTO LIVER FUNCTION AND AVENUES FOR FUTURE RESEARCH

Strategic positioning of the spleen within the hepatic portal circulation is a fascinating evolutionary adaptation to the functions served by the liver and the spleen. Due to the highly stagnant and sluggish circulation within splenic sinusoids, there is increased oxygen extraction within splenic sinusoids compared to other tissues of the gastrointestinal tract. It is reasonable to expect that there is a significant contribution from this stagnant circulation in the spleen to the generation of portal venous hypoxia. The quantification of the contribution of the spleen to portal venous hypoxia is an area for potential research. Furthermore, because of the strategic position of the spleen, the largest contributor to bilirubin production in the body, within the hepatic portal venous circulation, most of the bilirubin that is produced by the spleen is cleared by the liver and excreted to the gut, thus preventing large-scale entry of bilirubin into the systemic circulation. The advantages of this arrangement are twofold. First, bilirubin is a lipid-soluble antioxidant which has complementary cytoprotective roles to glutathione and it can penetrate into cells to provide hepatocytes with an additional defence mechanism against oxidative stress and lipid peroxidation[54]. Second, since a large fraction of the generated bilirubin is prevented from entering the systemic circulation, the neurotoxicity of bilirubin is minimized.

It may be worthwhile investigating the role of jejunal and ileal arcade vessels and their arterio-venous anastomoses in the maintenance of the anaerobic environment of the gut and the hypoxic environment of the liver.

There is a significant interaction between the gut and the liver through the portal vein and biliary secretions[55]. Whether there is a role for this gut–liver crosstalk in the regulation of the portal venous flow and, if so, the nature of it and the molecules involved in it is another area for potential research.

Further research is needed to identify antioxidants that can penetrate into hepatocytes and neutralize oxygen free radicals/reactive intermediates as these would provide novel therapeutic options for diseases originating through oxidative stress.

One of the reasons for the partial success of artificial liver support systems seems to lie in their inability to replicate the anatomical relationship of the liver to the portal vein in its detoxification function as in normal physiology. It could be expected that artificial liver support systems would perform better if this problem could be addressed through the development of a mechanism to feed portal venous blood into the artificial liver support system rather than feeding systemic venous blood into it in patients with liver failure.

CONCLUSION

The liver, which can be seen as the "chemical smithy" of the body, has to work with "fire" (the catalytic potential) to serve its vital functions (Figure 4). Multiple evolutionary defence mechanisms are in place to defend the liver from the damaging effects of the oxidative stress generated by this enormous catalytic potential (Figure 5). A better understanding of these mechanisms will shed light on unravelling the mysteries of liver function and novel therapeutic options.

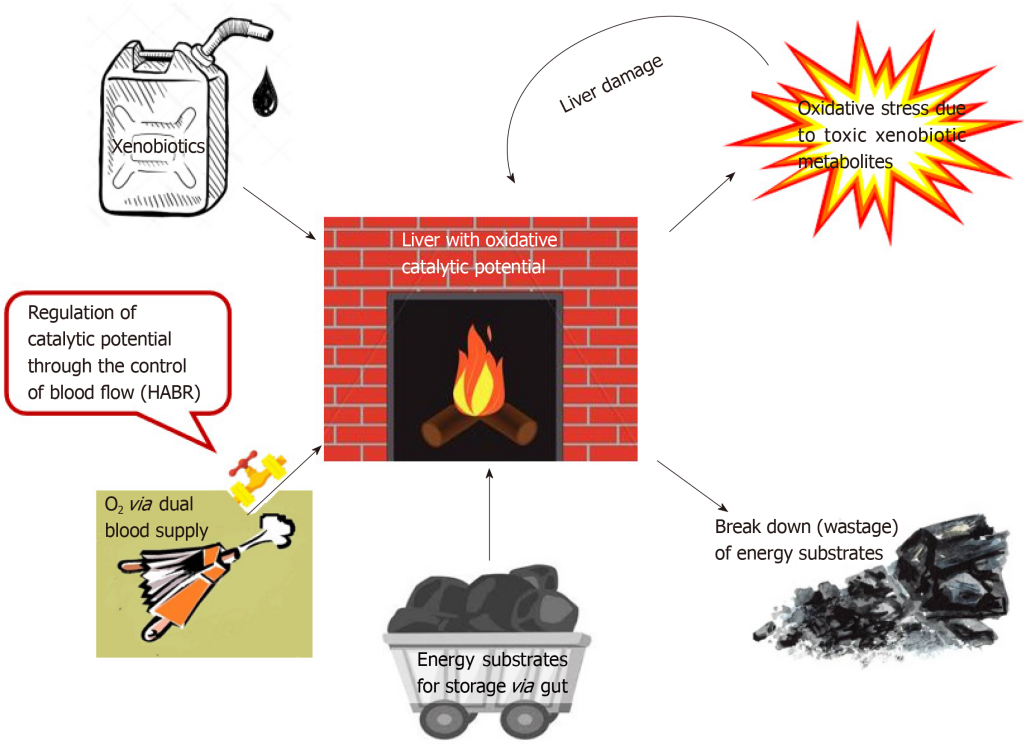


Figure 4 Liver as the "chemical smithy" of the body.

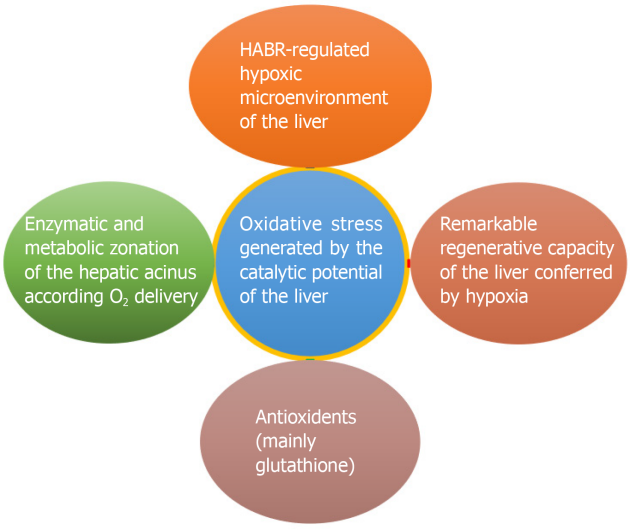


Figure 5 Protective adaptations of the liver against oxidative stress. HABR: Hepatic arterial buffer response.

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REVIEW

Helicobacter pylori and gastric cardia cancer: What do we know about their relationship?

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Abstract

The incidence of gastric cardia cancer is increasing around the world. Since the discovery of *Helicobacter pylori* (*H. pylori*), numerous studies have proved that it is a causative factor for many kinds of digestive system tumors. Although the literature on gastric cardia cancer and *H. pylori* is not scarce, there are still many controversies on the relationship between gastric cardia cancer and *H. pylori*. Many Western research results showed that there was a negative or no correlation between *H. pylori* infection and gastric cardia cancer, but in several studies in Asian countries, such as China, *H. pylori* was demonstrated to be a risk factor for gastric cardia cancer. Therefore, we intended to analyze the related studies to find out the relationship between *H. pylori* and gastric cardia cancer and find out the causes of the above controversies. We also conducted a meta-analysis of the relationship between cagA positive expression of *H. pylori* and gastric cardia cancer, to find out whether there is an effect between those two. The primary purpose of this paper was to explore the relationship between gastric cardia cancer and *H. pylori*. Through analysis, the study showed the reasons for the controversies mentioned above: (1) Geographical factors could affect the relationship between *H. pylori* and gastric cardia cancer; (2) The definition of gastric cardia cancer in various studies is inconsistent. The result of a meta-analysis about the relationship between *H. pylori* virulence factor cagA and gastric cardia cancer showed that there was no relationship between these two.

Key words: Gastric cardia cancer; *Helicobacter pylori*; Cytotoxin-associated gene A; Relationship; Risk factors; Meta-analysis

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Core tip: The relationship between gastric cardia cancer and *Helicobacter pylori* (*H. pylori*) is unclear. Therefore, this article focuses on the relationship between gastric cardia cancer and *H. pylori* and the reasons for this relationship. This paper also discusses the relationship between cagA and gastric cardia cancer, as well as the influence of different colonization sites of *H. pylori* on gastric cardia cancer and the influence of *H. pylori* on the prognosis of gastric cardia cancer, and such.

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INTRODUCTION

In modern society, cancer has become a significant cause of morbidity and mortality worldwide. At present, the incidence of gastric cardia cancer is increasing around the world[1,2]. Gastric cardia cancer stands out distinctly and is different from gastric cancer and esophageal cancer[3,4]. It is relatively insidious, and the degree of cancer cell differentiation is low. Gastric cardia cancer also has extensive invasion and rapid metastasis. Gastric cardia cancer has seriously endangered human health and has become a significant public health problem[5]. This cancer occurs in the region of the gastric cardia, which is located at the junction of the stomach and esophagus. It is the transitional zone between the distal esophageal mucosa and the proximal gastric mucosa. Gastric cardia cancer always occurs on the lesser curvature side of the gastric cardia (~75%), followed by the posterior and anterior walls, and the greater curvature side is rarely affected.

Gastric cardia cancer is neither esophageal cancer nor gastric cancer. There are many differences among the three. Scholars have found that the incidence of esophageal cancer and gastric cardia cancer increased, while the incidence of distal gastric cancer decreased[6-8]. Many epidemiological, histopathological, and molecular biological studies have showed that there are some similarities between gastric cardia cancer and distal esophageal adenocarcinoma, but gastric cardia cancer is different from distal gastric cancer and esophageal squamous cancer. Gastric cardia cancer and the other two cancers have not only different pathogenesis, but also have different prognostic factors. Besides, esophageal adenocarcinoma mainly spreads to the parastatal lymph nodes and the lower posterior mediastinum, while gastric cardia carcinoma has the characteristic of bilateral metastasis to the chest and abdominal cavity.

For the definition of gastric cardia cancer, there are few international definitions. Gastric cardia cancer is defined as cancer occurring at the anatomic site of the cardia, within 2 cm below the esophagogastric junction[9]. The Siewert[3] classification is another standard classification scheme. It differentiates the following three distinct tumor entities in the area of the esophagogastric junction: Esophageal tumor (type I), true cardia tumor (type II), and subcardial gastric carcinoma (types III). The World Health Organization (WHO) classification of tumors classified gastric cardia cancer as tumors of the esophagogastric junction in 2000. The literature states that "adenocarcinomas that cross the esophagogastric junction are called adenocarcinoma of the esophagogastric junction, regardless of where the bulk of the tumor lies."

Some scholars believe that the formation of gastric cardia cancer has undergone multi-stage pathological processes such as cardia inflammation, intestinal metaplasia, intraepithelial neoplasia, carcinoma *in situ*, and invasive cancer[10]. There are many reasons for the formation of gastric cardia cancer, and the development of gastric cardia cancer is the result of multiple factors interacting in various stages.

EPIDEMIOLOGY OF GASTRIC CARDIA CANCER

The age-standardized incidence of gastric cardia cancer (per 100000 cases) in different parts of the world was shown in the study of Colquhoun *et al*[11]. It showed that Eastern/Southeastern Asia had a higher incidence of gastric cardia cancer than other regions in the world, at 8.7 per 100000 for males and 2.4 per 100000 for females. The

incidence of gastric cardia cancer in Sub-Saharan Africa was lower than that in other regions, at 0.2 per 100000 for males and 0.1 per 100000 for females. Gastric cardia cancer was more common in males than in females.

China has a higher incidence of gastric cardia cancer in the world. Epidemiological data showed that the incidence of esophageal and gastric cardia cancer was consistent. China is a high incidence area of esophageal cancer, and many studies suggested that the incidence of gastric cardia cancer is also high in this area, where esophageal cancer has a high incidence. This phenomenon has been observed in China's Linxian (Henan Province)[12], Cixian (Hebei Province)[13], Chaoshan (Guangdong Province)[14], and other areas with a high incidence of esophageal cancer.

RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* INFECTION AND GASTRIC CARDIA CANCER

Helicobacter pylori (H. pylori), which colonizes specifically in the human stomach, was first identified from patients with peptic ulcer disease by Barry Marshall and Robin Warren[15]. The prevalence of *H. pylori* infection in most countries in the world remains high. According to Hooi *et al*[16], there were 4.4 billion cases of *H. pylori* infection worldwide in 2015. Africa had the highest percentage of *H. pylori* infection (70.1%; 95% confidence interval [CI]: 62.6-77.7), while the lowest percentage was observed in Oceania (24.4%; 95%CI: 18.5-30.4). Nigeria had the highest *H. pylori* infection rate of any country (87.7%; 95%CI: 83.1-92.2). The prevalence of *H. pylori* in Latin America cannot be underestimated. A meta-analysis in a study by Curado *et al*[17] suggested that *H. pylori* infection rates are high in all age groups in Latin America. Differences in social and economic conditions across different countries might also affect the infection rate of *H. pylori*[18]. It was associated with many diseases[19-21], especially gastric cancer[22-24].

H. pylori colonizes uniquely in the human stomach. Severe diseases caused by *H. pylori* infection are related to the host, bacteria, and environment, such as some gastrointestinal disorders[25]. There is also a link between gastric cardia cancer and *H. pylori*. Therefore, the purpose of this paper was to find out the relationship between them through literature review and meta-analysis (Supplementary Figure 1).

Data from GLOBOCAN 2018 showed that *H. pylori* infections account for 35.7% of cancers caused by infection-related factors worldwide, ranking first. It showed more details about the proportion of cancers caused by *H. pylori* infection in various regions of the world (Figure 1). The top three areas were: Central and Eastern Europe (49.3%), East Asia (47.6%), and West Asia (45.2%). These data showed the seriousness of the harm of *H. pylori* to humans.

Relationship between H. pylori infection and gastric cardia cancer by region

Table 1 shows the *H. pylori* infection rates and age-standardized incidence of gastric cardia cancer (per 100000 cases) in different parts of the world, based on the studies of Colquhoun *et al*[11] and Hooi *et al*[16]. The data showed that the infection rate of *H. pylori* was also high in several regions with a high incidence of gastric cardia cancer. However, although the infection rate of *H. pylori* was as high as 76.9% in Africa and Western Asia, the incidence of gastric cardia cancer was relatively low. Therefore, these data revealed a correlation between gastric cardia cancer and *H. pylori* to some extent.

Data from some Western countries showed that *H. pylori* was a protective factor for gastric cardia cancer, or there was no pathogenic relationship between these two. A nested case-control study of a Norwegian population by Hansen *et al*[26] and others found that gastric cardia cancer was negatively associated with *H. pylori* (odds ratio [OR] = 0.27, 95%CI: 0.12-0.59). Ye *et al*[27] found no correlation between gastric cardia cancer and *H. pylori* infection based on the native Swedish population who were younger than 80 years.

However, studies in China, Japan, and other Asian countries have shown that *H. pylori* was the pathogenic factor for gastric cardia cancer. A cohort study by Kamangar *et al*[28] on 29584 residents in Linxian (Henan Province, China) suggested that *H. pylori* infection was a risk factor for gastric cardia cancer (hazard ratio [HR] = 1.64; 95%CI: 1.26-2.14). Yasuo *et al*[29] also found that 75% of Japanese patients with gastric cardia cancer had *H. pylori* infection, and *H. pylori* infection was closely associated with gastric cardia cancer.

Marlene[30] and others conducted a meta-analysis of the research on the relationship between *H. pylori* infection and gastric cardia cancer. The population of this study included people from all over the world. The results of the study showed that for gastric cardia cancer, the pooled relative risk (PRR) was 1.08 (95%CI: 0.83-1.40; I^2 =

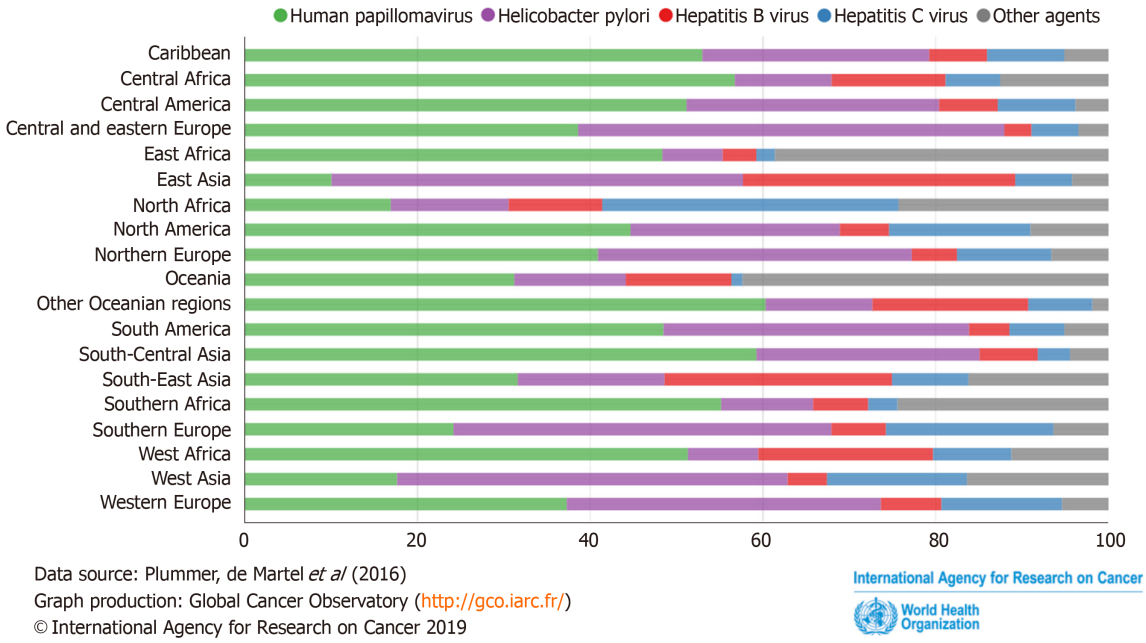


Figure 1 Proportions of cancer cases among both sexes attributable to infections in 2012 (by region).

52.8%), but the difference was not statistically significant. Subsequently, those authors divided the regions into high incidence areas and low incidence areas based on the incidence of gastric cancer. China, Japan, and South Korea were classified as high-risk settings, while Australia, Finland, Germany, United States, *etc*. were classified as low-risk Settings. The results showed that *H. pylori* infection was a risk factor for gastric cardia cancer in the high incidence areas of gastric cancer (RR = 0.78, 95%CI: 0.63-0.97; I^2 = 11.6%). This result suggested that geographical factors could affect the relationship between *H. pylori* and gastric cardia cancer.

Also, as mentioned above, although gastric cardia cancer was classified as a type of esophagogastric junction cancer by the WHO in 2000, there are still inconsistencies in the diagnostic criteria for gastric cardia cancer among many current studies. In the study of Hansen *et al*[26], the diagnosis of gastric cardia cancer was based on International Classification Atom of Diseases for Oncology (second edition). Inconsistencies in the diagnostic criteria for gastric cardia cancer may also lead to a wrong diagnosis, thus affecting the relationship between gastric cardia cancer and *H. pylori* and leading to inconsistent research results.

Relationship between H. pylori virulence factor cagA and gastric cardia cancer: A meta-analysis

The virulence factor genes of *H. pylori* include *vacA, cagA, cagE, oipA, babA2, babB*, and *iceA*, *etc*.[31,32]. *H. pylori* virulence factors play an important role in the progression of gastric cardia cancer. Cytotoxin-associated gene A (cagA) is a virulence factor of *H. pylori* that has been studied most in the world. CagA is located at one end of the cag-PAI (a 40-kb piece of DNA) and is likely to be incorporated into the *H. pylori* genome through a horizontal transfer process[33]. CagA was only found in *H. pylori* highly virulent strains. *H. pylori* cagA protein appears as a bacterial oncoprotein[34]. Lee *et al*[35] showed that people infected with *H. pylori* which contains the cagA protein produce more reactive oxygen species and have an increased risk of gastric cancer. CagA protein is the only bacterial oncoprotein identified to date. CagA contains two repeatable protein-binding motifs, the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif and the cagA multimerization (CM) motif. There are two major pathological and biochemical processes that contribute to *H. pylori* cagA-induced gastric cancer: Abnormal cancer-promoting signals caused by SHP2 imbalance *via* the EPIYA motif, and gastric epithelial destruction caused by CM-mediated PAR1 inhibition[36]. EPIYA motifs are divided into four categories (EPIYA-A, -B, -C, and -D), depending on the amino acid sequence surrounding each EPIYA motif, and they have different characteristics[37].

The current research results on the relationship between cagA and gastric cardia cancer are also controversial. In the study by Limburg *et al*[38], the adjusted OR value of cagA positive gastric cardia cancer patients compared with cagA negative patients was 1.79 (95%CI: 1.05-3.06), indicating that cagA positivity was a risk factor for patients with gastric cardia cancer. Some other studies showed that there was a

Table 1 Gastric cardia cancer age-standardized incidence rates (per 100000) and *Helicobacter pylori* infection rates

Region[1]	Gastric cardia cancer		*H. pylori* infection rate (%)
	Males	Females	General population
Eastern/Southeastern Asia	8.7	2.4	58.1
Eastern Europe	4.7	1.4	62.8
Central/Southern America & Caribbean	4.0	1.3	63.4
Central Asia	3.8	1.7	79.5
Northern & Western Europe	3.7	1.0	37.2
Oceania	3.4	1.1	24.4
Southern Europe	3.3	0.9	55.0
Northern American	2.7	0.7	37.1
Northern Africa & Western Asia	2.5	1.2	76.9[2]
Sub-Saharan Africa	0.2	0.1	

[1]Regions were based on the following UN geographical regions: Sub-Saharan Africa (including Eastern, Middle, Southern and Western Africa), Northern Africa and Western Asia, Central Asia (including India), Eastern and South-Eastern Asia (including China), Central/Southern America and the Caribbean, Northern America, Eastern Europe, Northern and Western Europe, Southern Europe and Oceania.
[2]Total *H. pylori* infection rate of Northern Africa, Western Asia, and Sub-Saharan Africa. *H. pylori*: *Helicobacter pylori*.

significant negative correlation between cagA positivity and the development of gastric cardia cancer. Ye *et al*'s[27] study showed that cagA positivity was not associated with the risk for gastric cardia adenocarcinoma (OR = 1.00, 95%CI: 0.70-1.60). Therefore, we performed a meta-analysis of the relationship between *H. pylori* cagA and gastric cardia cancer.

The study was based on the guidelines of Meta-analysis of Observational Studies in Epidemiology (MOOSE)[39] and the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA)[40]. PubMed, Web of Science, Embase, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), and Wanfang (China) electronic databases were searched for relevant articles published up to December 2019. The search items were "gastric cardia cancer" and "*Helicobacter pylori* cagA".

The quality of the eligible studies (Supplementary file 1) was evaluated according to the Newcastle-Ottawa Scale (NOS)[41] (Supplementary Table 2), and articles with a score higher than six were considered high-quality. The STATA (Version 13.1 MP, Stata Corp, College Station, TX, United States) was used to analyze the data. $P < 0.05$ or $I^2 > 50.0\%$ was considered to have significant heterogeneity. A fixed-effects model was used when there was no significant heterogeneity, otherwise a random-effect model was used. A sensitivity analysis was performed to evaluate the stability of the pooled results. Egger's test[42] and Begg's test[43] were used to assess the extent of publication bias. $P < 0.05$ was considered statistically significant, and all statistical tests were two-sided.

After screening, a total of 12 articles were included in the study[27,38,44-53]. The random-effects model ($I^2 = 42.2\%$, $P = 0.099$) and fixed-effects smodel ($I^2 = 42.2\%$, $P = 0.060$) were used for heterogeneity testing, respectively. The results of the heterogeneity test showed no significant difference. The sensitivity analysis showed that the combined OR did not change significantly, indicating that the combined OR was fairly stable. The P values of Egger's and Begg's tests were 0.277 and 0.244, respectively. Detailed results are shown in the Supplementary Materials. The fixed-effect model was eventually selected for use (Figure 2). The pooled OR of this study was 1.03 (95%CI: 0.84-1.26). The results could not indicate that *H. pylori* cagA positivity is a risk factor for gastric cardia cancer (Supplementary Figures 2-4).

Other relationships between H. pylori and gastric cardia cancer

H. pylori infection in different parts of the gastric cardia mucosa is different, which is consistent with the difference in the incidence of gastric cardia cancer in different regions. The distribution of *H. pylori* infection in the cardia mucosa is characterized by the invasion of both sides of the root of mucosal fold in the cardia. The high incidence area of gastric cardia cancer overlap with the high infection area of *H. pylori*. In the course of gastric cardia cancer, *H. pylori* infection in the cardia and gastric antrum

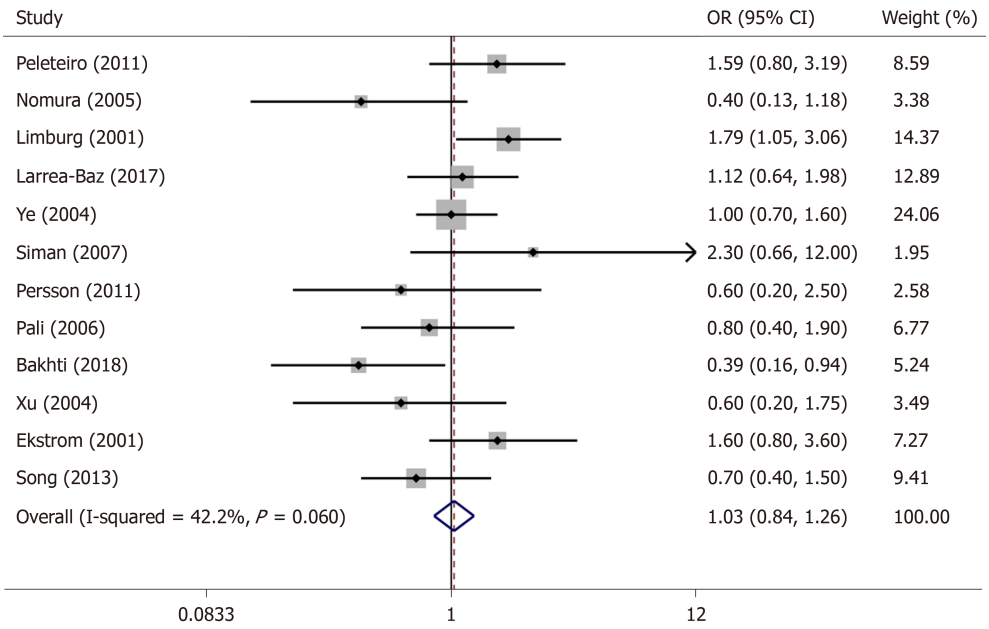


Figure 2 Forest plot of studies evaluating the odds ratios of *Helicobacter pylori* cagA positive expression for gastric cardia cancer.

mainly promotes the occurrence of the tumor. *H. pylori* infection also affects the prognosis of patients with gastric cardia cancer. *H. pylori* may be related to the prediction of gastric cardia cancer, but it is not an independent factor.

OTHER RISK FACTORS FOR GASTRIC CARDIA CANCER

Gastric cardia cancer is a multi-factorial ailment, which is the result of the interaction of multiple factors, including genetic factors, environmental factors, *etc*.

Demographic characteristics such as age, gender, and ethnicity are all factors influencing gastric cardia cancer. The incidence of gastric cardia cancer increases in the elderly, and the research by Chen *et al*[14] showed that the population of 50-80 years had a high incidence of gastric cardia cancer. Several other studies suggested that gastric cardia cancer is more prevalent in males. Colquhoun[11] and others showed that the incidence of gastric cardia cancer was significantly higher in males than in females (male: female = 3:1). Kubo *et al*[54] and others, through the analysis of five groups of cancer registration data (1992-1998), also found a high incidence of gastric cardia cancer in males.

Current studies have found that many tumors have a family genetic predisposition, and studies on the relationship between gastric cardia cancer and family history have found a correlation between these two. Yang *et al*[55] investigated 16605 patients with gastric cardia cancer and 26053 patients with non-cardia cancer through questionnaires. And after a long period of follow-up of 2000 patients, they found that positive family history significantly increased the risk of gastric cardia cancer.

Yang *et al*[55] found that smoking significantly increased the risk of gastric cardia cancer (OR = 1.98, 95%CI: 1.79-2.19). The results of the study by Zendehdel *et al*[56] also showed that compared to never-users of any tobacco, smokers had an increased risk for gastric cardia cancer (RR = 2.10, 95%CI: 1.50–3.00). Obese subjects (BMI ≥ 30 kg/m^2) had a higher risk of gastric cardia cancer than the average population (RR = 2.73, 95%CI: 1.56-4.79), according to the results of a prospective cohort study in the Netherlands[57]. Also, Jansson *et al*'s[58] study showed a correlation between covert coping strategies when maltreated at work and the risk of gastric cardia cancer.

Genetic risk factors, epigenetic risk factors, long noncoding RNAs, and microRNAs are all in the field of molecular biology. For example, a tumor suppressor protein encoded by the *p53* gene often mutates in many kinds of cancers and is related to cell proliferation and tumor growth[59]. Shao's[60] study showed that after Bonferroni correction, the association between TP53BP1 rs560191 G4C and gastric cardia cancer remained significant. The advent of multiple genome-wide association studies has led to the successful identification of many single nucleotide polymorphisms (SNPs), including those associated with gastric cardia cancer. Xiao *et al*'s[61] study also showed that the interaction between SNPs and *H. pylori* infection is related to the increased

risk of gastric cardia cancer. In Abdi *et al*'s[62] study, the factors of molecular biology of gastric cardia cancer were studied more specifically, including not only SNPsd but also long noncoding RNAs and microRNAs.

CONCLUSION

This article discusses the relationship between *H. pylori* and gastric cardia cancer; however, the relationship between *H. pylori* and gastric cardia cancer could not be analyzed generally. Accurate classification of gastric cardia cancer and patients' geographic factors can influence the relationship between *H. pylori* and gastric cardia cancer. Also, *H. pylori* has a large number of different virulence factors. In this study, only the relationship between the positive expression of cagA and gastric cardia cancer was meta-analyzed, but no correlation between these two was found. The effects of other virulence factors on gastric cardia cancer need to be further studied. Both *H. pylori* related hosts and the environment may have an impact on cardia cancer, which has not been discussed in depth in our research. In addition, the impact of family history on the relationship between *H. pylori* and gastric cardia cancer, and even the relationship between eradication of *H. pylori* and gastric cardia cancer were not included in this study, which need further research.

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REVIEW

Treatment strategies and preventive methods for drug-resistant *Helicobacter pylori* infection

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Abstract

The infection and drug resistance rates of *Helicobacter pylori* (*H. pylori*) are high and must be prevented and treated by better strategies. Based on recent research advances in this field as well as the results from our team and those on traditional Chinese medicine, we review the causes of drug resistance, and prevention and treatment strategies for drug-resistant *H. pylori* infection, with an aim to make suggestions for the development of new drugs, such as establishment of new target identification and screening systems, modification of existing drug structures, use of new technologies, application of natural products, and using a commercial compound library. This article may provide reference for eradication of drug-resistant *H. pylori*.

Key words: *Helicobacter pylori*; Drug-resistant; Strategies; Methods; Treatment; Prevention

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Core tip: The resistance rate of *Helicobacter pylori* is increasing and there is an urgent need to develop better treatment strategies. We review the current progress in the field with regard to prevention of drug-resistant bacterial infection, effective diagnosis and standardized treatment, rational application of antibacterial drugs, and prevention of drug-resistant bacterial transmission. The factors causing drug resistance are suggested to be eliminated so as to ensure the success of the first-line treatment of patients with drug resistance, whose accurate treatment is based on individual drug history and drug-sensitivity testing results. Traditional Chinese medicine has good effects in the treatment of drug-resistant bacterial infection and should be more widely applied.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is an important cause of chronic gastritis, peptic ulcers, gastric cancer, and other diseases[1-3]. In addition, *H. pylori* is associated with a variety of extra-intestinal diseases, such as periodontitis and secondary thrombocytopenic purpura[4]. *H. pylori* now infects more than half of the world's population, and the infection rate is higher in developing countries compared to developed countries, with more than 80% of cases diagnosed in underdeveloped areas[5-7]. Due to the high infection rate of *H. pylori* and the widespread use of antibiotics for treatment, the drug resistance rate of *H. pylori* is increasing along with a simultaneous decrease in eradication rate, which poses a serious threat to public health. There is an urgent need for better strategies to prevent and treat *H. pylori* infection. In this paper, we review the current advances in methods of prevention and treatment of drug-resistant *H. pylori* infection, aiming to provide reference for eradication of drug-resistant *H. pylori*.

CAUSES OF DRUG RESISTANCE OF *H. PYLORI*

The main methods of the international *H. pylori* eradication program include standard triple, non-bismuth quadruple, bismuth quadruple (a proton pump inhibitor + bismuth + two antimicrobial agents) treatments. The non-bismuth quadruple regimens consist of sequential, concomitant, and mixed therapies. Currently, bismuth quadruple treatment is preferentially recommended. The Kyoto consensus emphasizes that eradication is the first-line treatment for patients with *H. pylori* infection with dyspepsia[8]. The Toronto consensus provides recommendations on *H. pylori* eradication methods for adult patients[9]. The Maastricht V/Florence Consensus points out that when *H. pylori* is sensitive to clarithromycin (CLA), the eradication rate of the international standard triple protocol is 97.3%[10]. In areas with high resistance to CLA or double resistance, the eradication rate of quadruple therapy containing bismuth is up to 86%[11]. In the Fifth National Consensus Conference on the Management of *H. pylori* Infection held in China, the eradication rates of the seven regimens are all around 90%[12].

Although the efficacy of first-line treatment containing various antibiotics to which *H. pylori* is sensitive, the eradication rate is less than 100%, and with the failure of the first eradication, the rate of drug resistance increases and so radical treatment becomes more difficult. The causes of eradication failure and drug resistance mainly include history of antimicrobial use, improper use of antibiotics, course of digestive diseases, and certain drug characteristics[13], which are listed in Table 1. Although amoxicillin is not prone to resistance, the rate of resistance to amoxicillin has gradually increased in recent years[13], which highlights the severity of drug resistance of *H. pylori*. Consequently, in 2017, the World Health Organization listed CLA-resistant *H. pylori* as one of the 12 pathogens in urgent need of new antibiotics[14].

PREVENTION AND TREATMENT STRATEGIES FOR DRUG-RESISTANT *H. PYLORI INFECTION*

At present, strategies targeting drug-resistant *H. pylori* include prevention of drug-resistant bacterial infection, effective diagnosis and standardized treatment, rational application of antibacterial drugs, and prevention of drug-resistant bacteria transmission (Figure 1). Effective prevention is the source of control of drug-resistant *H. pylori* infections. Only with reasonable prevention can the incidence of infections be effectively decreased. When drug-resistant infections occur, effective diagnosis and standardized treatment are essential towards reducing the occurrence of secondary drug resistance and increasing the eradication rate of *H. pylori*. Invasive or non-invasive testing is the key to rapid and effective diagnosis. Selection of antibiotics

Table 1 Factors influencing drug resistance of *Helicobacter pylori*

Level	Causes
Main factors	History of antimicrobial use
	Antibiotics are not properly used
	Course of disease
	Drugs tend to develop resistance
	Transmission of drug-resistant plasmids
Secondary factors	Age
	Gender
	Race
Other factors	Territory
	Planting site and density

based on the sensitivity to antibacterial drugs and formulation of rational, standardized, and accurate treatment plans based on patient condition are also critical[12].

Effective antibacterial drugs are the key to *H. pylori* prevention and treatment. The government, health administration departments, drug regulatory departments, and hospital medical staff must proactively perform their respective duties to enhance antibacterial drug management. Other important aspects include forming a management system for rational drug use, formulating medication guidelines, establishing antibacterial drug guidelines and drug resistance monitoring networks, and supporting the development of new types of drugs. Furthermore, preventing transmission is an important step towards stopping the spread of drug-resistant *H. pylori*. Security control in laboratories and hospitals should be strengthened to prevent the spread of drug-resistant strains.

METHODS FOR PREVENTION AND TREATMENT OF DRUG-RESISTANT *H. PYLORI*

Based on the above-mentioned four strategies for the control of drug-resistant *H. pylori* and combined with research from our research group, we will conduct a detailed analysis of the prevention methods (Figure 2).

Prevention of infection

Prevention in adolescents: One third of children worldwide have been infected with *H. pylori*, highlighting the importance of prevention in adolescents[15]. Screening for the disease in adolescents can decrease the lifetime risk of gastric cancer. As *H. pylori* is transmitted mainly through fecal-oral and oral-oral routes, parents should pay attention to the diet of adolescents, keep oral hygiene, avoid mouth-to-mouth feeding, promote meal sharing, and avoid mixing water cups, toothbrushes, and mouthwash cups amongst family members.

Prevention in adults < 50 years old with a low gastric cancer risk: For this population, an *H. pylori* infection test and a gastric atrophy test should be combined to effectively prevent transmission to the next generation. In addition, tableware and toilets should be disinfected frequently, and people should develop good eating habits and pay attention to the hygiene of drinking water. Medical workers in hospitals should pay special attention to the transmission of *H. pylori* in hospitals.

Prevention in adults ≥ 50 years old with a high gastric cancer risk: *H. pylori* infection increases with age. One of the main reasons is cross-infection in the home or in the population. In addition to paying attention to health problems in the family, people with a high gastric cancer risk should also undergo screening for the disease. The combined detection of serum pepsinogen and *H. pylori* antibodies can increase the level of prevention for people with a high gastric cancer risk[16].

Effective diagnosis

Diagnosis of *H. pylori* infection is extremely important. Invasive or non-invasive detection methods are usually used in the individual diagnosis of *H. pylori* infection. Invasive detection methods include endoscopy[17], rapid urease test[18], histological

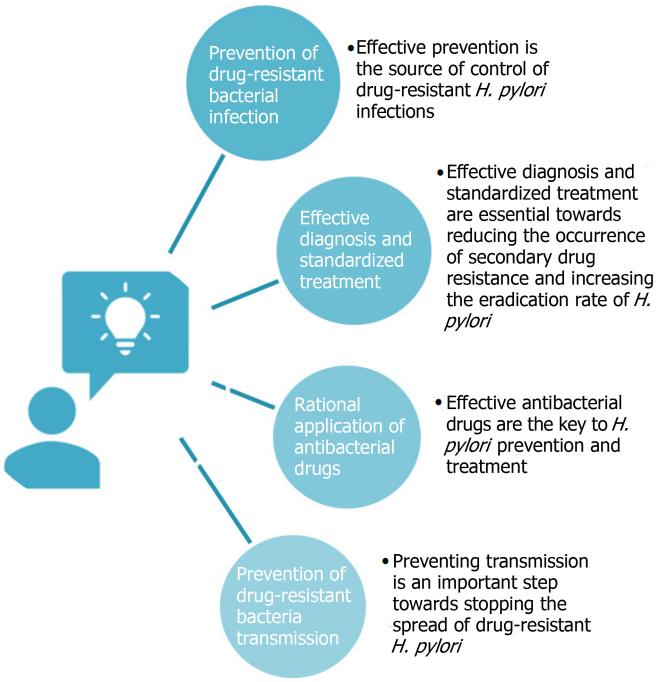


Figure 1 Strategies for prevention and treatment drug-resistant *Helicobacter pylori* infection.

method, and bacterial culture[19]. Non-invasive detection methods include ^{13}C urea breath test[20], serum antibody test[21], stool antigen test[22], and other molecular biology techniques. Each test method has its own advantages and disadvantages, which should be selected from person to person. Considering the accuracy and safety of diagnosis, non-invasive detection methods are generally recommended. There are also some other approaches used to assist diagnosis such as serum pepsinogen measurement[23] and gastric X-ray[24].

Standardized treatment

Adjusting standardized medication: Drug-resistant *H. pylori* is a strain that is not easily eradicated after the first standardized treatment. To treat this refractory disease caused by drug resistance, antibiotics should be adjusted in time for remedial treatment based on the results of drug sensitivity testing or medication history. Seven common antibiotic combinations[25], as shown in Figure 3, are used in the remedy in the treatment of antimicrobial drugs with low drug resistance. In the protocol, antibacterial drugs such as tetracycline, metronidazole, and amoxicillin have a high eradication rate[26]. *H. pylori* has high resistance to CLA, with a primary resistance rate of 20% to 50%. In areas with high rates of CLA, or high rates of metronidazole-CLA dual resistance, quadruple therapy is recommended in preference to CLA and metronidazole as first-line therapy[27]. Non-bismuth quadruple concomitant and sequential therapies can also be used as an eradication protocol. Patients were less well tolerated and had poor compliance to these two treatment regimens[28]. The 7-d concomitant therapy has been shown to be better than 7-d or 10-d triple therapy[29]. With the same treatment course, concomitant therapy is superior to sequential therapy[30]. Moreover, antimicrobial treatment should be controlled[31]. The actual trend is towards the use of quadruple instead of triple therapy and prolongation of the duration of each eradication regimen (10-14 d).

New quadruple therapy has a modification to the traditional triple and quadruple therapy that should be used as the first treatment option. Recent studies indicated that minocycline, cephalosporins[32], and rabeprazole can be used as alternatives to antibacterial agents in quadruple therapy[33]. However, new quadruple therapy is in its early stage, and the dosage and course of treatment should be further optimized.

Combination of Chinese and Western medicines: Antibiotics are broad-spectrum agents and prone to drug resistance, while traditional Chinese medicines are less prone to drug resistance, less toxic, and have complex mechanisms. Combining the two can effectively treat *H. pylori* infection. In a previous study, 162 patients with *H. pylori* infection were randomly divided into two groups. The treatment group received traditional Chinese medicine combined with Western medicine for anti-*H. pylori* therapy. After three courses, the effective rate was as high as 100%, which was

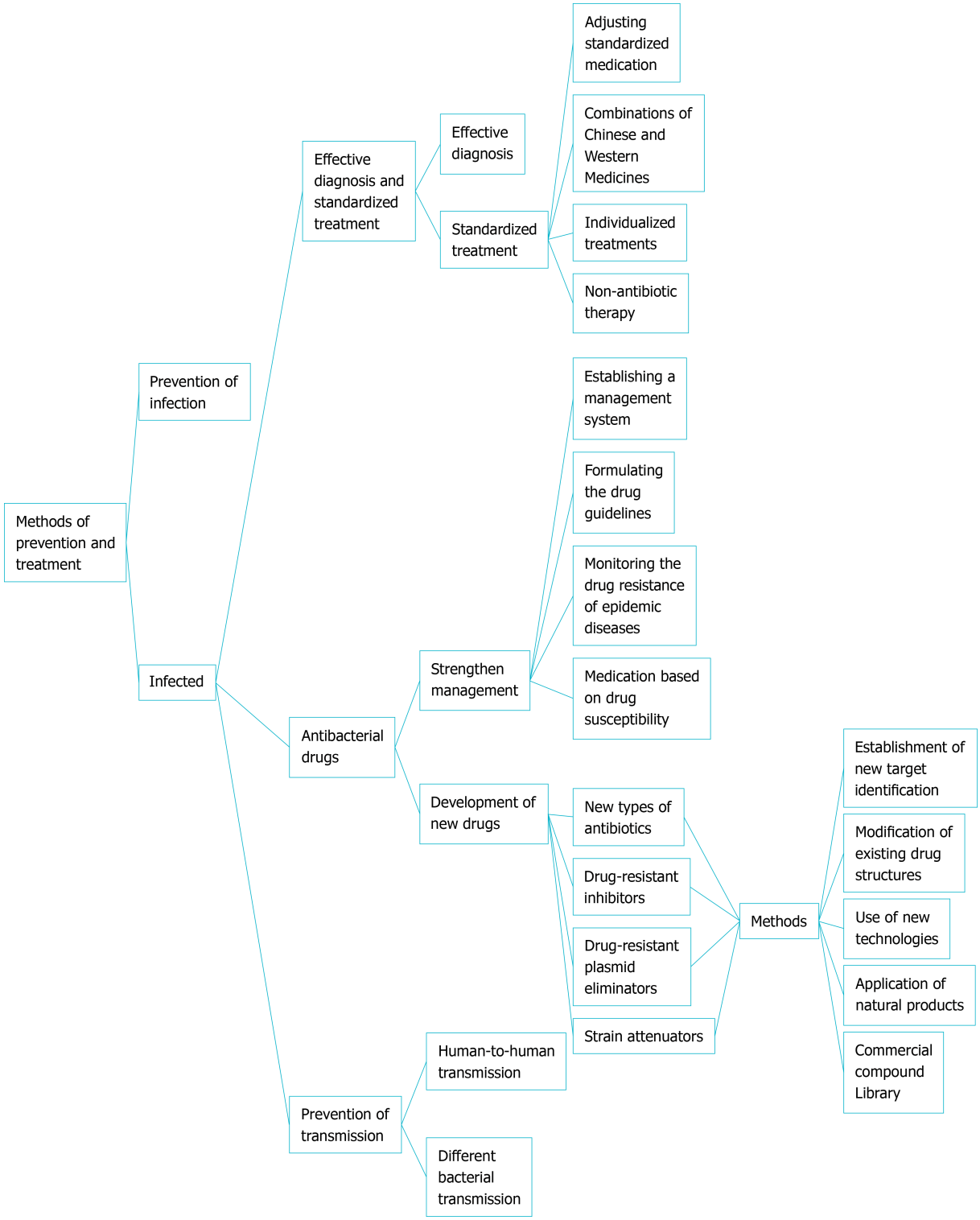


Figure 2 Methods for prevention and treatment of drug-resistant *Helicobacter pylori* infection.

significantly higher than that of the control group treated with Western medicine alone[34]. This result showed that the combination of traditional Chinese and Western medicines is significantly better than Western medicine alone, which may improve the prognosis of patients.

Individualized treatments in special pathological conditions: As amoxicillin is not prone to drug resistance and has few adverse reactions, it is often the first choice for *H. pylori* eradication therapy. However, when patients are allergic to penicillin, other antibiotics with low resistance should be selected instead of amoxicillin, such as tetracycline[35]. When choosing a regimen containing antibiotics to which *H. pylori* is highly-resistant, treatment time can be appropriately extended[28]. Patients with *H.*

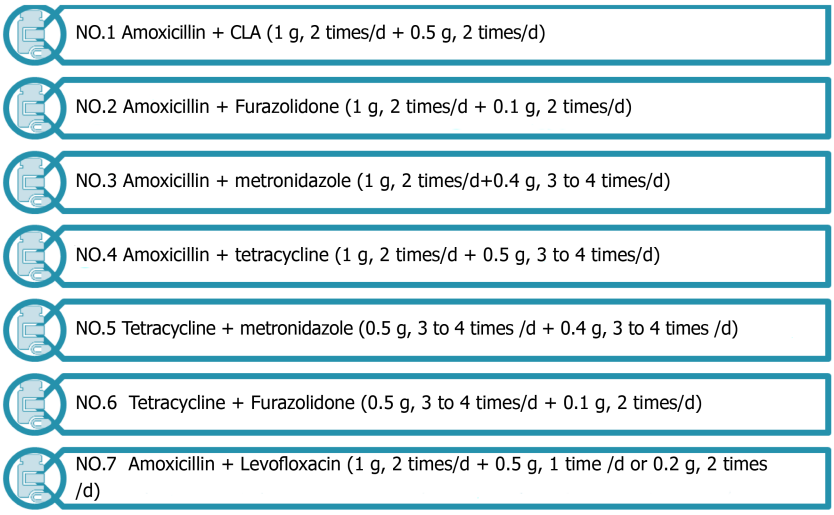


Figure 3 Combination of common antibacterial drugs. Notes: Nos. 5 and 6 are suitable options for those allergic to penicillin. Levofloxacin has a high drug resistance rate and 7 is not the first choice.

pylori infection should have their blood glucose well-controlled during treatment[36]. In the treatment of such patients with *H. pylori* infection accompanied by special pathological conditions, individualized treatment plans should be formulated based on the patient's condition.

Non-antibiotic therapy: Currently, triple or quadruple therapies are widely accepted as first-line *H. pylori* eradication treatment regimens; however, these regimens often lead to some adverse outcomes, such as intestinal flora imbalance and drug resistance. Probiotics are an important factor in the human body to maintain the micro-ecological balance. Combining probiotics with other therapies to treat *H. pylori* infection is safe, feasible, and beneficial. For example, Lactobacillus can influence the colonization of *H. pylori*[37], and the triple treatment combined with Blaird's yeast had a significantly higher eradication rate than the traditional triple treatment[38]. However, the adjuvant role of probiotics in the eradication of *H. pylori* remains controversial and more research is warranted[39].

Traditional Chinese medicine treatments are methods with Chinese characteristics. Some monomer components containing Chinese medicine mucosal-protective agents exhibit the characteristics of high eradication rate, low drug resistance, few adverse reactions, and low toxicity. These medicines even kill drug-resistant *H. pylori* and may therefore provide new tools for the eradication of *H. pylori*[40]. For example, some quinolone alkaloids in *Fructus Evodia* inhibit the growth of *H. pylori* and achieve eradication without affecting the intestinal flora[41]. According to epidemiological statistics, the total effective rate of traditional Chinese medicine treatment can reach 95.45%[42].

The bacteriostatic effect of berberine is strongest amongst the monomer components of Chinese medicine, followed by rhubarb, and scutellaria. Cortex, Radix Ginseng, Forsythia, and Hedyotis diffusa also exert a certain antibacterial effect[43]. The mechanism of action of traditional Chinese medicines may be related to the inhibition of functional protein synthesis[44], biofilm synthesis[45], inflammatory factor release[46], and virulence factor release[44] and the reduction of adhesion[47]. The mechanism of action for these medicines is complex. Also, the extraction and analysis of active ingredients have not fully completed, and the course of treatment is difficult to control.

Rational application of antibacterial drugs

Strengthening the management of antibiotics: According to the regulations covering the use of antibiotics in the "Administrative Measures for the Classification of Prescription Drugs and Over-the-Counter Drugs"[48], the management system for antibiotics should be strictly enforced. Measures include enhancing the management of antibiotics in hospitals, formulating a reasonable medication management system, and preventing antibiotic abuse. In particular, medical workers should ensure that patients use antibiotics safely, reasonably, and effectively, to ensure their health and well-being.

Following the principles of antibiotic use: The principles of antibiotic use should be

strictly followed: (1) Clear medication indications and corresponding antibiotics need to be used for *H. pylori* infection; (2) For targeted use, triple therapy is preferred; (3) The rational dosage of drugs and the sufficient course of treatment can not only ensure efficacy but also prevent the development of drug resistance; (4) The patients should be asked about the history of drug allergy in detail before the medication; (5) The appropriate method of administration needs to be selected so that the general *H. pylori* medication is orally administered; (6) The drug should be carefully changed along with the treatment plan after confirming failure of the triple or quadruple therapy; and (7) Patients with impaired liver and kidney function should be cautious in medicine taking.

Biological standards for rational drug use: Rational drug use refers to the selection of the best drug and the formulation of a dosing plan to effectively, safely, and economically prevent and cure diseases. The World Health Organization has established biological standards for rational drug use as follows: (1) Proper use of drugs needs to be ensured; (2) The drug information is appropriate; (3) The efficacy, safety, use, and price are appropriate for patients; (4) Dosage, usage, and course of treatment should be appropriate; (5) The subject is appropriate, without contraindications or significant adverse reactions; (6) The drug resource allocation is correct; and (7) Patients have good drug compliance.

Indications for antibiotic application: Antibiotics can be classified into first, second, and third-line drugs according to the antibiotics management classification. *H. pylori* infections are usually treated with first- and second-line drugs. First-line drugs refer to antibiotic drugs that are non-restricted, narrow-spectrum, and positive in effect, have slight adverse reactions and low prices, and are available in sufficient supply. Second-line drugs are the drugs that are restricted in use, have a broad antibacterial spectrum and good curative effects, but have obvious adverse reactions or are more expensive, or are drug varieties that may develop rapid resistance and have controlled use. Third-line drugs are generally used in a unique way as they exert curative effects but are relatively toxic and expensive. They are a class of drugs that will have serious consequences once drug resistance occurs. In the treatment of drug-resistant *H. pylori* infection, the most suitable antibiotic should be selected and used according to the best course of treatment. Narrow-spectrum, "low-grade" antibiotics should be used as much as possible.

Drug susceptibility testing for accurate treatment: Differences in the rates of drug resistance are closely associated with region[49], medical standards, economic development level, and quality of life[50]. The resistance rate of *H. pylori* to antibacterial drugs can determine the eradication rate of treatment options. The epidemic of drug resistance differ among different regions. Based on local drug resistance monitoring data, specific drug resistance conditions should be combined with drug sensitivity tests to make a reasonable plan to achieve the purpose of a precise treatment.

Development of new drugs and more drug candidates

H. pylori has serious drug resistance, particularly multiple drug resistance, for which there are not many drug candidates available. Therefore, new types of antibiotics, drug-resistance inhibitors, drug-resistance plasmid eliminators, and strain attenuators urgently need to be researched and developed based on the following methods:

Establishment of new target identification and screening systems: Screening new targets provides new avenues for the development of new antibacterial drugs. Some enzymes are involved in the biosynthesis of unsaturated fatty acids, such as *H. PYLORI*0773 (FabX), a decapeptide from the decanoyl-acyl carrier protein (ACP) in a parallel reaction with the first enzyme of acyl-CoA dehydrogenase of the fatty acid β-oxidation cycle. Also it isomerizes trans-2-decenol-ACP to form a key UFA synthesis intermediate, cis-3-decenoyl-ACP, which reverses the normal fatty acid synthesis cycle of *H. pylori* in the c10 phase[51]. However, there remains a certain distance from the screening of targets to drugs entering clinical trials.

Modification of existing drug structures: Modification, semi-synthesis, and synthesis of existing drugs are currently the recommended methods. Amoxicillin-UCS-2/tripolyphosphate (TPP) nanoparticles constructed with urea-modified chitosan derivatives UCS-2 and sodium tripolyphosphate (STPP) have more effective and specific effects in eliminating *H. pylori in vitro*. Amoxicillin UCCS-2/TPP nanoparticles reduced the levels of pro-inflammatory cytokines and decreased inflammatory damage caused by *H. pylori* infection[52]. Modification of drugs can ensure their activity and shorten the time of preparation and mechanistic exploration; however, the toxicity of newly modified drugs should also be tested.

Use of new technologies: MicroRNAs (miRNAs) are a class of small non-coding RNAs widely found in intergenic or intron regions. They play a role in suppressing cancer mainly by regulating the expression of tumor suppressor genes[53]. MiRNA210 is a candidate molecule that is often highly expressed in gastric cancer and mediates epithelial-mesenchymal transition. It is considered a viable molecular target in the treatment of gastric cancer and can inhibit the invasion and metastasis of gastric cancer[54].

Application of natural products: Natural products include plants, microbial secondary metabolites, and marine life. Both live cells and the supernatant of *Lactobacillus plantarum* ZDY201 can inhibit the growth and urease activity of *H. pylori*. Owing to its good lactic acid production and anti-inflammatory effects against *H. pylori* SS1 infection, it is expected to become a candidate strain of probiotics[55]. Traditional Chinese medicine has an *H. pylori* killing effect, low drug resistance, and low toxicity, and can be used as non-antibiotic drugs to treat *H. pylori* infection[56]. Some natural products and agents have been shown to affect the TLR4 and MAPK signaling pathway activation by *H. pylori*[57]. Screening active ingredients from plants is a fast and effective method for treating drug-resistant *H. pylori* infection.

Commercial compound library: Some old drugs that are used in clinical trials can be used. For example, furazolidone has been used as a replacement for CLA or metronidazole[58]. However, due to the limited types of old medicines, it is difficult to purchase such medicines, which limits their usage.

Prevention of transmission

Although drug-resistant *H. pylori* is weakly infectious, in terms of preventing transmission, in addition to preventing human-to-human transmission, the most important thing is to prevent transmission of drug-resistant plasmids. The spread of drug-resistant plasmids can occur between different bacteria, as well as between different individuals, strains, and animals, making humans susceptible to drug free *H. pylori* infection[59]. Livestock management[60], drug resistance testing[61], and management of antibiotics for other infectious diseases should also be implemented.

OUTLOOK

Through the unremitting efforts of scientific researchers and people from all works of life, some results in the prevention and treatment of drug-resistant *H. pylori* have been acquired. For example, zinc linolenate can specifically act on *H. pylori* and does not easily result in drug resistance[62]. However, there is still a long way to deal with the key problem of low *H. pylori* eradication rate. Drug resistance monitoring, application of drug susceptibility testing, and research and development of new drugs warrant further exploration. *H. pylori* associated gastritis is an infectious disease. Vaccines are the most effective method for *H. pylori* infection prevention, however, no vaccine is currently available. A vaccine that is expected to prevent *H. pylori* infection will be introduced to the market in the near future.

CONCLUSION

Currently, there is no effective way to prevent and treat drug resistance in *H. pylori* infection. To cope with this situation, we suggest comprehensive prevention and treatment measures. First, the factors of causing drug resistance are suggested to be eliminated to ensure the success of the first triple or quadruple treatment for patients with drug resistance, whose accurate treatment is based on individual drug history and drug-sensitivity testing results. Traditional Chinese medicine plays a unique role in the treatment of drug-resistant bacteria with relatively few side effects, which is worthy of further exploration.

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MINIREVIEWS

Utility of gastrointestinal ultrasound in functional gastrointestinal disorders: A narrative review

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Abstract

Gastrointestinal (GI) ultrasound (GIUS) is valuable in the evaluation of GI diseases such as inflammatory bowel disease, but its use in functional GI disorders (FGIDs) is largely unknown although promising. In order to review the current knowledge on current and potential uses of GIUS in FGIDs, information was obtained *via* a structured literature search through PubMed, EMBASE and Google Scholar databases with a combination of MESH and keyword search terms: "ultrasound", "functional GI disorders", "irritable bowel syndrome", "functional dyspepsia", "intestinal ultrasound", "point of care ultrasonography", "transabdominal sonography", "motility", "faecal loading", "constipation". GIUS is currently used for various settings involving upper and lower GI tracts, including excluding organic diseases, evaluating physiology, guiding treatment options and building rapport with patients. GIUS can be potentially used to correlate mechanisms with symptoms, evaluate mechanisms behind treatment efficacy, and investigate further the origin of symptoms in real-time. In conclusion, GIUS is unique in its real-time, interactive and non-invasive nature, with the ability of evaluating several physiological mechanisms with one test, thus making it attractive in the evaluation and management of FGIDs. However, there are still limitations and concerns of operator dependence and lack of validation data for widespread implementation of GIUS in FGIDs.

Key words: Ultrasound; Functional gastrointestinal disorders; Irritable bowel syndrome; Functional dyspepsia; Constipation

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Core tip: Functional gastrointestinal (GI) disorders are extremely common for every gastroenterologist. However, they are largely a heterogenous group of conditions and we do not have reliable modalities of investigational tools to evaluate origin of symptoms.

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GI ultrasound has increasing value for the evaluation of GI diseases. We are the first to perform a review on this topic of the utility of GI ultrasound in functional GI disorders. Our results show that though the potential uses are promising, more validation data is needed for widespread implementation.

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INTRODUCTION

Gastrointestinal (GI) ultrasound (GIUS) is increasingly recognised as a valuable tool in the evaluation of GI disease[1], especially in inflammatory bowel disease (IBD) where it has a similar diagnostic yield to endoscopy and cross-sectional imaging[2].

Functional GI disorders (FGIDs) are disorders of gut-brain interaction related to mechanisms such as motility disturbances, visceral hypersensitivity and altered central nervous system processing[3]. Their diagnoses rest on symptom-based criterias and exclusion of organic diseases. Although it is suggested that physicians make a positive diagnosis of FGID and minimize investigations[4], many perform a limited set of tests, with normal endoscopies often being the "confirmation" of a FGID[5]. GIUS have been recommended by some society guidelines to rule out organic diseases before diagnosing a FGID[6,7]. As such, the primary goal of this review is to discuss the current and potential utility of GIUS in the evaluation of FGID, focusing on common FGIDs such as IBS and functional dyspepsia (FD). As part of the review, we will discuss how clinicians can take advantage of the unique nature of GIUS to evaluate upper and lower GI physiology, exclude organic disease, guide treatment decisions and build rapport with patients.

A literature search was conducted using Medline (1946 to February 2019), EMBASE (1947 to February 2019) and Google Scholar databases using a combination of MESH and keyword search terms: "ultrasound", "functional GI disorders", "irritable bowel syndrome", "functional dyspepsia", "intestinal ultrasound", "point of care ultrasonography", "transabdominal sonography", "motility", "faecal loading" and "constipation". Papers not written in English were excluded.

EVALUATING GI PHYSIOLOGY

The GI tract is a unique system, with many organs performing multiple different functions. Thus, it is challenging to thoroughly assess GI function using currently available imaging techniques, which mainly evaluate anatomical structures. GIUS allows real-time evaluation of organ function, in addition to structure, and can provide a wide array of information on physiology such as motility, biomechanics, flow, and organ filling/emptying[8].

For example, GIUS allows real-time dynamic assessment of intestinal peristalsis to narrow down possible differential diagnoses as diminished peristalsis can indicate an unhealthy bowel seen in small bowel inflammation, obstruction, ischemia, and infiltrative processes[9]. If a transition point with a collapsed distal bowel is also seen, this may suggest mechanical obstruction[10]. Certain patterns can be seen in malabsorptive conditions like coeliac disease, where small bowel wall thickening is seen with hyperperistalsis causing a constant to-and-fro movement of luminal content[11].

FD is a common FGID with many putative pathophysiological mechanisms including antral hypomotility, antroduodenal dyscoordination, impaired accommodation, delayed gastric emptying and gastric hypersensitivity, each of which could contribute to the various subtypes[12,13]. It is important to consider the contributing pathophysiology to symptoms in each patient[13] as newer treatment options are available to target specific pathophysiological disturbances[12]. GIUS offers the benefit of evaluating more than one mechanism in a single test.

GIUS has been used to evaluate gastric emptying for more than 30 years[14] and has been shown to have good correlation to the "gold standard"[15] of gastric emptying measurement: Scintigraphy, with the benefit of no exposure to ionising radiation.

GIUS has shown good reliability and interobserver agreement in the measurement of gastric emptying rates[16], and is cheaper and more easily repeatable compared to other modalities such as wireless motility capsules, gastric emptying breath tests and MRI[17]. This process of gastric emptying is complex and affected by many factors such as antral contractions, antroduodenal coordination, proximal gastric relaxation and pyloric tone[18], but most investigational modalities look at only a single aspect of gastric emptying[19]. GIUS overcomes this by providing real-time information on multiple parts of gastric physiology[20]. For example, GIUS can demonstrate gastric contractions and measure the frequency and amplitude of these contractions[21,22], thus investigators can visualise whether delayed gastric emptying is due to an abnormally intense pyloric contractility or antral hypomotility[23], as treatment options may differ depending on the finding[24]. GIUS can also assess the stomach's ability to accommodate after meals[25], which is another mechanism contributing to FD symptoms, with dyspeptic patients showing smaller proximal stomach volumes after meals[26,27].

CLINICAL UTILITY IN EXCLUDING ORGANIC DISEASES

Differentiating between FGID and organic diseases can sometimes be difficult. One of the competing diagnoses for IBS is IBD which may also affect a similar demographic profile of patients[28]. It is particularly important for a timely diagnosis of IBD to be made, as delay in treatment can result in the development of complications. Laboratory markers such as C-reactive protein and faecal calprotectin are not always accurate predictors of inflammation[29,30]. Colonoscopy and cross-sectional imaging modalities have been longstanding stalwarts of the diagnostic armamentarium for IBD. However, colonoscopy is invasive and requires bowel preparation and sedation, CT imaging has the risks of ionising radiation, and MRI is costly and time-consuming.

GIUS has the benefit of being less costly, non-invasive and widely available, making it particularly useful in areas where healthcare resources are limited. It obviates the need for sedation, fasting or bowel preparation, making it ideal for repeated real-time use in clinics. GIUS also allows the managing clinician to perform the targeted examination, so decisions on therapy can be made in the right clinical context.

A prospective, real-world study[31] on consecutive patients presenting to a gastroenterology unit with symptoms suggestive of bowel disease, showed that the overall sensitivity and specificity of GIUS compared to radiological and endoscopic studies for bowel disorders was 85.4% and 95.4%, respectively. Another study[32] looked at 58 consecutive symptomatic patients presenting to a gastroenterology clinic and found GIUS to have an overall sensitivity and specificity of 80% and 97.8% respectively compared to endoscopy in identifying inflammatory causes of their symptoms. GIUS could differentiate IBS and IBD patients in a consecutive series of 313 patients[33] admitted to an outpatient clinic with non-specific chronic abdominal pain and bowel dysfunction with GIUS having 74% sensitivity and 98% specificity in detecting IBD compared to radiological and endoscopic studies. These studies suggest that GIUS can serve as a useful tool in differentiating IBD from FGID. Furthermore, GIUS can be used to triage patients and decide the urgency of investigations. GIUS can help support timing and urgency of endoscopy, as a negative GIUS together with a normal CRP and faecal calprotectin makes IBD very unlikely and therefore further investigations unnecessary[32,33]. Furthermore, the cases missed by GIUS were mild endoscopically, and often did not need urgent treatment[32,33]. In addition, they could be followed with serial GIUS scans to determine when treatment was warranted.

FGIDs also commonly result in severe abdominal pain requiring hospital admission, and emergency physicians not familiar with the patient's background may subject them to excessive investigations. GIUS scans have been suggested as first-line imaging tools in patients with an acute abdomen[34], and have been shown to be comparable to CT scans in the diagnosis of appendicitis[35], diverticulitis[34] and intestinal obstruction[10]. The ability of GIUS to evaluate both upper and lower GI tracts, as well as intestinal and extra-intestinal features makes it a valuable initial tool when the cause of patient's symptoms is not entirely clear. Furthermore, GIUS is able to visualise splanchnic vessels, mesentery, omentum and lymph nodes, and abnormalities in these areas lend weight to certain diagnoses[36]. GIUS can also be extended to evaluate other organs to include differentials such as ascites, ruptured ectopic pregnancies[37], nephrolithiasis[38] and gallstones[39].

ASSESSING SEVERITY AND SUBTYPING IN IBS

The diagnosis of FGID is centred solely on the patient history, but there are disparities between patient reported bowel habits and objective features of constipation or diarrhoea[40,41]. GIUS can objectively demonstrate constipation can help subtype IBS and educate patients, while the visualisation of the location of the faecal retention can aid the physician in choosing the appropriate treatment (Figure 1). Scoring systems[42] have been developed to report the severity of faecal loading on radiological images, by looking at the degree of faecal retention and bowel dilatation. Since GIUS has been shown to be comparable to CT scans in the visualisation of faecal loading[43], it can quantify faecal loading without exposing the patient to radiation. The severity of faecal loading can be determined on GIUS[44], with haustra-shaped acoustic reflections suggestive of "harder" faecal loading (Figure 1A), while has shown that a composite measurement of colonic diameters has been shown to be a surrogate measure of constipation[45].

There is also the potential for novel metrics to assess bowel contents. To date, MRI has been used to evaluate differences in small and large bowel content between healthy controls and patients[46], and a constricted small bowel was more commonly found in non-constipated IBS while a dilated transverse colon was more likely in IBS-C[47]. GIUS can potentially subtype IBS patients *via* these measurements, while offering the added benefits of being cheaper and more widely available.

There is also interest in using GIUS to assess colonic transit time which is recommended to be evaluated in chronic constipation[48]. At present transit time is measured either radiologically, *via* a nuclear medical study, or *via* wireless motility capsule which is expensive and not widely available. GIUS has been used to evaluate colonic transit time using water-filled latex balloons containing metal particles, and then following the metal particles through the stomach, small bowel and colon. In summary, GIUS has the potential to measure faecal loading, colonic diameter, and transit time in a single test.

USING GIUS TO BUILD RAPPORT WITH PATIENTS

GIUS plays a unique role in FGID patients by helping to strengthen the physician-patient relationship through several ways. IBS patients' have been shown[49] to yearn for (1) increased quality time spent with their healthcare provider; (2) education on their condition; and (3) reassurance that organic diseases were excluded. Since GIUS does not involve sedation, physicians can use the time during the procedure to build rapport with patients by involving them in the discussion of their condition. GIUS was shown[32] to improve patient's understanding of their condition and was generally preferred over endoscopy.

In a study by patients attending the emergency department were randomised to bedside ultrasound or standard clinical examination[50], and higher patient self-rated satisfaction score was found in the ultrasound group with decreased short-term health care consumption. Other studies have found similar results[51,52]. It has also been shown that serial imaging using ultrasound can strengthen doctor-patient relationships[53], which is a cornerstone in the management of FGID patients[3], where cognitive processes such as symptom-specific anxiety[54] may perpetuate symptoms. For example, GIUS can be used to evaluate the painful area, and then patients can be shown the images and reassured that no abnormality was detected. Subsequently, education and discussions with regard to visceral hypersensitivity can commence. Alternatively, demonstrating faecal loading can educate and convince patients of the origin of their symptoms, and pave the way to understanding treatment options.

Physicians have often been encouraged to make a positive diagnosis of FGID[55], however, the lack of biomarkers and the concern that organic causes are missed resonate with both patients and health care providers[56], further contributing to health seeking behaviour[57,58]. Using GIUS as an extension of the clinician's physical examination has been shown[39] to reduce unnecessary investigations. In their study of 1962 consecutive patients, GIUS ruled in or out diagnostic hypotheses in about two-thirds of the cases, and further testing was only required in around 37% of patients.

RESEARCH APPLICATIONS

GIUS can determine the respective contributions of physiological findings in the GI tract to the patient's overall symptoms by directly correlating the symptoms to these findings since the imaging is real time[59]. It can analyse temporal mechanistic relations

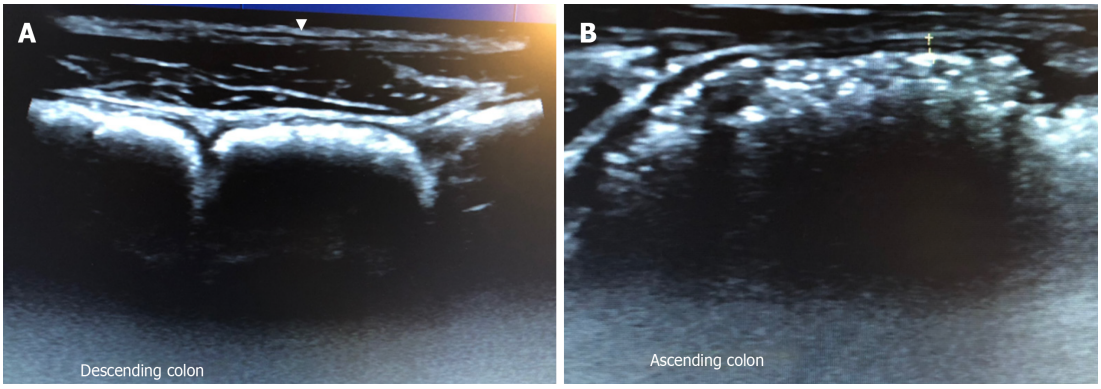


Figure 1 Gastrointestinal ultrasound images. A: Gastrointestinal ultrasound images of descending colon showing haustra-shaped reflections with acoustic shadow behind, suggestive of severe faecal loading B: Gastrointestinal ultrasound images of strong reflection with acoustic shadow behind, also suggestive of faecal loading.

of the GI tract with medications, meals[22,59] and even stress[60], and thus is a valuable tool for research in the field of GI motility. Also, one of the major challenges in assessing treatment outcomes in patients FGID is the lack of objective biomarkers, as symptoms often show wide variability[61]. Measurement of GIUS parameters can be safely and easily taken pre- and post- treatment intervention (Figure 2) to evaluate the efficacy and mode of action in real time.

An interesting use of GIUS is the evaluation of symptoms in FGID related to food intake. GIUS allows limited evaluation of the volume and content of the intestines, thus it can be used together with a food challenge to evaluate mechanisms for symptom generation. It has been shown that different types of FODMAPs generate different physiological effects on MRI scans[62]. In theory, GIUS has the potential to perform some of the MRI measurements such as colonic diameter and content, and thus can be used to correlate patient's symptoms after a substrate load, allowing greater understanding of the origin of symptoms after ingestion of certain foodstuffs, and also diagnose malabsorptive conditions such as lactose or fructose malabsorption. To demonstrate this, 32 patients with chronic abdominal complaints self-attributed to food intake were examined with GIUS after ingesting the suspected food item. The sonographic features were recorded before, during and after the food challenge, and the investigators found significant correlation between symptoms and intestinal wall thickness in the duodenal bulb and jejunum[63].

FUTURE DIRECTIONS

GIUS still does have its limitations. Not all patients are easy to evaluate using GIUS: obese patients and those with previous abdominal surgery are particularly challenging. Not all areas of the bowel are seen easily with GIUS and this may be result in over-diagnosis of pathology detected in sigmoid and terminal ileum areas, with missed pathologies in the transverse colon and rectum. Furthermore, although many studies mentioned in this review demonstrate the utility of GIUS in understanding pathophysiological mechanisms contributing to a patient's GI symptoms, these measurements have not gained clinical relevance[19], and future studies are needed to look into GIUS measurements that may predict a worse prognosis or a response to specific treatment options.

There may be resistance to the widespread adoption of GIUS because of concern of operator ability and thus the potential to miss an important diagnosis. However, there is a slow gain in acceptance that physicians can perform focused ultrasound examinations even without previous ultrasound experience[64]. It has been shown that even ultrasound-naïve clinicians can become competent after performing around 200 supervised examinations[65]. There is also a shift in perspective from a concern about missed findings to recognition that appropriate use of GIUS improves diagnostic accuracy compared to clinical examination alone[66].

CONCLUSION

FGID are extremely common conditions seen in gastroenterology practices worldwide, and we have described the current and potential uses of GIUS in the

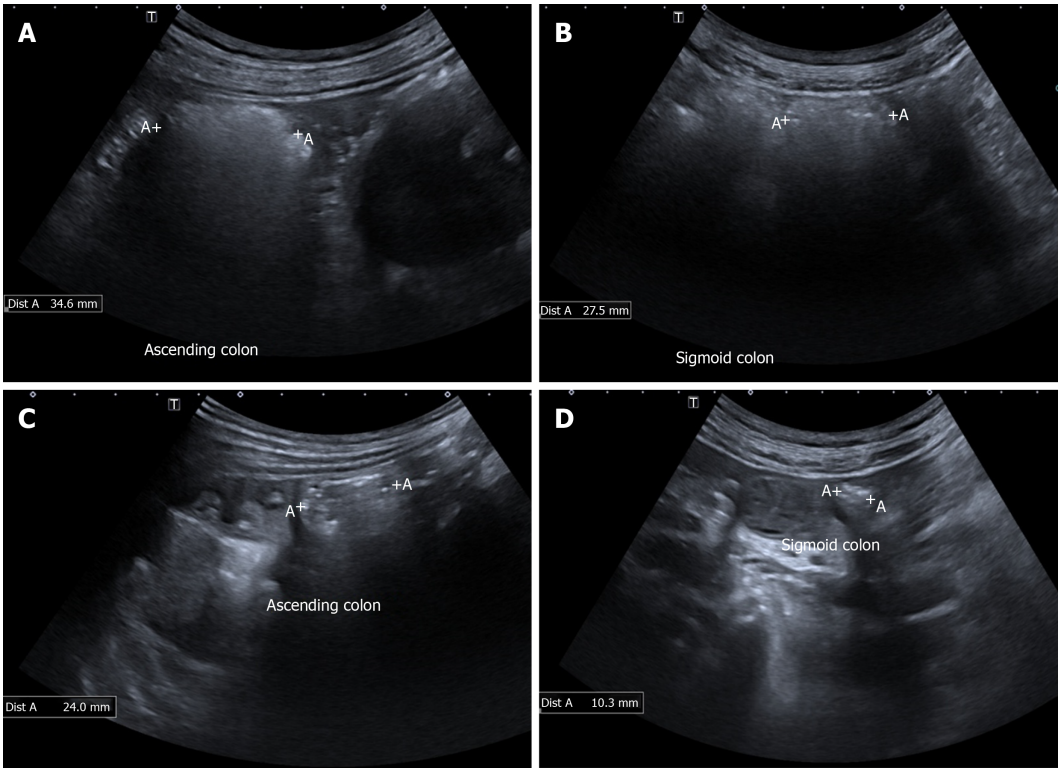


Figure 2 shows the ascending colon and sigmoid colon images of a constipated patients. A: Image of ascending colon pre-treatment; B: Image of sigmoid colon pre-treatment; Note the strong reflections with acoustic shadow behind, suggestive of severe faecal loading; Colonic diameters are 34.6 mm and 27.5 mm respectively for ascending and sigmoid colon; C: Image of ascending colon post-treatment; D: Image of sigmoid colon post-treatment; Note the absence of strong reflections or acoustic shadows suggestive of minimal faecal loading; Colonic diameters of both ascending and sigmoid colon are also reduced post-treatment.

evaluation and management of FGID (Table 1). GIUS is unique in being able to offer real-time physiological information, reliably exclude organic diseases, aid the physician in guiding treatment decisions, and also strengthen patient-physician relationships, thus it offers great potential in its use in FGID (Table 2).

Table 1 Current and potential uses of gastrointestinal ultrasound in functional gastrointestinal disorders

Current	Potential
Evaluating physiology	
Stomach	
Assess contribution of mechanisms to symptoms. Mechanisms include gastric emptying, gastric motility, gastroduodenal flow, gastric wall deformation, gastric accommodation and intragastric distribution of meals	Tailor management of patients based on contributing pathophysiology of symptoms to FGID (*e.g.* prokinetics for patients with FD and antral hypomotility)
Small bowel	
Assess peristalsis to evaluate small bowel obstruction and its causes (*e.g.* mechanical *vs* ileus); support diagnosis for malabsorptive conditions (*e.g.* coeliac disease)	Study interactions of mechanisms to one another and temporal relationships between mechanisms and food/treatment/stress. *e.g.* evaluate mechanism behind food intolerances/malabsorption by evaluating physiological changes with food challenges in real time
Large bowel	
Objectively assess constipation/faecal loading severity and location; assess colonic transit time	Objectively assess treatment outcomes (*e.g.* quantify improvement in constipation post treatment); scoring systems to quantify severity of faecal loading; objectively subtype IBS patients using luminal contents and diameters of large and small bowel; potentially measure colonic transit time, evaluate colonic contents and measure bowel diameters in a single test
Excluding organic diseases	
Reliably exclude IBD from FGID in combination with other biomarkers; Useful screening investigation for abdominal pain, including acute cases as able to exclude appendicitis, diverticulitis, intestinal obstruction *et al*; UMAT can be used as an initial workup tool for patients with dyspepsia; Determine urgency of endoscopy based on GIUS findings; Determine type of investigations (upper *vs* lower GI tract, endoscopy *vs* cross sectional imaging) based on GIUS findings	
Building rapport with patients	
Allows more interaction time between physician and patients, opportunity to educate patients and opportunity to provide real-time reassurance	

GI: Gastrointestinal; FGID: Functional gastrointestinal disorder; IBD: Inflammatory bowel disease; FD: Functional dyspepsia; GIUS: Gastrointestinal ultrasound.

Table 2 Advantages and disadvantages of gastrointestinal ultrasound

Advantages	Disadvantages
Widely available	Operator dependent
Relatively cheap	Not all areas of bowel equally visualised
Non-invasive	May be technically difficult in obese or previous surgery
Real-time	Expertise not widely available
Does not involve sedation, bowel prep or intravenous contrast agents	
Can assess patient in physiological state	
Able to assess multiple physiological mechanisms with a single test	
Able to visualise upper and lower gastrointestinal tract as well as intra-luminal and extra-luminal organs	
Builds rapport with patients as allows interaction between patient and physician	
Allows managing clinician to perform test, allowing test to be focused in answering pertinent clinical questions	

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SYSTEMATIC REVIEWS

Systematic review with meta-analysis of the epidemiological evidence relating smoking to type 2 diabetes

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Abstract

BACKGROUND

Evidence relating tobacco smoking to type 2 diabetes has accumulated rapidly in the last few years, rendering earlier reviews considerably incomplete.

AIM

To review and meta-analyse evidence from prospective studies of the relationship between smoking and the onset of type 2 diabetes.

METHODS

Prospective studies were selected if the population was free of type 2 diabetes at baseline and evidence was available relating smoking to onset of the disease. Papers were identified from previous reviews, searches on Medline and Embase and reference lists. Data were extracted on a range of study characteristics and relative risks (RRs) were extracted comparing current, ever or former smokers with never smokers, and current smokers with non-current smokers, as well as by amount currently smoked and duration of quitting. Fixed- and random-effects estimates summarized RRs for each index of smoking overall and by various subdivisions of the data: Sex; continent; publication year; method of diagnosis; nature of the baseline population (inclusion/exclusion of pre-diabetes); number of adjustment factors; cohort size; number of type 2 diabetes cases; age; length of follow-up; definition of smoking; and whether or not various factors were adjusted for. Tests of heterogeneity and publication bias were also conducted.

RESULTS

The literature searches identified 157 relevant publications providing results from 145 studies. Fifty-three studies were conducted in Asia and 53 in Europe, with 32 in North America, and seven elsewhere. Twenty-four were in males, 10 in females and the rest in both sexes. Fifteen diagnosed type 2 diabetes from self-report by the individuals, 79 on medical records, and 51 on both. Studies varied widely in size of the cohort, number of cases, length of follow-up, and age. Overall, random-effects estimates of the RR were 1.33 [95% confidence interval (CI): 1.28-1.38] for current *vs* never smoking, 1.28 (95%CI: 1.24-1.32) for current *vs*

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non-smoking, 1.13 (95%CI: 1.11-1.16) for former *vs* never smoking, and 1.25 (95%CI: 1.21-1.28) for ever *vs* never smoking based on, respectively, 99, 156, 100 and 100 individual risk estimates. Risk estimates were generally elevated in each subdivision of the data by the various factors considered (exceptions being where numbers of estimates in the subsets were very low), though there was significant ($P < 0.05$) evidence of variation by level for some factors. Dose-response analysis showed a clear trend of increasing risk with increasing amount smoked by current smokers and of decreasing risk with increasing time quit. There was limited evidence of publication bias.

CONCLUSION
The analyses confirmed earlier reports of a modest dose-related association of current smoking and a weaker dose-related association of former smoking with type 2 diabetes risk.

Key words: Smoking; Type 2 diabetes; Prospective studies; Meta-analyses; Dose-response; Review

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Core tip: Based on data from 145 follow-up studies of individuals free of type 2 diabetes at baseline, we confirm evidence of a modest association of smoking with subsequent onset of the disease. Meta-analysis showed relative risks of 1.33 [95% confidence interval (CI): 1.28-1.38] for current *vs* never smoking, 1.28 (95%CI: 1.24-1.32) for current *vs* non-smoking, and 1.13 (95%CI: 1.11-1.16) for former smoking. Risks increased with amount smoked and decreased with time quit. Elevated risks were consistently seen when the data were subdivided by various factors, suggesting that the associations are not a result of uncontrolled confounding.

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INTRODUCTION

Pan *et al*[1], 2015 published a meta-analysis and systematic review of the relationship of active, passive and quitting smoking with incident type 2 diabetes. Based on 88 prospective studies, they reported pooled relative risks (RRs) and 95% confidence intervals (CIs) compared to never smoking of 1.37 (95%CI: 1.33-1.42) for current smoking, 1.14 (95%CI: 1.10-1.18) for former smoking and 1.22 (95%CI: 1.10-1.25) for passive smoking, and evidence of a dose-relationship with amount smoked and years quit. This was an update of a previous review by the US Surgeon General, 2014[2], which based on 46 studies, had argued for a causal relationship. As evidence on tobacco smoking and type 2 diabetes has accumulated rapidly in the last few years, we wanted to investigate more extensively how this relationship may vary based on characteristics of the study or of the RR. We conducted our own updated review and meta-analysis, based solely on active smoking of cigarettes, with or without use of pipes, cigars or smokeless tobacco.

MATERIALS AND METHODS

Study inclusion criteria

Epidemiological prospective studies of populations without type 2 diabetes at baseline in which smoking was related to subsequent incidence of the disease.

The studies had to provide RR estimates for one or more defined major or dose-related smoking indices. The defined "major indices" compare ever, current or ex-smokers with never smokers, or current smokers with non-current smokers, and refer to smoking of any product (cigarettes, pipes, cigars and combinations) or to smoking

of cigarettes. The defined "dose related indices" concern the amount currently smoked and the duration of quitting.

Study exclusion criteria

Studies were excluded where the participants were restricted to those with diseases related to type 2 diabetes.

Literature searches

This was carried out in five steps.

Step 1 identified relevant papers from four previously published reviews of evidence from relevant prospective studies. The review in the 2014 United States Surgeon-General Report[2], presented an analysis based on 46 prospective studies, taking into account studies reported in an earlier review by Willi *et al*[3], 2007 and adding additional studies. Since that Report, which included studies published up to 2010, two further meta-analyses have been published. That by Pan *et al*[1], 2015 included 88 studies, all but five of those considered by the United States Surgeon-General, along with many other studies published up to May 3, 2015. Another review by Akter *et al*[4], 2017 was limited to studies in Japan, and also considered studies up to 2015.

Step 2, carried out on January 31, 2019, repeated the Medline searches described by Pan *et al*[1], 2015, but with the search date restricted to January 1, 2015 onwards.

Step 3 was based on a search on our in-house reference system for papers with keywords DIABETES.

Step 4, carried out on March 1, 2019, repeated the Embase searches described by Pan *et al*[1], 2015, with the search restricted to papers not on Medline.

Finally, Step 5 was based on reference lists of papers identified in Steps 2, 3 and 4, looking for additional potentially relevant papers published from 2015.

In Steps 2 and 4, abstracts were examined first, with full texts obtained only for papers which appeared likely to be relevant. This step was initially carried out by Coombs KJ, with a 20% check made by Lee PN.

At each step, papers (or abstracts) examined for potential relevance were only those not previously considered.

At the end of this process, a set of potentially relevant papers was obtained. Subsequently, more detailed examination of the full texts at the data entry stage revealed that some papers did not actually meet the inclusion criteria, leading to a reduction in the list of relevant papers.

Data recorded

Relevant information was entered onto a publication database and a linked RR database.

The publication database contains a record for each publication describing the following aspects: In-house reference ID of the publication; first author; publication year; location (continent/country); study name; study title; population studied; beginning and end year of baseline; end year of follow-up; length of follow-up; definition of type 2 diabetes (for both baseline exclusion and subsequent incidence) and source of diagnosis; cohort size; number of type 2 diabetes cases; age at baseline; sexes considered; races considered; definition of smoking; results available (current, former, ever, amount smoked, and years quit); details of results available for specific subsets [sex, age, body mass index (BMI), physical activity, alcohol, family history of type 2 diabetes, education, diet, and others]; and details of factors adjusted for in analyses (sex, age, BMI, physical activity, alcohol, family history of type 2 diabetes, education, diet, blood pressure, cholesterol, glucose, triglycerides, waist size, and others).

The RR database holds the detailed results, typically containing multiple records for each publication. Each record is linked to the relevant publication and refers to a specific comparison. The record includes details of the publication reference ID, study name, sex, age range at baseline, length of follow-up, BMI range, definition of smoking, and smoking status of the numerator (current, former or ever), and of the denominator (never or non). Where the smoking status is former, the range of years quit is entered. The range of amount smoked is also entered. For unadjusted RR estimates, the numbers of cases and at risk (or person years) are entered for both the numerator and denominator.

For adjusted RR estimates, the RR and 95%CI are entered, taken directly from the publication, or estimated using standard methods[5], with details also entered of the factors adjusted for.

Numbers of cases and at risk, or RRs and 95%CIs, are only entered for the whole population or for subgroups defined by sex, age group or BMI group. As noted above, the availability of results by other factors is recorded in the publication database, but

the detailed results have not so far been entered. Results are also only entered unadjusted for potential confounding variables and adjusted for the most confounding variables for which results were available.

All data were entered by Coombs KJ and checked by Lee PN, with any disagreements discussed and resolved.

Multiple publications for the same study

Once the data were entered, the list of publications was sorted into studies. Where the RRs from only one publication needed to be used in analysis, with the others providing no useful extra data (*e.g.*, providing similar data for a shorter follow-up), these "other" publications were rejected, with the reasons for rejection noted. Where more than one publication from the same study provided useful data (*e.g.*, for different aspects of smoking), one publication was nominated as the main reference for the study (typically, the publication providing the most detailed results) and others were nominated as subsidiary references. Thus, it was possible to have main, subsidiary and rejected references from the same study. Another possibility is that a publication may give a pooled analysis of several individual studies, including useful data for aspects not covered in the main publications of the separate studies. These pooled publications are also nominated as subsidiary references.

Meta-analyses

Fixed-effect and random-effects meta-analyses were conducted using the method of Fleiss and Gross, 1991[6], with heterogeneity quantified by H, the ratio of the heterogeneity chisquared to its degrees of freedom. H is directly related to the statistic I^2[7] by the formula $I^2 = 100\ (H-1)/H$. For all meta-analyses, Egger's test of publication bias[8] was included.

The major smoking indices

Meta-analyses were conducted using the available data for current *vs* never, current *vs* non, ever *vs* never, and former *vs* never smoking. Where there was a choice of estimates for a study, preference was given to results that were for the full range of amount smoked, the longest follow-up, the most adjusted, the widest age range, and the preferred product, with preference being given, in order to results for: Cigarettes; smoking excluding exclusive pipe/cigar; smoking; and tobacco; but not exclusive cigar, pipe or smokeless tobacco. For a study of both sexes, preference was also given to separate estimates for the two sexes, if available. While in most studies, the choice of estimates was straightforward, in others it was not (*e.g.*, between an unadjusted RR for a longer follow-up from one publication and an adjusted RRs for a shorter follow-up from another). Here Coombs KJ and Lee PN agreed and recorded the most relevant RR to choose (disregarding its magnitude). For a particular exposure (*e.g.*, current *vs* never) each study could provide only the estimate or two sex-specific estimates for inclusion in the meta-analysis.

Effect estimates were derived based on all the selected RRs as well as for those subdivided by various categorical variables: Sex (male, female, and sexes combined); continent (Asia, Europe, Americas, and Oceania); publication year (before 2005, 2005-14, 2015 or later); diagnosis of type 2 diabetes (self-reported, medical data only, both); population (general, pre-diabetics only, excludes pre-diabetics); total number of adjustment factors (0, 1-5, 6-10, 11+); cohort size (< 5000, 5000-20000, > 20000); number of type 2 diabetes cases (< 500, 500-999, 1000-2000, 2001+); highest baseline age (< 60, 60-74, 75+ years); length of follow-up (< 5, 5-10, > 10 years); definition of smoking [cigarettes, smoking (whether or not excluding exclusive pipe/cigar), tobacco]; and whether each of a range of different variables were adjusted for.

The dose-related smoking indices

When comparing RRs by amount currently smoked (with a reference group of never smokers) or non-smokers, or by years quit (with a reference group of never smokers), a study typically provides a set of non-independent RRs for each dose-category, expressed relative to a common base. To avoid double-counting, it is necessary to include only one in any one meta-analysis.

For amount smoked, three methods were used. One method used only for studies that reported results for two levels of amount smoked, was to compare results for 1-19 and 20+ cigs/d, the most common subdivision used. The second, used only for studies that reported results for three levels of amount smoked was to compare results for low, medium and high cigs/d regardless of the levels selected. The third involved defining a set of key values (10, 20 and 40 cigs/d) and carrying out a separate meta-analysis for each key value. For an RR to be allocated to a key value its dose category had to include that key value and no other. This method was only applied for studies reporting results by three or more levels, with all three key value

results available. These methods were used for data on current *vs* never smoking, and for current *vs* non-smoking.

For years quit, two methods were used. One simply used the shortest and longest categories. The other used the key values approach with values of 3, 7 and 12 years quit.

Results by BMI

For each of the studies that reported independent RR estimates separately for different subdivisions of the population by level of BMI, estimates were made, for each smoking index for which data were available, of the ratio of the RR for highest *vs* lowest BMI group, these ratios then also being meta-analysed.

Avoidance of overlap

When conducting meta-analyses care was taken to minimize overlap of cases. Thus, results from subsidiary papers were used only when the main paper did not provide the result required for the particular meta-analysis. Also, if an RR was available from three separate studies, and also from a combined analysis from the three studies, the individual results were preferred, only using the combined RR for a smoking index for which results were not reported in all the different studies.

RESULTS

Publications and studies identified

As summarized in Table 1[9-15], 221 publications were originally identified as likely to be relevant, with 42 later rejected during data entry, the reasons for rejection being given in Supplementary File 1. As seven of the publications provided results for two independent data sets (either presenting separate results for two studies or for two non-overlapping follow-up periods), data entry was carried out initially for 186 publication records. On investigation of studies with multiple records, 29 records were rejected as providing no useful information extra to those provided in other records) and 12 were classified as subsidiary, providing some limited extra information for records classified as main. This meant that there were 145 studies, 144 separate studies plus the combined analysis of three studies (HPFUS, NHS and NHSII). Table 2[9-14,16-161] summarizes some characteristics of these studies, while Supplementary file 1 also gives information on why some publications were rejected or only provided subsidiary information.

All stages of the identification of relevant papers, classification of papers with studies, and data entry were conducted initially by Coombs KJ and checked by Lee PN. Exceptionally, Lee PN only checked 20 percent of the abstracts for the Medline and Embase searches. This 20 percent check, of a total of 8798 hits, only resulted in four extra full-text papers being examined, only one of which proved to have relevant data. Given the very limited extra information obtained, and the time spent, it was decided not to extend this to a 100 percent check.

Study characteristics

Location: As shown in Table 2, 53 of the 145 studies were conducted in Asia (including 23 in Japan, 10 in South Korea, nine in China and 11 in other countries). Fifty-three were conducted in Europe (eight in Great Britain, eight in Finland, seven in Germany, six in Sweden, five in Spain, and 19 in other countries), with 32 in North America (all in the United States), six in Australia and one in Brazil.

Population: Ten of the studies were in females, 24 in males and 111 in both sexes. About half were of the relevant general population, with Table 2 showing further details.

Time: There was a clear increase in study frequency with time, with 17 starting before 1980, 23 starting in the 1980s, 47 in the 1990s, 42 in 2000-2005, and 16 from 2006 onwards.

Years follow up: Twenty-four studies involved less than 5 years follow-up; 62 studies involved 5-9.9 years follow-up; 36 studies involved 10-14.9 years follow-up; and 23 studies involved 15 years or more years follow-up, with the longest (NOVAK) involving 35 years.

Diagnosis: Fifteen of the studies diagnosed type 2 diabetes only on the basis of self-report of the individuals, 79 only on medical records, and 51 on both.

Size: The numbers in the cohorts studied varied from 182 to over eight million. Sixty-

Table 1 Literature searching

Step		Papers originally selected as probably relevant[1]	Papers rejected during data entry[2]	Papers providing separate results for multiple studies[3]
1	Previous reviews	98	10	3[9-11]
2	Medline search	74 (from 3365 hits)	23	4[12-15]
3	In-house database	1	0	0
4	Embase search	33 (from 5433 hits)	7	0
5	Secondary references[4]	15 (of 30 identified)	2	0
Total		221	42	7

[1]Numbers of papers originally selected exclude those already identified in a previous step.
[2]Reasons for rejection are summarized in Supplementary file 1.
[3]Or for separate periods of follow-up.
[4]From papers identified in steps 2 to 4.

three were under 5000, 39 in the range 5000 to 20000 and 43 larger than this.

Type 2 diabetes cases: The number of type 2 diabetes cases varied from 27 to almost 180000. Eighty-two involved fewer than 500 cases, 21 involved 500-999 cases, 13 involved 1000-2000 cases, and 28 involved more than this. The number was not available for one study.

Age: Most of the studies included some individuals of age 75 or older at baseline. However, 24 were restricted to those aged less than 60 and 30 more were restricted to those aged less than 74.

Meta-analyses

Current *vs* never smoking: The studies provided 99 RR estimates from 80 studies for the comparison of current *vs* never smoking. Nineteen studies provided estimates for both sexes, six for females only, 17 for males only and 38 only for sexes combined. Of the 99 estimates, 12 were below 1, 10 were above 2, with the remaining 77 in the range 1 to 2. The overall fixed-effect RR estimate was 1.25 (95%CI: 1.24-1.26) with highly significant heterogeneity between the estimates (Chisq. 816.8 on 98 df, $P < 0.001$, I^2 = 88.0%). The random-effects estimate was somewhat higher at 1.33 (95%CI: 1.28-1.38). There was limited evidence of publication bias ($0.01 < P < 0.05$).

Table 3 presents the overall random-effects estimate, together with a breakdown of the estimates by various factors, with fuller details given in Supplementary file 2. There was evidence ($P < 0.05$) that the estimates varied by population type with both the estimates from studies restricted to pre-diabetics exceeding 3. There was also evidence that estimates were higher in those that were more adjusted ($P < 0.05$) or adjusted for various other individual factors (age, alcohol, family history of diabetes, cholesterol, triglycerides – all $P < 0.05$ - and glucose – $P < 0.01$), but were lower in those that were adjusted for education ($P < 0.05$). It is notable, however, that with the exception of two estimates based on less than five RRs, all the RR estimates shown in Table 3 were significantly ($P < 0.05$) increased.

For the analysis subdivided by sex, Figure 1 (females), Figure 2 (males) and Figure 3 (sexes combined) summarize the data in forest plots, while Figure 4 (females), Figure 5 (males) and Figure 6 (sexes combined) present funnel plots to illustrate possible publication bias. No marked publication bias was evident.

Table 4 (and Supplementary file 3) summarizes the results of the dose-response analysis for current *vs* never smoking. Whichever of the three methods of dose-response grouping was used, the RR estimates clearly rose with increasing amount smoked, and the increase at each level remained significant ($P < 0.05$). Note that the sets of estimates are not independent, with all the studies providing results for the key value analysis also contributing to the low/medium/high split.

Current *vs* non-smoking: There were 156 RR estimates from 133 studies for the comparison of current *vs* non- smoking. Twenty-three studies provided estimates for both sexes, eight for females only, 24 for males only and 78 for sexes combined.

Of the 156 estimates, 27 were below 1, 11 were above 2, with the remaining 118 in the range 1 to 2. The overall fixed-effect RR estimate was 1.20 (95%CI: 1.20-1.21), with highly significant heterogeneity (Chisq. 1986.7 on 155 df, $P < 0.001$, I^2 = 92.2%), and the random-effects estimate was 1.28 (95%CI: 1.24-1.32), slightly lower than the

Table 2 Some characteristics[1] of the 145 studies of smoking and type 2 diabetes

Study Ref.	Main/ Other Ref.	Continent	Country, location[2]	Study Population[3]	Sex	Baseline	Follow-up (yr)[4]	Diagnosis code[5]	Cohort size	Diabetes cases	Age
3 studies[6]	[16]	North America	United States	Medical professionals	M+F	1984-1991	19.6	3	162807	12384	25-75
AICHI	[17]/[18]	Asia	Japan, Aichi	Civil servants	M+F	2002	9.0	3	3338	225	35-66
AIZAWA	[19]	Asia	Japan, Matsumoto	Participants from hospital (not otherwise defined)	M+F	2005	4.9	2	4159	279	Any
ALEIN	[20]	Asia	Taiwan (China), A-Lein	Persons undergoing community wide screening for hepatitis	M+F	1996-1997	8.0	2	3539	423	40-69
ALSWH	[21]	Oceania	Australia	General population	F	1998	12.0	1	12367	871	47-52
ANSAN	[22]/[23,24]	Asia	South Korea, Ansun and Ansan	Community based	M+F	2001-2002	4.0	2	4041	329	40-69
ARIC	[25]/[26]	North America	United States, North Carolina, Mississippi, Maryland	Probability sample from 4 US communities with exclusive sampling of African Americans in one of the four sites, Black or White	M+F	1987-1989	9.0	3	10892	1254	45-64
ASAN	[27]	Asia	South Korea, Asan	Attending voluntary medical check-ups	M+F	2000	5.0	2	5372	234	20-79
ATTICA	[28]	Europe	Greece, Athens	General population	M+F	2001-2002	10.0	2	1485	191	18-89
Ausdiab	[29]	Oceania	Australia	General population	M+F	1990-2000	5.0	2	5842	244	25+
BEDFORD	[30]	Europe	England, Bedford	Borderline diabetics with a 2h fasting glucose of 6.7-11.1 mmol/L	M+F	1962-1964	10.0	2	241	36	18+
BIP	[31]	Asia	Israel	Subjects with impaired functional capacity (New York Heart Association class II and III)	M+F	1990-1993	6.2	2	630	98	45-74
BMES	[32]	Oceania	Australia, West of Sydney	Non institutionalised residents	M+F	1992-1994	10.0	3	2123	165	49+

BOGA-LUSA	[33]	North America	United States, Bogalusa	General population	M+F	1973-2010	9.1	2	7725	176	< 18
BOTNIA	[9]/[34]	Europe	Finland, Botnia	Family members of diabetics	M+F	1990	7.6	2	2770	138	Any
BRHS	[35]	Europe	Britain	General population	M	1978-1980	16.8	3	7124	290	40-59
BRU-NECK	[36]	Europe	Italy, Bruneck	General population, White	M+F	1990	10.0	2	837	64	40-79
BURKE	[37]	Oceania	Australia Kimberley	General population, Aboriginal	M+F	1988-1989	12.9	2	493	104	15-88
BWHS	[38]	North America	United States	African American subscribers to magazine targeted at black women	F	1995	16.0	3	43003	4387	21-69
CASSAN	[39]	North America	United States	Majority were veterans, 98% Caucasian	M	1963	18.0	2	1972	226	20-80
CCHS	[40]	North America	United States, Cleveland	General population	M+F	2008	5.0	2	5084	872	18+
CDCdeC	[41]	Europe	Spain, Canaries	General population	M+F	2000-2005	3.5	3	5521	146	18-75
CEHSC	[42]	Asia	Hong Kong (China)	General population volunteers	M+F	1998-2001	9.8	2	53905	806	65+
CKB	[43]	Asia	China	General population	M+F	2004-2008	7.2	2	461211	8784	30-79
CoLaus	[44]	Europe	Switzer-land, Lausanne	General population	M+F	2003-2006	5.5	2	3166	47	35-75
CPSI	[45]	North America	United States	General Population	M+F	1959-1960	12.0	3	709827	25397	30+
CRISPS	[46]	Asia	Hong Kong (China)	General population, Chinese	M+F	2000-2004	9.0	2	1380	123	Any
CURES	[47]	Asia	India, Chennai	General population	M+F	2001-2003	9.1	2	1376	385	20+
DAQING	[48]	Asia	China	Care clinic patients with pre-diabetes, part of diabetes prevention inter-vention	M+F	1986	23.0	3	568	436	Any
DEHGHA	[49]	Europe	Nether-lands, Ommoord	General population	M+F	1990-1993	10.8	2	6935	645	55+
DE-PLAN	[11]	Europe	Spain, Navarra, Reus and Barcelona	Partici-pants in clinical trial on Mediter-ranean diet, Caucasian	M+F	2006	4.2	2	552	124	45-75
DESIR	[50]	Europe	France, Western	Volunteers for periodic health checks	M+F	1998	9.0	2	3817	203	30-64

DLCS	[51]	Europe	Nether-lands, Northern	General population, Western Europe	M+F	2007-2013	4.0	3	72880	1056	18-90
DNC	[52]	Europe	Denmark	Nurses	F	1993-1999	15.3	2	24174	1137	44+
DONGFENG	[53]	Asia	China, Da Qing	Retired employees	M+F	2008-2010	4.0	3	17690	1390	Any
DWECS	[54]	Europe	Denmark	Workers	M+F	1995-2005	5.0	2	6823	NA	30-59
EPIC-IN	[55]	Europe	8 countries[7]	Subset of partici-pants in EPIC-InterAct cohort	M+F	1991	11.7	3	23501	10327	Any
ESTHER	[56]	Europe	Germany, Saarland	General population	M+F	2000-2002	8.0	3	7462	718	50-75
FAGERB	[57]	Europe	Sweden, Göteborg	General population, Caucasian	F	2001-2004	5.5	2	341	69	64
FINNMARK	[58]	Europe	Norway, Finnmark	General population	M+F	1997-1978	12.0	2	11654	162	35-52
GLOSTRUP	[59]	Europe	Denmark, Glostrup	General population	M	1982-2001	18.9	2	5350	211	30-70
GNHIES	[60]	Europe	Germany	General population (non institutionalized)	M+F	1997-1999	5.0	2	3625	82	18-79
HDNNCDS	[12]	Asia	China, Harbin	General population, Chinese	M+F	2010	4.2	3	7133	578	20-74
HEALTH 2000	[10]	Europe	Finland	General population	M+F	2000-2001	7.0	2	4110	81	40-79
HEINZN	[61]/[62]	Europe	Germany, Western	General population	M+F	2000-2003	5.1	3	3547	319	45-75
HENAN	[63]	Asia	China, Henan	General population, N Chinese ancestry	M+F	2007-2008	6.0	3	12272	775	18+
HIPOP-OHP	[64]	Asia	Japan	Employees	M+F	1999	3.4	3	6498	229	Any
HIPPIS1	[65]	Europe	England and Wales	Primary care patients	M+F	1993-2008	8.0	2	2540753	78081	25-79
HIPPIS2	[66]	Europe	England	Primary care patients	M+F	2005-2016	3.9	2	8186705	178314	25-84
HISAYAMA	[67]	Asia	Japan, Hisayama	General population	M+F	1988	11.8	2	1935	286	40-79
HPFUS	[68]	North America	United States	Health profes-sionals	M	1986	6.0	3	41810	509	40-75
HPHS	[12]	Asia	China, Harbin	General population, Chinese	M+F	2008	4.2	3	3350	244	20-74
HUNT	[69]	Europe	Norway, Nord-Trøndelag	General population	M+F	1984-1997	11.0	3	90819	1860	20+
ICARIA	[70]	Europe	Spain	Spanish workers	M+F	2004-2007	4.1	3	380366	9960	18-65
ICS	[71]	Asia	Iran, Isfahan, Arak and Najafabad	General population	M+F	2001	7.0	2	2980	389	35+

IPC	[72]	Europe	France, Paris	Workers and those seeking employment who had undergone 2 health checks	M+F	1998-2010	5.3	2	22567	527	18+
IRAS	[73]	North America	United States, 4 areas[8]	General population	M+F	1992-1993	5.0	2	906	148	40-69
IWHS	[74]	North America	United States, Iowa	Community based	F	1986	13.2	1	36839	3281	55-69
JACC	[75]	Asia	Japan	Community based	M+F	1988-1990	5.0	1	16160	396	40-79
J-ECOH	[76]/[77]	Asia	Japan	Employees	M+F	2008-2010	3.9	2	53930	2441	15-83
JHS	[78]	North America	United States, Mississippi	General population, Black	M+F	2000-2004	8.0	2	2991	479	21-84
JPHC	[79]	Asia	Japan	General population	M+F	1990	10.0	1	28893	1183	40-59
JPHC2	[80]	Asia	Japan	General population	M+F	1995-1998	5.0	1	59834	1100	45-74
KANGBUK	[81]	Asia	South Korea, Seoul	Individuals undergoing health screening	M+F	2002	5.6	3	174314	5544	18+
KAWAHA	[82]	Asia	Japan, Kitakyushu City	City workers	M+F	2008	3.7	2	52781	4369	20-89
KAWAKA	[83]	Asia	Japan, electrical company	Employees of large electrical company	M	1984	8.0	2	2312	41	18-53
KMIC	[84]	Asia	South Korea	Government and school employees	M	1990-1986	8.0	2	27635	1170	35-44
KoGES-K	[85]/[86]	Asia	South Korea, Kangwha	Community based	M+F	2006-2011	4.0	2	2079	142	40+
KORA F4/FF4	[87]	Europe	Germany, Augsburg	General population	M+F	2006-2008	7.0	2	504	76	62-81
KORA S4/F4	[88]	Europe	Germany, Augsburg	General population	M+F	1999-2001	7.0	2	887	93	55-74
KPNW	[89]	North America	United States, Portland	Health care members	M+F	1997-2000	6.8	2	46578	1854	40+
LEICESTER	[90]	Europe	England, Leicester	With clinical diagnosis of polycystic ovary syndrome	F	1988-2009	5.2	2	2164	138	16-79
LIETO	[91]	Europe	Finland, Leito	General population	M	1998-1999	9.0	2	430	30	64+
LINDBE	[92]	Europe	Denmark, Copenhagen	General population	M+F	2001-2003	8.5	2	5349	136	20-94
LLP	[93]	Europe	England, Liverpool	General population	M+F	1998-2008	10.0	2	8753	763	45-79
MAILES	[94]	Oceania	Australia, Adelaide	General population	M	2002-2006	4.9	3	1597	232	35-80
MANSON	[95]	North America	United States	Physicians in randomized trial	M	1982	12.0	1	21068	770	40-84

MECC	[96]	North America	United States, Hawaii and California	General population, African American and Latino	M+F	1993-1996	14.0	3	48995	15833	50-75
MECH	[97]	North America	United States, Hawaii	General population, Caucasian, Hawaiian, Japanese, American	M+F	1993-1996	12.1	3	74970	8559	45-75
MESA	[98]/[99]	North America	United States, 6 states[9]	General population, White, Black, Hispanic or Chinese	M+F	2000-2002	10.2	2	5931	359	45-84
MFH	[10]	Europe	Finland	General population	M+F	1978-1980	10.0	2	4517	145	40-79
MJH	[100]	Asia	Taiwan (China)	Paid members of private health screening program, Chinese	M+F	2001-2014	6.7	3	147908	4781	18+
MONI-CAG	[101]	Europe	Germany, Augsburg	General population	M+F	1984-1995	12.5	3	10892	672	25-74
MONI-CAS	[102]	Europe	Sweden, Northern	General population	M	1986-1994	8.7	3	1275	27	25-74
MORIMO	[103]/[104]	Asia	Japan, Nagano prefecture	Volunteers in Nagano Prefecture	M+F	1990-1992	10.1	3	5872	595	40-69
MOZAFF	[105]	North America	United States, 4 states[10]	Ambulatory, noninstitutionalized subjects	M+F	1989-1992	10.0	2	4883	337	65+
MPBB	[106]	North America	United States, Michigan	Subjects who had injected food contaminated with polybrominated biphenyls, 99.8% White	M+F	1976	25.0	3	1384	180	20+
MPP	[9]	Europe	Sweden, Malmo	General population	M+F	1974-1992	24.8	2	16061	2063	Any
MUTUAL	[107]	Asia	Japan	Civil servants	M+F	2000	6.5	2	5848	287	30-59
MYHUS	[108]	Asia	Japan	Employees	M+F	2004	5.0	3	13700	408	36-55
NAGALA	[109]/[110]	Asia	Japan, Gifu	Subjects receiving medical check-ups	M+F	2004-2015	5.1	3	17810	804	Any
NAGAYA	[111]	Asia	Japan, Nagoya	Volunteer attendants of annual health check ups	M	1988-1990	7.4	3	16829	869	30-59
NAKANI	[112]	Asia	Japan, Osaka	Employees	M	1994	5.0	2	1266	54	35-59
NCDS	[113]	Europe	Britain	Birth cohort from March 1958	M+F	1974	17.0	1	4945	28	16
NHANES	[114]	North America	United States	General population	M+F	1971-1975	18.0	3	4830	443	25-74

NHIC	[115]	Asia	South Korea	Recipients of biennial medical check-ups	M+F	1992-1995	14.0	2	1236443	89422	30-95
NHIS-HEALS	[116]	Asia	South Korea	Recipients of national health screen test	M+F	2002-2003	10.8	2	359349	37678	40-79
NHIS-NCS	[117]	Asia	South Korea	Nationally represen-tative	M+F	2002	6.8	2	51405	2749	20+
NHS	[118]	North America	United States	Registered Nurses	F	1976-1982	24.0	3	100526	5392	30-55
NHSII	[13]	North America	United States	Registered Nurses	F	1989-1991	23.0	3	88086	5441	25-42
NIH - AARP	[119]	North America	United States, 6 states[11]	General population	M+F	1995-1996	11.0	1	207479	18000	50-71
NOMAS	[120]	North America	United States, North Manhattan	General population, White, Black or Hispanic	M+F	1993-2001	11.0	3	2430	449	40+
NOVAK	[121]	Europe	Sweden, Gothen-burg	General population (interven-tion group in ineffective trial)	M	1970-1973	35.0	2	6828	899	47-56
OLMS-TED	[122]	North America	United States, Rochester	General population who also took at least one medication	M+F	1999-2004	6.0	2	13508	1182	18+
ONAT	[123]	Asia	Turkey	Partici-pants in nationwide survey	M+F	1997-1998	5.9	3	3385	216	28+
OSAKA	[124]	Asia	Japan, Osaka	General population undergoing basic health check-ups	M+F	2001	4.0	2	9327	171	40-74
OSLO	[125]	Europe	Norway, Oslo	General population	M	1972-1973	28.0	3	6382	584	40-49
OSTENS	[126]	Europe	Sweden, Stockholm	General population	M	1992-1994	10.0	2	2383	99	35-56
PARK	[127]	Asia	South Korea, not known	Under-going health examina-tions	M	2002	4.0	2	1717	50	Any
PATJA	[128]	Europe	Finland, North Karelia and Kuopio	General population	M+F	1972-1992	21.0	2	41372	2770	25-64
PINGLIANG	[129]	Asia	China, Ping Liang	General population pre-diabetic at baseline	M+F	2002-2003	10.8	2	334	98	Any
PMMJS	[130]	Asia	China, Jiangsu	General population	M+F	2000-2004	5.0	2	3598	160	35-74

PREDI-MED	[11]	Europe	Spain, Navarra, Reus and Barcelona	Partici-pants in clinical trial on Mediter-ranean diet, Caucasian	M+F	2003-2009	4.8	2	1381	155	55-80
PREDI-MERC	[131]	Europe	Spain, Madrid	General population	M+F	2007	6.4	2	2048	44	30-74
PRE-VEND	[132]	Europe	Nether-lands, Groningen	General population	M+F	1997-1998	11.4	3	7953	447	Any
REGA-RDS	[133]	North America	United States	General population, Black or White	M+F	2003-2007	9.5	2	7758	891	45+
SABE	[134]	South America	Brazil, Sào Paulo	General population	M+F	2000	6.0	1	914	72	60+
SAIREN	[135]	Asia	Japan, Ibaraki-ken	General population undergoing annual health check-ups	M+F	1993	5.0	2	128141	7990	40-79
SALSA	[136]	North America	United States, Sacramento	General population, Latino	M+F	1998-1999	10.0	3	782	144	60+
SAM-SUNG	[137]	Asia	South Korea, Seoul	Under-going health examina-tions, Korean	M	2006	6.0	3	1774	180	20+
SAPA-LDIA	[138]	Europe	Switzer-land	General population	M+F	2002	8.3	3	2631	110	18+
SAWADA	[139]	Asia	Japan, Tokyo	Employees of Tokyo Gas Company	M	1985	14.0	3	4745	280	20-41
SAX45	[140]	Oceania	Australia, New South Wales	General population	M+F	2006-2008	3.4	1	54997	888	45+
SCCS	[14]	North America	United States, Southern	General population, Black or White	M+F	2002-2009	4.5	1	35892	3439	40-79
SCCS2	[14]	North America	United States, Southern	General population, Black or White	M+F	2012[12]	3.0	1	20712	1708	43-82
SHFS	[141]	North America	United States, 4 states[13]	Members of multiplex families, American Indians	M+F	2001-2003	5.5	2	431	133	14+
SHIP	[142]	Europe	Germany, Augsburg	Caucasian German citizens	M+F	1997-2001	11.1	2	2034	206	20-81
SMHS	[143]	Asia	China, Shanghai	General population	M	2002-2006	5.4	3	51464	1304	40-74
STILLW	[144]	Europe	Finland	Employees of Finnish Company	M	1986	17.0	2	5827	313	18-65
STRAND	[145]	Europe	Finland, Helsinki	Volunteer executives and busines-smen	M	1974-1975	20.0	3	1802	94	40-56

STRING	[146]/[147]	Europe	England, London	Civil service employees	M+F	1985-2002	23.7	2	8270	1286	50
SUGIMO	[148]	Asia	Japan, Tokyo	Partici-pants in MHTS	M+F	1976	16.0	2	2573	296	18-69
SULA-WESI	[149]	Asia	Indonesia, South Sulawesi	Three tribes	M+F	2013	3.0	2	182	58	16+
SWAN	[150]	North America	United States, Michigan	Partici-pants in study of menopause transition, Black or White	F	1996	16.0	3	424	157	42-52
TCS	[151]	Asia	Thailand	Students at Sukothai Thammi-thirat Open University	M+F	2005	8.0	1	39507	698	15-88
TERATA	[152]	Asia	Japan, Chiba	Steel-workers	M	2002	8.0	2	8423	464	Any
TLSA	[153]	Asia	Taiwan (China), Non-aboriginal areas	Partici-pants in ongoing survey on aging, Taiwanese	M+F	1999	4.0	1	2995	277	53+
TOPICS6	[154]	Asia	Japan, Toranomon	Govern-ment employees and some general population	M+F	1997-2002	5.0	3	7654	289	40-75
TROMSO	[155]	Europe	Norway, Tromsø	General population	M+F	1994-1995	10.8	3	26168	522	25-98
UCHIMO	[156]	Asia	Japan, Osaka	Employees of large company	M	1981-1991	10.0	2	6250	450	35-60
VETE-RAN	[157]	North America	United States	Veterans	M+F	2002-2003	4.0	2	239057	33453	18-99
VIP	[158]	Europe	Sweden, Västerbo-tten County	General population	M+F	1990-2012	9.9	3	32120	2211	35-55
WHI	[159]	North America	United States	Postmenop ausal women in a clinical trial or an observa-tional study	F	1993-1998	11.0	1	135906	15076	50-79
YOUNGF	[160]	Europe	Finland	Population based	M+F	1980	24.0	3	2298	79	3-18
ZUT-PHEN	[161]	Europe	Nether-lands, Zutphen	General population	M	1960	25.0	2	841	58	40-59

[1]Where relevant, characteristics are shown for the main reference, given first in the column Main/Other Ref.
[2]If location not stated, then national.
[3]All races are included unless stated otherwise.
[4]NA means not available. Some studies provided results for more than one follow-up time. Here the longest follow-up is indicated. The follow-up times are presented as means, medians or averages to various numbers of decimal places. The values shown are the best estimate available.
[5]1 = self-report only; 2 = medical records only; 3 = both.
[6]Studies HPFUS, NHS and NHSII.
[7]France, Italy, Spain, United Kingdom, Netherlands, Germany, Sweden and Denmark.
[8]Los Angeles, Oakland, San Antonio and San Juis Valley.
[9]Maryland, Illinois, North Carolina, California, New York and Minnesota.
[10]North Carolina, California, Maryland and Pennsylvania.
[11]California, Florida, Louisiana, New Jersey, North Carolina and Pennsylvania.
[12]Subset of SCCS who were diabetes free at end of SCCS follow-up. Unclear what the baseline date range of SCCS2 actually was.

[13]Arizona, North and South Dakota and Oklahoma. M: Male; F: Female.

estimate for current *vs* never smoking. As for current smoking, there was limited evidence of publication bias ($0.01 < P < 0.05$).

Table 3 also presents the overall random-effects estimate for current *vs* non-smoking, as well as a breakdown of the estimates by different factors (see also Supplementary file 4). As for current *vs* never smoking, the random-effects estimate was elevated in all subdivisions of the data, significantly so except where based on very few estimates. There was little evidence of variation in the RR in subdivisions of the data by level of the various factors studied, the most notable exceptions being the somewhat higher estimate in studies adjusted rather than unadjusted for family history of diabetes, and the variation by continent.

Table 4 (and Supplementary file 5) summarizes the results of the dose-response analysis for current *vs* non-smoking. As for current *vs* never smoking, there was clear evidence that risk rises with amount smoked, whichever dose-response grouping is used.

Forest and funnel plots for the analysis subdivided by sex are shown in Supplementary file 6.

Former *vs* never smoking: There were 100 RR estimates from 81 studies for the comparison of former *vs* never smoking. Nineteen provided estimates for both sexes, seven for females only, 17 for males only and 38 for sexes combined.

Of the 100 estimates, 18 were below 1, 7 were above 2, with the remaining 75 in the range 1 to 2. The overall fixed-effect estimate was 1.09 (95%CI: 1.08-1.10), with highly significant heterogeneity (Chisq. 263.6 on 99 df, $P < 0.001$, $I^2 = 62.4\%$). The random-effects estimate was 1.13 (95%CI: 1.11-1.16). Somewhat stronger evidence of publication bias ($0.001 < P < 0.01$) was seen than for current smoking.

Table 5 presents the overall random effects estimate, together with a breakdown of the estimates by different factors (see also Supplementary file 7). There was no strong evidence ($P < 0.01$) of variation in the RR by level of any factor, with estimates slightly elevated in all subgroupings except where based on very few estimates.

Table 6 (and Supplementary file 8) summarizes the results of the dose-response analysis for former *vs* never smoking. These showed clear evidence that the RR declined with increasing time since quitting.

Again, forest and funnel plots are shown in Supplementary file 6.

Ever *vs* never smoking: One hundred RRs were available from 82 studies. The overall fixed-effect RR estimate was 1.17 (95%CI: 1.16-1.18) with evidence of considerable heterogeneity (Chisq. 897.37 on 99 df, $P < 0.001$, $I^2 = 89.0\%$), the random-effect estimate being 1.25 (95%CI: 1.21-1.28). There was some evidence of publication bias ($0.001 < P < 0.01$). RRs were generally elevated in all subgroups, the strongest evidence of variation by any factor ($P < 0.001$) relating to adjustment for education, unadjusted estimates (RR = 1.29, 95%CI: 1.24-1.34) being higher than adjusted ones (RR = 1.17, 95%CI: 1.12-1.21). There was also weaker evidence ($P < 0.05$) that RRs were somewhat higher in Asia, and somewhat lower in populations with a baseline upper age limit of 75 or more, or if the RRs were unadjusted for glucose. See Table 8 and Supplementary File 9 for fuller details.

Only one of the studies provided information on risk by amount smoked, so no dose-response meta-analyses were possible.

Again, forest and funnel plots are shown in Supplementary file 6.

Ratio of RRs for highest to lowest BMI groupings: Six studies provided results by level of BMI, three of these giving results for each sex separately. One study provided data only for current *vs* never and former *vs* never smoking, while the others also provided data for current *vs* non-smoking and ever *vs* never smoking. None of the meta-analyses provided any evidence of variation in RR by level of BMI, the random effects meta-analysis estimate of the highest to lowest ratio being 1.20 (95%CI: 0.92-1.57) for current *vs* never smoking, 1.06 (95%CI: 0.82-1.36) for current *vs* non-smoking, 1.12 (0.95-1.32) for former *vs* never smoking, and 1.03 (95%CI: 0.87-1.23) for ever *vs* never smoking, based on, respectively, 9, 7, 9 and 7 estimates. (See Supplemen-tary file 10).

Supplementary files

Supplementary file 1 gives further details of the literature search, including a list of the 42 publications rejected during data entry, giving the reasons for rejection, and a description of how multiple publications from a study were dealt with.

Supplementary Files 2, 4, 7 and 9 give full details of the results for the main

Table 3 Meta-analysis random effect relative risks for current smoking

Grouping[1]		Current *vs* never smoking			Current *vs* non-smoking		
		n[2]	RR (95%CI)	*P*	*n*	RR (95%CI)	*P*
Overall		99	1.33 (1.28-1.38)	$P < 0.001$, $P < 0.05$	156	1.28 (1.24-1.32)	$P < 0.001$, $P < 0.05$
Sex	Female	25	1.30 (1.23-1.37)		31	1.26 (1.21-1.31)	
	Male	36	1.40 (1.32-1.49)		47	1.30 (1.24-1.36)	
	Combined	38	1.28 (1.18-1.39)	NS[3]	78	1.26 (1.18-1.34)	NS
Continent	Asia	44	1.36 (1.30-1.43)		57	1.36 (1.29-1.43)	
	Europe	32	1.34 (1.27-1.42)		60	1.25 (1.20-1.30)	
	North and South America	19	1.27 (1.18-1.37)		34	1.18 (1.12-1.25)	
	Oceania	4	1.05 (0.68-1.62)	NS	5	1.54 (1.28-1.85)	$P < 0.001$
Publication year	Up to 2005	13	1.41 (1.27-1.56)		23	1.24 (1.16-1.33)	
	2005-2014	47	1.36 (1.30-1.43)		66	1.31 (1.27-1.35)	
	2015 or later	39	1.27 (1.20-1.35)	NS	67	1.23 (1.17-1.30)	NS
Basis of diagnosis	Self-report only	12	1.32 (1.25-1.40)		17	1.34 (1.25-1.44)	
	Medical records only	49	1.32 (1.25-1.38)		86	1.29 (1.23-1.34)	
	Both	38	1.36 (1.27-1.46)	NS	53	1.24 (1.17-1.32)	NS
Population	General	93	1.32 (1.28-1.37)		147	1.28 (1.24-1.32)	
	Pre-diabetics only	2	3.29 (1.51-7.21)		3	1.23 (0.79-1.90)	
	Pre-diabetics excluded	4	1.61 (1.30-1.99)	$P < 0.05$	6	1.38 (1.15-1.67)	NS
Number of adjustment factors	0	17	1.15 (1.00-1.33)		33	1.19 (1.08-1.31)	
	1 to 5	18	1.36 (1.25-1.47)		30	1.38 (1.27-1.51)	
	6 to 10	43	1.40 (1.32-1.48)		64	1.29 (1.25-1.33)	
	11 or more	21	1.28 (1.20-1.37)	$P < 0.05$	29	1.22 (1.15-1.30)	$P < 0.1$
Cohort size	< 5000	35	1.36 (1.19-1.56)		58	1.31 (1.20-1.42)	
	5000 to 20000	20	1.38 (1.25-1.53)		43	1.24 (1.17-1.32)	
	> 20000	44	1.32 (1.26-1.37)	NS	55	1.29 (1.24-1.35)	NS
Number of type 2 diabetes cases	< 500	44	1.37 (1.23-1.52)		78	1.27 (1.19-1.35)	
	500-999	18	1.50 (1.34-1.67)		24	1.40 (1.27-1.55)	
	1000-2000	10	1.26 (1.15-1.38)		17	1.20 (1.11-1.30)	
	2001+	27	1.29 (1.22-1.35)	$P < 0.1$	37	1.26 (1.20-1.33)	NS
Highest age at baseline	< 60	13	1.36 (1.23-1.51)		22	1.24 (1.16-1.32)	
	60-74	27	1.44 (1.32-1.56)		38	1.36 (1.27-1.45)	

	75+	59	1.29 (1.24-1.35)	$P < 0.1$	96	1.26 (1.21-1.31)	NS
Length of follow-up (yr)	< 5	14	1.27 (1.19-1.35)		25	1.24 (1.15-1.34)	
	5-10	55	1.38 (1.30-1.47)		81	1.34 (1.28-1.40)	
	> 10	30	1.31 (1.22-1.39)	NS	50	1.22 (1.17-1.28)	$P < 0.05$
Definition of smoking	Cigarette	47	1.32 (1.27-1.38)		63	1.25 (1.21-1.29)	
	Smoking	50	1.36 (1.26-1.46)		89	1.30 (1.23-1.37)	
	Tobacco	2	1.10 (0.94-1.29)	$P < 0.1$	4	1.16 (1.06-1.27)	$P < 0.1$
Adjusted for age	No	20	1.17 (1.04-1.32)		41	1.22 (1.12-1.33)	
	Yes	79	1.35 (1.31-1.41)	$P < 0.05$	115	1.29 (1.25-1.33)	NS
Adjusted for sex	No	72	1.35 (1.29-1.41)		107	1.27 (1.23-1.32)	
	Yes	27	1.29 (1.20-1.39)	NS	49	1.29 (1.20-1.38)	NS
Adjusted for BMI	No	29	1.24 (1.11-1.38)		55	1.22 (1.13-1.32)	
	Yes	70	1.35 (1.30-1.41)	NS	101	1.30 (1.26-1.34)	NS
Adjusted for physical activity	No	41	1.27 (1.20-1.35)		87	1.27 (1.21-1.33)	
	Yes	58	1.36 (1.30-1.43)	$P < 0.1$	69	1.28 (1.23-1.33)	NS
Adjusted for alcohol consumption	No	42	1.26 (1.19-1.34)		87	1.26 (1.20-1.32)	
	Yes	57	1.37 (1.31-1.43)	$P < 0.05$	69	1.29 (1.25-1.33)	NS
Adjusted for family history of diabetes	No	61	1.28 (1.22-1.35)		99	1.23 (1.17-1.29)	
	Yes	38	1.41 (1.33-1.49)	$P < 0.05$	57	1.34 (1.29-1.40)	$P < 0.01$
Adjusted for education	No	63	1.37 (1.31-1.44)		115	1.29 (1.24-1.35)	
	Yes	36	1.28 (1.21-1.34)	$P < 0.05$	41	1.23 (1.18-1.28)	$P < 0.1$
Adjusted for diet	No	74	1.35 (1.29-1.41)		126	1.29 (1.24-1.34)	
	Yes	25	1.30 (1.22-1.38)	NS	30	1.23 (1.18-1.28)	$P < 0.1$
Adjusted for blood pressure	No	53	1.31 (1.24-1.40)		88	1.27 (1.21-1.34)	
	Yes	46	1.35 (1.29-1.41)	NS	68	1.28 (1.24-1.33)	NS
Adjusted for cholesterol	No	72	1.30 (1.25-1.35)		115	1.26 (1.22-1.31)	
	Yes	27	1.40 (1.32-1.48)	$P < 0.05$	41	1.32 (1.25-1.39)	NS
Adjusted for glucose	No	79	1.30 (1.25-1.35)		116	1.26 (1.22-1.31)	
	Yes	20	1.44 (1.35-1.54)	$P < 0.01$	40	1.34 (1.27-1.41)	NS
Adjusted for triglycerides	No	80	1.30 (1.25-1.36)		124	1.27 (1.22-1.31)	
	Yes	19	1.45 (1.33-1.58)	$P < 0.05$	32	1.34 (1.24-1.44)	NS
Adjusted for waist circumference	No	82	1.34 (1.29-1.40)		136	1.28 (1.24-1.32)	
	Yes	17	1.29 (1.19-1.41)	NS	20	1.25 (1.16-1.35)	NS

Adjusted for any other factors	No	37	1.30 (1.19-1.42)		62	1.28 (1.18-1.38)	
	Yes	62	1.34 (1.29-1.40)	NS	94	1.27 (1.23-1.30)	NS

[1]For sex, publication year, basis of diagnosis, number of adjustment factors, definition of smoking and age adjusted the grouping relates to characteristics of the relative risk. For other factors it relates to characteristics of the study.
[2]Number of estimates combined.
[3]NS means not significant, $P \geq 0.1$. For the overall analysis, the first P value relates to heterogeneity between estimates and the second to publication bias. For the other analyses it relates to a test of heterogeneity between the random-effects estimates at each level. Information on publication bias by level of each factor studied is given in Supplementary Files 2 and 4. NS: Not significant; CI: Confidence interval; RR: Relative risk.

analysis of, respectively, current *vs* never smoking, current *vs* non-smoking, former *vs* never smoking and ever *vs* never smoking. Each file is laid out similarly. Introductory pages describe the content and layout of the output, and explain the abbreviations used and the decisions made where multiple results were available for a single study. Table 1 of each Supplementary File then gives details of each candidate RR selected from the main and subsidiary publications for each study, while Table 2 of each file gives details of the RRs actually used in the analyses, and Tables 3-27 of each file give full results of the meta-analyses subdivided by each of the 25 factors considered (sex, continent, *etc*.).

Supplementary Files 3, 5 and 8 give full details of the dose-response analysis of respectively, current *vs* never smoking (by amount smoked), current *vs* non- smoking (by amount smoked) and former *vs* never smoking (by year quit). Each file includes separate blocks of description and results, similar to those for Supple-mentary Files 2, 4 7 and 9 , but only including Tables 1-3 of those files, with Table 3 only showing results subdivided by sex. Each block relates to a specific dose-response level (*e.g*., about 10 for amount smoked).

Supplementary file 6 presents forest and funnel plots for current *vs* non-smoking, former *vs* never smoking and ever *vs* never smoking, similar to those shown in Figures 1-6 of the paper for current *vs* never smoking.

Supplementary file 10 gives the results of meta-analyses of ratios of relative risks for the highest to lowest BMI groupings available.

DISCUSSION

According to the United States National Institute of Diabetes and Digestive and Kidney Diseases Health Information Center[162], risk factors for type 2 diabetes include overweight/obesity, age, a family history of diabetes, high blood pressure, low high-density lipoprotein cholesterol, high triglycerides, a history of gestational diabetes, giving birth to a baby weighing 9 pounds or more, physical inactivity, a history of heart disease or stroke, as well as being in certain ethnic groups or having certain diseases. Smoking is not mentioned as a risk factor.

The meta-analyses we conducted indicate a modest relationship of smoking to risk of type 2 diabetes. This can be seen for current smoking (whether compared with never or non-smokers), former smoking and ever smoking. While there was clear evidence of heterogeneity in the RRs, the random-effects RRs showed increased risks in males and females, in younger and older subjects, in all continents studied, regardless of the basis of diagnosis, and regardless of the definition of smoking used. Despite the evidence of heterogeneity between the individual estimates, a striking feature of the results presented in Tables 3 and 5 was the fact that the estimates were elevated in virtually every subdivision of the data, whichever factor the subdivision was based on. There was also clear evidence (see Tables 4 and 6) of an increasing risk with increasing amount smoked by current smokers and of decreasing risk with increasing time quit by former smokers. Though there was some evidence of variation in risk by level of some factors, this did not suggest that the elevation in risk was unique to some populations or could be explained by adjustment for specific confounding variables. Nor did the fact that some studies did not report an elevation affect the overall conclusion. With a relatively weak association (with RRs about 1.3 for current smoking and about 1.13 for former smoking) it might be expected that some smaller studies would not detect an elevated risk. However, this did not affect the overall conclusion. Indeed, it was notable that, of the 12 RR estimates for current *vs* never smoking that were below 1.0, only one was statistically significant (at $P < 0.05$), whereas, of the 87 estimates above 1.0, as many as 63 were.

Given the weight of evidence from this review and others, smoking may be a contributory factor to type 2 diabetes. Publication bias, for which some evidence was

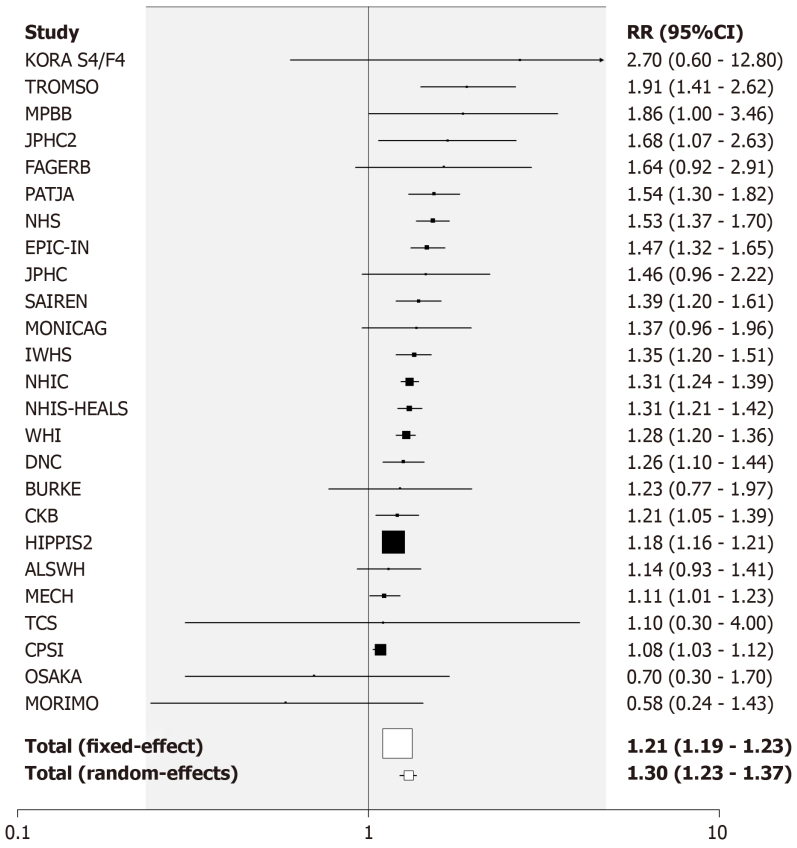


Figure 1 Forest plot for current *vs* never smoking, results for females. For each selected relative risk (RR), the figure shows the study ref. (see Table 2) and the RR and 95% confidence interval, both numerically and plotted as a line on a log scale from 0.1 to 10. The RRs are plotted from highest to lowest, with the RR estimate shown in the centre of the line as a square, with area proportional to the weight of the estimate. Lines showing RRs with wide confidence intervals may be truncated, as indicated by an arrow head at the truncated end. Also shown are the overall fixed-effect and random-effects estimates. The vertical line is at RR = 1 with an increased risk indicated by a preponderance of squares to its right. RR: Relative risk; CI: Confidence interval.

detected, might have led to some over-estimation of the association, due to some studies finding no relationship not presenting their results. Bias due to misclassification of smoking status would only tend to bias the observed relationship down, not produce an association that did not truly exist. Failure to control properly for diet, BMI or related factors would not seem to be an explanation of the association as elevated risks were seen in studies that adjusted for these factors. That said, it is clear from Table 3 that many of the studies did not adjust for various factors listed in the first paragraph of the discussion, so that the association seen between smoking and type 2 diabetes may have suffered from uncontrolled confounding to some extent.

This review has limitations, some unavoidable. Lack of access to individual person data limited the detail of the meta-analyses that can be carried out, but obtaining such data was not practical. Obtaining a reliable definition of outcome, exposure and adjustment variables was sometimes hindered by incomplete information in the source papers. Some studies involved relatively few type 2 diabetes cases, but associations were evident both in studies with small and large numbers. It is possible that our analyses did not make full use of all the data collected, but this is inevitable in a paper of reasonable length. We would be willing to make our database available to bona fide researchers for further analysis.

Our results are consistent with those of the earlier review by Pan *et al*[1] based on 88 prospective studies. Although our analyses were based on a considerably larger number of studies, 145, our estimated random-effect RRs of 1.33, 1.28 and 1.13 for current *vs* never, current *vs* non, and former *vs* never smoking were similar to their corresponding estimates of 1.40, 1.35 and 1.14. Like us, they also found dose-response relationships with amount smoked and years since quitting. The interested reader is referred to that paper for further discussion of limitations of the data and interpretation of the results.

That paper refers to "the high prevalence of smoking in many countries and the

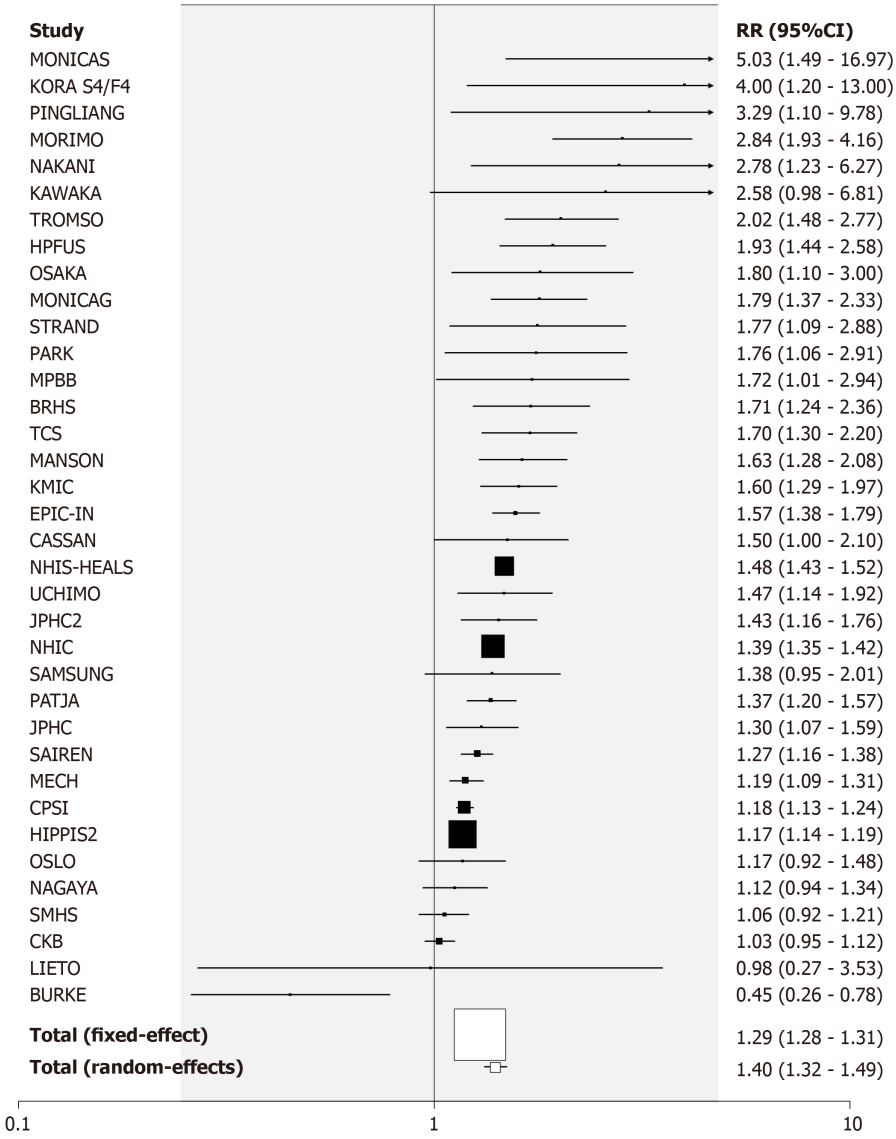


Figure 2 Forest plot for current *vs* never smoking, results for males. For each selected relative risk (RR) the figure shows the study ref. (see Table 2) and the RR and 95% confidence interval, both numerically and plotted as a line on a log scale from 0.1 to 10. The RRs are plotted from highest to lowest, with the RR estimate shown in the centre of the line as a square, with area proportional to the weight of the estimate. Lines showing RRs with wide confidence intervals may be truncated, as indicated by an arrow head at the truncated end. Also shown are the overall fixed-effect and random-effects estimates. The vertical line is at RR = 1 with an increased risk indicated by a preponderance of squares to its right. RR: Relative risk; CI: Confidence interval.

increasing number of diabetes worldwide" and considers that "reducing tobacco use should be prioritized as a key public health strategy to prevent and control global epidemic of diabetes". Though reduction of smoking is clearly important to limit a range of diseases such as lung cancer, chronic obstructive pulmonary disease and cardiovascular disease, one must question this prioritization, in the light of the range of other risk factors for type 2 diabetes noted above, and the evidence that diabetes incidence is rising fast worldwide[56], while smoking is declining[2]. As a strategy, controlling diet may be much more beneficial. The work of Taylor *et al*[163] suggests that, in many people, type 2 diabetes can be completely reversed quite rapidly by appropriate diet and weight loss.

In conclusion, the analyses confirm earlier reports of a modest dose-related association of current smoking and a weaker dose-related association of former smoking with risk of type 2 diabetes.

Table 4 Dose-response analyses for current smoking

Grouping[1]	Current *vs* never smoking		Current *vs* non-smoking	
	n[2]	RR (95%CI)	*n*	RR (95%CI)
Using key values:				
About 10 cigs/d	13	1.10 (1.03-1.18)	13	1.04 (0.98-1.10)
About 20 cigs per d	13	1.31 (1.19-1.44)	13	1.27 (1.16-1.39)
About 40 cigs per d	13	1.55 (1.39-1.72)	13	1.54 (1.37-1.72)
Low	23	1.17 (1.11-1.23)	22	1.13 (1.07-1.19)
Medium	23	1.30 (1.22-1.39)	22	1.26 (1.18-1.34)
High	23	1.53 (1.41-1.65)	22	1.48 (1.37-1.60)
1-19 cigs/d	18	1.32 (1.20-1.45)	17	1.20 (1.10-1.30)
20+	18	1.58 (1.42-1.76)	17	1.44 (1.31-1.59)

[1]The key value analysis is based on all studies which provide estimates for each key value (*i.e.*, for a range which included the key value and no other key value). The low/medium/high analysis is based on all studies which provide estimates for exactly three levels. The 1-19, 20+ analysis is based on those studies which reported results only for these two levels.
[2]Number of estimates combined. CI: Confidence interval; RR: Relative risk.

Table 5 Meta-analysis random effects relative risks for former (*vs* never) smoking

Grouping[1]		*n*[2]	RR (95%CI)	*P*
Overall		100	1.13 (1.11-1.16)	$P < 0.001$, $P < 0.01$
Sex	Female	26	1.13 (1.08-1.18)	
	Male	36	1.12 (1.08-1.16)	
	Combined	38	1.16 (1.09-1.22)	NS[3]
Continent	Asia	44	1.16 (1.10-1.22)	
	Europe	32	1.13 (1.09-1.18)	
	North and South America	20	1.11 (1.07-1.16)	
	Oceania	4	1.07 (0.93-1.23)	NS
Publication year	Up to 2005	13	1.13 (1.06-1.21)	
	2005-2014	47	1.16 (1.11-1.22)	
	2015 or later	40	1.11 (1.08-1.15)	NS
Basis of diagnosis	Self-report only	12	1.17 (1.05-1.29)	
	Medical records only	49	1.11 (1.08-1.13)	
	Both	39	1.16 (1.11-1.22)	$P < 0.1$
Population	General	94	1.13 (1.11-1.16)	
	Pre-diabetics only	2	0.97 (0.08-12.64)	
	Pre-diabetics excluded	4	1.11 (0.86-1.44)	NS
Number of adjustment factors	0	18	1.11 (1.01-1.23)	
	1 to 5	18	1.20 (1.11-1.30)	
	6 to 10	42	1.12 (1.08-1.17)	
	11 or more	22	1.13 (1.09-1.17)	NS
Cohort size	< 5000	35	1.21 (1.11-1.32)	
	5000 to 20000	20	1.19 (1.09-1.29)	
	> 20000	45	1.12 (1.09-1.15)	NS
Number of type 2 diabetes cases	< 500	44	1.21 (1.12-1.30)	
	500 to 999	18	1.11 (1.03-1.20)	
	1000 to 2000	10	1.26 (1.10-1.45)	
	2001+	28	1.11 (1.08-1.14)	$P < 0.1$
Highest age at baseline	< 60	14	1.20 (1.10-1.30)	
	60-74	27	1.19 (1.10-1.29)	
	75+	59	1.11 (1.09-1.14)	NS
Length of follow-up (yr)	< 5	14	1.13 (1.08-1.19)	
	5-10	55	1.16 (1.10-1.23)	

	> 10	31	1.11 (1.08-1.15)	NS
Definition of smoking	Cigarette	48	1.12 (1.09-1.15)	
	Smoking	50	1.15 (1.10-1.21)	
	Tobacco	2	0.95 (0.83-1.08)	$P < 0.05$
Adjusted for age	No	21	1.13 (1.05-1.22)	
	Yes	79	1.13 (1.10-1.16)	NS
Adjusted for sex	No	75	1.13 (1.10-1.16)	
	Yes	25	1.13 (1.07-1.19)	NS
Adjusted for BMI	No	31	1.15 (1.07-1.24)	
	Yes	69	1.12 (1.10-1.15)	NS
Adjusted for physical activity	No	41	1.15 (1.11-1.20)	
	Yes	59	1.12 (1.09-1.16)	NS
Adjusted for alcohol consumption	No	43	1.15 (1.10-1.19)	
	Yes	57	1.13 (1.09-1.16)	NS
Adjusted for family history of diabetes	No	61	1.13 (1.10-1.17)	
	Yes	39	1.13 (1.09-1.17)	NS
Adjusted for education	No	65	1.16 (1.12-1.19)	
	Yes	35	1.09 (1.05-1.14)	$P < 0.05$
Adjusted for diet	No	75	1.14 (1.11-1.17)	
	Yes	25	1.12 (1.07-1.16)	NS
Adjusted for blood pressure	No	54	1.14 (1.10-1.19)	
	Yes	46	1.13 (1.09-1.16)	NS
Adjusted for cholesterol	No	73	1.13 (1.10-1.16)	
	Yes	27	1.14 (1.08-1.20)	NS
Adjusted for glucose	No	80	1.13 (1.10-1.16)	
	Yes	20	1.15 (1.07-1.23)	NS
Adjusted for triglycerides	No	81	1.12 (1.10-1.15)	
	Yes	19	1.17 (1.08-1.27)	NS
Adjusted for waist circumference	No	83	1.13 (1.10-1.16)	
	Yes	17	1.14 (1.05-1.24)	NS
Adjusted for other factors	No	38	1.15 (1.08-1.23)	
	Yes	62	1.13 (1.10-1.15)	NS

[1]For sex, publication year, basis of diagnosis, number of adjustment factors, definition of smoking and age adjusted the grouping relates to characteristics of the RR. For other factors it relates to characteristics of the study.
[2]Number of estimates combined.
[3]NS means not significant, $P \geq 0.1$. For the overall analysis, the first P value relates to heterogeneity between estimates and the second to publication bias. For the other analyses it relates to a test of heterogeneity between the random-effects estimates at each level. Information on publication bias by level of each factor studied is given in Supplementary file 6. CI: Confidence interval; RR: Relative risk; NS: Not significant; BMI: Body mass index.

Table 6 Dose-response analyses for former *vs* never smoking (years quit)

Grouping[1]		*n*[2]	RR (95%CI)
Using key values:	About 3 yr quit	8	1.39 (1.21-1.60)
	About 7 yr quit	8	1.17 (1.07-1.27)
	About 12 yr quit	8	1.11 (1.01-1.22)
Shortest		14	1.46 (1.31-1.63)
Longest		14	1.13 (1.01-1.27)

[1]The key value analysis is based on all studies which provide estimates for each key value (*i.e.*, for a range which included the key value and no other key value). The shortest/longest analysis is based on all studies which provide estimates by years quit.
[2]Number of estimates combined. CI: Confidence interval; RR: Relative risk.

Table 7 Meta-analysis random effects relative risks for ever (*vs* never) smoking

Grouping[1]		*n*[2]	RR (95%CI)	*P*
Overall		100	1.25 (1.21-1.28)	$P < 0.001$, $P < 0.01$
Sex	Female	24	1.25 (1.18-1.31)	
	Male	36	1.25 (0.20-1.31)	
	Combined	40	1.22 (1.14-1.31)	$P < 0.05$
Continent	Asia	41	1.30 (1.25-1.36)	
	Europe	36	1.21 (1.17-1.26)	
	North and South America	20	1.19 (1.13-1.26)	
	Oceania	3	0.87 (0.48-1.57)	$P < 0.05$
Publication year	Up to 2005	13	1.25 (1.16-1.34)	
	2005-2014	47	1.26 (1.20-1.33)	
	2015 or later	40	1.23 (1.18-1.28)	NS[3]
Basis of diagnosis	Self-report only	10	1.35 (1.17-1.56)	
	Medical records only	51	1.22 (1.18-1.27)	
	Both	39	1.26 (1.19-1.33)	NS
Population	General	95	1.24 (1.21-1.28)	
	Pre-diabetics only	1	3.30 (1.24-8.77)	
	Pre-diabetics excluded	4	1.43 (1.17-1.76)	$P < 0.1$
Number of adjustment factors	0	23	1.18 (1.06-1.32)	
	1 to 5	16	1.28 (1.20-1.36)	
	6 to 10	40	1.24 (1.19-1.30)	
	11 or more	21	1.22 (1.16-1.28)	NS
Cohort size	< 5000	39	1.26 (1.14-1.38)	
	5000 to 20000	17	1.27 (1.17-1.38)	
	> 20000	44	1.24 (1.20-1.28)	NS
Number of type 2 diabetes cases	< 500	46	1.26 (1.16-1.36)	
	500 to 999	17	1.32 (1.19-1.47)	
	1000 to 2000	9	1.28 (1.14-1.43)	
	2001+	28	1.22 (1.17-1.26)	NS
Highest age at baseline	< 60	13	1.35 (1.23-1.47)	
	60-74	27	1.32 (1.23-1.41)	
	75+	60	1.21 (1.17-1.25)	$P < 0.05$
Length of follow-up (yr)	< 5	14	1.21 (1.15-1.26)	
	5-10	56	1.29 (1.22-1.35)	
	> 10	30	1.21 (1.15-1.28)	NS
Definition of smoking	Cigarette	48	1.22 (1.18-1.26)	
	Smoking	50	1.28 (1.20-1.36)	
	Tobacco	2	1.09 (0.94-1.25)	$P < 0.1$
Adjusted for age	No	27	1.19 (1.09-1.31)	
	Yes	73	1.24 (1.20-1.28)	NS
Adjusted for sex	No	77	1.26 (1.22-1.30)	
	Yes	23	1.20 (1.13-1.27)	NS
Adjusted for BMI	No	35	1.23 (1.13-1.34)	
	Yes	65	1.24 (1.20-1.28)	NS
Adjusted for physical activity	No	43	1.26 (1.20-1.32)	
	Yes	57	1.24 (1.19-1.29)	NS
Adjusted for alcohol consumption	No	46	1.24 (1.19-1.30)	
	Yes	54	1.25 (1.20-1.30)	NS
Adjusted for family history of diabetes-	No	62	1.22 (1.17-1.27)	
	Yes	38	1.28 (1.23-1.33)	NS
Adjusted for education	No	67	1.29 (1.24-1.34)	
	Yes	33	1.17 (1.12-1.21)	$P < 0.001$
Adjusted for diet	No	76	1.26 (1.22-1.31)	

	Yes	24	1.21 (1.15-1.26)	NS
Adjusted for blood pressure	No	57	1.25 (1.19-1.32)	
	Yes	43	1.23 (1.19-1.27)	NS
Adjusted for cholesterol	No	75	1.24 (1.20-1.28)	
	Yes	25	1.27 (1.19-1.36)	NS
Adjusted for glucose	No	79	1.23 (1.19-1.27)	
	Yes	21	1.31 (1.25-1.37)	$P < 0.05$
Adjusted for triglycerides	No	83	1.23 (1.20-1.27)	
	Yes	17	1.31 (1.22-1.41)	NS
Adjusted for waist circumference	No	84	1.25 (1.21-1.30)	
	Yes	16	1.21 (1.12-1.31)	NS
Adjusted for other factors	No	42	1.24 (1.15-1.33)	
	Yes	58	1.23 (1.20-1.28)	NS

[1]For sex, publication year, basis of diagnosis, number of adjustment factors, definition of smoking and age adjusted the grouping relates to characteristics of the relative risk. For other factors it relates to characteristics of the study.
[2]Number of estimates combined.
[3]NS means not significant, $P \geq 0.1$. For the overall analysis, the first P value relates to heterogeneity between estimates and the second to publication bias. For the other analyses it relates to a test of heterogeneity between the random-effects estimates at each level. Information on publication bias by level of each factor studied is given in Supplementary file 8. CI: Confidence interval; RR: Relative risk; NS: Not significant.

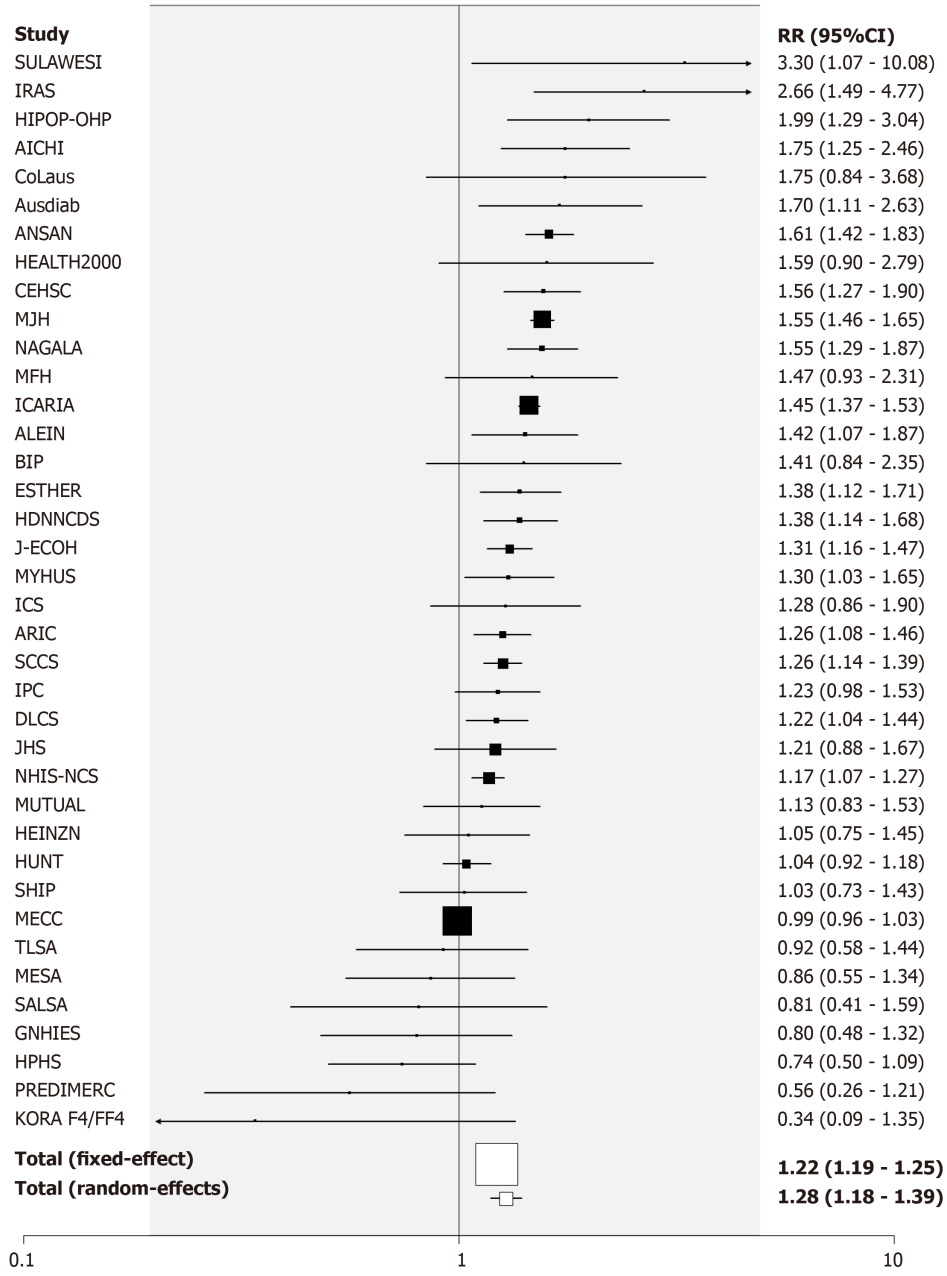


Figure 3 Forest plot for current *vs* never smoking, results for sexes combined. For each selected relative risk (RR), the figure shows the study ref. (see Table 2) and the RR and 95% confidence interval, both numerically and plotted as a line on a log scale from 0.1 to 10. The RRs are plotted from highest to lowest, with the RR estimate shown in the centre of the line as a square, with area proportional to the weight of the estimate. Lines showing RRs with wide confidence intervals may be truncated, this being indicated by an arrow head at the truncated end. Also shown are the overall fixed-effect and random-effects estimates. The vertical line is at RR = 1 with an increased risk indicated by a preponderance of squares to its right. RR: Relative risk; CI: Confidence interval.

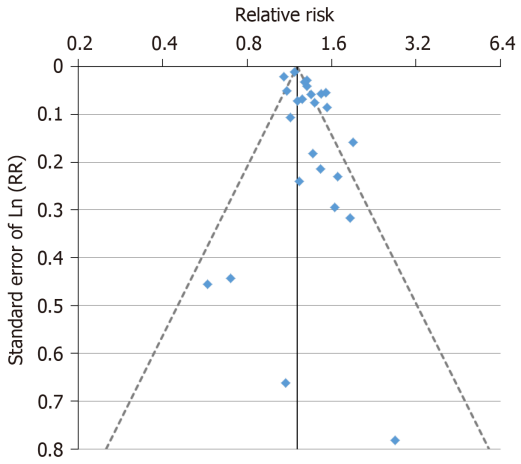


Figure 4 Funnel plot for current *vs* never smoking, results for females. Each of the selected relative risks (RRs) is shown as a diamond, plotted against its value on the x-axis (on a log scale) and the standard error of $\log_e$ RR on the y-axis. The vertical line indicates the overall fixed-effect estimate, while the diagonals indicate where 95% of the values should lie, given the standard error of $\log_e$ RR. Evidence of publication bias is indicated by a tendency for the smaller (high standard error) studies to show larger treatment effects. RR: Relative risk.

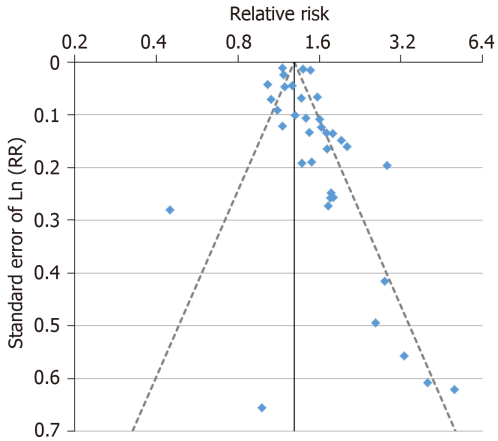


Figure 5 Funnel plot for current *vs* never smoking, results for males. Each of the selected relative risks (RRs) is shown as a diamond, plotted against its value on the x-axis (on a log scale) and the standard error of $\log_e$ RR on the y-axis. The vertical line indicates the overall fixed-effect estimate, while the diagonals indicate where 95% of the values should lie, given the standard error of $\log_e$ RR. Evidence of publication bias is indicated by a tendency for the smaller (high standard error) studies to show larger treatment effects. RR: Relative risk.

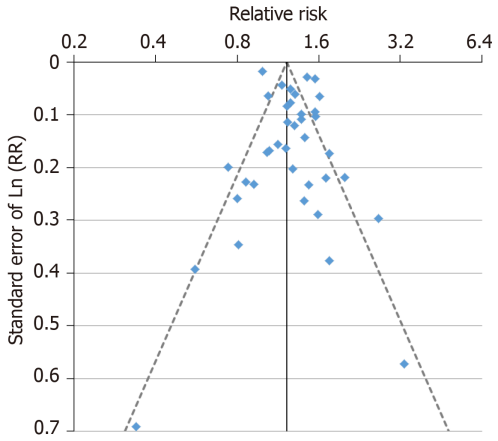


Figure 6 Funnel plot for current *vs* never smoking, results for sexes combined. Each of the selected relative risks (RRs) is shown as a diamond, plotted against its value on the x-axis (on a log scale) and the standard error of $\log_e$ RR on the y-axis. The vertical line indicates the overall fixed-effect estimate, while the diagonals indicate where 95% of the values should lie, given the standard error of $\log_e$ RR. Evidence of publication bias is indicated by a tendency for the smaller (high standard error) studies to show larger treatment effects. RR: Relative risk.

ARTICLE HIGHLIGHTS

Research background

A systematic review of the relationship between smoking and incident type 2 diabetes, based on 88 epidemiological prospective studies, was published in 2015. Much new evidence on this relationship has become available since then.

Research motivation

To obtain up-to-date evidence relating smoking to type 2 diabetes.

Research objectives

To systematically review available evidence from prospective studies on the relationship of type 2 diabetes onset to ever, current or former smoking of cigarettes or of any tobacco product, including dose-response data.

Research methods

Attention was restricted to prospective studies of populations free of type 2 diabetes at baseline which related subsequent incidence of the disease to one or more defined major or dose-related smoking indices. The major indices compared ever, current or former smokers to never smokers and current smokers to non-current smokers. The dose-related indices concerned amount currently smoked and years quit. Literature searches identified relevant papers from previous reviews, from Medline searches and from references lists of relevant papers identified. Data were extracted on study details and on the relative risks required, estimated if required using standard methods. Care was taken to avoid overlap of data from the same study from multiple publications. Fixed-effect and random-effects meta-analyses were conducted, including tests of heterogeneity and publication bias. Where a study provided multiple estimates, a preference scheme was used involving factors such as level of adjustment for confounding factors, length of follow-up and age range considered. Sex-specific results were used, if available. Effect estimates were derived based on all the selected RRs, and also for those subdivided by various categorical variables – sex, continent, year of publication, basis of diagnosis of diabetes, initial diabetes status of the population, age, length of follow-up, definition of smoking, and whether a range of different variables were adjusted for.

Research results

The literature searches identified 157 relevant publications providing results from 145 studies. Overall random-effect RR estimates were 1.33 [95% confidence interval (CI): 1.28-1.38] for current *vs* never smoking, 128 (95%CI: 1.24-1.32) for current *vs* non-smoking, 1.13 (95%CI: 1.11-1.16) for former *vs* never smoking and 1.25 (95%CI: 1.21-1.28) for ever *vs* never smoking, each combined estimate being based on at least 99 individual estimates. Estimates were generally elevated in each subdivision of the data by the categorical variables considered, though in some cases RR estimates varied significantly ($P < 0.05$) by level. The dose-response analysis showed that risk increased with increasing amount smoked, and reduced with increasing time quit.

Research conclusions

Our analyses confirmed and extended reports of a modest dose-related association of current smoking and a weaker dose-related association of former smoking with risk of type 2 diabetes. The evidence suggests smoking may contribute to the risk of type 2 diabetes, though our estimates may be affected by publication bias and some uncontrolled confounding. Although reduction of smoking is clearly important to limit risk of diseases such as lung cancer, chronic obstructive pulmonary disease and cardiovascular disease, the worldwide rise in incidence of type 2 diabetes, coupled with a decline in smoking, suggests that control of other factors, such as diet, may be much more beneficial in reducing type 2 diabetes risk.

Research perspectives

Our analyses suggest strongly that there is a modest increased risk of type 2 diabetes associated with current smoking which is greater in heavier smokers and reduced following quitting. Further large prospective studies could characterize this more precisely by more detailed assessment of smoking history and by more fully accounting for the range of other factors known to be related to type 2 diabetes. Care should be taken to determine the accuracy of all the data used, and to assess the effect that any possible inaccuracy might have on the estimated association.

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META-ANALYSIS

Single-balloon and spiral enteroscopy may have similar diagnostic and therapeutic yields to double-balloon enteroscopy: Results from a meta-analysis of randomized controlled trials and prospective studies

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Abstract

BACKGROUND

Double-balloon, single-balloon, and spiral enteroscopy (DBE, SBE, and SE) have revolutionized the management of intestinal diseases. However, evidence about efficacies of these methods is lacking. We aimed to conduct a meta-analysis comparing the clinical outcomes among DBE, SBE, and SE.

METHODS

We searched randomized controlled trials and prospective studies in MEDLINE, PubMed, EMBASE, Cochrane Library, and Chinese CQVIP database. Studies referencing the comparison of at least two of these three methods were included. Primary outcome was diagnostic yield. Other outcomes were therapeutic yield, total enteroscopy, examination time, time to maximum insertion, and depth of maximal insertion (DMI).

RESULTS

Eleven studies including 727 patients were identified: DBE *vs* SE (n = 6), DBE *vs* SBE (n = 4), and SBE *vs* SE (n = 1). The diagnostic and therapeutic yields did not differ significantly when comparing DBE with SE [odds ratio (OR) = 1.19, 95% confidence interval (CI): 0.68-2.08; OR = 1.17, 95%CI: 0.61-2.23] and DBE with SBE

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(OR = 0.85, 95%CI: 0.55-1.33; OR = 1.71, 95%CI: 0.64 - 4.60). Total enteroscopy, examination time, time to maximum insertion, and DMI were similar between SBE and DBE. DBE was superior to SE with regard to DMI [mean difference (MD) = 36.76, 95%CI: 5.09-68.43], with longer time to maximum insertion (MD = 15.14, 95%CI: 12-18.27) and examination time (MD = 12.98, 95%CI: 9.57-16.38).

CONCLUSION
DBE and SBE have similar clinical outcomes. Compared with DBE, SE seems to have similar diagnostic and therapeutic yields, but shorter procedural time in cost of less depth of insertion. SE needs further evaluation *vs* SBE. DBE is recommended for complete enteroscopy.

Key words: Double-balloon enteroscopy; Single-balloon enteroscopy; Spiral enteroscopy; Meta-analysis; Randomized controlled trials

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Core tip: We conducted a meta-analysis to compare the effectiveness of double-balloon, single-balloon, and spiral enteroscopy. We found that double-balloon enteroscopy may have similar clinical outcomes to single-balloon enteroscopy, and spiral enteroscopy seems to have similar diagnostic and therapeutic yields, but shorter procedural time in cost of less depth of insertion when compared with double-balloon enteroscopy.

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INTRODUCTION

When double-balloon enteroscopy (DBE) was introduced into the clinic by Yamamoto in 2001[1], dramatic changes have happened in the diagnosis and management of disorders in the distant part of small bowel, which were dependent on surgically assisted enteroscopy or rope guided enteroscopy before. Currently, there are another two different systems: Single-balloon enteroscopy (SBE) and spiral enteroscopy (SE). Although the balloon-assisted enteroscopy techniques follow the push-and-pull principle, SE is dependent on a new system: Pleating of the small bowel *via* rotation[2].

There have been several randomized controlled trials (RCTs) comparing DBE with SBE[3-6]. In these studies, DBE has been shown to be similar to SBE with regard to diagnostic/therapeutic yield, depth of maximal insertion, and procedure time, but superior to SBE in terms of total enteroscopy. There have been seven studies including four RCTs and three prospective studies comparing DBE with SE[2,7-12]. In these studies, diagnostic yield was supposed to be similar between DBE and SE. Two recent meta-analyses only compared DBE with SBE, and did not evaluate SE[13,14]. Another meta-analysis compared balloon enteroscpy (combined DBE with SBE) with SE[15]. Moreover, these three meta-analyses included May's study, in which SBE was not really applied but by detaching one balloon from double-balloon enteroscope[16,17]. In addition, they did not include Chinese studies due to language barrage.

Therefore, we conducted a meta-analysis aimed to evaluate the efficacy of DBE, SBE, and SE for the management of small bowel diseases.

MATERIALS AND METHODS

The methodology and reporting of this study followed the PRISMA statement guidelines recommended by Cochrane collaboration for meta-analysis[18].

Data sources and searches

A comprehensive literature search was independently performed by two authors. The

search was performed on MEDLINE, PubMed, EMBASE, Cochrane Library, and Chinese CQVIP database (http://en.cqvip.com/) from January 2008 to October 2018, using the search terms "balloon enteroscopy", "single balloon enteroscopy", "double balloon enteroscopy", and "spiral enteroscopy". Boolean operators AND, OR, and NOT were used. Meanwhile, abstracts from the conference proceedings were also retrieved.

Study selection

RCTs and prospective studies comparing DBE, SBE, and SE were eligible for inclusion. Retrospective studies and non-comparative studies that used only DBE, SBE, or SE were excluded. The studies were restricted to studies performed in human and published in English and Chinese. And a manual search of the references of all retrieved studies was performed in case of omission of the ongoing or completed trials. Two investigators independently assessed the eligibility and validity of each study. Queries concerning inclusion were resolved by discussion and consensus between the two reviewers.

Outcome measures

The primary outcome evaluated in this study was diagnostic yield rate, defined as the percentage of enteroscopy procedures with findings relevant to the indication of the procedure[10]. Other outcomes were therapeutic yield rate, total enteroscopy rate, depth of maximal insertion (DMI), time to maximum insertion, and examination time (per-oral and per-anal).

Data extraction

The following data from each study were extracted: First author's name, publication year, country, study design, interventions, patient demographics, indications, number of endoscopies, diagnostic and therapeutic yields, DMI, time to maximum insertion, and examination time (per-oral and per-anal). Different opinions between the reviewers were revolved through discussion.

Risk of bias assessment

As described in the Cochrane Handbook for Systematic Reviews of Interventions[19], we assessed the quality of RCT based on random sequence generation, allocation concealment, blinding of participants and investigators, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. We used the Newcastle-Ottawa Scale to assess the quality of prospective study[20]. Any disagreement was resolved *via* discussions.

Statistical analysis

Statistical analyses were performed using RevMan software (version 5.3; Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Standard deviation was obtained from the range and interquartile range as recommended[21,22]. Results are expressed as odds ratio (OR) for categorical variables or mean difference (MD) for continuous variables with 95% confidence interval (CI). Heterogeneity was assessed using I^2 test. $P < 0.05$ or $I^2 > 50\%$ was considered as significant heterogeneity. A fixed- or random-effects model was used, depending on the absence or presence of significant heterogeneity. Potential for publication bias was assessed by funnel plot. $P < 0.05$ was considered statistically significant.

RESULTS

Description of studies

The detailed steps of our literature search are shown in Figure 1. Briefly, a total of 384 articles were identified. After screening, 22 potentially relevant articles and abstracts were retrieved from the electronic databases. After excluding 11 studies based on the predefined inclusion criteria, 11 studies were identified. Six trials compared DBE with SE[2,8-12], four compared DBE with SBE[3-6], and one compared SBE with SE[7], which were evaluated separately in the meta-analysis. Baseline characteristics and outcome measures are shown in Tables 1 and 2. The risks of bias of the RCTs included in this meta-analysis are detailed in Supplementary Figure 1. Each prospective study achieved eight points in assessing quality according to the Newcastle-Ottawa Scale.

DBE vs SE

There were six comparisons of DBE *vs* SE: Five from Europe and one from Asia[2,8-12]. Three were RCTs[2,8,11] and three were prospective studies[9,10,12]. A total of 370 procedures were analyzed, including 255 DBE and 115 SE procedures.

Table 1 Study characteristics

Ref.	Country	Study design	Intervention	No. of patients	Male/Female	Age	Indications (OGIB/others)
Moran *et al*[7], 2018	United States	RCT	SBE	17	3/14	55.8 ± 20.3	8/9
			SE	13	10/3	53.1 ± 18.3	7/6
Oka *et al*[8], 2015	Japan	RCT	DBE	10	6/4	63.9 ± 12.8	0/10
			SE	10	6/4	63.9 ± 12.8	0/10
Despott *et al*[9], 2015	United Kingdom	Prospective	DBE	15	5/10	51.4 ± 5.4	0/15
			SE	15	5/10	51.4 ± 5.4	0/15
Messer *et al*[2], 2013	Germany	RCT	DBE	13	6/7	47.6 ± 20/7	7/6
			SE	13	7/6	61.2 ± 18.6	12/1
Rahmi *et al*[10], 2013	France	Prospective	DBE	191	76/115	59.0 ± 15.9	151/40
			SE	50	25/25	58.4 ± 14.3	43/7
May *et al*[11], 2011	Germany	RCT	DBE	10	6/4	69 ± 12	10/0
			SE	10	6/4	69 ± 12	10/0
Frieling *et al*[12], 2010	Germany	Prospective	DBE	17	7/10	61 ± 17	14/3
			SE	18	10/8	58 ± 25	11/7
Efthymiou *et al*[3], 2012	Australia	RCT	DBE	57	24/33	61 (49-68)[1]	44/13
			SBE	50	19/31	67 (51-72)[1]	40/10
Domagk *et al*[6], 2011	Germany	RCT	DBE	65	32/33	52 (18-84)[2]	29/36
			SBE	65	35/30	53 (21-80)[2]	26/39
Takano *et al*[5], 2011	Japan	RCT	DBE	20	15/5	62.7 ± 16.1	13/7
			SBE	18	13/5	64.9 ± 14.7	10/8
Ren *et al*[4], 2011	China	RCT	DBE	50	35/15	42.3	25/25
			SBE				

[1]Median (IQR).
[2]Median (range). RCT: Randomized controlled trial; SBE: Single-balloon enteroscopy; SE: Spiral enteroscopy; DBE: Double-balloon enteroscopy; OGIB: Obscure gastrointestinal bleeding.

As shown in Figure 2, there was no significant difference in diagnostic yield (OR = 1.19, 95%CI: 0.68-2.08) or therapeutic yield (OR = 1.17, 95%CI: 0.61-2.23) between DBE and SE. DBE resulted in deeper DMI (MD = 36.76, 95%CI: 5.09-68.43), corresponding to longer time to maximum insertion (MD = 15.14, 95%CI: 12.00-18.27) and examination time (MD = 12.98, 95%CI: 9.57-16.38). There was no statistical heterogeneity between the studies with regard to diagnostic yield, therapeutic yield, DMI, time to maximum insertion, and examination time ($P > 0.05$). The funnel plot did not show publication bias in terms of diagnostic yield between DBE and SE (Supplementary Figure 2).

DBE vs SBE

There were four RCTs comparing DBE with SBE[3-6]. A total of 327 procedures were analyzed, including 171 DBE and 156 SBE procedures.

As shown in Figure 3, there were no significant differences in diagnostic yield (OR = 0.85, 95%CI: 0.55-1.33), therapeutic yield (OR = 1.71, 95%CI: 0.64-4.60), total enteroscopy (OR = 6.10, 95%CI: 0.31-118.52), DMI (MD = -20.42, 95%CI: -89.29-48.45), per-oral examination time (MD = -7.45, 95%CI: -36.60-21.16), or per-anal examination time (MD = 2.48, 95%CI: -6.49-11.44). There was no statistical heterogeneity between the studies with regard to diagnostic yield, therapeutic yield, or per-anal examination time ($P > 0.05$). The funnel plot did not show publication bias in terms of diagnostic yield between DBE and SBE (Supplementary Figure 3).

SBE vs SE

We could not conduct a meta-analysis since there was only one trial comparing SBE *vs* SE. In the single trial[7], patients needed for an anterograde enteroscopy were randomized to receive SBE ($n = 17$) or SE ($n = 13$), thus evaluating a direct comparison between SBE and SE. However, this was insufficient to compare these modalities using meta-analysis. In this trial which ceased without reaching the required sample size, there were no significant differences identified between SBE and SE with regard to diagnostic yield (41% *vs* 69%, $P = 0.16$), therapeutic yield (24% *vs* 46%, $P = 0.26$), DMI (285.3 ± 80.8 cm *vs* 330.0 ± 88.2 cm, $P = 0.16$), or procedure time (38.3 ± 12.4 *vs*

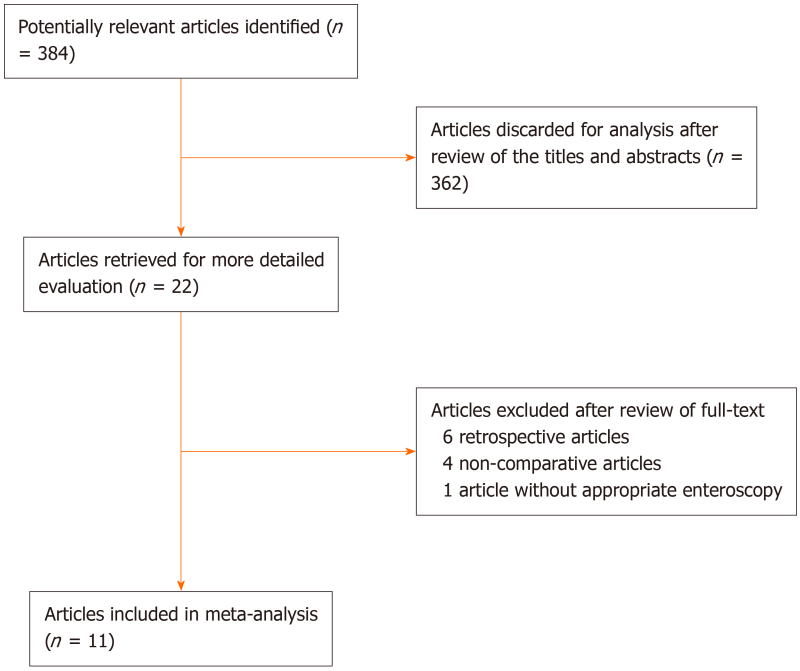


Figure 1 Flow chart.

37.0 ± 10.5, $P = 0.76$).

Sensitivity/subgroup analysis

We did not perform additional sensitivity or subgroup analysis to assess the observed heterogeneity due to limited number of studies.

DISCUSSION

Although the small intestine has long been considered the final frontier of endoscopy, significant progress in small-bowel enteroscopy techniques has led to increased diagnostic and therapeutic capabilities[23]. This meta-analysis including 727 patients, to our knowledge, is the first systemic meta-analysis comparing DBE, SBE, and SE with regard to clinical outcomes. Our study showed that DBE and SBE had similar clinical outcomes. We also identified that the diagnostic and therapeutic yields were comparable between DBE and SE. It was noteworthy that compared with DBE, SE had shorter procedural time but less depth of insertion. However, SE needs further evaluation vs SBE for clinical outcomes.

In our meta-analysis, double- and single-balloon systems showed similar performance in diagnostic and therapeutic yields, DMI, time to maximum insertion, and examination time. As for diagnostic yield, these four RCTs included in our study showed no significant difference. But in two retrospective studies we excluded[24,25], diagnostic yields were significantly higher in SBE, compared to the DBE group (61.7% *vs* 48.2%, $P < 0.001$; 62.0% *vs* 35.6%, $P = 0.014$). Nevertheless, retrospective studies easily suffered from patient selection bias, which was also declared by the author. These findings are consistent with previous meta-analyses[13,14]. However, the two meta-analyses included May's study[17] which was based on the P-type Fujinon instrument for both DBE and SBE. Although the P-type enteroscope allowed deeper intubation in the small bowel, therapeutic maneuvers were difficult to perform using this instrument in comparison with the T-type[26]. Lipka *et al*[14] mentioned that the P-system was less comparable to the Olympus system used in the other trials. Although results were similar to the previous, our study excluding that trial may be more credible. It is interesting that these two meta-analyses included the same four RCTs but reached the controversial conclusion in terms of total enteroscopy rate. Wadhwa *et al*[13] concluded that DBE was superior to SBE with regard to complete small bowel visualization (relative risk [RR] = 0.37, 95%CI: 0.19-0.73), while Lipka not (RR 1.73, 95%CI: 0.86-3.48). The recommendation to prefer DBE in patients in whom total enteroscopy is desired may be based on the feeling of many endoscopists. In our study, total enteroscopy rate tended to be higher in DBE than SBE, although the difference was not significant (OR = 6.10, 95%CI: 0.31-118.52). Complete enteroscopy

Table 2 Outcome measures of enteroscopy

Ref.	Intervention	No. of endoscopy	Diagnostic yield, *n* (%)	Therapeutic yield, *n* (%)	Total enteroscopy, *n* (%)	DMI, mean ± SD (cm)	Time to maximum insertion, mean ± SD (min)	Examination time, mean ± SD (min)	
								Oral	Anal
Moran *et al*[7], 2018	SBE	17	7/17 (41.2)	4/17 (24)	NA	285.3 ± 80.8	NA	36.1 ± 11.4	NA
	SE	13	9/13 (69.2)	6/13 (46)	NA	330.0 ± 88.2	NA	34.8 ± 4.7	NA
Oka *et al*[8], 2015	DBE	10	10/10 (100)	NA	NA	228 ± 22.0	32.9 ± 5.2	38.9 ± 5.0	NA
	SE	10	9/10 (90)	NA	NA	214 ± 16.9	19.5 ± 2.6	28.2 ± 4.3	NA
Despott *et al*[9], 2015	DBE	14	NA	NA	NA	265 (227-324)[3]	45 (35-53)[3]	54 (45-62)[3]	NA
	SE	14	NA	NA	NA	175 (132-212)[3]	24 (20-28)[3]	28 (27-36)[3]	NA
Messer *et al*[2], 2013	DBE	13	6/13 (46)	12/13 (92)	12/13 (92.3)	346.2 ± 63.3	46.2 ± 12.5	59.6 ± 14.5	76.1 ± 21.8
	SE	13	9/13 (69)	12/13 (92)	1/13 (7.7)	268.5 ± 76.3	26.2 ± 7.7	43.4 ± 10.2	51.9 ± 11.0
Rahmi *et al*[10], 2013	DBE	191	143/191 (74.9)	133/191 (69.6)	NA	200 (150-300)[2]	NA	60 (45-80)[2]	NA
	SE	50	35/50 (70.0)	33/50 (66)	NA	220 (200-300)[2]	NA	55 (45-80)[2]	NA
May *et al*[11], 2011	DBE	10	NA	14[1]	NA	310 (220-430)[3]	46.5 ± 19.3	65.0 ± 12.8	NA
	SE	10	NA	9[1]	NA	250 (200-340)[3]	24.3 ± 11.8	43.3 ± 9.3	NA
Frieling *et al*[12], 2010	DBE	17	8/17 (47.1)	NA	NA	265 ± 88.1	NA	41.8 ± 9.5	
	SE	18	6/18 (33.4)	NA	NA	216 ± 54.1	NA	46.5 ± 12	
Efthymiou *et al*[3], 2012	DBE	66	35/66 (53)	17/66 (26)	0/5	234.1 ± 99.3	NA	60 (45-70)[2]	
	SBE	53	30/53 (57)	16/53 (30)	0/1	203.8 ± 87.6	NA	60 (45-67)[2]	
Domagk *et al*[6], 2011	DBE	65	28/65 (43)	6/65 (9)	12/65 (18)	253 (120-450)[3]	NA	105 (40-140)[3]	
	SBE	65	24/65 (37)	3/65 (5)	7/65 (11)	258 (100-560)[3]	NA	96 (35-135)[3]	
Takano *et al*[5], 2011	DBE	20	10/20 (50)	7/20 (35)	8/14 (57)	NA	NA	70.4 ± 26.5	90.4 ± 13.7
	SBE	18	11/18 (61)	5/18 (28)	0/14	NA	NA	92.8 ± 20.6	93.1 ± 22.6
Ren *et al*[4], 2011	DBE	20	12/20 (60)	NA	0/6	328 (200-450)[3]	NA	70.3 (41-123)[3]	81.4 (55-141)[3]
	SBE	20	18/20 (90)	NA	0/5	360 (300-450)[3]	NA	63.7 (38-112)[3]	73.1 (45-132)[3]

[1]Angiodysplasias treated with APC.
[2]Median (IQR).
[3]Median (range). SBE: Single-balloon enteroscopy; SE: Spiral enteroscopy; DBE: Double-balloon enteroscopy; NA: Not available; DMI: Depth of maximal insertion; SD: Standard deviate.

may not be necessary to make a diagnosis, but it plays a role in determining the extension of a known disease and the number of the lesions, especially for the fact that many small bowel diseases are not solitary but multiple, such as vascular malformations, polyps in polyposis syndrome, or Crohn's stenosis[27]. Similarly, previous studies have shown that the rate of complete enteroscopy by using the DBE method is approximately 40% to 80%[28], while the rates of SBE decreased to approximately 5% to 25%[29,30]. If complete enteroscopy is needed for identifying the extent and number of the lesions, specifically for those once diagnosed by capsule endoscopy with multiple lesions, we recommended DBE technique because it provides a higher rate of complete enteroscopy at present[27].

Spiral enteroscopy, first reported in 2009[31], is another newly developed enteroscopy, which was found to have the advantages of shorter examination time and being more stable within the bowel, thus allowing controlled examination of the intestinal mucosa[31-33]. The previous meta-analysis conducted by Baniya *et al*[15] combined prospective and retrospective studies, demonstrating that balloon enteroscopy (DBE + SBE) and SE were similar with regard to diagnostic and therapeutic success rates and DMI, but the procedure time was significantly less for the SE group. Excluding retrospective study, we identified three RCTs and three prospective studies comparing DBE with SE, and found that there were no significant differences between DBE and SE with regard to diagnostic and therapeutic yields. However, our study showed that DBE resulted in deeper DMI, in cost of longer procedure time, which is consistent with previous studies[2,9,11]. Although the difference was not significant, DBE tended to achieve deeper small bowel insertion compared to SE[8,12]. Only one study compared DBE and SE in terms of total enteroscopy, and the total enteroscopy rate was 92% in the DBE group and 8% in the SE group (P = 0.002)[2]. Compared with SE, we also recommended DBE if complete

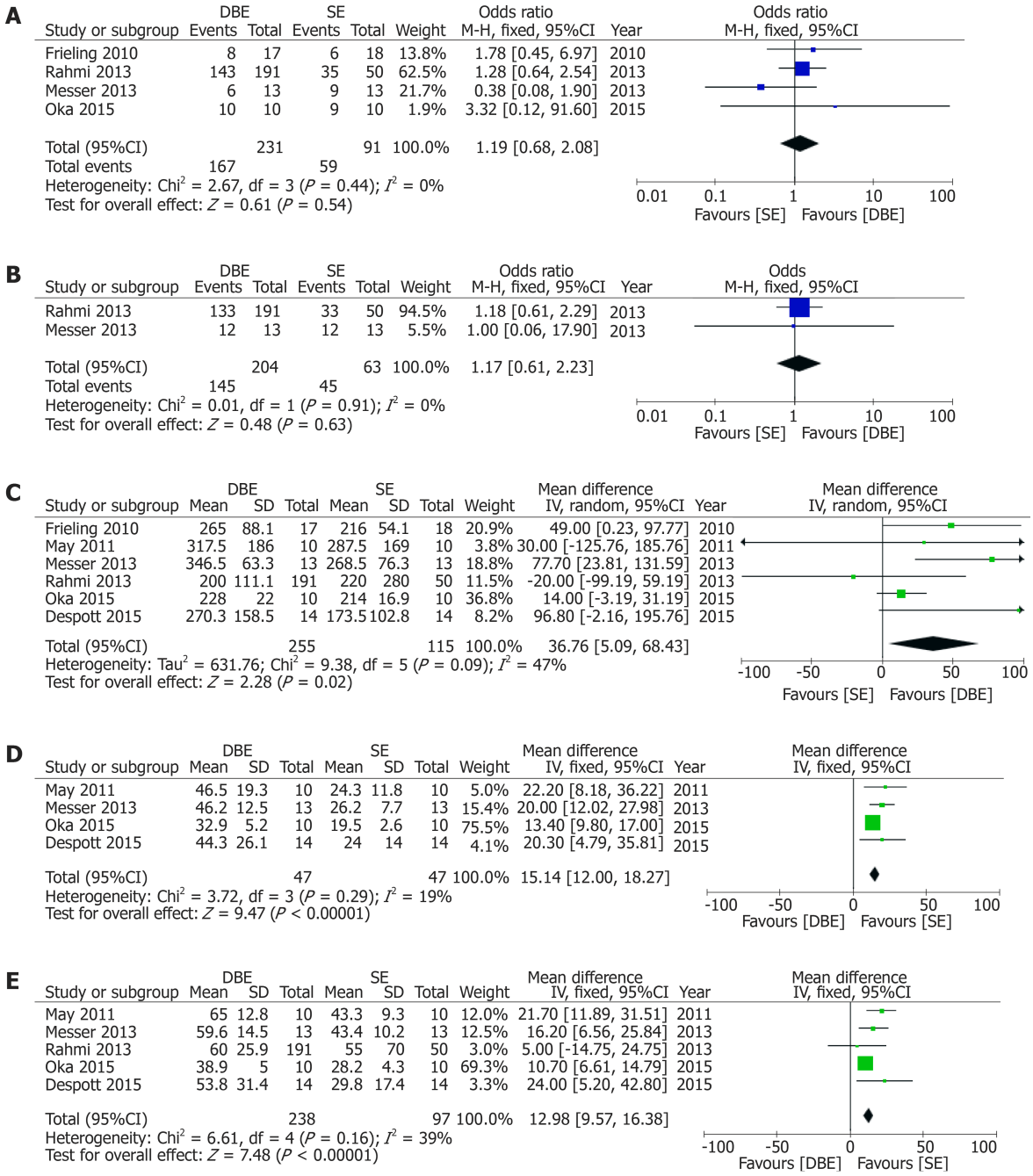


Figure 2 Outcome measures between double-balloon enteroscopy and spiral enteroscopy. A: Diagnostic yield; B: Therapeutic yield; C: Depth of maximal insertion; D: Time to maximum insertion; and E: Examination time.

enteroscopy was needed.

Up to now, there have been only two studies testing SE *vs* SBE. One RCT conducted by Moran *et al*[7] showed no significant differences between SE and SBE with respect to diagnostic yield, therapeutic yield, DMI, and procedure time. One retrospective study conducted by Khashab *et al*[23] showed that SE and SBE were similar in diagnostic yield but the insertion depth was significantly higher in SE. Small sample size and retrospective design prevented giving a definitive judgement, and future adequately powered prospective study is required to confirm these findings.

Recently, a novel motorized spiral endoscope has been introduced into clinical evaluation[34]. This novel enteroscopy is currently been evaluated for its efficacy and safety in three prospective clinical trials. It seems to provide a faster and deeper approach to the small bowel with similar safety and efficacy to balloon enteroscopy[35].

There were some limitations to the current study. First, the sample sizes of studies we included were small and we lacked high-quality prospective randomized multicenter trials. Second, a limited number of studies obstructed subgroup and sensitivity analyses. Third, there was no uniform method for insertion depth

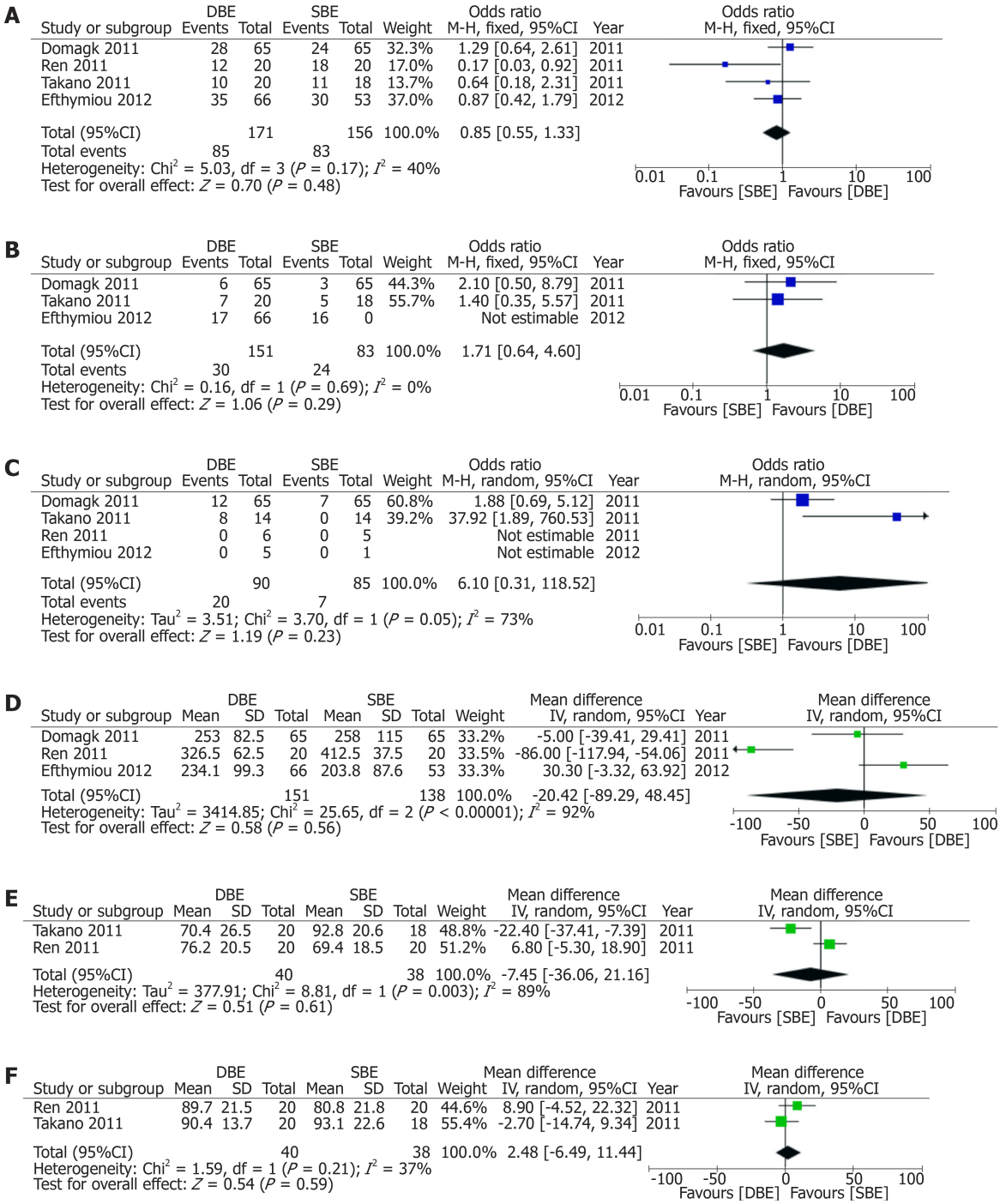


Figure 3 Outcome measures between double-balloon enteroscopy and single-balloon enteroscopy. A: Diagnostic yield; B: Therapeutic yield; C: Total enteroscopy; D: Depth of maximal insertion; E: Examination time (per-oral); and F: Examination time (per-anal).

estimation and different methods of enteroscopic distance estimation were used, which may have an impact on the results[23]. Fourth, due to limited studies comparing SBE with SE, we could not conduct a meta-analysis. Fifth, we only identified and included those studies that were published in English and Chinese because of the language barrier for the authors. Therefore, our meta-analysis may not include some useful published in other kinds of languages. Finally, peroral enteroscopy tended to provide higher diagnostic and therapeutic yields than peranal method. However, a limited number of studies also obstructed separate analysis.

In conclusion, the present meta-analysis has demonstrated that DBE and SBE have similar clinical outcomes. Compared with DBE, SE seem to have similar diagnostic and therapeutic yields, but shorter procedural time in cost of less depth of insertion. SE needs further evaluation *vs* SBE for clinical outcomes. DBE is recommended for complete enteroscopy.

ARTICLE HIGHLIGHTS

Research background

There are three different methods for the management of disorders in the distant part of small bowel: Double-balloon enteroscopy (DBE), single-balloon enteroscopy (SBE), and spiral enteroscopy (SE).

Research motivation

The efficacy of the three methods in terms of diagnostic and therapeutic yield rates, total enteroscopy rate, depth of maximal insertion, time to maximum insertion, and examination time remains unclear.

Research objectives

We aimed to compare the efficacy of DBE, SBE, and SE for the management of small bowel diseases.

Research methods

We searched randomized controlled trials and prospective studies in MEDLINE, PubMed, Embase, Cochrane Library, and Chinese CQVIP database. Statistical analyses were performed *via* RevMan software.

Research results

The diagnostic and therapeutic yields did not differ significantly when comparing DBE with SE and DBE with SBE. Total enteroscopy, examination time, time to maximum insertion, and depth of maximal insertion were similar between SBE and DBE. DBE was superior to SE with regard to depth of maximal insertion, with longer time to maximum insertion and examination time.

Research conclusions

DBE and SBE may have similar clinical outcomes. Compared with DBE, SE seems to have similar diagnostic and therapeutic yields, but shorter procedural time in cost of less depth of insertion.

Research perspectives

SE needs further evaluation *vs* SBE. DBE is recommended for complete enteroscopy.

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SCIENTOMETRICS

Chinese research into ulcerative colitis from 1978 to 2017: A bibliometric analysis

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Abstract

BACKGROUND

Over the last 40 years, with accumulating evidence demonstrating a significant increase in the incidence of ulcerative colitis (UC) in China, the number of studies on UC has been rapidly increasing. But it still lacks a comprehensive meta-analysis of publications regarding UC for the last four decades in China. Thus, a bibliometric analysis of UC is warranted to investigate the trend and distribution of the publications on UC in China in recent years. And it is supposed that the number of the papers related to UC increased by year.

AIM

To investigate the current status of research output from Chinese studies related to UC during the period of 1978 to 2017, with special attention paid to the distribution of publication dates, journals, regions, and research organizations.

METHODS

Publications on UC were searched in the Chinese periodical database SinoMed from January 1978 to December 2017. The search term used for retrieval was "ulcerative colitis". The language of the publications was restricted to English or Chinese. The studies have to be performed in China. Then, a bibliometric analysis was performed on the distribution of publication dates, journals, regions, and research organizations with EndNote, Excel, MySQL, and GraphPad Prism.

RESULTS

A total of 16257 papers matched the search criteria, which included 7561 papers published in core journals, 4641 evidence-based articles, and 4177 publications of randomized controlled trials. These papers were mainly published in *Chinese Journal of Coloproctology, World Chinese Journal of Digestology, Chinese Journal of Digestion, Chinese Journal of Integrated Traditional and Western Medicine on Digestion,*

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and *Modern Journal of Integrated Traditional Chinese and Western Medicine*. In particular, the majority of these organizations were located in Jiangsu, Henan, Shandong, and Guangdong Provinces which are rich areas or have the largest population per province. Most of these studies were conducted by academic institutions.

CONCLUSION
Over the past four decades, the output of research into UC in China has increased significantly, with academic institutions playing a central role in the academic field, but the number and quality of these researches vary substantially among different regions.

Key words: Ulcerative colitis; Bibliometric analysis; SinoMed; Literature research; Core journal; Academic institutions

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Core tip: Ulcerative colitis is a chronic inflammatory disorder of the gastrointestinal tract. This bibliometric analysis indicated a significant increase in the number of Chinese ulcerative colitis publications over the last 40 years. Meanwhile, the numbers of both evidence-based articles and publications of randomized controlled trial were also increased, but the proportions of evidence-based articles and publications of randomized controlled trial were inadequate. In addition, region distribution of these publications was unbalanced, Jiangsu, Henan, Shandong, Guangdong Provinces featured significantly in the research filed, and most of the studies were conducted by academic institutions.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract, characterized by a tendency of chronic and relapsing mucosal inflammation[1-4]. The precise etiopathogenesis of UC remains unclear, and it is generally considered as the result of maladjustment of immune responses to intestinal microorganisms in individuals with genetic susceptibility[5,6]. The primary symptoms of UC include abdominal pain, bloody diarrhoea, weight loss, and fever. The complications include anemia, megacolon, and colitis related colon cancer.

UC was generally considered to be prevalent in Western countries. The highest prevalence of UC was reported in Europe (UC 505 per 100000 in Norway), according to a systematic review of the worldwide incidence and prevalence of inflammatory bowel diseases (IBD) based on population data[7-9]. However, recent epidemiological studies showed that the incidence of UC is increasing rapidly in China and other newly industrialized countries[10-12]. Although the prospective population-based epidemiological studies were rare in China, the prevalence of UC was estimated to be 11.6 per 100000 in China according to a multi-center retrospective study[13-15]. It could be concluded that the incidence of UC has been dramatically increased. These studies demonstrated that UC is a common disease in the 21st century. Furthermore, with the increasing incidence of UC, the corresponding number of studies on UC was increasing steadily.

Bibliometrics is the quantitative analysis of academic literature by comprehensively applying methods of mathematics, philology, and statistics. Through exploring the characteristic information of literatures, bibliometric analysis can reveal the occurrence, history, research status, and future development in certain academic fields. In the field of IBD, a bibliometric analysis has been performed to analyze the 50 top-cited articles focused on IBD[16]. As the largest developing countries, China has witnessed a dramatic increase in the incidence of IBD during the last four decades. However, there is no comprehensive bibliometric analysis concerning the features of

publications on UC in China.

Therefore, the aim of this study was to quantitatively analyze the Chinese literature on UC over the past 40 years, trying to facilitate a comprehensive understanding of the prior research on UC in China. We hope that our study can shed some light on the development of IBD management for other developing countries in the world.

MATERIALS AND METHODS

The statistical review of the study was performed by a biomedical statistician. Generally, the ethical approval was not required in this study since the data in this study was downloaded from a public database and no patient was enrolled.

Data acquisitions

Publications were explored from the Chinese periodical database SinoMed (http://www.sinomed.ac.cn/) which is the most famous database regarding publications from China in the field of biology and medicine. SinoMed is open access and contains both Chinese and English publications. Most of English publications are simultaneously indexed in PubMed. The database was retrieved on April 30, 2018. The search term applied for retrieval was "ulcerative colitis", which had been searched in the Article Title or Abstract of the literature. The language of the publications was restricted to English or Chinese. The studies have to be performed in China. The publication dates were restricted between 1978 and 2017, since the first consensus about diagnosis and treatment of IBD was held by Chinese Medical Association for digestive diseases in 1978. Publications in 2018 were excluded since there may be accepted research outputs that were not available in the database. The category of core journal, evidence-based article, and randomized controlled trial (RCT) publications are automatically classified and output by the SinoMed database. The search yielded a complete list of papers on UC, and 18948 papers were obtained.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) Publications which focused on UC and regarded UC as the main topic; and (2) Papers published by Chinese research organizations, including Hong Kong, Macao, and Taiwan. In addition to the exclusion on period, other exclusion criteria were as follows: (1) Publications which focused on other diseases (*e.g.*, CD, colorectal cancer, melanosis coli, and irritable bowel syndrome); (2) Papers published by foreign research organizations; and (3) Duplicated publications.

Statistical analysis

Publications matching the search criteria of our study were retrieved and reviewed. Multiple characteristics of these publications, including publication dates, journals, regions, and research organizations, were collected. All statistical data were analyzed by applying EndNote X8 (Thomson Reuters, United States), Microsoft Office Excel 2016 (Microsoft, United States), and MySQL 8.0.15 (Oracle, United States). GraphPad Prism 6.01 (GraphPad Software, United States) was applied to draw the charts.

RESULTS

Quantity and quality distribution of publications

A total of 16257 publications during the period from 1978 to 2017 were included. Amongst the publications matching the criteria of our study, 7561 papers were published in core journals, which were classified by the SinoMed database. The first evidence-based article was published in 1990, and 4641 papers met the requirements of evidence-based articles during the period from 1990 to 2017. In 1992, the first paper of RCT was published. Then, a total of 4177 papers of RCT were published for the period of 1992–2017 (Figure 1).

Quantity distribution of publications

The number of papers published per decade was 119 (from 1978 to 1987), 1005 (from 1988 to 1997), 5001 (from 1998 to 2007), and 10132 (from 2008 to 2017), respectively. Amongst them, 55 (from 1978 to 1987), 502 (from 1988 to 1997), 2420 (from 1998 to 2007), and 4584 papers (from 2008 to 2017) were published in core journals.

Quality distribution of publications

Out of the publications, the number of evidence-based articles per decade was 0, 35

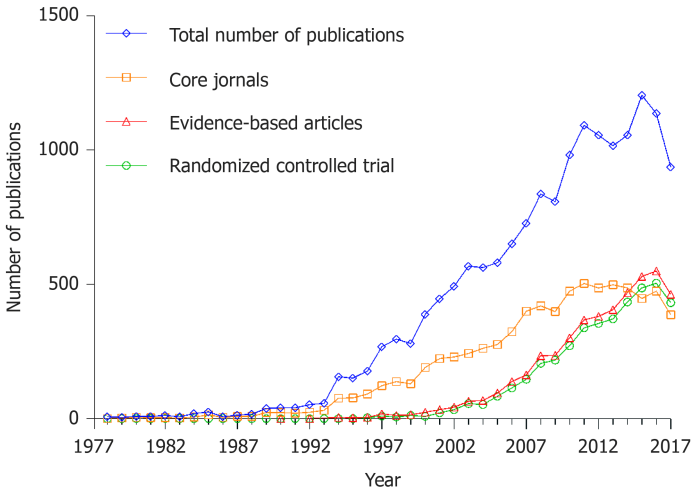


Figure 1 Number of papers focused on ulcerative colitis in China from 1978 to 2017. A total of 16257 papers were published for the period of 1978-2017. Amongst them, 7561 papers were published in core journals, 4641 met the requirements of evidence-based articles, and 4177 were publications on randomized controlled trial. The number and proportion of evidence-based articles and randomized controlled trials were increased significantly. Both the quantity and quality of the literature focused on ulcerative colitis were improving.

(3.48%), 662 (13.24%), and 3944 (38.93%), respectively. The number of RCT publications per decade was 0, 16 (1.59%), 538 (10.76%), and 3623 (35.76%), respectively.

Distribution of journals

Considering the journal distribution of the publications, 16257 papers were published in 1119 kinds of academic journals. Among these journals, *Chinese Journal of Coloproctology* accounted for the largest number of papers on UC (532 papers, 3.27%), followed by *World Chinese Journal of Digestology, Chinese Journal of Digestion, Chinese Journal of Integrated Traditional and Western Medicine on Digestion, Modern Journal of Integrated Traditional Chinese and Western Medicine*. Taken together, these journals published 1489 (9.16%) of the total 16257 papers as presented in Figure 2A.

Five hundred and twenty-four journals were classified as core journals by the SinoMed database, which published 7561 papers over the last 40 years. *World Chinese Journal of Digestology* accounted for the largest number of papers (302 papers, 3.99%), followed by *Chinese Journal of Digestion, Chinese Journal of Integrated Traditional and Western Medicine on Digestion, Modern Journal of Integrated Traditional Chinese and Western Medicine*, and *Hebei Journal of Integrated Traditional Chinese Medicine*. These journals contributed to 1156 (15.29%) papers; these data are presented in Figure 2B.

Distribution of organizations

A total of 15683 publications were analyzed in this section; meanwhile, the organizations of 574 publications could not be identified through the papers. A total of 7356 papers published in the core journals were analyzed in this section; while the organizations of 205 publications could not be identified from the papers.

Proportion of publications by academic institutions

Prior to the year 2000, only 1267 (8.08%) papers were published in total, with 651 (8.85%) papers published in core journals. So, we analyzed the papers published during 2000 to 2017 in this section.

From 2000 to 2005, the proportion of papers published by academic institutions fluctuated from 25.47% to 34.63% and those published by academic institutions in core journals fluctuated from 28.57% to 50.20%. During the years of 2006 to 2011, the proportion of papers published by academic institutions ranged between 39.28% and 46.68%, while the proportion of papers published by academic institutions in core journals ranged between 52.39% and 63.35%. Finally, during the years of 2011 to 2017, the proportion of papers published by academic institutions fluctuated from 44.53% to 50.16% and the proportion of papers published by academic institutions in core journals fluctuated from 61.04% to 68.90% (Figure 3).

Distribution of regions

A total of 15683 publications were analyzed in this section. These publications were distributed across 23 provinces, 4 municipalities (Beijing, Shanghai, Tianjin, and

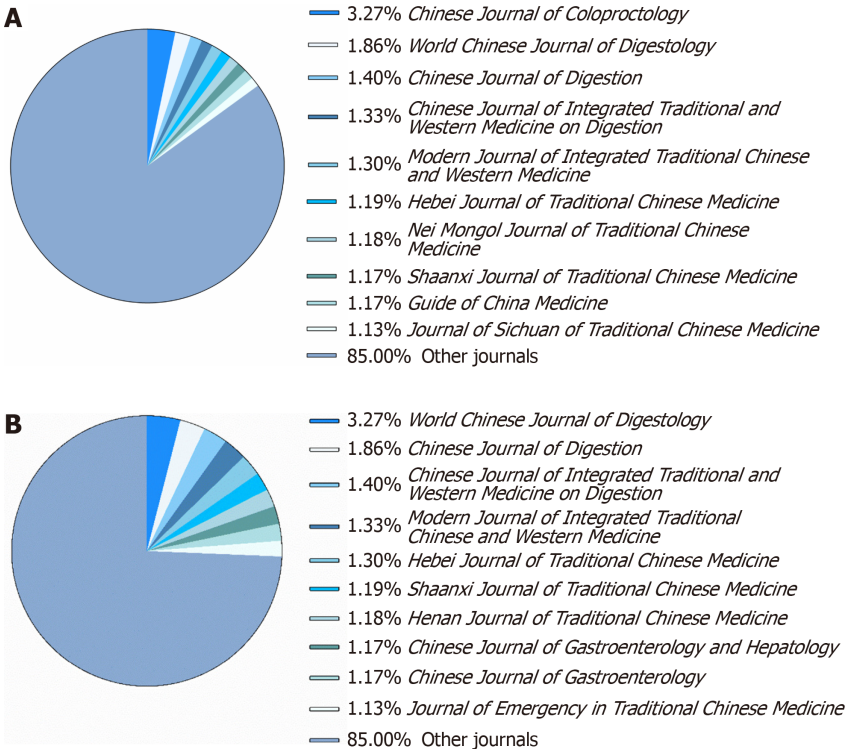


Figure 2 Journal distribution of publications focused on ulcerative colitis. A: Proportion of papers focused on ulcerative colitis (UC) published by each journal; B: Proportion of papers addressing UC published by each core journal. A total of 16257 papers focused on UC were published in 1119 journals. Amongst them, 7561 papers were published in 524 core journals. Papers on UC were mainly published by the *Chinese Journal of Coloproctology*, *World Chinese Journal of Digestology*, *Chinese Journal of Digestion*, *Chinese Journal of Integrated Traditional and Western Medicine on Digestion*, and *Modern Journal of Integrated Traditional Chinese and Western Medicine*.

Chongqing), 5 autonomous regions (Inner Mongolia, Ningxia Hui Autonomous Region, Xinjiang Uygur Autonomous Region, Tibet, and Guangxi Zhuang Autonomous Region), and 2 special administrative regions (Hong Kong and Macao) (Figure 4C). Jiangsu Province accounted for the most publications in this field (1317 publications, 8.40%), followed by Henan Province (1298 publications, 8.28%), Shandong Province (1253 publications, 7.99%), and Guangdong Province (1229 publications, 7.84%) (Figure 4A).

A total of 7356 papers published in core journals were analyzed in this section, while the organizations responsible for 205 publications could not be identified through the papers. The papers published in core journals were distributed in 22 provinces, 4 municipalities, 4 autonomous regions, and 2 special administrative regions (None of the papers were published in Tibet or Taiwan Province) (Figure 4B). Out of these, Jiangsu Province published the most papers in core journal (696 publications, 9.46%), followed by Beijing (586 publications, 7.97%), Guangdong Province (551 publications, 7.49%), and Hebei Province (515 publications, 7.00%) (Figure 4C).

Among the 15683 papers, 15584 (which were published during the period of 1993 to 2017) were analyzed in this section; while only 99 papers were published from 1978 to 1992. From Figure 4D, a total of 4796 papers were published in East China (30.78%). Again, 2606 papers were published in Central China (16.72%), while 2491 papers were published in North China (15.98%). Furthermore, 1658 papers were published in Northeast China (10.64%), while 1638 papers were published in South China (10.51%). Lastly, 1253 papers were published in Northwest China (8.04%), whereas 1142 papers were published in Southwest China (7.33%).

In summary, it is interesting to find that more publications come from rich areas. The number of publications is predominantly dependent on the incidence of IBD. This phenomenon is in line with the opinion that industrialization and economy advance can contribute to the development of IBD.

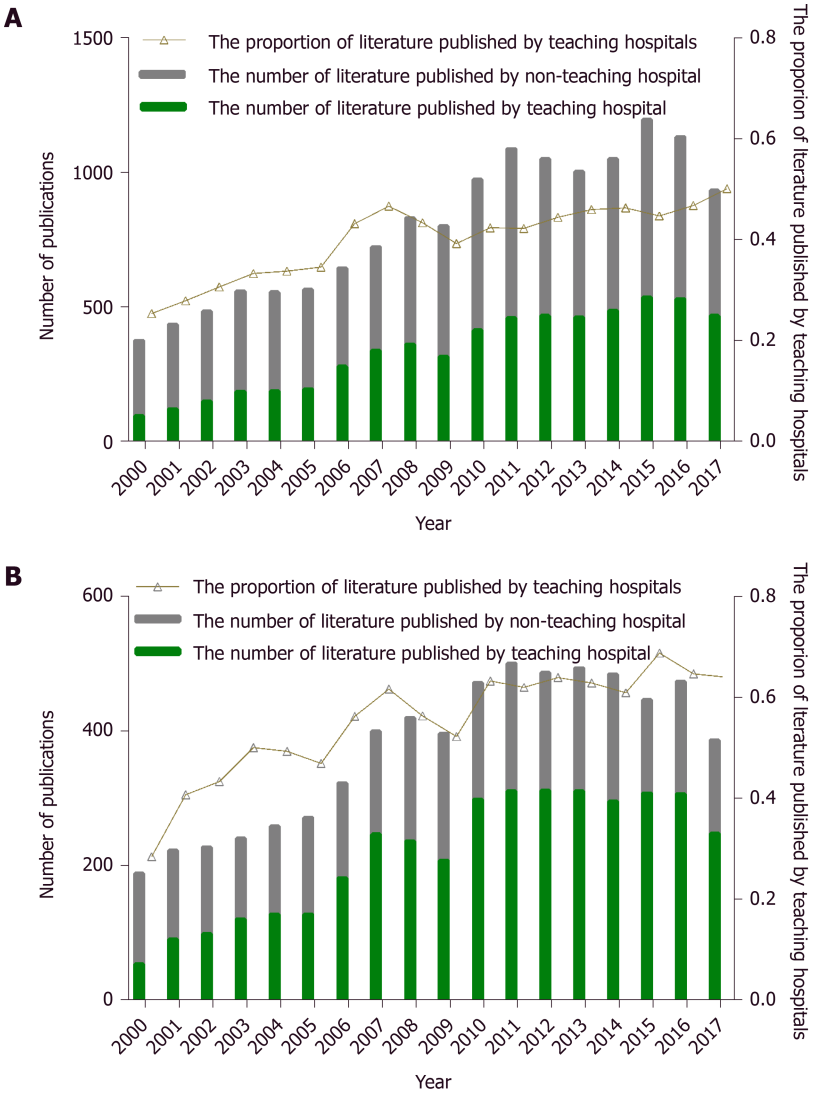


Figure 3 Organization distribution of publications focused on ulcerative colitis. A: Proportion of papers published by academic institutions from 2000 to 2017; B: Papers published by academic institutions in core journals from 2000 to 2017. In recent years, the proportion of papers published by academic institutions fluctuated from 44.53% to 50.16% and the proportion of papers published by academic institutions in core journals fluctuated from 61.04% to 68.90%. The general trend indicated that academic institutions exerted a fundamental role in the research related to ulcerative colitis.

DISCUSSION

The first report about UC in China was published in 1956. However, publications related to UC has been on the rise since the 1970s. Early investigates were focused on observational studies (*e.g.*, case reports, treatment experience, and retrospective literature), which help researchers to have a deeper understanding of the diagnosis and treatment of UC. Since the first consensus on the diagnosis and treatment of IBD was reached in 1978, a large number of studies focused on UC were performed by scholars and doctors in China. Furthermore, the reform and opening up in China provided a strong driving force of economic development, thus providing additional financial support for scientific studies. Consequently, increasing studies were focused on the molecular mechanism and animal studies. Meanwhile, as the number of publications related to UC was increased, the number of evidence-based articles was also increased, suggesting that Chinese scholars started to pay attention to the etiology and pathogenesis of UC. The number and proportion of evidence-based articles and publications of RCT were increased according to the analysis performed in this study. In subsequent studies, the focus should be on prospective studies to improve the rigorousness and quality of the publications.

The papers focused on UC were published in 1119 journals, including 524 core journals. Amongst them, *Chinese Journal of Coloproctology*, *World Chinese Journal of Digestology*, *Chinese Journal of Digestion*, *Chinese Journal of Integrated Traditional and*

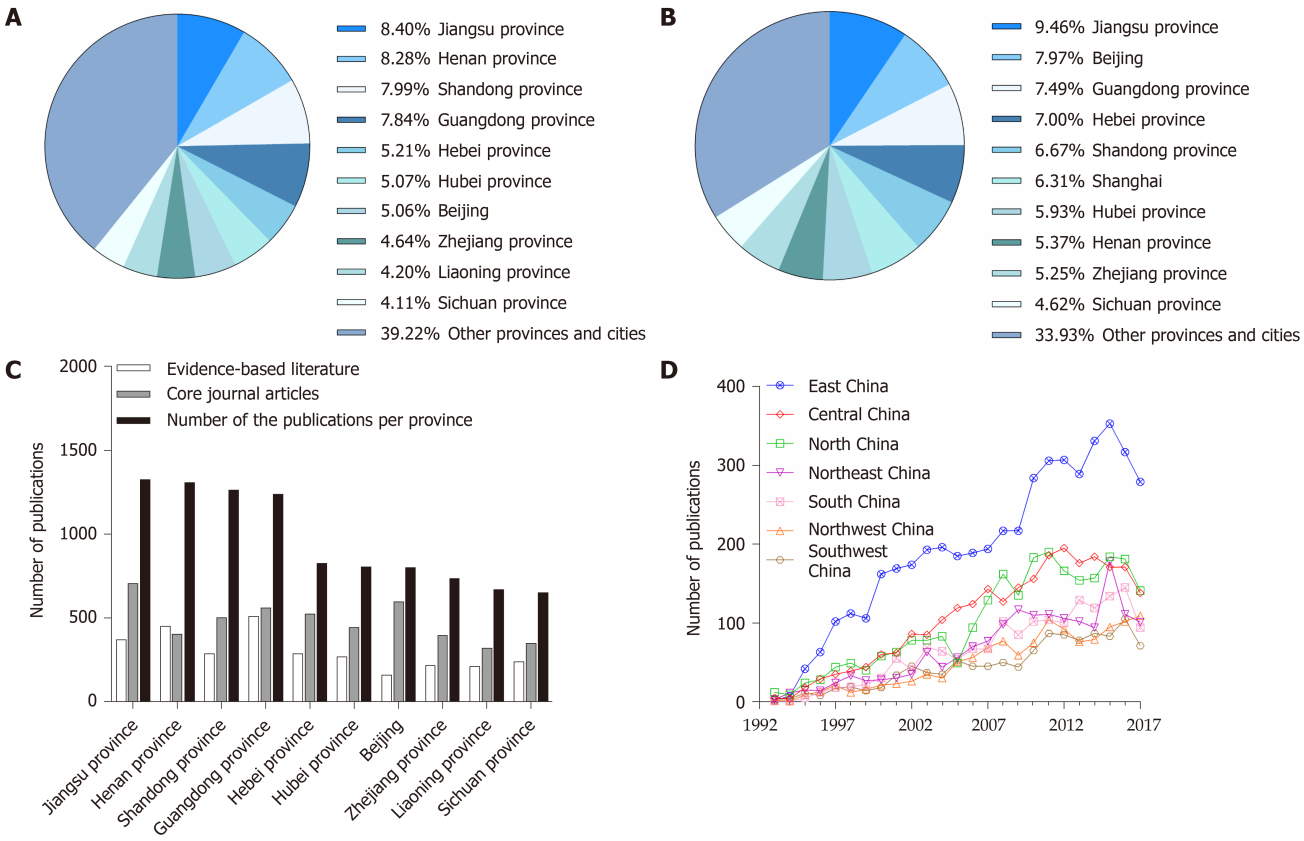


Figure 4 Reginal distribution of publications focused on ulcerative colitis. A: Proportion of publications in each province; B: Proportion of papers published by core journals in each province; C: Province distribution for the literature on ulcerative colitis (UC) in China; D: Regional distribution of the literature focused on UC in China from 1993 to 2017. A total of 15683 papers were analyzed in **Figure 4A-C**. Amongst them, 7356 papers were published in core journals. Jiangsu, Henan, Shandong, and Guangdong Provinces featured significantly in the research filed related to UC. A total of 15584 papers were analyzed in **Figure 4D**. Out of these, the most papers were published in East China.

Western Medicine on Digestion, and *Modern Journal of Integrated Traditional Chinese and Western Medicine* featured significantly in the research filed, and devoted the largest number of publications (1489 publications, 9.16%). Furthermore, with an increasing incidence and prevalence of UC in the past four decades, the researches related to UC also increased. It was observed that amongst clinical departments, department of gastroenterology, department of integrated traditional Chinese and Western medicine, department of general surgery, and department of pediatrics contributed to the largest number of publications on UC[17-19]. Meanwhile, studies on laboratory testing, imaging, endoscopic, pathology, nursing, and mental health guidance related to UC were also on the rise[18,20-22]. Since UC is characterized by complexity, unspecific manifestations, relapsing-remission, chronicity, and destructiveness, the management and treatment of UC are challenging both for the physician and the patient. It is also suggested that further studies should be undertaken with regard to the fact that the treatment of UC does not only depend on physicians from gastroenterology doctors, but also on the cooperation of experts from other departments, such as surgeons, radiologists, laboratory doctors, and professor of evidence-based medicine[23]. Such multidisciplinary collaboration could provide the best opportunity for the accurate diagnosis and treatment of UC patients in China. Moreover, Chinese traditional medicine, and the combination of traditional Chinese and Western medicine were special research methods in China, accounting for about one-third of all the publications[24-27]. This was indicative of the fact that more attention should be paid to the essence of traditional Chinese medical technology, and the rigidity and rationality of experimental designs. Multi-centric studies exploring the epidemiology and treatment of UC are also warranted to achieve an early breakthrough for the diagnosis and treatment of UC.

According to our research, the research capacity varied greatly among different regions in China. Financial support from government played an essential role in the quantity and quality of publications. The economy in East China, Central China, and North China was prosperous and people in these areas also have easier access to higher education and medical care resource; as a result, both advantages provide a

suitable scientific research condition for scientists. Therefore, among the various subregions, East China, Central China, and North China accounted for the largest number of publications, while only a few studies were conducted in Southwest China and Northwest China, especially in Tibet, Qinghai Province, Hainan Province, and Ningxia Hui Autonomous Region. As per our expectations, Jiangsu Province, Henan Province, Shandong Province, and Guangdong Province were the most fruitful provinces in the UC-related fields and made up the largest number of publications (5097 publications, 32.50%). In addition, many high-quality RCTs were conducted in Beijing, Jiangsu Province, Guangdong Province, Zhejiang Province, and Shanghai. Therefore, regions with strong scientific research capabilities should devote more resources to further explore the pathogenesis and treatment of UC. Meanwhile, the economic development, medical treatment, and education in remote areas should also be improved. More importantly, remote areas should be given access to advanced medical technology from developed regions, thereby narrowing the gap of medical standards in different regions.

Our research demonstrated that the proportion of papers published by academic institutions was more than 44.53% in recent years, and the proportion of papers published by academic institutions in core journals was more than 61.04%, showing that the academic institutions have served as a leading role in studies related to UC. Studies conducted by academic institutions were rather related to the latest international cutting-edge research, and the experiments were designed to be more rigorous, especially for high-quality RCTs and multi-center studies. However, with regard to research capacity, there was a considerable gap between China and developed countries. Therefore, academic institutions should be aware of the latest breakthroughs in international research and explore new research directions in studies related to UC. At the same time, academic institutions should exert a fundamental effect in organizing teaching activities, and promoting the spread of information correlated to UC.

However, this study had some limitations. The data was obtained exclusively from China, and thus did not reflect the research status of UC worldwide. Second, some papers were excluded on account that the full texts of these studies were unavailable, resulting in selection bias which may have influenced our results. However, we included over 16000 studies which were published in the SinoMed, a widely used Chinese database. Therefore, we believed that the data did provide a representative profile of UC in China.

In conclusion, this bibliometric analysis indicated a significant increase in the number of Chinese UC publications over the last 40 years. Moreover, the numbers and the proportions of evidence-based articles and RCT publications were also increased significantly according to the analysis in this study. The *Chinese Journal of Coloproctology, World Chinese Journal of Digestology, Chinese Journal of Digestion, Chinese Journal of Integrated Traditional and Western Medicine on Digestion*, and *Modern Journal of Integrated Traditional Chinese and Western Medicine* were the main journals that published the literature related to UC. Additionally, the regional distribution of these publications was unbalanced, and most of the studies were conducted by academic institutions. The research focused on UC has developed rapidly in China over the years. With the development of science and technology, it is rather promising for scholars to further explore the etiology and pathogenesis of UC, and these explorations may provide novel insights on more effective treatment for UC. Therefore, scholars and physicians should focus on the rigorousness and science of experimental design. Besides, academic institutions should work as a fundamental effect in promoting teaching activities and the cooperation between different departments, to improve the research capabilities of UC in China.

ARTICLE HIGHLIGHTS

Research background

Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract. Its accurate diagnosis and effective treatment remain a challenge for physicians. Along with the increasing morbidity of inflammatory bowel diseases (IBD) in China, even in Asia, the epidemiologic research and bibliometric analysis of UC in China are crucial in the world range. Thus, a comprehensive study of UC related publications is warranted to investigate the trend and distribution of the publications in China for the last four decades.

Research motivation

A bibliometric analysis of publications focused on UC will provide information about the current status of research outputs related to UC in recent years. There was a bibliometric analysis focused on the 50 top-cited articles in IBD. But no comprehensive bibliometric analysis of UC during four decades was conducted. Such a study contributes to the blank of bibliometric

analysis of UC in China, and the trend of literature and research will be instructive to clinicians and scientists in other countries.

Research objectives
The aim of this study was to demonstrate the trend and distribution of the publications focused on UC in China over the past 40 years. And a bibliometric analysis of the number and proportion of evidence-based articles and randomized controlled trial publications can provide the quality distribution of the publications. Such a study can establish comprehensive information on the UC studies in China, provide the information that scholars should pay attention, and help improve the quality of the studies in future research.

Research methods
We searched the Chinese periodical database SinoMed for the related literature of UC published between January 1978 and December 2017. Papers should focus on UC as the main topic and studies performed in China. And a bibliometric analysis was used to demonstrate the distribution of publication date, journal, region, and research organization of these papers, especially papers published in core journals, evidence-based articles, and randomized controlled trial publications.

Research results
A total of 16257 papers met the searching criteria, including 7561 papers published in the core journals. There were only 4641 evidence-based articles and 4177 randomized controlled trial publications, but the proportion of these papers in all publications was increased. In terms of the regional distribution, a total of 4796 (30.78%) papers have been published in the Eastern area of China. Jiangsu, Henan, Shandong, and Guangdong Provinces have witnessed 5097 (32.50%) papers. In addition, our research found that most of the studies were conducted by academic institutions.

Research conclusions
This bibliometric analysis indicated a significant increase in the quantity and quality of UC research in China for the period from 1978 to 2017. Although the proportion of evidence-based articles and randomized controlled trial publications has increased, the total number was still inadequate. Besides, the regional distribution of the literature was unbalanced, and academic institutions played a leading role in the relevant research of UC. Further research is warranted to investigate the epidemiological surveys for the general population in China, so as to provide information comparing directly an increase in UC incidence and the number of publications.

Research perspectives
The research focused on UC has developed rapidly in China over the years. As the accurate diagnosis and effective treatment of UC remain a challenge for physicians, it is promising for scholars to further explore the etiology and pathogenesis of UC, and thus identify new insights for more effective treatment options. Therefore, scholars should focus on the rigorousness and science of experimental design. Besides, academic institutions should work as a fundamental effect in promoting teaching activities and the cooperation between different departments, to improve the research capabilities of UC in China.

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COVID-19: Off-label therapies based on mechanism of action while waiting for evidence-based medicine recommendations

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Abstract

The world pandemic due to coronavirus disease 2019, known as COVID-19, embodies a high rate of disease transmission that causes a critical hospitalization overload. As of May 15, 2020, the disease has been the cause of more than 4 million infections and more than 280000 deaths all over the world. At the beginning, we underestimated the disease; now, we have sufficient information and it is clear that it is not just a respiratory disease. In fact, if a prompt treatment is not initiated, the disease may evolve towards an abnormal immune response and cytokine storm with an important thrombophilic pattern. Therefore, we think that while waiting for certainties to be established by evidence-based medicine, it is not ethical to not try off-label therapies for some of the well-known drugs, as they could have some efficacy based on their mechanisms of action.

Key words: COVID-19; Evidence based medicine; Hydroxycloroquine; Azithromycine; Indomethacine; Doxycycline

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Core tip: The world pandemic due to coronavirus disease 2019, known as COVID-19, embodies a high rate of disease transmission that has caused a critical hospitalization overload. While waiting for certainties to be established by evidence-based medicine, it is not ethical to not try off-label therapies with some of the well-known drugs that could show some efficacy based on their mechanisms of action.

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COMMENTARY ON HOT TOPICS

At the end of 2019, some cases of pneumonia of unknown etiology were observed in Wuhan (China). A few weeks later, this disease was discovered to be due to a virus of the coronavirus family, that was named severe acute respiratory syndrome-coronavirus-2 (SARS-Cov2), and the related coronavirus disease 2019 (COVID-19) was named accordingly.

COVID-19 embodies a high rate of disease transmission that has caused a critical hospitalization overload. By May 15, 2020, it had produced more than 4 million documented positive cases and about 280000 deaths all over the world. At the beginning, we underestimated and misunderstood COVID-19. Now, however, we have sufficient information of the disease pathophysiology, and it is clear that we are not dealing with just a respiratory disease. In fact, in many cases, if a prompt treatment is not undertaken, the infection may evolve towards a more severe disease and the occurrence of a cytokine storm with multi-organ damage[1-3].

Multi-organ damage should be investigated in patients recovered from moderate-severe COVID-19. Follow-up studies should be conducted to verify the higher risk of developing autoimmune diseases due to the uncontrolled and abnormal immune response to the virus. Finally, the possibility of developing neoplastic disease should be examined[4].

It is mandatory to promptly initiate a therapy at the onset of symptoms to stop the progression of the COVID-19 disease. This will ultimately reduce the risk of cytokine storm and, consequently, a hospitalization overload.

From the beginning of the pandemic, due to the lack of specific and approved therapies for the disease, the medical community was split among two currents of thought. The interventionists suggested that antiviral drugs, immunomodulators and low molecular weight heparin should be used off-label. In contrast, the evidence-based medicine (EBM) supporters preferred to wait for a treatment backed by scientific studies.

In only 4 mo, this pandemic has severely tested hospitals and health organizations all over the world, particularly under the absence of specific therapies. However, while waiting for EBM indications, we believe that during an emergency, even with a lack of specific approved therapies, it is not ethical to not at least try some therapies based on medical rationale.

Thus, we suggest a therapeutic scheme based on drugs that have an indication according to their mechanisms of action to treat patients in home health care at the onset of symptoms. This may allow for the avoidance of disease progression as well as hospital overload.

An important issue, to which we need to pay attention, is the great difference among the mechanisms of different antiviral drugs. Some drugs work by inhibiting the entry of the virus into the host cells and others by inhibiting the viral replication inside the host cells. The first will be efficacious if promptly administrated at the onset of symptoms, to avoid the entry of the virus, while the others are useful once the virus has penetrated. On the basis of these observations, it is not surprising that, in some studies the use of hydroxychloroquine, which is an inhibitor of virus entry, has been unsuccessful due to the late onset of the therapy.

FEASIBLE AT-HOME THERAPIES FOR COVID-19

At-home treatment for patients with beginning mild-moderate disease without risk factors, based on Italian experience (not published data)

Hydroxychloroquine 400 mg b.i.d. p.o. on first day and 200 mg b.i.d. after for 7 d: Hydroxychloroquine blocks viral internalization into host cells and it is also effective in terms of modulating the immune response to COVID-19 infection[5-9](Table 1).

Table 1 Mechanisms of some anti-coronavirus disease 2019 drugs

Drug	Inhibition of viral internalization into host cells	Inhibition of viral replication in the host cells	Modulation of immune response to virus infection	Effectiveness against virus
Hydroxychloroquine	Yes	No	Yes	Probably effective in the initial stage of the disease
Azithromycin	Yes	Yes	Yes	Probably effective in all stages of the disease
Indomethacin	No	Yes	Yes	Probably effective in the initial and intermediate stages of the disease
Doxycycline	Yes	Yes	Yes	Probably effective in all stages of the disease
Sofosbuvir with ledipasvir or with velpatasvir	No	Yes	No	Probably effective in all stages of the disease
Ivermectin	No	Yes	No	Probably effective in all stages of the disease
Remdesivir	No	Yes	No	Probably effective in all stages of the disease

Azithromycin 500 mg, 1 tablet/d p.o. the first day and then 250 mg/d for 4 d: Azithromycin blocks viral internalization into host cells. It probably inhibits viral proteases and it's also effective in terms of modulating the immune response to COVID-19 infection. Furthermore, it can protect patients from bacteria superinfections[7-9].

Alternatives: When the above two drugs are contraindicated (particularly in patients with retinopathy, diabetes, or heart disease because of risk of prolonged QT tract), we suggest indomethacin as an alternative to hydroxychloroquine and doxycycline as an alternative to azithromycin.

Indomethacin should be given as 50 mg b.i.d. p.o. (associated with gastric protection) for 7-10 d. It acts by blocking viral RNA synthesis *in vitro* and this effect is independent of cyclooxygenase inhibition. In addition, indomethacin has anti-inflammatory properties[10].

Doxycycline should be given as 100 mg 2 tablet/d p.o. on the first day and 1 tablet/d p.o. for another 6 d. It has antiviral properties based on chelating zinc compounds on matrix metalloproteinases which are important for COVID-19 survival and cell infiltration. Furthermore, it can block cell-to-cell adhesion and viral replication. Doxycycline also has anti-inflammatory properties and can protect the patients from bacteria superinfections[11].

Treatment for patients with beginning mild-moderate disease associated with risk factors and for patients with moderate disease

Low molecular weight heparin (4000 to 8000 u.i. b.i.d.) should be added according to the patient's weight and condition, in order to block intravascular coagulation correlated to the cytokine storm[12,13].

Antiviral drugs should also be added. In addition to the antiviral drugs currently being tested (lopinavir-ritonavir, remdesivir and favipiravir), there are other promising antiviral drugs not being tested. Antiviral drugs used successfully against hepatitis C virus (HCV) are sofosbuvir associated with ledipasvir or with velpatasvir[14-16]. HCV is a single-stranded RNA+ virus, like the coronavirus; as such, the antiviral drugs against HCV (RNA-dependent RNA polymerase inhibitors and protease inhibitors) could be effective against the COVID-19.

Ivermectin (an anti-helminthic drug) has already shown antiviral properties against human immunodeficiency virus, Zika, Dengue and West Nile. It has also shown *in vitro* antiviral properties against the COVID-19, likely based on its nuclear transport inhibitory activity that stops the viral capacity to reduce the host cell's antiviral response[7,17].

Recently, the Federal Drug Administration gave emergency-use authorization for remdesivir in adults and children hospitalized with severe COVID-19[18]. Remdesivir is a nucleotide analogue, and the RNA-dependent RNA polymerase incorporates the

active triphosphate form of remdesivir into viral RNA. Incorporation produces termination of viral RNA synthesis and inhibits viral replication[19].

Currently, among the antiviral drugs, the most used off-label is hydroxychloroquine, particularly in combination with azithromycin. But this combination seems controversial because of its arrhythmogenic risk. However, recently, a paper reported the results of a study in which were used data from the United States Food and Drug Administration Adverse Event Reporting System (on about 13 million total reports) and concluded that hydroxychloroquine use was not associated with safety signals, while azithromycin alone was associated with TdP/QT prolongation events and should be used with caution[20]. To avoid the problem of additive cardiotoxicity of hydroxychloroquine plus azithromycin, we can replace azithromycin with doxycycline in at-risk patients.

It is also very interesting that in some countries, such as Peru, in which hydroxychloroquine and ivermectin are largely in use, the lethality of COVID-19 is among the lowest (2.7%). Perhaps this good result could be due to their different mechanisms of action, which are empowered one with the other.

CONCLUSION

The take-home message we want give is: while waiting a specific vaccine or EBM indications, upon which old or novel drugs will be certainly useful in the treatment of COVID-19, and on the basis of the large experience acquired in the first 4 mo of this pandemic, we think that a prompt treatment should be started at home in those patients with mild-moderate disease using off-label drugs with a medical rationale. In this way, we could avoid disease progression, hospital overload and, possibly, reduce mortality.

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FIELD OF VISION

Learning and competence development *via* clinical cases – what elements should be investigated to best train good medical doctors?

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Abstract

In European higher education, application of information technology, concentration on the learning-processes, consistent implementation, transfer learning, case-based learning, autonomous learning has been extensively studied in the last decade. Educational sciences based on neuroscientific findings use brain-based learning and teaching, including integrated thematic instructions and emotion-theory. Elements essential to this strategy, such as theory and methods for learning, competencies, attitudes, social reality, and a metadiscourse are described herein. Research on learning tends to focus on declarative knowledge, associative learning with conditional stimuli, and procedural knowledge with polythematic/crosslinking thinking. Research on competencies: In research on competencies (*e.g.*, for clinical reasoning, decision-making), intuitive and analytical components are studied. As repeated presentation and exercising of clinical cases is crucial for an efficient learning process, the implementation of interactive scenarios including affectively involving didactics is considered. For competence-development observational methods, questionnaires/item sets or factors have to be targeted and empirically validated. Attitudes and social reality: Clinical decision-making, identification processes and attitudes ("Hidden curriculum"), as well as secondary socialization processes (integration of social norms, values, preparation of role-acquisition, occupational role) are studied *via* process research, conceptual research, and observational methods. With respect to social reality research, conscious and unconscious bargaining processes have to be taken into account. Methodology: Neuroscience – memory, neuronal, molecular biology, and computer science (Neurocircuits) are integrated into observational process research (*e.g.*, affective-cognitive interface, identification processes) and conceptual research is added and studied on the meta-level, including discussion

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of research paradigms. This discussion provides ongoing feedback to projects in a hermeneutic circle.

Key words: Social neuroscience; Case-based learning; Mixed-method design; Hidden curriculum; Socialization; Research

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Core tip: Consequent application of evidence based didactics based on social neuroscientific findings is necessary to develop good medical doctors and therapeutic professionals. An overview of the higher education history and development throughout the past decades is given. An up to date description of the current knowledge regarding higher education and research strategies to enhance the evidence-based components to optimize teaching and learning are proposed.

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INTRODUCTION

For a long time, educational sciences were grounded in neuroscience, whereas "Neurodidactics" were based on computer/mathematical models[1,2]. Constructivist-didactic perspectives called for revision of such archaic positions and research paradigms, stating that controlled intervention cannot be possible[2,3] nor be studied reliably, as the trajectory and outcome of learning processes are not assessable[2,4]. The next great shift in research methodology involved the expansion of the descriptive and interpretative perspectives[2,5], which was suggested in order to adequately investigate gains in acquisition of knowledge. As a result, the utility of Brain-Based Learning and Teaching[6], along with Integrated Thematic Instructions[7] (including Emotion-Theory) were researched in the context of European higher education.

In parallel with discussions on adequate research methodology, the learning context was refined, and applied research in the domain led to several new strategies in European Higher Education. First, the Declaration of Prague (2001)[8] suggested the application of Information- and Communication-Technology. Later, it was recommended that a focus on the learning-processes (Declaration of Leuven 2009)[9] should take place and that research should be implemented consistently (Declaration of Vienna-Budapest 2010)[10]. In the Lancet Report (2010)[11], a lack of research on Transfer-Learning was stated, and so as a result, Case-based learning (CBL) together with workplace-based assessment was implemented. With growing evidence for the applicability and advantages of CBL, several subsequent efforts focused on autonomous learning, leading to the LLL-Strategy (Lifelong Learning). In general, with respect to research methodology, once again the basis of Educational sciences was firmly and prominently grounded in neuroscientific findings[12].

We support the claim that brain-based learning and teaching[6] using integrated thematic instructions[7] has to include Emotion-Theory, and should not exclude the constructivist perspective of taking the learning environment into account[13].

For this strategy to work, we need to concentrate theoretical underpinnings and research in the following areas: Learning, competencies, attitudes, social reality, and inclusion of a Metadiscourse.

THEORETICAL CONSIDERATIONS

Learning

Learning and competence development is based on research according to the research paradigm of the educational sciences and neurosciences, including social neurosciences.

Declarative knowledge: Declarative knowledge has to be acquired concerning basic facts that include knowledge which is available and can be accessed on the conscious level, and in general is stored as unconscious knowledge in long-term memory. Teaching and learning etiological concepts to understand basic principles of specific domains is therefore bound to conscious and unconscious mechanisms.

Associative learning: In this type of learning, the temporal relationship of the two stimuli has to be recognized: The person responds to the first stimulus in anticipation of the second (a neural link/association is its foundation). The result of the reclassification of stimuli and responses are drives (conditional appetence, conditional action, conditional aversion, and/or conditioned inhibition). Associative learning is the fundamental basis of memory.

Procedural knowledge: To attain, train, foster, and confirm procedural knowledge, poly-thematic crosslinking thinking first has to take place: The ability to link information (thoughts, symbols, images, scenes) in a meaningful way and to know how to master this information has to be followed by creatively linking and combining previously seemingly unrelated areas (domains) with each other. The result is to attain the competence *e.g.*, to write a novel instead of reading a novel. The latter is of course a less complex task.

For these three steps, evidence shows that both relational factors and feedback enhance learning outcomes, especially working alliance and motivation. Feedback over the course of the learning process enhances the effect of the teaching intervention. Personalized feedback systems fit into the unique profile of the student and monitor progress in his or her learning priorities. Conducting studies on creating a personalized feedback system adapted to the cultural diversity of the adolescent population in Europe could be an interesting task. Previous research shows that students' establishment of personal goals and the use of visually attractive mobile interfaces enhance the adherence of students/adolescents to learning processes. Moreover, a bottom-up definition of learning tools increases its acceptability among learners and teachers. Accordingly, a participatory approach, meaning working collaboratively with learners and teachers to co-design the features, is a recent development employed in order to strengthen the learning outcome.

Competencies

As attaining competence in a subject is a quite differentiated and complex task, a conceptual and methodological approach has to be developed. Several studies reached the consensus that the concept of personalized reasoning consists of intuitive and analytical components[14-16]. Therefore, the methodological approach to teaching (*e.g.*, personalized reasoning) that can be investigated in research studies on mental processes consists of providing problem descriptions or patterns that can then be stored in "frames," "scenarios", "semantic networks/qualifiers", or in health professions, "illness scripts." Repeated presentation and training of real-life situations and cases is furthermore crucial for an efficient learning process[16,17]. These considerations led to the implementation of interactive scenarios in many didactic consideration and teaching efforts[16-19]. Advanced statistical techniques that enable us to discover patterns in data and make predictions on natural phenomena and human interactions with their environment may be used. Pattern recognition, consolidation of relevant phenotypes, and development of prediction and classification algorithms for developing decision rules can, for example, be refined.

Research strategies to investigate competence development can either use observational methods, questionnaires, and / or follow the development of item sets that can be condensed and studied further as factors[14] and empirically validated.

Attitudes

Attitudes influence competence development. They provide insights into how biographical experience, motivation, and personality traits have changed in response to social changes. Who we are today is in part a result of our collective responses to social and cultural change and may have potentiated a decline or an increase in prosocial traits in students. The challenges of preparing students are better understood when viewed within the broader social context.

An attitude especially in health or pedagogic professions normally contains empathic, precise, ethically sound and scientifically grounded decision making and authentic care. Identification processes play an important role in reaching this ideal attitude ("Hidden curriculum")[20] as do secondary socialization processes (*i.e.*

integration of social norms, values, training of most important roles, preparation of role-acquisition, and occupational role).

In this context, research might include process research, conceptual research, as well as questionnaire/observational methods with each of their own respective research paradigms.

For research in this context, drawing upon influences from psychotherapy research and social neuroscience is helpful in order to target and understand learning processes: For example, Sharpless *et al*[21] measured the program performance of universities and teaching institutes in higher education by calculating prediction models for the Examination for Professional Practice (EPPP) at the end of a university program. Pass rates and program performance of universities in the United States and Canada were higher when the total amount of internships and emphasis on practical aspects were high and very prominent in the curriculum. Overall, the EPPP showed numerous advantages and better results in case the higher students had scored in the PreGraduate Record Examination in total, or the higher the percentage of Cognitive Behavioural Training Elements - CBT orientation was. Interestingly, program performance correlated positively with higher percentages of ethnic minority students in the program.

In addition to environmental factors, precise biological factors and structures may be investigated in order to understand learning processes: The encoding of subjective value is directly related to emotional regulation as well as neuro-structurally related to the ventral prefrontal cortex[22], and psychological mindedness is connected with metabolism in the right precuneus[23]. Fear influences decision-making, especially in narcissistic states[24], as links between affective and cognitive functioning may influence the sense of self-agency. These examples of results show the diverse factors that play a role in the complex puzzle of research on learning and training.

Identity, identification, and social reality

Attitudes are to a great extent influenced by identification processes that start very early in life. In addition, neuro/psycho-developmental aspects also have to be considered: It is well established that psychoanalysis provides knowledge that helps us understand the development of personality. Researchers have begun to emphasize more and more the role of affect regulation in personality, development, and individualization and identification processes. Affect regulation refers to cognitive and behavioral strategies people use to maximize pleasant emotions and minimize unpleasant ones. These strategies may be explicit (coping mechanisms) or implicit (defenses). It has been proposed that feelings are mechanisms for the selection and retention of behavioral and mental responses. To the extent that particular behaviors, coping strategies, or defensive strategies become associated with regulation of aversive affective states and maximization of pleasurable ones, they will be encoded as "solutions" to affective problems. From this viewpoint, affect regulation strategies are a form of procedural knowledge and are activated under specific circumstances, such as the presence of particular affects. Affect regulation strategies can be adaptive or maladaptive. Some regulation strategies are affect-specific, whereas others can be used to regulate multiple affects of similar valence. These procedures are often activated to resolve discrepancies between perceived and desired states of self, significant others, and external circumstances. Emotions and other sensory feeling states are evolved mechanisms for channeling behavior in directions that foster adaptation. The avoidance of unpleasant states and the pursuit of pleasant ones leads to goal-directed mental and behavioral processes, including defenses and compromise formations. Affects provide a flexible motivational mechanism in humans, as they become associated with representations of perceived, feared, wished-for, or otherwise valued states through the interaction of environmental events and highly specific, naturally selected biological proclivities. The investigation of affect and its regulation also refers to the detection of coping styles. Vollrath *et al*[25] showed that dispositional coping styles prospectively influence change in personality. Observing affect parameters should not be left alone, as affects are activated under specific circumstances, *i.e.* in object-relationship. The concept of object relations has played an increasingly important role in psychoanalytic theorizing, as well as in clinical psychoanalysis, psychotherapy and medicine[26]. A short summary of a few pertinent issues will provide a context for describing what is of interest: Ogden[27] traced the contributions of Freud, Abraham, Melanie Klein, Fairbairn, Winnicott, and Bion to the conceptualization of internal object relations. The original model of all internal objects is Freud's model of the normal development of the superego through the process of identification, as the ego assimilates aspects of the personality and functions of external objects. This newly established psychic agency acquires its own set of

motivations and actions, including object relatedness. Ogden[27] also drew on Freud's extension of the role of splitting of the ego, beyond the formation of the superego, in the development of internal objects. For Ogden[27], another core concept is Fairbairn's assertion that it is an aspect of the relationship with the object, rather than aspects of the object, that becomes internalized. In addition, Ogden[27] incorporated into his thesis Bion's description of the potential for the defensive splitting of the mind into active suborganizations capable of engaging in specific forms of object relatedness. Ogden[27] 's elaboration of these concepts indicates that splitting of the ego into new subdivisions is necessary for early interpersonal relationships to be internalized. Each sub-organization – being a component of the ego – has a dynamic capacity to semi-autonomously generate experience and leave its stamp on the quality of object relations. This psychoanalytically informed view of object relations stipulates that they are the product of intrapsychic sub-organizations of the ego (internal object representations) and not of external interpersonal relationships. However, the quality of object relations is manifested in the interpersonal situation[28]. Despite the enduring quality of object relations, these intrapsychic structures are modifiable by experiences during healthy development. It is suggested that secure attachment is the basis of the acquisition of metacognitive or mentalizing capacity. Horner cited that the concepts of internalization and of object relations are fundamental to the developmental psychology of psychoanalysis, especially in terms of therapeutic technique. The investigation of object relation styles benefits our understanding of problems that people with identity disturbances and/or problems in their mentalizing capacity have. Mentalization and reflective functioning is essential for learning. Primary socialization is the precursor for secondary occupational socialization and therefore plays a distinct role in education[29].

When the influence of secondary socialization on attitudes is targeted, we have to provide research data on integration of social norms and values, training of the most important roles, considerations of the preparation of role-acquisition, and definitions or possibilities for operationalization of the occupational role, which is not correlated with development or education, but more influenced by social reality (Figure 1).

Investigation of social reality can be described as a specific situation experienced by a person that leads to actions and interaction. The person experiences a situation on the basis of his/her ego-id-superego (*i.e.* with ego-functions, wishes, moral values, *etc* .). Within the person, as well as within the process of connection between two or more persons, bargaining between each other's ego, id, and superego structure takes place. Both conscious and unconscious bargaining processes need to be taken into account.

METHODOLOGY

To investigate complex phenomena, several different research paradigms must be taken into account in order to identify as many influencing factors as possible. Which methodologies might be considered?

Neuroscience – memory (place neurons), neuronal, molecular levels, and computer sciences (Neurocircuits) have to be integrated into observational process research (*e.g.*, affective-cognitive interface, identification processes, *etc*.), and conceptual research has to be added and repeatedly discussed on a meta-level, with inclusion of discussions of research paradigms. These discussions should inform ongoing projects in a hermeneutic circle. In the same way as clinical theories have been developed on the basis of clinical cases, observations, and continuous discussion among experts, the way forward is to ground these theories *via* empirical research work as scientific theory. Thus, an interdependence of constructivist and empirical research paradigms may be synergistically combined.

Basic sciences relevant to neurosciences

On a molecular level, as has been investigated by Eric Kandel, the marine snail Aplysia californica was an important animal model for studying the molecular mechanisms of learning because of the very few, but very large nerve cells he uncovered. The objective was to study the gill withdrawal reflex of the animal, as it had been shown that it can be attenuated (habituation), enhanced (sensitization) or durably reinforced (conditioning). The basis of such changes is the interaction of different molecules in the nerve cells and transmitters in the synaptic cleft. These and other similar investigations on a molecular level are conducted in the neurosciences, fostering scientific knowledge with respect to brain- or memory-function (*e.g.*, research on place-cells[30]), resting state- or DMN investigations, and *e.g.* research concerning the neuronal anxiety

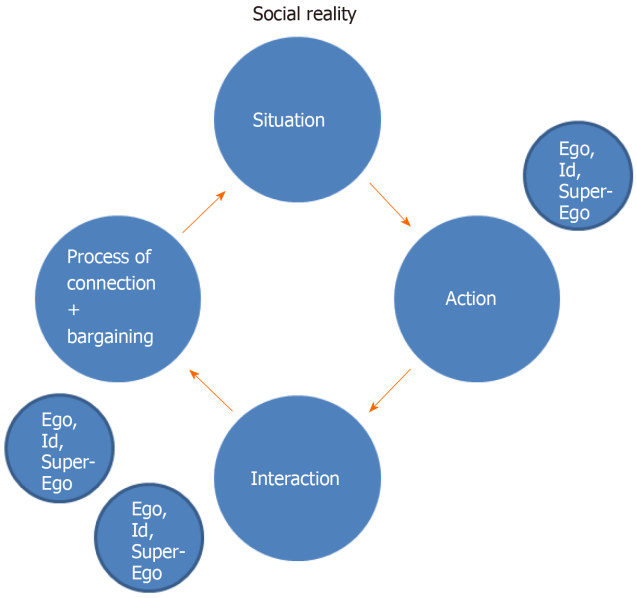


Figure 1 Social reality.

networks, all of which contribute to our understanding of learning processes.

Mathematics

As the measurement of change processes concern a number of actors (*e.g.*, therapists, clinicians, doctors, candidates, students) and their learning efforts, relevant variables, teaching aims, learning achievements, and factors must first be defined and operationalized, as these will determine a student's learning progress. For the purposes of evaluation of these learning and change processes, implementing a user-friendly applied model and software-package on different data (*i.e.*, on students' learning processes) will prove useful. In order to optimize the visualization software, regular monthly evaluations involving subtests have to be conducted. Afterwards, algorithms for model-building with the help of the computer algebra program MATLAB can be implemented and model parameters can be optimized; thus, the theory of the Hidden-Markov-Models is useful. Teaching experts almost universally agree that the definition of the requirements for user software and its implementation has to be discussed and specified in interprofessional teams. Hidden Markov models are stochastic signal models which consist of two random processes, wherein the first process is hidden and can only be accessed through the second. Initially, the first random process is defined as a Markov chain in order to widen it *via* the second into a Hidden Markov model. A discrete Markov chain consists of N states, whereby the system is always in one of these states. At discrete time points, the system states change. Over the course of these events, the probability that an actual state changes into a definite state is described with the aid of the state transition coefficient (see also[31]). The most essential points are to work out descriptions of precise evidence-based physical (bio-psycho-social) parameters regarding the learning progress and to set up descriptions of mathematical logic, *i.e.*, data processing in a recurring interprofessional discussion process.

Both methodological approaches follow a natural sciences research paradigm and have to be integrated into a process research design. Some qualitative methods have to be added into a mixed-methods approach in order to justify the often diverse, subjective parameters of human beings.

Observational methods

In order to understand identification processes (relevant for research on attitudes), the affect-cognitive interface, or subliminal affect perceptions and their influence on learning in process research, observational methods have to be integrated. The idiographic design complements nomothetic research strategies, providing more differentiated answers to behavioral questions. For example, the meaning of nonverbal communication is widely known in psychotherapy research. So it is thus astonishing that nonverbal interaction has not yet been established in initial and further training of clinicians. Different patient and therapist variables need to be taken into account (*e.g.*,

via video analysis) in order to understand a number of complex aspects of the relationship. Within the scope of this research field, investigated micro-process-units are the clinicians' and patients' micro-expressions, as measured by the emotion facial action coding system[32]. The ability to recognize facial expressions enables novel applications in human-computer interaction and other areas. Consequently, there has been active research in this field, with several recent works utilizing convolutional neural networks (CNNs) for feature extraction and inference. Being able to recognize facial expressions is key to nonverbal communication in human beings, and the production, perception, and interpretation of facial expressions has been widely studied[33]. Advanced statistical techniques that enable us to discover patterns in data and make predictions about natural phenomena and human-environmental activity can be used. Pattern recognition, condensation of educationally relevant phenotypes, and development of prediction and classification algorithms for development of preventive decision-rules for states or problems like trainer-trainee feedback strategies may be developed.

Qualitative-quantifying research designs shed light into the ingredients relevant for change. Investigations that focused on trainers showed for instance the influence of a person/the therapist on treatment outcome when fruitful learning and change take place. Therapist effects explain up to 20% of the variance in outcomes[34-37]. For research purposes, it is necessary to study the clinician/therapist dynamic in much more detail: We know that attitudes differ, or more precisely, that therapeutic attitudes differ between potential trainees (students)[38], trainees in psychotherapy (propedeutics), and qualified psychotherapists. Attitudes are related, in that therapeutic attitudes relate to interpersonal problems and emotional reactions[39]. Therapeutic attitudes also change over the course of additional training[40]. Such research approaches of qualitative, interview-driven, quantifying designs advance operationalization of different relevant variables and parameters. Recording and transcription is another approach to be mentioned[41].

Another finding was presented *via* experimental case-series design[14], pointing out two different modes of thinking. Considering factors relevant for clinical reasoning, clinical decision-making, and authentic clinical care, factor analysis approaches showed three relevant factors and aspects: (1) Conscious analytic processing consisting of application of rules, conscious processing, reflecting upon reasons for procedure, meta-analytic information processing, and search for alternative; (2) Positive, holistic intuition consisting of variables like complexity, holistic processing, global rating, and clinician's emotional arousal; and (3) Automatization. Conscious-analytic processing and automatization varies with the level of training and the years of clinical experience.

In addition to these mixed-methods designs, conceptual considerations may be added.

Conceptual research

Transformative education[42] based on disruption, action, reflection is one concept for learning – a mostly performative one. Taking unconscious/subliminal perception, fast and slow thinking[43] processes, and implicit aspects into account, learning concepts could be widened - either *via* empirical conceptual research[44] or *via* a theoretical, more rational epistemic approach.

METADISCOURSE

Conceptual research can be added and repeatedly discussed on a meta-level, also including discussion of research paradigms. This discussion gives feedback to ongoing projects according to a hermeneutic circle. New theoretical considerations could be implemented and investigated in a timely fashion, so that improvements can be adopted and pursued, and hindering factors can be avoided. Digitization, therefore, opens up new opportunities and avenues.

Designs

The main concern is how to combine empirical data with constructivist objectives, which can be solved within a combination of both qualitative and quantitative research methods. Nevertheless, when it comes to training of aspects such as authentic care, decision making, training in history taking, or training of other types negotiations, we have to take into account intuitive factors[14], and/or in a broader sense, the social brain and mind. It consists of perceptions, *i.e.* conscious and

subliminal ones, attachment, commitment, refusal, mirror neurons, or mentalization. Findings show that perception, attachment, rejection, and mentalization network are perturbed by direct and indirect social stress. Therefore, the learning and teaching environment should be supported by didactically sound and affect-involving ingredients[45]. One possibility to affectively involve students is the training of communication skills[46] and discussion of cases in a structured and secure atmosphere. When social behavior is disrupted, a dysfunction in social perception, attachment, and rejection may be discerned. Thus, an adequate research design for learning has to take affect research into account. Case-based teaching can be assessed and studied along guidelines for case-studies[47], and simulation and virtual-reality paradigms can be used to contribute to environmental validity.

Affect research: Theory and practical application

Based on the question of what empathy[26] and especially what affects are, the relevant metapsychological formulations in S. Freud's work are examined within the framework of recent developments in psychoanalysis and neurosciences. The theoretical structure of a psychoanalytic affect theory relates, among other things, to the results of emotion research, endocrinology, and neurophysiology on the one hand, and to cognitive psychological, behavioral, and linguistic studies on the other[48].

Recent research results from the neurosciences indicate that affects and their sequence of actions are clearly anchored in neurophysiology or require clearly, anatomically identified neuronal circuits. The facial expressions linked to affective expressions, gestures, posture, voice, as well as visceral patterns are part of subcortically integrated motor reaction patterns. Facial muscle movements and neuron firing do not reveal anything about the underlying intrapsychic dimension, which is our main interest. Affects are a central determinant of inner and outer reality.

Solms[49] shows that the question of affects forces one to recognize the inner connection between the soul and the somatic and to bring this state of affairs into line with the theoretical designs of psychoanalysis. This leads to the conclusion that affect is a primary sense modality. While sensory modalities such as seeing or hearing represent aspects of the outer, objective world, affect is that primary sense modality in which the inner, subjective world is perceived, which in principle is unconscious. The scientific task is not only to describe and classify the superficial qualities of the inner perception, but also to gain an understanding of the "real facts" that underlie the sensory phenomena of the affect according to the psychoanalytic objective (see also[32]).

Historically, Freud[50] conceptualized affects in the context of his motivation theory, namely the drive theory, and defined the affects in a specific relation to the drives. In Freud's earliest drafts of affect theory, which are primarily to be read as fear-theoretical writings, an event that takes place in external reality triggers an affective reaction that becomes meaningful due to the connection with a certain idea.

In modern affect theory designs, a connection between instinctual and affect events is assumed, but other sources of motivation appear earlier in addition to the instincts. Modern psychoanalytical affect-theoretical designs can be divided into the subject areas "affects and drives", "affects and ego development" and "affects and object relationships". According to Krause *et al*[51], one has to differentiate between Freud's drive theory and his biological foundation of the drive concept and psychological drive theory. Although it turned out that Freud's attempt to find a biological foundation for the concept of instinct using ideas from the stimulus physiology of the time was insufficient, it should be noted that the derivation of the concept of instinct from biology was considered necessary. In the psychological part of instinct theory, Freud differentiated between instinctual source, instinctual goal, and instinctual object, and thus tried to grasp "instincts" by their goals and not by their causes. Starting from an ethological understanding, many instinctual processes are social processes in which the characteristics of the object have the same power to trigger behavior as internal sources of stimulus. Freud's social component only comes into play through the so-called drive object. Bowlby[52] revised the classical instinct theory, taking into account the approaches of ethological instinct theory in their application to attachment motivation.

Following the discussion of Freudian concepts of affect, affects were considered from the aspect of the instinctual event, the object relationships, as well as in connection with ego functions. In their affect-theoretical discussion, contemporary psychoanalytic theories mostly address the connection between affect and ego functions as well as affects and object relationships - including the object relationship theories of M. Klein and D. Winnicott. Knapp's exemplary compilation of affect-theoretical designs from the fields of psychology, neuropsychology and psychoanalysis offers optimal guidance and understanding[48,53] for the basic building

blocks of psychic life and, above all, poses neurophysiological and psychosomatic questions.

One possible research design

Application of methods to better measure and interpret non-verbal communication in personalized interactions may aid in the search for modulators of efficacy in dyadic encounters. One hypothesis could be formulated: Affective micro-expressions at the encounter's beginning predict working alliance. The objective is to develop a method of examining facial affects in therapeutic settings and interpreting the underlying emotions they represent, with a perspective on future exploitation in the form of computer-based treatment/monitoring tools. Methodologically, personalized encounters or treatment sessions (aiming at change) are videotaped, and verbal content is evaluated with computer assistance from ATLAS.ti. Visual content is screened for facial action units/micro-expressions using the Emotional Facial Action Coding System and reevaluated utilizing CNNs for feature extraction and inference. Working Alliance Inventory scores are compiled to assess their impact on the quality of the therapeutic relationship. Reliability of facial action unit coding is ensured by a training course and an independently-evaluated, standardized test. Interrater reliability can be calculated. Recognizing such expressions under naturalistic conditions is likely, however, more challenging. In order to highlight the methodological differences between these works, we have to break down each method into the three components: (1) Preprocessing; (2) CNN architecture; and (3) CNN training and inference. Amongst other things, a major finding could be that confrontations are linked to the display of specific affects and micro-expressions in both therapist and patient alike. Interestingly, it may be that the display of some micro-expressions also correlates with a higher WAI score. Results could be a consequence of the complexity of affects and the interplay of primary/subliminal and secondary emotions. Finally, we may be able to describe the circumstances of the routine evaluation and feedback elements to the interviewer *via* a computer-based feedback slope if we can develop a such a system for regular evaluation purposes.

Looking ahead, as many recent activities concentrate on training issues, as well as change- and learning-processes, further collaboration between neuroscientific (subliminal perception) and imaging techniques (associative learning, DMN) is a logical way to go. As educational sciences are based on (social) neuroscientific findings[12], such an approach could shed light on relevant interaction processes. Indeed, feedback-studies or randomized controlled trials on supervision processes could be conducted. Another area of particular interest might be micro-expressions, focusing on subliminal/unconscious interaction. Drawing upon lessons learned from conditions that impair social cognition (Digeorge 22q11DS-syndrome/Moderator-MA for treatment efficacy), this may help further training (Mentalisation Based Treatment + training) of educators /mentors in simulation scenarios (compare BeSiC/Bern, tEACH).

While an evidence-based approach to the practice of medicine has become accepted as an absolute requirement, (medical) educators have been slow to apply a similar approach to the educational process. Yet there exists a wealth of evidence-based learning strategies which have been studied in seminar settings and reported in social neuroscience and cognitive science literature. As the knowledge base grows exponentially, with the result that students need to learn ever-increasing amounts, the very best strategies and the tools necessary to apply those strategies should be provided. These will be essential to facilitating this essential transformational process and to describing some of the most effective learning strategies and their application to teaching processes. The approach leading to evidence-based teaching, dependent on evidence-based findings that emerged from carefully performed basic science studies and which were subsequently validated in clinical trials, now forms the basis of nearly all (clinical) decision-making today.

A scientific approach to studying which methods or strategies were most effective in the learning process has been validated in real-life classroom studies leading to the following key findings.

As the doubling rate for knowledge production for 2020 has been estimated to be just 74 d and the effectiveness of *e.g.*, medical treatments vastly improve when treatments were directed by evidence-based science, a similar approach has to be implemented if our students are to be adequately prepared to be doctors in the 21st century. In order to make the learning process as efficient as possible, we have to provide: (1) Spaced learning: Spaced repetition algorithms spread learning opportunities over time to improve knowledge retention. Students should have access to delivery-optimized recall questions, while the learning format itself spreads out the

topics being studied so that there is space between the study of the same medical concepts; (2) Interleaving: Knowledge should be broken down into 'easily digestible' sessions. This enables students to learn more effectively by easily switching between different topics. Doing this helps students learn the similarities and differences between different medical concepts, giving them a holistic and interconnected knowledge base; (3) Didactics videos: Dual coding, which combines verbal and visual representations of the same information, should be utilized as students learn best when they have multiple representations of the same idea. Learning science also shows that shorter length representations (3-9 min) are important for increasing knowledge retention; and (4) Active retrieval: Quiz questions at the end of each learning unit should ensure learner engagement utilizing frequent low-stakes or no-stakes assessments.

Furthermore, readiness assessments should be implemented, as well as briefings and debriefings of active learning formats supported by assignments with detailed drill-down analytics that bring data-driven leaning to the forefront for every instructor. An example was shown at: https://my.ltb.io/www/#/ or https://moodle.meduniwien.ac.at/course/view.php?id=682. Data-driven solutions are the key to improving the educational process.

CONCLUSION

Neuroscience[54] defined the non-conscious and allowed some connections between biology and behavior to be made. Mathematics and computer science have put some effort into simulating the mind[55]. Considering the speed, breadth, and depth of technical/technological advances today, it is necessary to develop specialized domain knowledge, and therefore interdisciplinary working groups need to develop glossaries. The main domains could then be tested and discussed again in a hermeneutic circle to influence and see the impact on the learning process per se. Research into the subjectivity of human beings (students, trainees) may be empirical, qualitative, or inductive, but should ideally be re-evaluated by the subject in an idiographic manner.

To give an example illustrating the current dilemma as well as the need for at least two perspectives, consider that in psychoanalytic literature, countertransference is usually described in a self-report format by the therapist. For example, the most recent psychometrically sound self-report measure, the Countertransference Questionnaire[56,57] asks the therapist to report his or her feeling/reaction towards the patient by indicating whether sentences such as "I feel guilty about my feelings toward him/her." (Item 24) are true. However, even in retrospect, these reports fall prey to the unavoidable "blind spots" of the therapist. Therefore, psychotherapy process research has searched for other, more rigourous ways to capture countertransference, which requires gauging the ongoing therapeutic process in a multimodal manner, encompassing self-report and observer-rated measurement. The most frequently applied observer-rated instruments are the Countertransference Factors Inventory (CFI[58]) and the Inventory of Countertransference Behavior (ICB[59]). The ICB can be used by supervisors to assess therapists' positive and negative countertransference behaviour in a specific session, whereas the CFI has a broader focus on therapists' competencies that may be important to countertransference management. The dilemma of both perspectives, self-report and observer-rated, is that the former is vulnerable to the therapist's "blind spots", while the latter is confined to the therapist's observable verbal behaviour, or, as Hayes has cogently expressed: "therapists' self-reports are limited by what they are willing and able to reveal, and raters' observations are restricted to overt displays of countertransference"[60]. Ideally, one could triangulate further *via* video-based investigations into unconscious/subliminal perceptions.

Personalized feedback systems promote change, and the more subjective perception and experience is assessed and reconsidered, the better significant change can take place. Differentiated steps may be undertaken to promote motivation, provide more security in disruptive times, and make change possible. Triangulated research designs, domain knowledge, and knowledge on data reduction might be considered in conjunction with idiographic assessment of subjective values, subliminal affect perceptions and attitudes, and values and beliefs.

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REVIEW

Immunotherapy in hepatocellular carcinoma: Combination strategies

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Abstract

Liver cancer is one of the most common causes of cancer death globally, and its incidence in the United States is increasing. Patients with advanced hepatocellular carcinoma (HCC) who are not candidates for surgical resection, liver transplant, or locoregional therapies can be treated with systemic therapies. Multiple agents, including sorafenib, lenvatinib, and regorafenib are approved for use as either first- or second-line therapy in this patient population, but all have relatively modest survival benefits. HCC is potentially susceptible to therapy with checkpoint inhibitors, including agents such as nivolumab and pembrolizumab, which are both approved by the Food and Drug Administration for patients previously treated with sorafenib but have not demonstrated superior overall survival in phase III trials. It is clear that more effective approaches are needed to potentiate the effects of checkpoint inhibitors in patients with HCC. This review will outline and appraise the current literature on the use of checkpoint inhibitors in HCC as part of a combination treatment involving an additional mode of therapy. The list of agents that can be paired with checkpoint inhibitors includes an additional checkpoint inhibitor, vascular endothelial growth factor or vascular endothelial growth factor receptor inhibitors, tyrosine kinase inhibitors, OX-40 agonists, and PT-112 inhibitors. The main non-pharmacologic therapies currently being studied for inclusion in a combination strategy include radiation therapy, trans-arterial chemoembolization, and ablation.

Key words: Hepatocellular carcinoma; Liver neoplasms; Antineoplastic agents; Immunological; Protein kinase inhibitors; Angiogenesis inhibitors

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Core tip: Immunotherapy remains a viable option for the systemic treatment of patients

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with unresectable hepatocellular carcinoma, although nivolumab and pembrolizumab failed to meet their endpoints in phase III studies. Combining immunotherapy with other treatment modalities may improve treatment responses. Multiple clinical trials are evaluating the safety and efficacy of these new combination strategies, which involve pairing PD-1 or PD-L1 inhibitors with CTLA-4 inhibitors, or pairing checkpoint inhibitors with alternative agents or non-pharmacologic therapies.

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INTRODUCTION

Worldwide, liver cancer is the fourth leading cause of cancer death and the seventh most common cancer in terms of incidence[1]. The most common liver cancer subtype is hepatocellular carcinoma[1]. Over the last 40 years, hepatocellular carcinoma (HCC) incidence has risen by approximately three-fold in the United States[2]. Between 2000 and 2009, the incidence rose by approximately 4.5% per year and 0.7% per year from 2010 to 2012[3]. Between the years 2018 and 2040, global liver cancer incidence is expected to rise by approximately 62%, while the number of liver cancer deaths worldwide will rise by 64%[2]. If HCC is detected at an early stage (Barcelona Clinic Liver Cancer stage 0 or A), surgical resection or ablation can be performed in select groups of patients[4]. Approximately 70% of patients will develop evidence of recurrence following resection[4,5]. If patients are not candidates for surgical resection, liver transplantation is offered to patients who meet the Milan criteria and provides a possibility of cure[4,5]. Patients who are not eligible for surgery or liver transplantation are candidates for locoregional therapies, including trans-arterial chemoembolization (TACE) and ablation, if they have early- or intermediate-stage disease (Barcelona Clinic Liver Cancer stage 0-B), or systemic therapies if they have advanced disease (Barcelona Clinic Liver Cancer stage C)[4]. Multiple options for systemic therapy exist (Table 1). The tyrosine kinase inhibitors sorafenib and lenvatinib are approved for use as first-line therapy[6]. Ramucirumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF) receptor 2, and the tyrosine kinase inhibitors regorafenib and cabozantinib are approved for patients previously treated with sorafenib, however, candidates for ramucirumab therapy must also have an alpha-fetoprotein (AFP) level of 400 ng/mL or greater[6,7]. With the exception of lenvatinib, which produced an objective response rate (ORR) of 24.1%, the rest of the approved systemic therapies could only achieve an ORR in the range of 2 to 11%[8-12]. Sorafenib, ramucirumab, regorafenib and cabozantinib were directly compared to placebo and increased overall survival by only 1.2 to 2.8 months[8,10-12].

In hopes of discovering therapies that could produce greater responses, investigators began to utilize checkpoint inhibitors in HCC patients, given their success in other malignancies and the contribution of the PD-1/PD-L1 and CTLA4 pathways to creating an immunosuppressive tumor microenvironment[13-15](Table 2). Nivolumab demonstrated an ORR of 15% and 20% in the dose-escalation and dose-expansion phases of the CheckMate 040 trial, respectively, while pembrolizumab produced an ORR of 17% in the KEYNOTE-224 trial[13,14]. Both agents were then Food and Drug Administration (FDA)-approved for use in patients who had previously received sorafenib[16,17]. However, neither nivolumab or pembrolizumab demonstrated a statistically significant improvement in overall survival (OS) in patients with unresectable HCC when compared to sorafenib or placebo in phase III trials, respectively[18,19]. Other checkpoint inhibitors studied in HCC patients in completed phase II trials include the PD-1 inhibitors camrelizumab, the PD-L1 inhibitor durvalumab, and the CTLA4 inhibitor tremelimumab[20-22]. In a phase II multicenter study (NCT02989922) involving 217 patients from Chinese medical centers with HCC who had failed or could not tolerate prior systemic therapy who were treated with camrelizumab, the ORR was 13.8%, with a six-month OS rate of 74.7%[20]. Durvalumab demonstrated an ORR of 10.3% with a median OS of 13.2 months in a multi-center phase I/II study in a cohort of 40 patients with HCC, most of whom had received prior sorafenib[21]. In a phase II study of tremelimumab in 21 patients from Spanish medical

Table 1 List of systemic therapies utilized in combination therapy

Drug class	List of agents	Mechanism of action
PD-1 inhibitors	Nivolumab, Pembrolizumab, Camrelizumab, Tislelizumab	Inhibits PD-1 receptor from binding to PD-L1
PD-L1 inhibitors	Atezolizumab, Durvalumab, Avelumab	Inhibits PD-L1 ligand from binding to PD-1 receptor
CTLA-4 inhibitors	Ipilimumab, Tremelimumab	Inhibits CTLA-4 receptor from binding to CD80 or CD86
OX40 agonists	INCAGN01876, INCAGN01949	Activates OX40 receptor *via* direct binding
Multiple tyrosine kinase inhibitors	Sorafenib, Cabozantinib, Lenvatinib, Regorafenib, Apatinib	Inhibits signaling from multiple tyrosine kinases
VEGF or VEGFR inhibitors	Ramucirumab, Bevacizumab	Inhibits VEGF interaction with VEGFR
Phosphaplatins	PT-112	Activates tumor cell apoptosis, inhibits angiogenesis

VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor.

centers with advanced HCC and chronic hepatitis C virus (HCV) infection, the ORR was 17.6% with a median OS of 8.2 months[22]. Tremelimumab caused a decrease in AFP levels of more than 50% in slightly more than one-third of all patients, and a reduction in HCV viral load in most patients[22].

The relatively modest benefits of currently-available systemic therapies for patients with advanced or unresectable HCC underscore the need for novel and improved therapies. Although nivolumab and pembrolizumab did not reach their endpoints in phase III trials, checkpoint inhibitors in general remain the focus of multiple active trials[18,19] (Tables 3-6). Updated results from the KEYNOTE-224 trial demonstrated that median OS was much greater in patients who responded to pembrolizumab compared to non-responders at the time of the first post-treatment scan, and that 30.8% of patients were alive at a median follow-up of 31.2 months[23]. These results suggest that patients who respond to checkpoint inhibitors may have a durable response. Combination therapies involving the use of both a checkpoint inhibitor and another therapy may provide a greater benefit than single-agent immunotherapy if they can substantially improve overall response rates. This review will outline the main types of combination therapies currently under investigation, discuss the rationale behind their design, and summarize the main clinical trials evaluating their safety and efficacy in HCC patients.

COMBINATION THERAPY WITH PD-1/PD-L1 INHIBITORS PLUS CTLA-4 INHIBITORS

The combination of nivolumab plus ipilimumab has proven successful in improving treatment responses in multiple malignancies when compared to standard-of-care therapy[24-27]. As a first line regimen, the combination of nivolumab and ipilimumab has demonstrated superior ORR, OS, and progression-free survival (PFS) when compared to either agent alone in patients with metastatic melanoma, even after 48 months of median follow-up[24,28]. Given what is known regarding the immune microenvironment of HCC, these results raised the question about whether or not the combination of a PD-1 inhibitor and CTLA-4 inhibitor can demonstrate durable clinical responses in advanced HCC patients that are superior to those seen with single-agent checkpoint inhibitors or targeted therapies in HCC patients.

Nivolumab plus ipilimumab

The CheckMate 040 study (NCT01658878) randomized 148 HCC patients who were previously treated with sorafenib to three separate arms comparing various treatment regimens utilizing ipilimumab and nivolumab[29,30]. Patients in Arm A received nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every three weeks for four doses followed by nivolumab 240 mg every two weeks, patients in Arm B received nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every three weeks for four doses followed by nivolumab 240 mg every two weeks, and patients in Arm C received nivolumab 3 mg/kg every two weeks plus ipilimumab 1 mg/kg given every six weeks[29]. Initial results indicated that 37% of patients developed grade 3 or 4 toxicity, with rash and pruritus being the most frequently reported adverse effect[29]. The ORR

Table 2 Reported results of successful clinical trials evaluating immunotherapy in hepatocellular carcinoma patients

Therapy	Comparator	Therapy indication	Phase	Approximate data collection period	Patients enrolled	Primary endpoints	Trial identifier
Nivolumab	None	Not specified	I/II	November 26, 2012-August 8, 2016	214 (dose-escalation), 48 (dose-expansion)	ORR (dose-escalation phase - 15%), (dose-expansion phase – 20%)	NCT01658878 (CheckMate 040)
Pembrolizumab	None	Second-line	II	June 22, 2016- February 20, 2017	104	ORR – 18.3%, median OS 13.2 mo	NCT02702414 (KEYNOTE-224)
Camrelizumab	Camrelizumab (regimen 1) *vs* camrelizumab (regimen 2)	Second-line	II	November 15, 2016–November 16, 2017	220	ORR 13.8%, median OS not reached	NCT02989922
Durvalumab	None	Not specified	I/II	August 29, 2012-October 24, 2016	40	ORR – 10.3%, median OS 13.2 mo	NCT01693562
Tremelimumab	None	Not specified	II	December 2008-May 2012	21	ORR - 17.6%, DCR 76.4%	NCT01008358
Pembrolizumab	Placebo	Second-line	III	May 31, 2016-November 23, 2017	413	Median OS 13.9 *vs* 10.6 months – HR: 0.781; 95%CI: 0.611 to 0.998; P = 0.0238, PFS 3.0 *vs* 2.8 months – HR: 0.718; 95%CI, 0.570 to 0.904; P = 0.0022 (pembrolizumab *vs* placebo)	NCT02702401
Nivolumab plus ipilimumab	Nivolumab plus ipilimumab (regimen 1 *vs* regimen 2 *vs* regimen 3)	Second-line	I/II	Minimum follow-up-28 months at time of data cutoff	148	ORR – (32 - Arm A, 31- Arm B, 31 - Arm C), median DOR (17.5 - Arm A, 22.2 - Arm B, 16.6 - Arm C)	NCT01658878 (CheckMate 040)
Durvalumab plus tremelimumab	None	Second-line	I/II	October 19, 2015-January 10, 2017	40	ORR – 15%	NCT02519348
Atezolizumab plus bevacizumab	Sorafenib	First-line	III	March 15, 2018-November 2019	501	OS HR: 0.58 (95%CI: 0.42- 0.79; P = 0.0006), PFS 6.8 *vs* 4.3 mo, P < 0.0001 (atezolizumab plus bevacizumab *vs* sorafenib)	NCT03434379(IMbrave 150)

ORR: Overall response rate; OS: Overall survival; DCR: Disease control rate; PFS: Progression-free survival; DOR: Duration of response; OS HR: Overall survival hazard rate.

was 31%, and after 24 months of follow-up, the OS rate was 40%[29]. This study was updated at the International Liver Cancer Association Conference in Sep 2019[31]. All 3 arms achieved a similar ORR (Arm A - 32%, Arm B - 31% and Arm C - 31%), while Arm A achieved the longest median OS (22.8 months *vs* 12.5 months for Arm B and 12.7 months for Arm C) and the highest OS rate at 30 months (44%)[31](Table 2). The study remains active[30].

The rather innovative study design allowed investigators to compare the impact of various doses of nivolumab and ipilimumab on treatment response[29-31]. The dosing schedule in Arms A and B was similar, however, the dose of ipilimumab was three times higher in Arm A, and patients in Arm C received ipilimumab less frequently than the other two arms[29]. The higher dose of ipilimumab received by patients in Arm A compared to patients in Arms B or C may have been responsible for the improved median overall survival[29]. Unsurprisingly, Arm A also had the highest number of treatment-related adverse effects, possibly due to the larger doses of ipilimumab the

Table 3 Summary of active clinical trials evaluating checkpoint inhibitor combination therapy

Therapy	Comparator	Therapy indication	Phase	Estimated study duration	Estimated patient enrollment	Primary endpoints	Trial identifier
Nivolumab plus ipilimumab	None	Neoadjuvant	I/II	March 1, 2019-September 1, 2022	32	Delay to surgery, incidence of treatment-related adverse events	NCT03682276 (PRIME-HCC)
Nivolumab plus ipilimumab	Nivolumab *vs* nivolumab plus ipilimumab (regimen 1) *vs* nivolumab plus ipilimumab (regimen 2)	Neoadjuvant	II	September 28, 2017-September 30, 2022	45	Incidence of adverse events	NCT03222076
Nivolumab plus ipilimumab	None	Neoadjuvant	II	June 1, 2018-December 31, 2022	40	Percentage of subjects with tumor shrinkage after therapy	NCT03510871
Nivolumab and ipilimumab plus INCAGN01876 (OX-40 Agonist)	Nivolumab plus INCAGN01876 *vs* ipilimumab plus INCAGN01876	Not specified	I/II	April 13, 2017-October 28, 2021	285	Safety and tolerability, ORR	NCT03126110
Nivolumab plus ipilimumab	Sorafenib or lenvatinib	First-line	III	September 16, 2019 – September 16, 2023	1084	OS	NCT04039607 (CheckMate 9DW)
Durvalumab plus tremelimumab	Durvalumab *vs* tremelimumab *vs* durvalumab plus tremelimumab (regimen 1) *vs* durvalumab plus tremelimumab (regimen 2) *vs* durvalumab plus bevacizumab	Second-line (first-line for patients receiving durvalumab plus bevacizumab)	II	October 19, 2015-January 6, 2021	433	Number patients experiencing adverse events and dose-limiting toxicities	NCT02519348
Durvalumab plus tremelimumab	Durvalumab *vs* durvalumab plus tremelimumab (regimen 1) *vs* durvalumab plus tremelimumab (regimen 2) *vs* sorafenib	First-line	III	October 11, 2017-October 30, 2020	1310	OS	NCT03298451 (HIMALAYA)

HCC: Hepatocellular carcinoma; ORR: Overall response rate; OS: Overall survival.

patients received, highlighting the inherent toxicity of this combination[31]. Twenty-two percent of patients discontinued the combination due to drug-related adverse events, compared to 6% and 2% of patients in Arms B and C, respectively[31]. Based on the results of CheckMate 040, the FDA has granted a priority review for nivolumab plus ipilimumab in the treatment of patients with advanced HCC who progressed on sorafenib as of November 2019[32].

There are multiple active clinical trials in addition to CheckMate 040 evaluating nivolumab plus ipilimumab for various treatment indications in HCC patients (Table 3). These include the phase III CheckMate 9DW clinical trial (NCT04039607) evaluating nivolumab plus ipilimumab as first-line therapy in comparison to sorafenib or lenvatinib in patients with advanced HCC[33]. The primary endpoint is overall survival[33]. If the combination of nivolumab and ipilimumab demonstrates significantly improved OS compared to standard-of-care sorafenib or lenvatinib, it may become the new standard-of-care first-line therapy. However, the increased

Table 4 Summary of active clinical trials evaluating combination therapy of checkpoint inhibitors plus vascular endothelial growth factor/factor receptor inhibitors

Therapy	Comparator	Therapy indication	Phase	Estimated study duration	Estimated patient enrollment	Primary endpoints	Trial identifier
Atezolizumab plus bevacizumab	None	First-line	II	January 1, 2020-June 30, 2022	48	Best overall response rate	NCT04180072
Atezolizumab plus bevacizumab	Sorafenib	First-line	III	March 15, 2018-June 29, 2022	480	OS, PFS	NCT03434379 (IMbrave 150)
Atezolizumab plus bevacizumab	Active surveillance	Adjuvant	III	December 31, 2019-July 12, 2027	662	RFS	NCT04102098 (IMbrave 150)
Durvalumab plus bevacizumab	Durvalumab alone *vs* tremelimumab alone *vs* durvalumab plus tremelimumab (regimen 1 *vs* regimen 2) *vs* durvalumab plus bevacizumab	First-line	II	October 19, 2015-January 6, 2021	433	Number patients experiencing adverse events and dose-limiting toxicities	NCT02519348
Durvalumab plus bevacizumab	Durvalumab plus placebo *vs* placebo alone	Adjuvant	III	April 29, 2019-June 16, 2023	888	RFS	NCT03847428 (EMERALD-2)
Camrelizumab plus apatinib	None	Second-line	II	June 1, 2019 – October 1, 2020	40	ORR	NCT04014101
Camrelizumab plus apatinib	Hepatic arterial infusion of chemotherapy	Adjuvant	II	February 15, 2019-February 28, 2023	200	RFS	NCT03839550
Camrelizumab plus apatinib and hepatic arterial infusion of FOLFOX chemotherapy regimen	None	Not specified	II	April 13, 2020-December 31, 2025	84	ORR	NCT04191889
Camrelizumab plus apatinib	Sorafenib	First-line	III	June 10, 2019-June 2022	510	OS, PFS	NCT03764293

OS: Overall survival; PFS: Progression-free survival; RFS: Recurrence-free survival; ORR: Overall response rate.

toxicity seen with this combination, especially if doses are similar to those used in Arm A of the CheckMate 040 trial, may lead to higher rates of therapy discontinuation[29]. At least three separate studies will evaluate the safety and feasibility of neoadjuvant nivolumab plus ipilimumab administered prior to surgical resection[34-36]. The phase II study (NCT03222076) sponsored by Anderson Cancer Center will randomize 45 patients with resectable HCC to receive adjuvant nivolumab or nivolumab plus ipilimumab prior to resection[34]. If successful, further studies may explore whether neoadjuvant nivolumab plus ipilimumab can decrease the high recurrence rates observed after surgical resection[4,5].

Durvalumab plus tremelimumab

The combination of durvalumab and tremelimumab was studied in a phase I/II trial (NCT02519348) in patients with unresectable HCC[37]. The safety profile was deemed

Table 5 Summary of active clinical trials evaluating combination therapy of checkpoint inhibitors plus tyrosine kinase inhibitors

Therapy	Comparator	Therapy indication	Phase	Estimated study duration	Estimated patient enrollment	Primary endpoints	Trial identifier
Nivolumab plus sorafenib	Nivolumab plus sorafenib (regimen 1 *vs* regimen 2)	First-line	II	April 16, 2018-May 31, 2020	40	MTD, ORR	NCT03439891
Pembrolizumab plus sorafenib	None	First-line	Ib/II	September 13, 2017-September 13, 2021	27	ORR	NCT03211416
Nivolumab plus lenvatinib	None	First-line	II	June 12, 2019-October 2021	50	ORR, safety/tolerability	NCT03841201
Pembrolizumab plus lenvatinib	Placebo plus lenvatinib	First-line	III	December 31, 2018-May 13, 2022	750	PFS, OS	NCT03713593 (LEAP-002)
Nivolumab plus cabozantinib	Nivolumab plus Ipilimumab plus cabozantinib	Not Specified	I/II	September 26, 2012-April 29, 2022	1097	Safety and tolerability	NCT01658878 (CheckMate 040)
Nivolumab plus Ipilimumab plus cabozantinib	Nivolumab plus cabozantinib	Not Specified	I/II	September 26, 2012-April 29, 2022	1097	Safety and tolerability	NCT01658878 (CheckMate 040)
Atezolizumab plus cabozantinib	Sorafenib *vs* cabozantinib	First-line	III	December 10, 2018-December 1, 2021	740	Duration of PFS, duration of OS	NCT03755791 (COSMIC-312)
Nivolumab plus regorafenib	None	Second-line	I/II	March 16 2020-December 2022	60	Rate of adverse events, rate of death	NCT04170556 (GOING)
Nivolumab plus regorafenib	None	First-line	II	May 30, 2020-May 30, 2023	42	Response rate	NCT04310709 (RENOBATE)

MTD: Maximum tolerated dose; ORR: Overall response rate; PFS: Progression-free survival; OS: Overall survival.

tolerable and an ORR of 15% was noted, according to results from the phase I portion of the study[37](Table 2). Common adverse effects included pruritus, fatigue, and elevated transaminases, which are similar to those noted in patients treated with durvalumab in phase II studies[21,37]. The phase II portion of the study seeks to evaluate the safety and feasibility of durvalumab plus tremelimumab as second-line therapy[38] (Table 3). This study will randomize 433 patients into five separate arms, in which patients with advanced HCC will receive either durvalumab or tremelimumab alone, durvalumab plus tremelimumab, or durvalumab plus bevacizumab[38]. Two arms of the study will compare different regimens of durvalumab plus tremelimumab[38]. Additional active studies involving this combination include the phase III HIMALAYA clinical trial (NCT03298451) which will compare durvalumab plus tremelimumab to sorafenib or durvalumab alone as first-line therapy in approximately 1310 advanced HCC patients from multiple countries[39](Table 3). Overall survival is the primary endpoint[39].

It remains to be seen whether the combination of durvalumab plus tremelimumab will have a similar toxicity profile as nivolumab plus ipilimumab. If durvalumab plus tremelimumab can demonstrate a high ORR with a comparatively lower rate of immune-related adverse effects, then it may become a viable alternative for HCC patients who have failed prior systemic therapies and cannot tolerate nivolumab plus ipilimumab due to adverse effects. If the dosing schedule utilized in the phase I/II clinical trial (NCT02519348) is adopted, this may minimize toxicity given the relatively infrequent dosing schedule of every four weeks[37]. Although the most common side-effects seen in patients treated with either combination include liver function test abnormalities and skin ailments such as pruritus or rash, the CheckMate 040 study demonstrated that 22% of patients discontinued therapy with nivolumab and ipilimumab due to treatment-related toxicity, compared to 7.5% of patients receiving durvalumab and tremelimumab in the NCT02519348 trial[31,37]. Given the ability of tremelimumab to reduce HCV viral loads, this combination may be preferred for patients with chronic hepatitis C infections[22].

Table 6 Summary of active clinical trials evaluating combination therapy of checkpoint inhibitors plus ablation, trans-arterial chemoembolization, or radiation

Therapy	Comparator	Therapy indication	Phase	Estimated study duration	Estimated patient enrollment	Primary endpoints	Trial identifier
Pembrolizumab plus RFA or MWA	None	First-line	II	May 9, 2019-September 2022	30	ORR	NCT03753659 (IMMULAB)
Durvalumab plus tremelimumab plus RFA	Durvalumab plus tremelimuumab, durvalumab plus tremelimumab plus TACE, or durvalumab plus tremelimumab plus cryoablation	Second-line	II	July 5, 2016, December 31, 2021	90	PFS	NCT02821754
Nivolumab plus TACE	None	First-line	II	June 14, 2018-September 2022	49	ORR	NCT03572582 (IMMUTACE)
Pembrolizumab plus TACE	None	First-line	I/II	January 28, 2018-December 31, 2020	26	Incidence of adverse events	NCT03397654 (PETAL)
Durvalumab plus tremelimumab plus TACE	Durvalumab plus tremelimuumab, durvalumab plus tremelimumab plus RFA, or durvalumab plus tremelimumab plus cryoablation	Second-line	II	July 5, 2016-December 31, 2021	90	PFS	NCT02821754
Durvalumab plus tremelimumab plus DEB-TACE	Durvalumab plus tremelimumab plus DEB-TACE (regimen 1 *vs* regimen 2)	Not Specified	II	June 12, 2019-November 2020	30	ORR	NCT03638141
Durvalumab plus bevacizumab plus TACE	Durvalumab plus bevacizumab plus TACE (one TACE procedure *vs* possibility of multiple procedures)	Second-line	II	April 27, 2020-December 31, 2022	22	PFS	NCT03937830
Durvalumab and bevacizumab plus TACE	Durvalumab plus placebo plus TACE *vs* placebo plus TACE	Not specified	III	November 30, 2018-November 29, 2023	600	PFS	NCT03778957 (EMERALD-1)
Pembrolizumab plus SBRT	None	Second-line	II	February 15, 2018-April 2, 2022	30	ORR	NCT03316872
Durvalumab plus tremelimumab and radiation	None	Second-line	II	May 14, 2018-October 31, 2025	70	ORR	NCT03482102

RFA: Radiofrequency ablation; MWA: Microwave ablation; ORR: Overall response rate; TACE: Transarterial chemoembolization; PFS: Progression-free survival; DEB-TACE: Drug-eluting bead transarterial chemoembolization; SBRT: Stereotactic body radiotherapy.

COMBINATION THERAPY WITH CHECKPOINT INHIBITORS PLUS OX40 AGONISTS

OX40 is a co-stimulatory receptor that is expressed by CD4 and CD8+ T-cells after antigen stimulation[40]. Treg cells can also express OX40[40]. OX40 agonists, which are monoclonal antibodies that bind OX40, induce T-cell expansion and persistence and may be able to suppress Treg activity[40]. The clinical use of a monoclonal antibody targeting OX40 was deemed safe following a phase I study (NCT01644968) in 30 patients with various malignancies, with the most common adverse effects including fatigue, rash, lymphopenia, fever, and pruritus[41]. Twelve patients in demonstrated a reduction in the size of at least one individual metastasis[41].

A combination strategy utilizing OX-40 agonists in conjunction with checkpoint inhibitors may be a viable option in the treatment of HCC. PD-1/PDL1 or CTLA4 blockade and OX40 agonism administered together may produce a greater activation of the immune system due to the targeting of distinct pathways. The use of an OX-40 monoclonal antibody in conjunction with an anti-PD-1 monoclonal antibody in mice models of ovarian cancer produced responses that were superior than those from either agent alone[42]. A phase I/II clinical trial (NCT03241173) was performed to determine whether this form of combination therapy is safe and effective in patients with solid malignancies including HCC[43]. The study contained three separate arms, including two arms where nivolumab or ipilimumab alone were given with the OX-40 inhibitor INCAGN01949, and another arm where both checkpoint inhibitors and INCAGN01949 were administered together[43]. Results are pending[43]. An additional

phase I/II trial sponsored by Incyte Biosciences (NCT03126110) is active and is similarly designed to the first trial, but is employing the OX40 agonist INCAGN01876 in patients with solid malignancies, including HCC[43,44](Table 3).

COMBINATION THERAPY WITH CHECKPOINT INHIBITORS PLUS VASCULAR ENDOTHELIAL GROWTH FACTOR OR VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR INHIBITORS

Anti-angiogenic agents have been a focus of research in HCC due to the relatively high vascularity of HCC tumors, however, studies suggest they may also have beneficial effects on the immune system[45-47]. VEGF expression can modulate the immune system *via* various mechanisms, leading to immunosuppression[46,47]. VEGF molecules can inhibit leukocyte adherence to the endothelium, inhibit the development of dendritic cells, and promote Treg proliferation[46,47]. The combination of checkpoint inhibitors and VEGF or vascular endothelial growth factor receptor (VEGFR) inhibitors may cause a greater net activation of the immune system than checkpoint inhibitors alone due to the added effect of VEGF inhibition.

Atezolizumab plus bevacizumab

Bevacizumab, a VEGF inhibitor, is currently approved for the treatment of multiple malignancies, including metastatic colorectal cancer, non-squamous non-small cell lung cancer, and ovarian cancer[48]. Bevacizumab was studied in a phase II trial in 46 patients with unresectable HCC and treatment resulted in a median PFS of 6.9 months and an OS rate of 53% at 1 year, 28% at 2 years, and 23% at 3 years[49]. However, 11% of patients developed clinically significant bleeding, including one patient who suffered a variceal bleed that was ultimately fatal[49]. Another phase II study evaluating single-agent bevacizumab in advanced HCC patients found that 9% of patients developed gastrointestinal bleeding[45]. Atezolizumab is a humanized monoclonal antibody that targets PD-L1 and prevents its binding to the PD-1 receptor and the B7.1 molecule[50]. It has been approved either as a single agent or in combination with chemotherapy for the treatment of patients with non-small cell lung cancer, small-cell lung cancer, urothelial carcinoma, and breast cancer[51]. The combination of atezolizumab plus bevacizumab demonstrated prolonged progression-free survival in metastatic renal cell carcinoma patients in a phase III trial with an acceptable safety profile[52].

Initial data testing this combination in HCC patients originates from a phase Ib study (NCT02645531) evaluating this combination as first-line therapy in 26 patients with advanced HCC[53]. Approximately 35% of patients developed grade 3-4 toxicities with hypertension being the most frequently reported adverse event, with an ORR of 62%[53]. In late 2019, the study authors reported that patients who received the combination therapy in Arm F demonstrated significantly better median progression-free survival (5.6 *vs* 3.4 months, $P = 0.0108$) when compared to atezolizumab alone[54]. The most common side effects seen in the patients randomized to combination therapy included proteinuria, fatigue and rash[55]. Patients in Arm A had a median overall survival of 17.1 months[55]. Initial phase III data has been reported from the IMBRAVE 150 trial that randomized approximately 501 systemic treatment-naïve patients with unresectable HCC to receive atezolizumab plus bevacizumab or sorafenib alone[56]. Preliminary data published in November 2019 demonstrated an improved PFS (6.8 *vs* 4.3) and OS hazard ratio (0.58) with the combination *vs* sorafenib alone[56](Table 2). Recent quality-of-life data from IMbrave150 presented in January 2020 revealed that patients taking atezolizumab plus bevacizumab had delayed time to deterioration of quality-of-life[57]. Patients in the combination arm reported greater time to deterioration of physical functioning, diarrhea, loss of appetite, fatigue, and pain[57](Table 4).

These data suggest that atezolizumab plus bevacizumab may become an alternative regimen for patients with advanced HCC due to its relatively acceptable toxicity profile when compared to a dual checkpoint inhibitor regimen, and improved efficacy when compared to sorafenib alone. The study remains active[58].

Other active clinical trials evaluating this combination include the NCT04102098 phase III trial, a part of the IMbrave 150 study, which will randomize 662 patients with resectable HCC and a high risk of recurrence to receive atezolizumab plus bevacizumab or surveillance as adjuvant therapy[59](Table 4). Given the significant improvement in PFS seen when this combination is used as first-line therapy, it may also be successful as adjuvant therapy and reduce the high recurrence rates often seen post-resection[4-5,56,59]. A single-arm phase II trial (NCT04180072) will enroll 48 patients

with advanced HCC and chronic HBV infection, allowing the investigators to determine whether HBV infection has any significant effect on the safety and effectiveness of atezolizumab plus bevacizumab[60](Table 4).

Durvalumab plus bevacizumab

The results of the studies testing atezolizumab and bevacizumab may be generalizable to other checkpoint inhibitors, such as durvalumab, if used in combination with bevacizumab or other VEGF inhibitors given similar mechanisms of action. The combination of durvalumab plus bevacizumab is being studied in multiple different trials, including the aforementioned phase II study (NCT02519348) in patients with advanced HCC as first-line therapy[38](Table 3). The phase III EMERALD-2 trial (NCT03847428) will compare durvalumab plus bevacizumab to either durvalumab alone or placebo as adjuvant therapy after either ablation or resection in HCC patients with a high risk of recurrence[61](Table 4).

Durvalumab plus ramucirumab

A phase I trial (NCT02572687) is evaluating the safety of the combination of ramucirumab and durvalumab in patients with advanced gastrointestinal or thoracic malignancies including hepatocellular carcinoma[62]. Although no reported phase II or III trials are currently active, if this combination proves to be safe with a tolerable side-effect profile, further study is warranted based on the results of the REACH-2 trial to determine if this combination is most effective in patients with an AFP greater than 400[10].

Camrelizumab plus apatinib

Apatinib is a tyrosine kinase inhibitor of VEGFR2 that binds to its target with ten-fold more affinity than sorafenib, and is currently approved in China for use in advanced gastric cancer patients[63,64]. Apatinib has demonstrated a tolerable safety profile with evidence of anti-tumor activity in HCC patients as single-agent therapy[64]. In a murine model of lung cancer, the combination of apatinib and an anti-PD-L1 monoclonal antibody inhibited tumor growth in a synergistic fashion with a notable increase in tumor-infiltrating lymphocytes[65]. The combination of apatinib and camrelizumab was studied in a phase Ia and Ib trial (NCT02942329) that enrolled 43 Chinese patients with various gastrointestinal malignancies, including gastric cancer, esophagogastric junction cancer, and advanced hepatocellular carcinoma[63]. This combination was thought to have a tolerable safety profile, with the most common side-effects being hypertension and an elevated AST[63]. Half of all HCC patients demonstrated a partial response, a response similar in magnitude to results from the initial Phase 1b trial (NCT02715531) testing first-line atezolizumab and bevacizumab[53,63]. Multiple studies are evaluating this combination in distinct HCC patient populations[66-69](Table 4). A phase II study (NCT04014101) seeks to determine whether camrelizumab plus apatinib is safe and effective in patients with advanced HCC as second-line therapy[66]. Another phase II study (NCT03839550) will determine whether this combination is superior to hepatic arterial infusion of chemotherapy in the adjuvant setting, with recurrence-free survival as the primary endpoint[67]. The phase II TRIPLET study (NCT04191889) will evaluate the safety and efficacy of the combination of FOLFOX chemotherapy infused directly into an artery perfusing the tumors, followed by camrelizumab and apatinib in 84 patients with advanced HCC[68]. A phase III study (NCT03764293) evaluating camrelizumab and apatinib as first-line therapy in advanced HCC patients will report both OS and PFS as primary endpoints[69]. The aforementioned studies are primarily being carried out in Chinese medical centers, which will limit the external validity of the results and require additional studies before their findings can be generalized to other patient populations[66-69].

COMBINATION THERAPY WITH CHECKPOINT INHIBITORS PLUS MULTI-TARGETED TYROSINE KINASE INHIBITORS

Sorafenib, lenvatinib, cabozantinib, and regorafenib all have activity against VEGF receptors and may mitigate the immunosuppressive activity of VEGF[6]. Although the exact mechanisms of action are unclear, TKIs can modulate the immune system[70-72]. Sorafenib decreased the populations of Tregs and CD8+ T cells that expressed PD-1 in the tumors of mice models of HCC[70]. An *in-vitro* study demonstrated that sorafenib could increase effector-T cell activation and inhibit Treg suppression of effector-T cells,

albeit at sub-pharmacologic doses[71]. Sorafenib has also been shown to restore the ability of dendritic cells to activate T cells *in vitro*[72]. In mouse models of HCC, lenvatinib demonstrated greater antitumor activity in immunocompetent mice when compared to sorafenib but not in immunodeficient mice, suggesting that the increase in activity may be related to immunomodulatory effects[73].

Given that the mechanism of action of TKIs differs from that of checkpoint inhibitors, pairing a TKI with a checkpoint inhibitor may produce responses that are either additive or synergistic. Additionally, responses to combination therapy with checkpoint inhibitors and TKIs may be more effective than responses to VEGF or VEGFR inhibitors alone because TKIs inhibit multiple distinct signaling pathways. If these combination therapies are proven effective, their safety may be of concern. Common toxicities observed with the various TKIs include diarrhea, skin rashes, fatigue, nausea, elevated aspartate aminotransferase levels, and rash[8,9,11,12]. The side-effect profiles of checkpoint inhibitors partially overlap with those of the TKIs, and it is unclear whether this may lead to greater toxicity when compared to single-agent regimens[13,14,24,31].

Sorafenib plus checkpoint inhibitors

The safety and effectiveness of sorafenib plus pembrolizumab as first-line therapy will be evaluated in 27 patients with advanced or metastatic HCC participating in a phase Ib/II study (NCT03211416) by the Roswell Park Cancer Institute[74](Table 5). One phase II trial (NCT03439891) will study the combination of nivolumab plus sorafenib as first-line therapy in patients with advanced HCC[75](Table 5). ORR is one of the primary endpoints for both studies[74,75].

Cabozantinib plus checkpoint inhibitors

Studies testing treatment regimens involving cabozantinib plus immunotherapy include the aforementioned CheckMate 040 study (NCT01658878), which contains two separate arms in which patients are receiving nivolumab plus cabozantinib and nivolumab and ipilimumab plus cabozantinib[30](Table 2). Initial data from this portion of the CheckMate 040 study has been reported[76]. Seventy-one patients with advanced HCC who were sorafenib-naïve or who had previously been treated with sorafenib were randomized to receive nivolumab plus cabozantinib or nivolumab plus ipilimumab and cabozantinib[76]. The ORR was 26% *vs* 17% for those who received all three drugs or nivolumab plus cabozantinib, respectively[76](Table 2). Median PFS was 6.8 for the three-drug regimen arm and 5.5 for the two-drug regimen arm[76](Table 2). The three-drug regimen caused significantly more toxicity, with 71% of patients in that arm reporting grade 3-4 adverse effects, *vs* 42% of patients in the two-drug arm[76]. Approximately 20% of patients in the three-drug arm discontinued the drug secondary to toxicity, compared with 3% of the patients in the two-drug arm[76]. Although the three-drug regimen demonstrates promise based on these early results, its high rate of toxicity may prohibit widespread adoption as standard-of-care therapy. Other checkpoint inhibitors, such as atezolizumab, are being studied as part of combination regimens involving cabozantinib[76](Table 5). The phase III COSMIC-312 trial (NCT03755791) will study the combination of cabozantinib and atezolizumab *vs* sorafenib as first-line therapy in a multi-national group of patients with advanced HCC[77](Table 5).

Lenvatinib plus checkpoint inhibitors

A phase 1b trial (NCT03006926) in which 18 patients were given a combination of lenvatinib and pembrolizumab demonstrated an acceptable safety profile, with hypertension and poor appetite reported as the most common adverse effects[78]. One phase II study (NCT03841201) will test the combination of lenvatinib and nivolumab in a cohort of patients from a German medical center with ORR and safety and tolerability measures as the primary endpoints[79](Table 5). A phase III study (NCT03713593) sponsored by Merck will test the combination of lenvatinib plus pembrolizumab in an international cohort of approximately 750 patients with advanced HCC with PFS and OS as the primary endpoints[80]. Patients will be randomized to receive either lenvatinib plus pembrolizumab or lenvatinib plus placebo[80](Table 5).

Regorafenib plus checkpoint inhibitors

Multiple studies evaluating regorafenib in conjunction with PD-1 inhibitors are active. They include a phase Ib trial sponsored by Bayer[81] that will elucidate the safety profile of regorafenib plus pembrolizumab. Recent results were presented at the American

Society of Clinical Oncology Gastrointestinal Cancers Symposium in January 2020[82]. Thirty-five patients had been treated, with 29 in the dose-defining cohort and 6 in the dose-expansion cohort[82]. Fifteen patients had discontinued treatment, with either clinical or radiologic disease progression as the most common reason[82]. Eighty-nine percent of patients experienced grade 3 or 4 treatment-emergent adverse events[82]. The multi-center phase II (GOING) trial (NCT04170556) and the single-center phase II (RENOBATE) trial from South Korea will evaluate the combination of regorafenib and nivolumab as second-line and first-line therapy, respectively[83,84](Table 5).

COMBINATION THERAPY WITH CHECKPOINT INHIBITORS PLUS LOCOREGIONAL THERAPIES

Locoregional therapies can activate the immune system through various mechanisms[85-87].

Treatment with either radiofrequency ablation (RFA) or TACE can stimulate the expansion of T-cells recognizing tumor-associated antigens, while RFA can lead to natural killer cell activation[85-87]. The ability of locoregional therapies to stimulate the immune system makes them potential candidates for an effective combination strategy with checkpoint inhibitors[85-87]. The side-effect profiles of immunotherapy and these locoregional therapies differ significantly, which may lead to relatively low toxicity compared to other combination regimens previously discussed[14,15,31,88,89].

Checkpoint inhibitors plus ablation

Patients who will likely be eligible for combination therapies involving ablation will have tumors 3 cm or less with early-stage, BCLC class 0 or A disease who are not candidates for surgical resection[4,5]. Although multiple types of ablation exist, RFA is the usual standard of care and adverse events may include intraperitoneal bleeding, intrahepatic abscess, grounding pad burns, bile duct injury, thermal damage to organs in close proximity to the tumor, pneumothorax, pain, and tumor seeding of tissues[4,88]. RFA is not optimal for tumors that are larger than 5 cm, numerous with 3 or more lesions, poorly visible *via* ultrasound, or adjacent to structures such as the biliary tree, bowel, and vital organs such as the heart[4,5,90].

Multiple studies evaluating combinations of radiofrequency ablation and immunotherapy are ongoing[91-93](Table 6). Patients with unresectable HCC underwent treatment with tremelimumab and radiofrequency ablation or chemoablation in a phase I study in which 32 patients were enrolled[91]. A partial response was noted in 5 out of 19 patients, and 12 out of 14 patients with confirmed HCV infection were noted to have a decrease in their viral load, consistent with prior studies involving tremelimumab[22,91]. The phase II clinical trial (NCT02821754) sponsored by the National Cancer Institute (NCI) includes an arm where patients with advanced HCC will receive durvalumab plus tremelimumab and RFA, and another where patients will receive cryoablation in conjunction with the checkpoint inhibitors as second-line therapy[92]. Progression-free survival is the primary outcome[92]. Studies involving pembrolizumab include the IMMULAB phase II study (NCT03753659), which will test the combination of RFA or microwave ablation plus pembrolizumab in 30 patients who had not received prior systemic therapy and report ORR as the primary outcome[93].

Checkpoint inhibitors plus TACE

TACE plus immunotherapy may be appropriate for patients with intermediate-stage disease, including those with BCLC class B disease based on current indications for TACE[4]. Some patients who are ineligible for RFA, such as those with larger tumors, multiple lesions, or smaller tumors which cannot be safely ablated may be candidates for TACE[4]. Potential adverse events of TACE therapy include damage to the hepatic artery, bile duct injury, acute liver failure, variceal bleeding, and cholecystitis[89,94]. Specific protocols for performing TACE may differ, and the types of chemotherapy, embolic agents, and schedule of TACE sessions may vary between institutions, among other factors[89]. This variation in how TACE is performed may lead to variation in side-effect profiles, and could affect the efficacy of combination therapies when compared across medical centers[89].

Multiple studies testing the safety of treatment combinations involving checkpoint inhibitors and TACE are active[95-99](Table 6). The phase II, single-arm IMMUTACE study from Germany (NCT03572582) will study the effectiveness and safety of

nivolumab plus TACE for patients with intermediate-stage HCC who have not received prior systemic therapy or TACE, with ORR as the primary endpoint[95]. The combination of TACE and pembrolizumab will be studied as first-line therapy in the phase I/II, single-arm PETAL trial (NCT03397654), which will report the incidence of adverse effects as the primary outcome measure[96]. A phase II clinical trial (NCT03638141) will evaluate the combination of durvalumab plus tremelimumab and drug-eluting bead TACE (deb-TACE) in approximately 90 patients with advanced HCC[97]. Patients with active HCV infection will be excluded, which may hinder the ability to detect any effects of the combination therapy on HCV viral load possibly due to tremelimumab[22,97]. The previously mentioned NCT02821754 phase II trial sponsored by the NCI contains an arm in which patients will receive durvalumab plus tremelimumab and TACE[92]. The combination of durvalumab plus bevacizumab and TACE will be evaluated as second-line therapy in a phase II trial (NCT03937830) sponsored by the NCI in 22 patients with advanced HCC[98]. The combination of durvalumab, bevacizumab and TACE will be compared to TACE alone or TACE plus durvalumab in the phase III, multi-center EMERALD-1 clinical trial (NCT03778957) which will report PFS as a primary outcome measure[99]. Secondary outcome measures include overall survival[99].

COMBINATION THERAPY WITH CHECKPOINT INHIBITORS PLUS RADIATION

The effect of checkpoint inhibitors on T-cells can be amplified by radiation[100]. Radiation therapy (RT) has been shown to induce antitumor immune responses through the formation of antitumor antibodies[100]. RT directed at one tumor site can induce responses in other tumor sites not directly targeted, a phenomenon known as the abscopal effect, which is related to immune system activation[100,102,103]. In mouse models of melanoma, radiation therapy can increase both antigen presentation to T-cells recognizing tumor antigens and infiltration of tumors by those T-cells[101]. RT can increase the expression of MHC-1 (major histocompatibility complex-1) molecules in tumors and increase T-cell and natural killer cell tumor infiltration[102,103]. Stereotactic body radiotherapy (SBRT) can be used to treat early-stage, BCLC A disease that is not amenable to treatment with RFA, and can achieve local control rates of approximately 80% or greater[90]. SBRT is a possible alternative treatment option after TACE has been unsuccessful, with local control rates of approximately 87%-99% in multiple studies[90]. Adverse events as a result of SBRT may include upper gastrointestinal bleeding from esophageal varices or gastric and duodenal ulcers, and biliary strictures[104]. SBRT generally has a more tolerable side-effect profile when compared to TACE, with rates of grade 3 or higher toxicity estimated at 6% to 27% in patients with unresectable HCC who receive SBRT[90,104]. If proven safe, SBRT plus immunotherapy could become a reasonable treatment option for HCC patients who are not candidates for RFA or who have failed TACE.

Multiple studies testing the combination of ionizing radiation and immunotherapy are ongoing in patients with unresectable HCC[105-107](Table 6). One phase I study (NCT03203304) will enroll 50 participants with advanced HCC who will first receive SBRT[105]. Patients will be randomized into two arms, one of which will receive nivolumab alone after SBRT, while the second will receive nivolumab and ipilimumab[105]. If the results from this study suggest that administering nivolumab plus ipilimumab after SBRT is safe, better responses may be seen with that combination than with nivolumab alone given after SBRT due to the synergistic effects of nivolumab plus ipilimumab[24,28,105]. A phase II study (NCT03482102) sponsored by Massachusetts General Hospital will evaluate the safety and efficacy of durvalumab and tremelimumab administered in combination with radiation as second-line therapy[106]. As with prior studies examining durvalumab plus tremelimumab, a central question of the NCT03482102 study is whether this combination will be less toxic than those utilizing nivolumab plus ipilimumab in addition to SBRT, and whether tremelimumab will lower the viral load in patients infected with HCV[22,31,106]. Another phase II study will report the overall response rate after enrolling approximately 30 patients, and treating them with pembrolizumab and SBRT in the second-line setting[107]. Both radiation dosing and the time intervals between radiation treatment and immunotherapy treatment may affect the efficacy of combination therapies, and should be considered when the results of these various studies are available[108].

FUTURE DIRECTIONS

PD-1 and PT-112 inhibitors

PT-112 is a platinum-based drug of the phosphaplatin class currently under development by Phosphaplatin Therapeutics with reported anti-tumor effects both *in-vivo* and *in-vitro*[109-111]. It promotes apoptosis in tumor cells and may have anti-angiogenic effects[110]. An *in-vitro* study demonstrated that PT-112 treatment led to increased phosphorylation of a wide variety of targets, including VGFR1, EGFR, and CDC2, suggesting it may have activity against multiple distinct pathways[111]. Tumor cells may not be able to evade the drug's antitumor effects through traditional drug resistance pathways because it binds to transmembrane proteins rather than DNA[110,112]. An *in-vitro* study in ovarian cancer cells demonstrated that cisplatin enters cells more readily than phosphaplatins, and may suggest that phosphaplatin treatment may produce fewer side-effects when compared to cisplatin therapy due to reduced intracellular accumulation[113]. A phase I clinical trial involving 62 patients receiving PT-112 (NCT2266745) demonstrated no maximum tolerated dose, with fatigue as the most frequently-reported adverse event[114]. A patient with small cell lung cancer who progressed after prior treatment with both a CTLA-4 inhibitor and PD-1 inhibitor was progression-free at 7.5 months, while a patient with non-small cell lung cancer who progressed on a PD-1 inhibitor was progression-free at 6 months, indicating the potential benefit of PT-112 therapy[114]. The ORR was approximately 10.7%[114]. A phase I/II trial testing PT-112 in patients with advanced solid tumors, including patients with advanced HCC, remains active[115]. The safety and efficacy of combining PD-1 therapy and PT-112 is being explored in a multi-center, non-randomized, phase I/II trial (NCT03409458) by Phosphaplatin Therapeutics that will administer PT-112 in combination with avelumab in patients with solid tumors, not including HCC[116]. If combination therapy involving PT-112 and immunotherapy is safe and effective, the natural properties of phosphaplatins may prevent drug resistance, leading to more durable responses. If these regimens are truly less toxic than other combination regimens due to low levels of intracellular accumulation of phosphaplatins, PT-112-based combination therapy may become an alternative option for HCC patients who cannot tolerate other regimens due to adverse effects if it is proven safe[113].

CONCLUSION

Although immunotherapy remains a promising strategy for HCC patients with unresectable disease, checkpoint inhibitors used as single-agent therapy produce relatively modest overall response rates without significant improvements in survival[18,19]. As we have outlined in this review, a combination treatment strategy pairing checkpoint inhibitors with additional pharmacologic agents, locoregional therapy or radiation therapy may produce greater responses than single-agent immunotherapy. Multiple active clinical trials are underway to determine which combination strategies can safely produce durable clinical responses, ideally with significant improvements in overall survival and overall response rates.

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REVIEW

Combined endoscopy/laparoscopy/percutaneous transhepatic biliary drainage, hybrid techniques in gastrointestinal and biliary diseases

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Abstract

In recent years, a wide range of gastrointestinal endoscopy techniques have been developed, such as endoscopic submucosal dissection (ESD) and endoscopic retrograde cholangiopancreatography (ERCP). Although ESD and ERCP have an important role in gastrointestinal and biliary diseases, each technique has its limitations. Hybrid techniques that combine endoscopic and surgical procedures have emerged that have the advantages of different procedures and negate their limitations at the same time. Laparoscopic endoscopic cooperative surgery and modified laparoscopic endoscopic cooperative surgery combine ESD and laparoscopic techniques to resect submucosal tumors with minimum resection area. Air leak test by intraoperative endoscopy can effectively identify a mechanically insufficient anastomosis and decrease the complication rate. The rendezvous technique that combines percutaneous transhepatic biliary drainage and endoscopy can be performed as a rescue approach for the treatment of biliary obstruction, stenosis and bile duct injuries. For patients with simultaneous presence of stones in the gallbladder and the common bile duct, the laparo-endoscopic rendezvous technique can perform ERCP and laparoscopic cholecystectomy at the same time and reduces the risk of pancreatic injury caused by ERCP. Biliobiliary and bilioenteric anastomosis using magnetic compression anastomosis is another choice for biliary obstruction. The most common used approach to deliver the magnets is by percutaneous-peroral tract. Laparoscopic-assisted ERCP is a safe and highly effective therapy for patients who develop biliary diseases after Roux-en-Y gastric bypass surgery.

Key words: Hybrid technique; Laparoscopic and endoscopic cooperative surgery; Endoscopic retrograde cholangiopancreatography; Laparoscopic-assisted endoscopic retrograde cholangiopancreatography; Rendezvous technique; Magnetic compression anastomosis

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Core tip: A wide range of hybrid techniques that combine two or more of endoscopy, laparoscopy and percutaneous transhepatic biliary drainage have been developed. The hybrid techniques include laparoscopic and endoscopic cooperative surgery, air leak test by intraoperative endoscopy, magnetic compression anastomosis, the rendezvous technique and laparoscopic assisted endoscopic retrograde cholangiopancreatography. This review aims to introduce these hybrid techniques and their applications for the treatment of gastrointestinal and biliary diseases.

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INTRODUCTION

In recent years, a wide range of gastrointestinal endoscopy techniques have been developed, such as endoscopic submucosal dissection (ESD) and endoscopic retrograde cholangiopancreatography (ERCP). ESD is now widely carried out for early neoplastic lesions of the gastrointestinal tract and has advantages of minimal invasion, low cost, patient tolerance and better quality of life of patients[1]. However, ESD is confined to incision of mucosal and submucosal layers. Laparoscopy is able to perform the full thickness resection, but sometimes laparoscopy cannot determine the precise incision line from the peritoneal cavity. ERCP has matured into an essential technique for managing biliary and pancreatic disorders, but it can be technically difficult in some situations (*e.g.*, completely biliary obstruction and altered anatomy) where percutaneous transhepatic biliary drainage (PTBD) may get access to the biliary tree.

In brief, none of these techniques can overcome all the difficulties encountered in the clinical practice. Therefore, many hybrid techniques that combine two or more of endoscopy, laparoscopy and PTBD have been developed that have the advantages of different procedures and negate their limitations at the same time. This review aims to introduce these hybrid techniques and their applications for the treatment of gastrointestinal and biliary diseases.

COMBINATION OF ENDOSCOPY AND LAPAROSCOPY

Resecting the gastrointestinal tumors

Gastrointestinal submucosal tumors (SMTs) are frequently seen in patients undergoing upper gastrointestinal endoscopy[2], and gastrointestinal stromal tumor is the most common type of SMT[3]. Usually, SMTs are treated by surgical approaches. Laparoscopic wedge resection has been confirmed a feasible option for SMT < 5 cm[4]. However, localization of small and intraluminal growing SMTs is difficult from the peritoneal cavity. As a result, excessive resection is needed to ensure the negative surgical margins, which can cause the deformity of the remaining stomach and gastric malfunction.

In order to decrease the resection area as much as possible, Hiki *et al*[5] firstly reported the conventional laparoscopic and endoscopic cooperative surgery (LECS) where the resection is performed jointly by the endoscopy and laparoscopy. Endoscopic submucosal dissection is used in this surgery. Firstly, the periphery of the tumor is marked by coagulation. Then three-fourths of the marked areas are cut down to the submucosal layer after submucosal injection. Next, a perforation of the gastric wall is created artificially, and the tip of the ultrasonically activated device is inserted into the perforation hole. Then three-fourths of the seromusclar layer is dissected along the incision line. After the tumor is inverted into the abdominal cavity, the serosa of the unresected tumor is grasped and retracted, and finally the incision line is closed by a laparoscopic stapler (Figure 1).

After the emergence of LECS, several modified LECS were developed excessively,

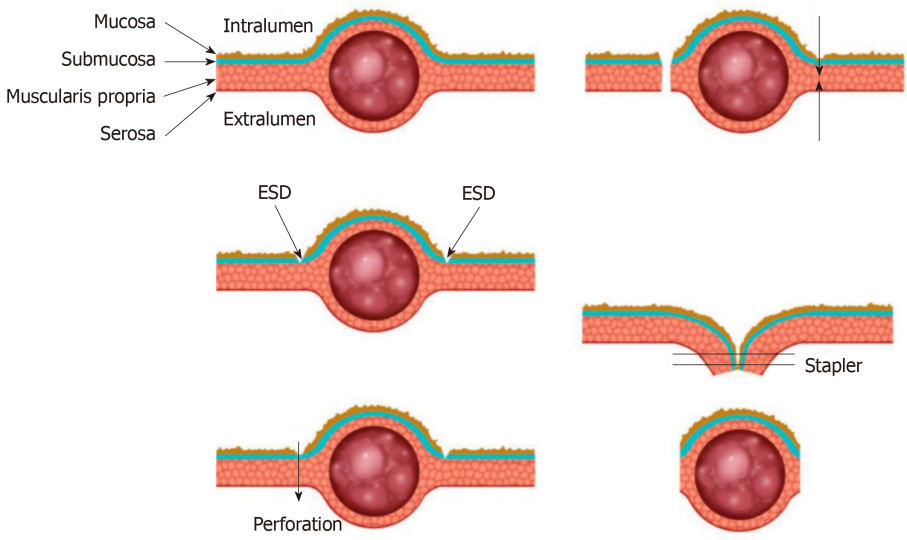


Figure 1 Conceptual diagram of the classical laparoscopic endoscopic cooperative surgery procedure[14]. ESD: Endoscopic submucosal dissection. Used with permission from John Wiley and Sons.

including inverted LECS[6], laparoscopic assisted endoscopic full thickness resection[7,8], combination of laparoscopic and endoscopic approaches for neoplasia with non-exposure technique[9], non-exposed wall invasion surgery (NEWS)[10] and closed-NEWS[11]. Based on whether the gastric wall is open during the surgery, these techniques can be divided into exposed technique and non-exposed technique. Table 1 compares characteristics of these two techniques. Though there are differences among these techniques, in general they all consist of two main parts that are the ESD technique and the laparoscopic surgery. The endoscopist determines the precise margin of the tumor, and then the resection is performed jointly by the endoscopy and laparoscopy.

As a less invasive approach, LECS has advantages of minimum resecting area and reserving function of organs at the greatest extent. In addition, LECS can be applied to tumors located in the esophagogastric junction or pyloric ring that cannot be removed by laparoscopic wedge resection[12,13]. The exposed LECS has a risk of tumor seeding and contamination of gastric juice in the peritoneal cavity due to the artificial perforation of the gastric wall[14]. The non-exposed LECS avoids the gastric open during the surgery and thus expands the indication of LECS for gastric epithelial neoplasms[15]. A series of studies on LECS and modified LECS have been conducted, showing that these techniques are feasible and safe for gastric SMTs[2,16-19].

Besides gastric tumors, LECS has been used to resect tumors in other parts of the gastrointestinal tract. There are a few reports of LECS for early superficial duodenal tumors (SDT), showing that this technique may be safe and feasible and could be an option for surgical SDT resection[37-25]. Standard treatment for SDT has not been established. Though ESD has been considered safe and effective for early gastric tumors, ESD for early duodenal cancer is associated with a high risk of perforation during and after surgery as a result of the narrow lumen and thin walls of the duodenum[26,27]. In LECS, the laparoscopic suture and monitoring may help to prevent the occurrence of perforation. Therefore, compared to ESD, LECS might be a safer approach for the treatment of SDT. For colon polyps and colorectal tumors that cannot be removed by conventional endoscopic techniques, LECS may also be an alternative choice[28].

Localization of gastrointestinal tumors

Endoscopic localization is essential in both endoscopic procedures and surgeries. In laparoscopy, endoscopic tattooing that uses suspensions of carbon particles is a commonly used approach to localize the tumor during laparoscopy. However, intraoperative endoscopic localization may be difficult to arrange between endoscopic and surgical teams. Hu *et al*[29] reported performing tumor resection using an ultrasonic scalpel through a gastric fistula formed by percutaneous endoscopic gastrostomy. Some novel methods may provide other choices for preoperative localization of tumors. Ohdaira *et al*[30] applied a magnet-string-clip system to gastric mucosa in 15

Table 1 Comparison between the characteristic of exposed laparoscopic endoscopic cooperative surgery and non-exposed laparoscopic endoscopic cooperative surgery

	Exposed LECS	Non-exposed LECS	
Techniques	Conventional LECS, Inverted LECS, LAEFR	CLEAN-NET	NEWS, Closed-NEWS
Opening of gastric wall	Yes	No	No
Advantages	Minimum resection area; more evidence of efficacy and feasibility	Non-exposed	Precise resection of both serosal and mucosal layers
Limitations	Possibility of tumor seeding and gastric juice contamination into the abdominal cavity	Potential risk of margin positive or excessive resection	Not applicable to tumor > 3 cm due to peroral retrieval

LECS: Laparoscopic endoscopic cooperative surgery; LAEFR: Laparoscopic assisted endoscopic full thickness resection; NEWS: Non-exposed wall invasion surgery; CLEAN-NET: Combination of laparoscopic and endoscopic approaches for neoplasia with non-exposure technique.

patients with early gastric cancer, and the tumor site was detected in all cases during laparoscopic gastrectomy. Hyun *et al*[31] introduced an endoscopic fluorescent band ligation method. The fluorescent rubber bands were endoscopically placed on the mucosa of porcine stomachs and colons, and the bands were clearly identified using the near-infrared fluorescence laparoscopy system during subsequent surgery.

Air leak test by intraoperative endoscopy

Anastomotic leak (AL) is one of the most frequent and devastating complications after many gastrointestinal surgeries[32,33]. Among measures that have been used to prevent AL, intraoperative air leak test (ALT) is the most widely used to identify a mechanically insufficient anastomosis[32]. The bowel proximal to the anastomosis is clamped, and then air is insufflated into the bowel lumen using a syringe or endoscope with the anastomosis under irrigation of saline. Leakage is detected by the bubbles arising from the anastomosis.

Compared to syringe, the intraoperative endoscopy can simultaneously provide air insufflation with adequate and steady pressure for ALT[33]. More importantly, it enables real-time assessment of anastomotic integrity, bleeding, vascular insufficiency and allows for repeatability if a leak is repaired[34]. The intraoperative ALT is easy, quick and associated with little or no risk[35]. One prospective randomized controlled trial showed that intraoperative endoscopy had significant lower rate of AL and lower need for reoperation than simple visual inspection in laparoscopic Roux-en-Y gastric bypass (RYGB)[36]. For colorectal surgeries, intraoperative endoscope has also been confirmed safe and effective[37-39].

COMBINATION OF ERCP AND PTBD

Rendezvous technique

ERCP has become the first choice of treatment for many biliary diseases, including bile duct injuries, obstruction and stenosis. Endoscopic treatment of the biliary stricture relies on initial passage of a guidewire across the stricture, followed by subsequent stricture dilation and stent placement[40]. However, this maneuver is not possible when the biliary duct is completely obstructed or transected. The rendezvous technique could be a choice for the recanalization of bile duct in this situation.

The rendezvous technique that combines endoscopic and percutaneous transhepatic approach was initially described for duodenoscopic sphincterotomy in the 1980s[41,42]. A guide wire is placed *via* the PTBD route, advanced into the duodenum, then grasped by grasping forceps or snares of the duodenoscope and pulled out of the duodenoscope. Then a catheter is advanced into the bile duct over the guidewire for drainage. Stents or balloons can also be placed to dilate the stricture of the bile duct. The procedure can also be completed in a reverse way where a guide wire placed endoscopically is grasped and pulled out through the PTBD route[43,44].

Because the guide wire may be damaged during withdraw and the procedure is cumbersome, a few modified techniques have been developed to avoid these problems, such as parallel cannulation technique[45-47]. With the advance in endoscopic ultrasonography (EUS) technology, the EUS guided rendezvous technique has been

developed, where the bile duct is punctured under the EUS guidance, and a guide wire is advanced antegrade through the papilla to perform a transpapillary procedure[48].

The rendezvous technique increases the success rate of biliary duct cannulation and facilitates the treatment of biliary tract diseases. It is reported with a high technical success rate of 80%-100%[49-53] and a significantly lower complication rate when compared to percutaneous transhepatic cholangiography[53]. The rendezvous technique can also be used to establish the continuity of the bile duct when surgical bile duct injury occurs, with a high primary success rate and a long term success rate of 55%[44].

Besides recanalization of bile ducts, the rendezvous technique is also reported to remove stones in the bile ducts[43,54,55]. Lithotomy by percutaneous transhepatic approach was performed firstly, but there were stones remaining in the intrahepatic duct or common bile duct (CBD). After the guide wire was grasped, the endoscopy was inserted further with the guide wire into the hepaticojejunostomy anastomotic region or CBD and lithotomy was performed for the remaining stones.

Magnetic compression anastomosis

Besides the rendezvous technique, biliobiliary and bilioenteric anastomosis using magnetic compression anastomosis (MCA) is another choice for the treatment of severe biliary strictures or complete obstructions. The working principle of MCA is that the magnetic compression force leads to gradual tissue necrosis within magnets while with tissue healing at the edge of the magnet simultaneously[56].

Two magnets are needed for the procedure, parent and daughter magnet. These two magnets can be delivered by a variety of methods, but the most common route is by the percutaneous-peroral approach[57]. One magnet is delivered through the PTBD route into the anastomosis site, and the other magnet is delivered endoscopically. When inserting a magnet into the CBD, full sphincterotomy or balloon dilation is usually required, and a metal stent may be inserted to facilitate further magnet delivery[58,59]. After recanalization and magnets removal, biliary stents can also be placed to prevent restenosis[59].

Bilioenteric anastomosis is a common operation to bypass extrahepatic biliary obstructions[60]. The conventional hand-sewn is time-consuming and associated with a high risk of complications[60]. In contrast, the MCA is considered to be associated with little complication because fistula formation after MCA requires a relatively long time. Also, there is no dilation of fibrotic tissue in the progress of fistula formation, so the risk of restenosis upon recoiling of fibrotic tissue is low[57].

COMBINATION OF ERCP AND LAPAROSCOPY

Laparoscopic-assisted ERCP

RYGB surgery is one of the most common bariatric procedures to treat obesity[61]. However, the patients have a high risk of biliary disease with up to 40% developing symptomatic cholelithiasis[61,62]. In addition, ERCP is challenging due to the surgically altered anatomy. Laparoscopic-assisted ERCP (LA-ERCP) is an option for these patients.

A gastrostomy is performed by the laparoscopy, and a port is placed into the remnant stomach. Then ERCP is performed by a conventional side-view duodenoscope *via* this port (Figure 2). After completion of the procedure, the port is removed, and the defect is closed by a suture or stapler. The transgastric route is commonly used to perform the LA-ERCP, and transjejunal route has also been reported[63]. Because the jejunal loop can easily reach the abdominal wall, the transjejunal LA-ERCP can be performed in all Roux-en-Y cases, even when the gastric remnant is not attainable. However, the transjejunal route needs a colonoscope to reduce the risk of intestinal injuries as a result of limited visual field of side-viewing of the duodenoscope.

LA-ERCP is a safe and highly effective therapy for patients who develop biliary diseases after RYGB surgery[64]. One advantage of LA-ERCP is the high successful rate, which was reported to be approximately 90%-100%[65]. Another one is that the successful rate remains high in long-limb reconstruction cases because a limb length of > 150 cm is associated with a high failure rate in other ERCP techniques[66]. In addition, LA-ERCPs would be favored if the patient also requires cholecystectomy. Therefore, LA-ERCP is preferred in patients with long limbs who require concomitant cholecystectomy[65].

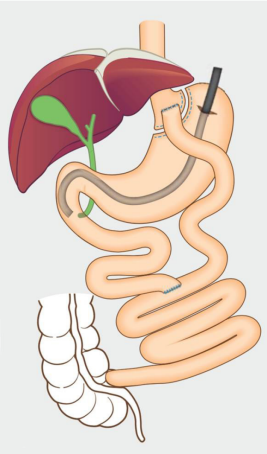


Figure 2 Laparoscopic-assisted endoscopic retrograde cholangiopancreatography in patients with Roux-en-Y anatomy[73].

Laparo-endoscopic rendezvous technique

The simultaneous presence of stones in the gallbladder and the CBD is a common clinical circumstance[67]. ERCP and laparoscopic cholecystectomy (LC) are considered as standard approaches to treat CBD stones and gallstones, respectively[68,69]. To perform ERCP and LC at the same time, the rendezvous intraoperative ERCP with transcystic guide-wire-assisted cannulation technique was developed as a one-stage intervention[70,71]. An antegrade guidewire is inserted and advanced through Vater's papilla into the duodenum by a surgeon. Subsequently, the guidewire is grasped by a snare and pulled out through the working channel of the duodenoscope and then cannulation of the CBD is performed.

The major advantage of the rendezvous procedure is a lower risk of pancreatic injury caused by the ERCP. The transcystic guide wire facilitates the endoscopic procedure and thus ensures elective CBD cannulation and avoids the inadvertent cannulation of the pancreatic duct. In addition, the antegrade approach avoids the problem of discordant patient positioning encountered when ERCP and LC are performed at the same time but separately. A recent meta-analysis compared different combinations of laparoscopic and intraoperative techniques (LC plus preoperative, intraoperative and postoperative ERCP and LC plus laparoscopic CBD exploration) and showed that the rendezvous approach was associated with the highest rates of safety and success[67]. The major limitation is that an experienced endoscopist may not be available for the procedure, and it may be difficult to arrange and carry out the rendezvous procedures in the operating room[68,72]. Moreover, using intraoperative cholangiography to detect CBD stones is essential before performing the rendezvous procedure[68]. Therefore, in centers where preoperative ERCP is routinely used to detect CBD stones, this technique is not applicable.

CONCLUSION

A wide range of hybrid techniques have been developed for the treatment of gastrointestinal and biliary diseases. These techniques expand the indications of therapeutic endoscopy, make it easier and safer to perform difficult procedures and decrease the agony of patients. Some of the techniques are only reported in few cases and further detailed evaluation of feasibility and efficacy is needed. For those that have been confirmed safe and effective, how to choose between hybrid techniques and conventional methods could be difficult. Further prospective investigations should be conducted to determine the best treatment options.

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MINIREVIEWS

Thrombopoietin-receptor agonists in perioperative treatment of patients with chronic liver disease

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Abstract

Thrombocytopenia is a multifactorial disorder that is common in patients with chronic liver disease (CLD), leading to challenging perioperative planning. As thrombocytopenia in CLD is associated with thrombopoietin (TPO) deficiency, the use of TPO-receptor agonists (TPO-RAs) to increase platelet counts is a promising approach. This has led to the development of various TPO-RAs, including romiplostim, eltrombopag, avatrombopag, and lusutrombopag. Of these, only avatrombopag and lusutrombopag are approved by the United States Food and Drug Administration for the perioperative treatment of thrombocytopenia in patients with CLD. Platelet transfusion is commonly used for the clinical management of thrombocytopenia in patients with CLD undergoing invasive procedures. However, the limitations and possible risks of transfusion, including short duration of efficacy, development of antiplatelet antibodies, risk of infections and such complications as transfusion-related acute lung injury or circulatory overload, and possibility of refractoriness, limit its use. Moreover, there is no consensus among guidelines as to the platelet count at which transfusions are indicated. Results from studies using TPO-RAs perioperatively in patients with thrombocytopenia and CLD are promising and provide an alternative to platelet transfusions in the pre- and post-operative setting. These TPO-RAs are the subject of this review, with focus on their use in the perioperative setting in patients with thrombocytopenia, associated supporting clinical trials, efficacy and safety data, and their use with respect to platelet transfusions.

Key words: Chronic liver disease; Thrombocytopenia; Thrombopoietin; Receptor agonist; Avatrombopag; Lusutrombopag; Romiplostim; Perioperative

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Core tip: Thrombocytopenia in patients with chronic liver disease complicates perioperative planning. Platelet transfusions are typically used as periprocedural treatment in these patients, but their use is complicated due to risk factors such as the development of infections and refractoriness. This has led to the development of thrombopoietin-receptor agonists, such as avatrombopag and lusutrombopag, that can increase platelet counts in patients with compromised thrombopoietin production and chronic liver disease. These thrombopoietin-receptor agonists can provide physicians with a safe and effective alternative to platelet transfusions and their use in clinical practice is the focus of this review.

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INTRODUCTION

Thrombopoietin (TPO) is a hormone produced by the liver that regulates platelet production by binding to and activating TPO receptors that are present on the surface of megakaryocytes. This induces a series of signaling cascades, leading to increased platelet production[1]. As a result, TPO levels typically decrease as liver disease progresses. This impaired TPO production, along with other factors, including platelet sequestration due to hypersplenism[2], bone marrow suppression, altered TPO metabolism, and development of anti-platelet antibodies, can contribute to thrombocytopenia[3].

Recombinant TPOs, as a treatment option, were developed to counter the lowered TPO levels by stimulating the TPO receptor. These pharmacologic agents mimic the action of TPO by binding to and activating different receptors on the megakaryocytes. The first generation of TPO-receptor agonists (TPO-RAs) included recombinant human thrombopoietin (known as rhTPO) and pegylated-human recombinant megakaryocyte growth and development factor (known as Peg-rHuMGDF). These resulted in the development of neutralizing autoantibodies that cross-reacted with endogenous TPO and were therefore discontinued. The next-generation TPO-RAs developed did not have homology to endogenous TPO and did not, therefore, produce an antigenic effect. These included romiplostim (NPLATE®) and eltrombopag (Promacta®/Revolade®), and most recently, avatrombopag (Doptelet®) and lusutrombopag (Mulpleta®)[4-7]. Of these, avatrombopag and lusutrombopag are approved by the United States Food and Drug Administration for the treatment of thrombocytopenia in patients with chronic liver disease (CLD) who are scheduled to undergo a procedure. Hetrombopag is another TPO-RA that is currently undergoing Phase 2 trials.

Patients with thrombocytopenia can have a negative perioperative outcome and platelet transfusions can be used to manage platelet counts during this period. Bleeding risk associated with low platelet counts has been demonstrated in several studies; however, the literature is equivocal, as some studies do not support an association. The optimal platelet count prior to performance of surgery varies, depending on the procedure and on patient variables, and while various guidelines advocate different platelet count thresholds for different invasive procedures, there are inadequate data to support these recommendations. For instance, the British Society of Hematology proposes thresholds of 50, 80 and 100 × 10^9/L according to the type of invasive procedure[8].

This review will provide an overview of the currently available TPO-RAs, with a focus on their use in the perioperative setting in patients with thrombocytopenia and CLD, associated supporting clinical trials, including the efficacy and safety data, and their use with respect to platelet transfusions.

ROMIPLOSTIM

Romiplostim is a recombinant fusion protein (peptibody)[4] containing two identical single-chain subunits, each consisting of a human immunoglobulin IgG1 Fc domain covalently linked to a peptide, itself containing two TPO receptor binding domains. It has no sequence homology to endogenous TPO and is produced using recombinant DNA technology in *Escherichia coli*. Romiplostim increases platelet production in a dose-dependent manner by the binding and activation of the TPO receptor, c-Mpl. As it is given intravenously, romiplostim avoids first-pass metabolism and is cleared by its binding to c-Mpl receptors and *via* the renal pathway. A single subcutaneous dose of 1 mcg/kg to 10 mcg/kg in patients with immune thrombocytopenia (ITP) results in a peak platelet count 1.3-14.9 times greater than the baseline platelet count over a 2-wk to 3-wk study period[4].

Efficacy and safety in clinical studies

Romiplostim is a subcutaneously administered drug indicated in the treatment of thrombocytopenia in patients with chronic ITP who have shown an insufficient response to corticosteroids, immunoglobulins, or splenectomy. Several studies have explored the efficacy of romiplostim for the management of perioperative thrombocytopenia in patients with CLD (Table 1). However, the small sample size in these studies is a major limitation. A 3-mo, single-center, single-arm, open-label study by Moussa *et al*[3] evaluated the efficacy of preoperative romiplostim treatment in 35 male patients with chronic hepatitis C, liver cirrhosis, and thrombocytopenia secondary to hepatitis C virus infection over 90 d. Romiplostim administered at 2 μg/kg once a week for up to 1 mo before the scheduled surgeries resulted in an increase in platelet counts (≥ 70 × 10^9/L) in 33 of the 35 patients, making them eligible for the procedure. Additionally, no serious adverse events (AEs) were observed during the treatment period (up to day 30) and none of the patients experienced postoperative bleeding or a thrombotic event within 60 d of the operation[3]. The authors concluded that preoperative treatment with romiplostim could be a viable and cost-effective alternative for patients who are unresponsive to standard therapy.

A recent single-center retrospective review of 47 patients treated with romiplostim perioperatively showed that the median platelet counts improved from 47 × 10^9/L at the time of romiplostim initiation to 164 × 10^9/L at the time of surgery. Romiplostim at a dose of 3 μg/kg per week for 2 wk increased the platelet count to over 100 × 10^9/L in 79% of patients. Additionally, bleeding and thromboembolic events were within acceptable limits in these patients[9]. The large cohort of patients, the fact that patients in this study had a wide variety of etiologies of thrombocytopenia, and that they underwent major surgical procedures, such as open cardiac surgery (unlike previous studies where procedures were minor in nature), makes this study stand out from the rest. However, the facts that this was a retrospective study without randomization, that no uniform platelet count threshold was set prior to proceeding with the medical procedure, that romiplostim doses were not standardized, and that bone marrow evaluation was not performed limits the conclusions that can be drawn[9]. Nevertheless, this study provides evidence for the use of romiplostim perioperatively in patients with chronic thrombocytopenia; although, this remains an off-label use for romiplostim.

Romiplostim treatment was also found to cause partial portal vein thrombosis (PVT), as reported for a 50-year-old woman with Child class B liver cirrhosis and hepatitis C-associated ITP; upon discontinuation of treatment complete recanalization of the portal vein was noted[10]. Based on this case study, caution must be exercised in the use of romiplostim in patients with advanced liver cirrhosis.

ELTROMBOPAG

Eltrombopag is a small-molecule non-peptide TPO-RA that interacts with the transmembrane domain of the TPO receptor on megakaryocyte precursors and megakaryocytes, leading to increased platelet production[5]. Eltrombopag is metabolized *via* cleavage, oxidation, and conjugation with glucuronic acid, glutathione, or cysteine. The cytochrome P450 enzymes involved in the oxidation pathway include CTP1A2 and CYP2C8, whereas UGT1A1 and UFT1A3 are involved in the glucuronidation pathway[5]. Eltrombopag increases platelet production after 7 d of repeated dosing, and platelet counts decrease to baseline 1 wk to 2 wk after discontinuing treatment[1]. Eltrombopag is approved for the treatment of

Table 1 Efficacy and safety results from clinical trials studying the effect of perioperative use of thrombopoietin-receptor agonists in patients with thrombocytopenia

Ref.	Dosing	Efficacy results	AEs
Romiplostim			
Basu *et al*[38], 2012	65 patients with CLD and thrombocytopenia randomized 1:1:1 to 500 μg romiplostim: 75 mg eltrombopag: 7 units of platelet transfusion	Improved platelet count > 180 × 10^9/L in all groups	Nausea, vomiting, dry mouth, headache, insomnia, irritability, local skin rash, shortness of breath, myalgia, arthralgia, erythema
Moussa *et al*[3], 2013	35 male patients with thrombocytopenia and CLD secondary to hepatitis C infection, dosed 2 μg/kg romiplostim weekly	Improved platelet count ≥ 70 × 10^9/L	No serious AEs reported
Marshall *et al*[39], 2015	18 patients with various etiologies of thrombocytopenia, including CLD, undergoing wide range of procedures	Improved platelet counts in all patients; all patients could receive surgery without delay or cancellation	No venous thromboembolic events
Al-Samkari *et al*[9], 2018	48 patients with various etiologies of thrombocytopenia, including CLD, undergoing 51 procedures, dosed 3 μg/kg romiplostim weekly (range 1-10 μg/kg/wk)	Improved platelet counts achieved in all patients after 1, 2 or 3 doses	Bleeding and thromboembolic events within acceptable limits
Eltrombopag			
ELEVATE (Eltrombopag); Afdhal *et al*[11], 2012	292 patients with CLD administered 75 mg/d or placebo for 14 d	Platelet transfusion not required in 72% of patients in the eltrombopag group and 19% of patients in the placebo group	Early study termination due to six portal vein thrombotic events in the eltrombopag group; headache, pyrexia, abdominal pain, diarrhea, nausea, *etc.*
Avatrombopag			
ADAPT-1; Terrault *et al*[14], 2018	231 patients with CLD and thrombocytopenia divided into low (<40 × 10^9/L) and high (40 to < 50 × 10^9/L) baseline platelet count cohorts Both cohorts were randomized 2:1 to receive avatrombopag or placebo; the low baseline cohort was treated with 60 mg and the high baseline cohort was treated with 40 mg avatrombopag, or placebo	Platelet transfusion, or rescue procedure for bleeding not required in 65.6% of avatrombopag-treated patients and 22.9% of placebo-treated patients in the low platelet count cohort and 88.1% of avatrombopag-treated patients and 38.2% of placebo-treated patients in the high platelet count cohort	Mild to moderate in severity in all treatment groups; most common TEAEs: abdominal pain, dyspepsia, nausea, pyrexia, dizziness, and headache; no thromboembolic TEAEs
ADAPT-2; Terrault *et al*[14], 2018	204 patients with CLD and thrombocytopenia; same dosing as ADAPT-1	Platelet transfusion, or rescue procedure for bleeding not required in 68.6% of avatrombopag-treated patients and 34.9% of placebo-treated patients in the low platelet count cohort and 87.9% of avatrombopag-treated patients and 33.3% of placebo-treated patients in the high platelet count cohort	Same as ADAPT-1; three thromboembolic TEAEs reported
Lusutrombopag			
L-PLUS-1; Hidaka *et al*[18], 2018	97 patients with thrombocytopenia and CLD randomized to receive 3 mg of lusutrombopag or placebo once daily for up to 7 d	Platelet transfusion, or rescue procedure for bleeding not required in 79.2% of lusutrombopag-treated patients and 12.5% of placebo-treated patients	Most common TEAEs: Nausea, pyrexia, headache, pain, and portal vein thrombosis; most common SAE with lusutrombopag was portal vein thrombosis
L-PLUS-2; Peck-Radosavljevic *et al*[17], 2019	215 patients; same dosing as L-PLUS-1	Platelet transfusion, or rescue procedure for bleeding not required in 64.8% of lusutrombopag-treated patients and 29% of placebo-treated patients	Most TEAEs were mild or moderate in severity; four asymptomatic thrombotic events, two each in the lusutrombopag and placebo groups, respectively; none attributed to lusutrombopag

AE: Adverse event; CLD: Chronic liver disease; SAE: Serious adverse event; TEAE: Treatment-emergent adverse event.

thrombocytopenia in adults and children with chronic ITP who have an insufficient response to corticosteroids, immunoglobulins, or splenectomy, in patients with chronic hepatitis C virus infection to allow initiation and maintenance of interferon-based therapy, and in patients with severe aplastic anemia who have an insufficient response to immunosuppressive therapy[5].

Efficacy and safety in clinical studies

The Eltrombopag Evaluated for Its Ability to Overcome Thrombocytopenia and Enable Procedures (referred to as "ELEVATE") study was a double-blind, randomized, placebo-controlled, Phase 3 trial conducted in 288 patients with CLD and platelet counts below 50 × 10^9/L to study the ability of eltrombopag to increase platelet counts and to reduce the need for platelet transfusions in patients undergoing an elective invasive procedure[11]. Patients received either 75 mg eltrombopag once daily or placebo. The primary efficacy endpoint was the proportion of patients who did not require a platelet transfusion before, during, and up to 7 d after the elective invasive procedure, and the secondary endpoint was the proportion of patients with bleeding before, during, and up to 7 d after the procedure. A majority of patients in the eltrombopag and placebo groups (62% and 56%, respectively) underwent elective invasive procedures that were in the lowest bleeding risk category. At the end of 2 wk, 59% of patients treated with eltrombopag and 5% of those receiving placebo had an increased platelet count, and primary endpoint was achieved in 72% and 19% of patients, respectively. With the exception of thrombotic events being higher in the eltrombopag group (4% *vs* 1% in the placebo group), the incidence and severity of AEs were similar in both groups. Serious AEs occurred in 13% and 12% of patients in the eltrombopag and placebo groups, respectively. A total of 8 patients, comprising 6 in the eltrombopag group and 2 in the placebo group, experienced 10 thrombotic events, with 9 of those events involving the portal venous system. It must be noted that none of the 8 total patients with a thrombotic event had received both eltrombopag and a platelet/blood product transfusion, and of the 26 patients who received both eltrombopag and a transfusion, none had a thrombotic event. Additionally, 5 out of the total 8 patients who had a thrombotic event were found to have cancer. However, a separate post hoc analysis identified an association between higher platelet counts (> 200 × 10^9/L) and an increased risk of thrombotic events[11], and the study was terminated early due to the increased incidence of PVT in the eltrombopag arm. Key efficacy and safety data from the study are outlined in Table 1.

In the absence of better identification of risk factors for the development of thrombotic events, dose optimization, and the risk of PVT in patients with thrombocytopenia and CLD undergoing an elective procedure, eltrombopag must be used with caution and is not recommended as an alternative to platelet transfusions in

this group of patients; this is an off-label use of eltrombopag.

AVATROMBOPAG

Avatrombopag is an oral, small-molecule non-peptide TPO-RA that, like eltrombopag, binds to the TPO receptor to bring out a number of cellular reactions that give rise to megakaryocytic proliferation and differentiation and increased platelet production. Similar to eltrombopag and lusutrombopag[7], it does not compete with TPO for binding to the TPO receptor, and may thus have an additive effect with TPO on platelet production[6,12]. Cytochrome P450 enzymes CYP2C9 and 3A are responsible for metabolizing avatrombopag to corresponding 4-hydroxylated products, with the former enzyme playing the predominant role[13]. It is the first TPO-RA to be approved for use perioperatively to treat thrombocytopenia in adult patients with CLD scheduled to undergo a procedure[6]. Avatrombopag is an orally administered drug that is taken with food for 5 d consecutively prior to an elective procedure. Avatrombopag results in a dose- and exposure-dependent increase in platelet counts in adults within 3-5 d of treatment, with a peak effect occurring after 10-13 d and counts remaining elevated over 50 × 10^9/L for at least 7 d post-procedure. Baseline levels are achieved after 35 d[6].

Efficacy and safety in clinical studies

There have been two randomized, double-blind, placebo-controlled Phase 3 trials[14] - ADAPT-1 and ADAPT-2 - with identical designs that have studied the effectiveness and safety of avatrombopag in reducing the need for platelet transfusion and rescue from bleeding in patients with CLD scheduled for a procedure. Patients were screened to exclude several criteria, including presence of arterial or venous thrombosis and a portal vein blood flow below 10 cm/s. Included patients were divided into low and high baseline platelet count cohorts and received 60 mg/d and 40 mg/d of avatrombopag, respectively. The primary endpoint of these studies was the proportion of patients who did not require a platelet transfusion or rescue procedure for bleeding after randomization and up to 7 d after a scheduled procedure. Secondary efficacy endpoints included the proportion of patients achieving the target platelet count of ≥ 50 × 10^9/L on the day of the procedure and the mean change in platelet count from baseline to procedure day. Patients treated with avatrombopag demonstrated increased platelet counts that were approximately 30-50 × 10^9/L higher on average compared with patients treated with placebo before minor invasive procedures, with a net doubling of counts on average. There were no meaningful differences in AEs of special interest among the avatrombopag-treated and placebo-treated patients, including thromboembolism, and the rates of occurrence of AEs across both the studies were comparable in the avatrombopag- and placebo-treated groups[14]. A treatment-emergent AE (TEAE) of partial PVT was observed in 1 avatrombopag-treated patient in the high baseline platelet count cohort in the ADAPT-2 trial at 13 d after the last avatrombopag dose and was assessed as nonserious and possibly related to the drug. Thromboembolic events were also observed in 2 placebo-treated patients in the ADAPT-2 trial, one being an acute myocardial infarction and the other a disseminated intravascular coagulation/ pulmonary embolus. Thromboembolic TEAEs were not noted in the ADAPT-1 trial. One non-TEAE of PVT was observed in the avatrombopag group (60 mg) at 31 d after the final dose of avatrombopag and was assessed as serious but not related. Table 1 outlines the key efficacy and safety results from these trials.

Results from a pharmacokinetic modeling study have shown that repeated dosing of avatrombopag as early as 12 d after completion of the first dosing can increase platelet counts to within safe limits[15]. Saab *et al*[16] have recently reported that repeated dosing of avatrombopag in 4 patients with CLD and thrombocytopenia led to a 2.3-fold mean increase in platelet count after the first dosing and 2.6-fold mean increase after the second dosing. The patients underwent procedures within 9-13 d after starting avatrombopag, with repeat dosing performed at least 30 d after completing the first dose. None of the patients required platelet transfusions or rescue therapy, or had TEAEs, and repeated dosing did not reduce the efficacy of avatrombopag[16]. Avatrombopag dosing has the ability to limit the magnitude and duration of increase in patient platelet counts and this predictable pharmacokinetic/pharmacodynamic profile could potentially be contributing to the limited risk of thromboembolic events associated with this treatment[14].

LUSUTROMBOPAG

Lusutrombopag is an orally administered, small-molecule non-peptide TPO agonist that activates the signal transduction pathways in a manner similar to endogenous TPO (while not competing for the same binding site), leading to increased platelet production[6-7]. Lusutrombopag is metabolized *via* the β- and ω-oxidation pathways and glucuronidation. The cytochrome P450 enzymes are mainly responsible for its metabolism, including CYP4A11[13]. The mean maximum platelet count following treatment with 3 mg/d lusutrombopag was 86.9 × 10^9/L and the median time to reach this was 12 d[7].

Efficacy and safety in clinical studies

Data from two Phase 3 double-blind studies, L-PLUS-1 and L-PLUS-2, have shown lusutrombopag to be effective in raising platelet counts in patients with CLD and thrombocytopenia prior to undergoing an elective invasive procedure. The primary endpoint of both trials was the proportion of patients who avoided pre-procedure platelet transfusions and rescue therapy for bleeding, as assessed from randomization through 7 d post-procedure[17,18]. Key secondary endpoints included the number of patients who required no platelet transfusions during the study, the proportion of patients who achieved a platelet count of ≥ 50 × 10^9/L with an increase of ≥ 20 × 10^9/L from baseline at any time during the study, and the number of days during which the platelet count was maintained at ≥ 50 × 10^9/L[17]. Safety assessments included the incidence of AEs, adverse drug reactions, bleeding-related AEs, and thrombotic events. Patients treated with lusutrombopag perioperatively demonstrated improvements in platelet counts similar to those seen with avatrombopag after 7 d of treatment. In the L-PLUS-1 trial, one serious AE of PVT, possibly related to lusutrombopag treatment, was reported in the lusutrombopag-treated group and one mild incidence of superior mesenteric vein thrombosis was reported in the placebo group that was considered not related to the study drug. As neither event was associated with an extremely high platelet count, and the incidence of thrombotic events were similar in both treatment groups, the authors concluded that no further monitoring for thromboembolic events was required in lusutrombopag-treated patients. In the L-PLUS-2 trial, four TEAEs were reported, two each in the lusutrombopag- and placebo-treated groups, respectively. All events were asymptomatic and deemed to be consistent with the findings of a meta-analysis that suggested the prevalence of PVT in patients with CLD and thrombocytopenia. Table 1 outlines the key safety and efficacy results from these trials.

A recent retrospective report of 1760 cirrhotic patients with low platelet counts[2] found that 66% of patients with platelet counts below 50 × 10^9/L required platelet transfusions for radiofrequency ablation, 43% for transarterial chemoembolization, and 55% for endoscopic injection sclerotherapy/endoscopic variceal ligation. When 25 of these patients were administered lusutrombopag prior to the procedures, platelet counts increased significantly compared with baseline levels (82 × 10^9/L *vs* 41 × 10^9/L), and of these patients, only 4 needed platelet transfusions before the procedures. The proportion of lusutrombopag-treated patients who required platelet transfusions was significantly lower (16%) compared with patients not treated with lusutrombopag (54%)[2]. Lusutrombopag was also found to be more effective in raising platelet counts in patients with baseline platelet counts above 30 × 10^9/L compared with patients with baseline platelet counts □less than or equal to 30 × 10^9/L and spleen index greater than or equal to 40 cm^2[2]. The authors of this retrospective study reported 1 case of portal thrombosis in the lusutrombopag group, which was revealed *via* a routine CT scan taken on the 12th d of treatment. The causal relationship of thrombosis with lusutrombopag treatment was unclear[2].

Sato *et al*[19] were the first to report the efficacy of repeated administration of lusutrombopag (3 mg daily) in a patient with hepatocellular carcinoma who had two planned invasive procedures, radiofrequency ablation and transarterial chemoembolization. In the first instance, lusutrombopag was discontinued after 5 d, as the platelet count increased to 98 × 10^9/L by day 5, while in the second instance, the patient required the full 7-d treatment to achieve a platelet count above 50 × 10^9/L. No AEs such as portal thrombus were reported during either of the treatments. More recently, Ishikawa *et al*[20] reported the safety and efficacy of repeated use of lusutrombopag to increase platelet counts in 8 patients with hepatocellular carcinoma and thrombocytopenia prior to repeated scheduled radiofrequency ablation procedures. Platelet counts increased from 42500 ± 5200/μL at baseline to 103100 ± 22800/μL at day 14 after first treatment, and from 43800 ± 6000/μL at baseline to 110700 ± 17800/μL at day 14 after repeat treatment, demonstrating that the repeated

use of lusutrombopag does not decrease its effect on platelet counts. None of the patients developed clinical symptoms such as thrombosis or PVT[20].

HETROMBOPAG

Hetrombopag is a small-molecule non-peptide TPO-RA, with a mechanism of action similar to eltrombopag but with an *in vivo* pharmacological effect that is 8-10 times that of eltrombopag[21]. Based on animal studies, it appears that hetrombopag is primarily metabolized *via* glucuronidation and hydrolyzed into aglycone following excretion with bile acid[21]. A Phase 1 study demonstrated that hetrombopag is safe and well-tolerated in healthy subjects and can be a potential candidate for the treatment of patients with chronic ITP[21]. It is now in Phase 2 trials for the treatment of ITP. Currently, there are no clinical studies underway to examine the effectiveness and safety of hetrombopag as perioperative treatment of thrombocytopenia in patients with CLD.

TPO-RAs FOR OTHER INDICATION UNDER INVESTIGATION

Romiplostim and eltrombopag are currently approved for management of chronic ITP but there are studies (like the ones outlined earlier) demonstrating their efficacy in other indications. For instance, romiplostim has demonstrated efficacy in treating chemotherapy-induced thrombocytopenia and myelodysplastic syndromes[22]. Eltrombopag has been shown to be effective in the treatment of hepatitis C infection (where it raises the platelet counts enough to allow continued treatment with ribavirin and pegylated-interferon), myelodysplastic syndrome, acute myeloid leukemia, and severe aplastic anemia[23]. Both avatrombopag and lusutrombopag have been approved for use perioperatively to treat thrombocytopenia in patients with CLD. Recently, a supplemental New Drug Application was filed for avatrombopag as therapy for the treatment of chronic ITP; meanwhile, the trial for avatrombopag as therapy in chemotherapy-induced thrombocytopenia (NCT03471078) is still underway. A trial to study the safety and efficacy of hetrombopag (NCT03222843) for the treatment of chronic ITP is currently underway.

RECOMMENDATIONS ON USING PLATELET TRANSFUSIONS BY SCIENTIFIC AND GOVERNING BODIES

Patients with thrombocytopenia and CLD are at an increased risk of bleeding. Platelet transfusions can be administered in these patients prophylactically, prior to scheduled medical procedures to minimize the risk of bleeding, or therapeutically to control bleeding. The goal of any prophylactic treatment is limiting hemorrhagic events in the peri- and post-operative surgical setting. However, there is uncertainty in prophylactic treatment of thrombocytopenia, as there are no effective tools that can predict bleeding risk in patients, and data on the correlation between thrombocytopenia and the risk of bleeding are equivocal[24,25]. While platelet transfusion is a primary option in these patients, the decision to undergo transfusion can be a complicated one.

Lack of consensus among guidelines

There is a lack of consensus among guidelines related to platelet transfusion in patients, primarily due to lack of sufficient and supportive data to substantiate the recommended platelet transfusion triggers[26]. Further, there are no guidelines that provide recommendations for platelet transfusion specifically for patients with CLD prior to an elective procedure. The variations in the recommended platelet threshold values before invasive procedures often depend on the type of patient and the perceived risk of the procedure. The American Society for Gastrointestinal Endoscopy (ASGE) guidelines[27], which are not specific to gastrointestinal bleeding, state that the minimum threshold platelet count for undergoing upper gastrointestinal endoscopy has not been established, but they also point out that there are publications supporting 20×10^9/L and 50×10^9/L as the threshold for platelet counts prior to diagnostic procedures and biopsies, respectively. The American Association for the Study of Liver Diseases guidelines for transcutaneous or transvenous liver biopsy state that

platelet transfusions should be considered when platelet counts are less than 50-60 × 10^9/L[28], and the ASGE guidelines recommend platelet transfusions only in case of severe thrombocytopenia[29]. The current American Society of Hematology guidelines recommend the use of corticosteroids as first-line treatment in newly diagnosed adults with a platelet count less than 30 × 10^9/L who are symptomatic or have minor mucocutaneous bleeding. In adults with ITP ≥ 3 mo who are corticosteroid-dependent or unresponsive to corticosteroids, the guidelines suggest treatment with TPO-RA eltrombopag or romiplostim. The guidelines recommend second-line treatment to be individualized, based on duration of disease and patient preferences; however, they provide no recommendations regarding the use of therapies prior to undergoing an invasive procedure or treatment[30].

The updated (2019) International Consensus Report on Management of ITP recommends target platelet counts above 50 × 10^9/L for minor surgery, above 80 × 10^9/L for major surgery, and above 100 × 10^9/L for major neurosurgery[31]. The National Institute for Health and Care Excellence guidelines recommend prophylactic platelet transfusions in patients who are having invasive procedures or surgery, in order to raise the platelet counts above 50 × 10^9/L. They advocate a higher threshold (50-60 × 10^9/L) for patients with a high risk of bleeding who are having invasive procedures or surgery after taking into account the type of procedure, the cause of thrombocytopenia, any coexisting causes of abnormal hemostasis, and whether the patient's baseline platelet count is falling. These guidelines also recommend prophylactic platelet transfusions to raise the platelet counts above 100 × 10^9/L in patients having surgery at critical sites, such as the central nervous system, whereas they do not recommend transfusions for patients having procedures with a low risk of bleeding, such as central venous cannulation or bone marrow aspiration[32]. The American Association of Blood Banks guidelines recommend prophylactic platelet transfusion for minor invasive procedures, such as central venous catheter placement at platelet counts ≤ 20 × 10^9/L and for major non-neuraxial surgery at platelet counts ≤ 50 × 10^9/L, but do not make recommendations for bleeding patients with thrombocytopenia[29].

Downsides of platelet transfusions

The choice of initiating platelet transfusions can also be complicated due to the associated risks. Platelet transfusions have been associated with a number of blood transfusion reactions, the possibility of fatal complications (*i.e.* sepsis), proinflammatory responses, febrile non-hemolytic reactions, and acute lung injury[33]. There is also the risk for development of refractoriness that can prevent further platelet transfusions which can, in turn, lead to decreased survival, prolonged hospital stays, and increased healthcare costs[13,34]. A major limitation of platelet transfusions is the short lifespan of platelets, which require use to occur within 4 d of obtainment to prevent bacterial growth[33]. There is also a shortage of platelets currently, due to the decline in blood collection and utilization[35].

While platelet transfusions can effectively increase platelet counts in the perioperative setting, factors such as alloimmunization or personal beliefs (*e.g.* Jehovah's Witnesses), suggest a need for alternatives[9].

GUIDANCE ON USE OF TPO-RAs

The treatment of patients with thrombocytopenia who require a medical procedure is variable and uncertain, as there are limited data to inform decisions. The aim of the treating physician is to minimize bleeding and improve clinical outcomes. Even though guidelines have no consensus on the threshold for platelet transfusion, most do support raising the platelet levels (to varying degrees) prior to procedures. This, combined with the risk associated with platelet transfusions, may make TPO-RAs an alternative to platelet transfusions in patients with CLD.

An ideal treatment for thrombocytopenia in patients with CLD undergoing an invasive procedure would have the following characteristics: (1) Effectiveness; (2) Oral bioavailability; (3) Minimal adverse effects; and (4) Cost-effectiveness. Neither platelet transfusions nor TPO-RAs satisfy all these requirements. Table 2 lists the key safety and tolerability information for each TPO-RA approved for use. Unlike platelet transfusions, TPO-RAs are not capable of increasing platelet counts rapidly, as the platelet counts increase approximately 5-7 d after and peak approximately 10-14 d after administration of a TPO-RA[36]. Therefore, one potential disadvantage of TPO-RAs is the lag time between dosage and increased platelet numbers, as it prevents the

Table 2 Key safety and tolerability information for thrombopoietin-receptor agonists approved for use[4-7]

Drug	Indication	Boxed warning	AEs	Use in special populations
Romiplostim	Treatment of thrombocytopenia in patients with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy	None	Thrombotic and thromboembolic events; bone marrow fibrosis; most common AEs: Arthralgia, dizziness, insomnia, myalgia, pain in extremity, abdominal pain, shoulder pain, dyspepsia, and paresthesia, headache	Not to be used in pregnancy and during lactation; safety and effectiveness in pediatric patients has not been established; use in geriatric patients with dose adjustment
Eltrombopag	Treatment of thrombocytopenia in adult and pediatric patients 1 yr and older with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy; treatment of thrombocytopenia in patients with chronic hepatitis C to allow the initiation and maintenance of interferon-based therapy; treatment of patients with severe aplastic anemia who have had an insufficient response to immunosuppressive therapy	Risk for hepatic decompensation in patients with chronic hepatitis C; risk of hepatotoxicity	Thrombotic and thromboembolic events; hepatoxicity; monitor liver function before and during therapy; increased risk of death and progression of MDS to AML; common AEs in patients with ITP: Nausea, diarrhea, upper respiratory tract infection, vomiting, increased ALT, myalgia and urinary tract infection	Not to be used in pregnancy and during lactation; safety and effectiveness in pediatric patients 1 yr and older with chronic ITP has been established; use in geriatric patients has not been studied in clinical trials; reduced initial dose recommended for patients of East Asian ancestry with ITP
Avatrombopag	Treatment of thrombocytopenia in adult patients with chronic liver disease who are scheduled to undergo a procedure	None	Thrombotic and thromboembolic complications: Monitoring platelet counts essential; common AEs: Pyrexia, abdominal pain, nausea, headache, fatigue, and edema peripheral	Not to be used in pregnancy and during lactation; safety and effectiveness in pediatric patients has not been established; use in geriatric patients has not been studied in clinical trials
Lusutrombopag	Treatment of thrombocytopenia in adult patients with chronic liver disease who are scheduled to undergo a procedure	None	Thrombotic and thromboembolic complications: Monitoring platelet counts essential; most common AE: headache	Not to be used in lactating patients; use in geriatric patients has not been studied in clinical trials; safety and effectiveness in pediatric patients has not been established

AE: Adverse event; AML: Acute myeloid lymphoma; ITP: Immune thrombocytopenia; MDS: Myelodysplastic syndrome.

use of TPO-RAs in emergent situations. The advantages of TPO-RAs include oral bioavailability (except in case of romiplostim, which is administered subcutaneously), their ability to increase platelet counts endogenously, and sustaining the increase over a longer duration compared to that produced by platelet transfusions and thereby reducing the need for rescue therapy while causing fewer procedure-related complications than platelet transfusions. Avatrombopag and lusutrombopag are currently the only TPO-RAs approved for the perioperative treatment of thrombocytopenia in patients with CLD, and compared with romiplostim and eltrombopag, they are associated with a decreased risk of thromboembolic and hepatic events. Nevertheless, platelet counts must be measured during treatment, as counts higher than 200 × 10^9/L have been shown to be associated with serious AEs, such as PVT. Both avatrombopag and lusutrombopag may be used in cirrhotic patients with low platelet counts scheduled to undergo an elective procedure with bleeding risks and in patients who cannot undergo platelet transfusions (Figure 1). Depending on the pre-procedure platelet counts, patients should be treated with either 40 mg or 60 mg of avatrombopag; the dose of lusutrombopag is always 3 mg. The window for undergoing procedures following dosing with avatrombopag and lusutrombopag is between 9-13 d and 8-14 d, respectively. It must be noted that while clinical trials with avatrombopag and lusutrombopag excluded patients with PVT, a PVT-prescreen is

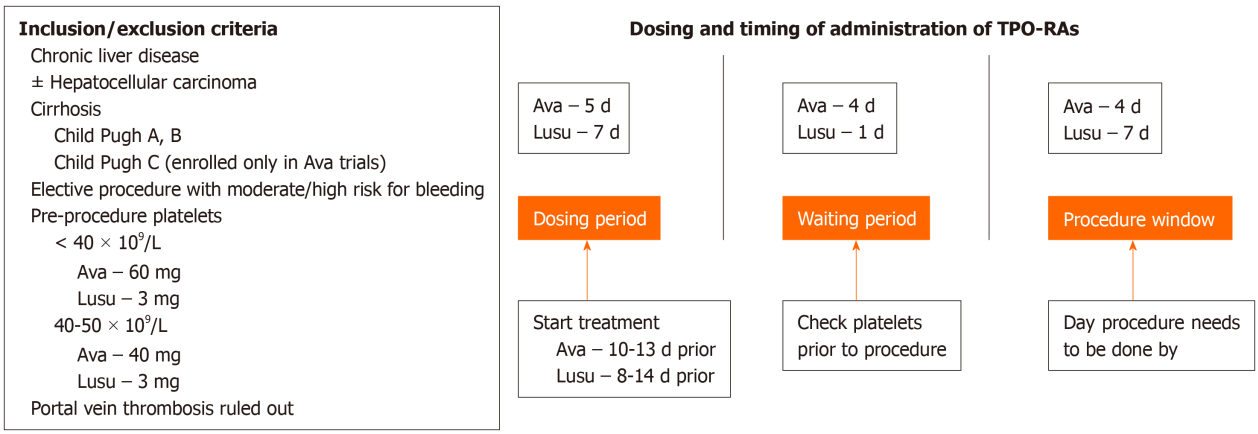


Figure 1 Patient criteria and general schema for administration of oral thrombopoietin-receptor agonists in patients with chronic liver disease and thrombocytopenia scheduled to undergo a procedure. Ava: Avatrombopag; Lusu: Lusutrombopag; TPO-RAs: Thrombopoietin-receptor agonists.

not a requirement as per labeling guidelines for both these TPO-RAs. Patients with liver cirrhosis and high Child-Turcotte-Pugh scores are more likely to have severe thrombocytopenia and also require invasive diagnostic procedures. Extreme caution must be taken when using TPO-RAs in such patients, as they are at high risk of developing PVT; currently, no data on the effects of the TPO-RAs avatrombopag and lusutrombopag in this class of patients are available, as they were excluded from the clinical trials[14,16-18].

There are also pricing differences between avatrombopag and lusutrombopag; in the United States, the wholesale acquisition cost (WAC) of avatrombopag for a 5-d course is $2970 for the 40-mg dose and $4455 for the 60-mg dose. The WAC for a 7-d course of lusutrombopag is $8500[37].

CONCLUSION

Recommendations for procedure-specific minimum platelet counts prior to a scheduled procedure often have inadequate supporting evidence, and the benefit of prophylactic platelet transfusion remains unclear. Physicians typically assess the need for platelet transfusions on a patient-to-patient basis, relying on their own experience and patient comorbidities to inform their decisions. TPO-RAs are a safe and effective alternative treatment option for patients with thrombocytopenia who are undergoing a medical procedure. They are associated with increased platelet counts, decreased bleeding events, and a reduced need for rescue treatments. Recent studies also support the safe and efficacious repeated use of TPO-RAs in patients with CLD. The exact fit of TPO-RAs, as compared with platelet transfusions, in patients with thrombocytopenia prior to undergoing a medical procedure is still unclear. Comparative clinical trial data in patients with thrombocytopenia are required to assess the efficacy, safety, cost-effectiveness, and impact on patient quality of life of TPO-RA use compared with the traditional use of platelet transfusions prior to scheduling a medical procedure. In the meantime, treatment decisions must be individualized by establishing the advantages and disadvantages of both these treatment options on a case-by-case basis.

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MINIREVIEWS

Role of non-coding RNAs in pathogenesis of gastrointestinal stromal tumors

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Abstract

Gastrointestinal stromal tumors (GISTs) are considered the model solid malignancies of targeted therapy after the discovery of imatinib effectiveness against their tyrosine kinase inhibitors. Non-coding RNAs are molecules with no protein coding capacity that play crucial role to several biological steps of normal cell proliferation and differentiation. When the expression of these molecules found to be altered it seems that they affect the process of carcinogenesis in multiple ways, such as proliferation, apoptosis, differentiation, metastasis, and drug resistance. This review aims to provide an overview of the latest research papers and summarize the current evidence about the role of non-coding RNAs in pathogenesis of GISTs, including their potential clinical applications.

Key words: Gastrointestinal stromal tumors; Non-coding RNA; MicroRNA; Transcriptomics; Biomarker; Long non-coding RNAs

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Core tip: There are several excellent reviews at the last decade contributed the role of non-coding RNAs in gastrointestinal stromal tumors (GISTs) carcinogenesis. However, until now, most of them focused only on the microRNAs characteristics. Recently there has been a substantial motion in understanding the role of other non-coding RNAs in GIST progress, like the long non-coding RNAs. This review provides an overview of both microRNAs and long non-coding RNAs role in GIST progression, their potential

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therapeutic use, their ability to predict drug sensitivity and many other aspects concerning GIST development.

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INTRODUCTION

Non-coding RNAs

The discovery of transfer RNA (tRNA), and ribosomal RNA (rRNA), in the 1950s is the beginning of the history of the non-coding RNAs (ncRNAs) that play functional roles in the eukaryotic cells[1]. James Watson imagined the one gene, one ribosome and one protein hypothesis (central dogma). Therefore, RNA changed from being a just information carrying molecule, to having three flavors. rRNA, tRNA and everything else was assumed to be mRNA[2]. Later on, in the 70s Stark *et al*[3] published the existence of other functional RNAs like ribonuclease P and snRNAs[4]. One of the prominent examples about how huge was the surprise at this period of time, was the eventual renaming of signal recognition protein to signal recognition particle (SRP-RNA). That happened after the discovery, that it contains a 7S RNA (by Walter *et al*[5]). In the early 90s, other long intergenic non coding RNAs were discovered, like XIST, by Brockdorff *et al*[6] Nowadays, it is generally known, according to the encyclopedia of DNA elements (published by the ENCODE Project[7]) consortium that the 80% of the human genome is transcripted for RNA molecules that have no protein coding capacity[8]. In the past, it was believed that this huge amount of RNA molecules was a transcriptional noise. Contrariwise, they appear to have direct function as regulators in several endocytic molecular paths. They seem to play crucial role in differentiation, development, and apoptosis of normal cells[9], so even in the era of complete genome sequences, non-coding RNAs gene have been eventually invisible. These features of non-coding RNAs have turned them into one of the most promising fields of scientific research.

ncRNAs are classified into two big subgroups according to their size[10].

Short ncRNAs, with < 200 nucleotides (nts) in length and include: MicroRNAs (miRNAs) usually bind to a specific molecular locum at the mRNA to induce degradation or block the prosses of translation. In addition, this may be done in the context of a feedback mechanism that involves chromosome methylation.

Small interfering RNAs (siRNAs) have a similar function as miRNAs with the additional feature of inducing heterochromatin formation through RNA transcriptional silencing complex which, when bound to siRNA, promotes H3K9 methylation and chromatin condensation.

Piwi-interacting RNAs seem to interact with the piwi family proteins. They involve in chromatin regulation and suppression of transposon activity in germline and somatic cells[11].

Long ncRNAs (lncRNAs) are longer than 200nt and may comprise thousands of nucleotides[12]**:** This group includes the long intergenic ncRNAs (lincRNAs), the natural antisense transcript, the transcripted ultraconserved regions and non-coding pseudogenes[13]. It seems to be transcribed mostly by RNA polymerase 2 as the mRNA does but they do not undergo the standard processing steps[14]. The mechanism of their function is generally unknown, but it is suggesting that it is similar to that of HOX antisense intergenic RNA (HOTAIR) which is the most studied lncRNA. It regulates chromatin methylation of the HOXD locus through polycomb repressive complex 2. HOTAIR was recently reported to play a crucial role in metastatic disease and may be a good prognostic marker in patients with breast cancer[15].

Post-transcriptional modifications that occur in RNA molecules started being explored at the recent years and therefore led to a new field of research called epitranscriptomics. Equivalent to epigenetics, which analyzes the post-transcriptional events occurring in DNA, epitranscriptomics investigates modifications resulting from all RNA processing events, such as RNA splicing, RNA editing, or methylation[16].

Gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GISTs) are specific, generally c-Kit (CD117)-positive, mesenchymal tumors of the gastrointestinal tract, encompassing a majority of tumors previously considered gastrointestinal smooth muscle tumors[17]. They are believed to originate from interstitial cells of Cajal or related stem cells. Interstitial cells of Cajal and GIST cells express the hematopoietic progenitor cell marker CD34 and the growth factor receptor c-Kit. Expression of the c-Kit gene protein product, CD117, has emerged as an important defining feature of GISTs[18,19]. Using these criteria, the incidence of GISTs has been estimated to be 6 to 15 cases per million individuals per year[20]. They constitute a significant percentage ranging from 1%-2% of all the gastrointestinal neoplasms. The most common genetic alterations found in GISTs include mutations of growth factors genes such as c-Kit (70–80%) and PDGFRA (platelet-derived growth factor A, 5%-8%). Several features of GISTs have been postulated in the past to predict their clinical behavior. Nowadays, much is known about the histological, immunohistochemical and molecular aspects of GISTs especially in diagnostic purposes[21,22]. However, little is known about the clinicopathological features that can predict the biological behavior of these tumors.

At the recent years, plenty of studies have revealed the specific molecular characteristics of GISTs. Nowadays, these tumors are considered among the best genetically understood human cancers[23].

Especially after the discovery of their sensitivity to tyrosine kinase inhibitors, GISTs tend to be referred as ideal tumor for novel molecular targeted therapies. Apart from that, the fact that many studies have been published specific chromosomal changes (*e.g.* loss of 14q), genetic mutations (*e.g. KIT*, *PDGFRA*), gene expression profiles (*e.g. ETV1*, *fascin1*) and miRNA expression profiles, have contributed to make them one of the well-recognized tumors[24]. It is important to mention that *KIT* and *PDGFRA* mutations are almost exclusive in GISTs, which makes them specific biomarkers of these tumors. The gold standard therapy in primary localized GISTs is a R0 surgical resection[25]. First line therapy for the advanced disease is Imatinib that offers a dramatic response, in most of the cases, for about 2-3 years[26]. After long term treatment, resistance is quite common. Sunitinib and regorafenib are the second line agents in imatinib resistant GISTs with also unsatisfactory outcomes in progressive disease[27]. Therefore, further fundamental clinical studies are being conducted in order to provide improved diagnostic modalities to increase the possibility for the patients to be diagnosed in early disease, and furthermore provide novel therapeutic options for the advanced disease cases.

NcRNAs in GISTs

At the present, a clear relationship with GISTs has been reported for only a few ncRNA classes, especially miRNAs and some lncRNAs such as the ultra-conserved genes, *HOTAIR*, *H19*, *MALAT1* and *CCDC26*[28,29]. The other types of ncRNAs it seems to participate in the genetic puzzle that gives rise to carcinogenic phenotype[13].

miRNAs are the most widely studied class of ncRNAs in GISTs and generally in human cancer. These small ncRNAs of approximately 22 nucleotides, mediate post-transcriptional gene silencing by controlling the translation of mRNA into proteins. miRNAs are estimated to regulate the translation of more than 60% of protein-coding genes[30]. They are involved in regulating many processes, including proliferation, development, differentiation, and apoptosis. Alterations of miRNAs expression profile has been reported in GISTs, and is associated with tumor location, mutation status, tumor risk, and chromosomal changes[31]. Two excellent reviews by Nannini *et al*[32], and Kupcinskas *et al*[33] have perfectly analyzed relevant miRNA profiling studies. Since then several papers came out concerning ncRNA and GISTs.

STUDIES SELECTION

This review included all studies published in PubMed database related to the role of ncRNAs in GIST published from 2008 to 2020. The keywords we used to retrieve the papers were GIST, ncRNAs, miRNAs and lncRNAs. 82 papers selected using these keywords. According the selection criteria, only 52 of them were relevant to the topic, 32 profiling studies, 9 reviews, 11 other studies (Figure 1).

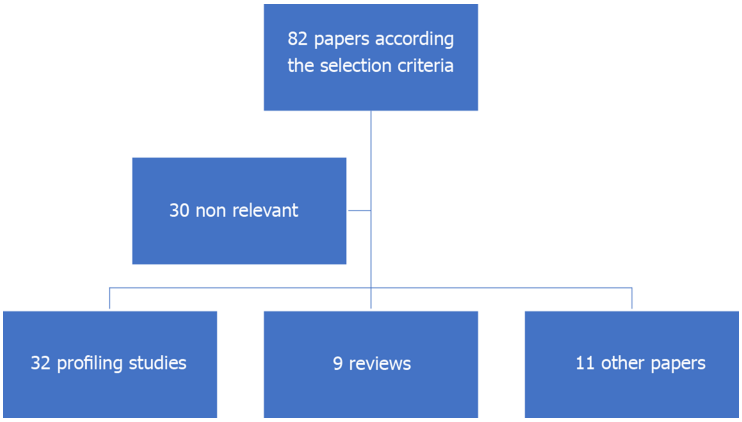


Figure 1 Studies selection.

CHROMOSOMAL LOSS OF 14q AND MIRNA EXPRESSION

Chromosomal deletions have been reported as frequent and characteristic aberrations and are related to the carcinogenesis of the GISTs[34]. The most common described are in 14q, 22q, and 1p. Among them, partial or entire chromosomal loss of 14q is the most frequently found (60%–70%) and represents the majority of gastric GISTs, while 1p loss is usually present in small bowel GISTs[35] and its characterized by poor clinical outcome[36]. None of the other common chromosome eliminations[37] (22q, 1p) seems to affect the miRNAs expression profile. Table 1 summarizes the studies related to chromosomal loss of 14q and miRNAs expression. miRNAs seem to form two distinct clusters on the 14q chromosome. A study by Choi *et al*[38] published in 2010, identified a clear correlation between the 14q loss and deregulation of miRNA expression profile in 20 tumors. They noticed that, 6 GISTs that did not have 14q loss, formed a separate cluster. Furthermore, they found 73 deregulated miRNAs at a significant level according to 14q loss status. Among the 73 miRNAs, 38 were encoded on 14q. Kelly *et al*[39] studied a cluster of miRNAs on 14q32 region and revealed similar downregulated miRNAs according to 14q loss statue, in both adult and pediatric patients, but distinguish miRNA expression pattern between the adult and pediatric GISTs. They suggest that this happens due to the different methylation state of the maternal and paternal allele during the aging. Another study by Haller *et al*[40] identified 44 miRNAs located at 14q32.31 chromosomal region. Moreover, in a qRT-PCR analysis of additional 49 GIST, the authors observed a significant lower expression of miRNA-134 and miRNA-370 in GIST with 14q loss. As mentioned above these miRNAs found to affect the mutational status of *KIT* and *PDGRFA*, and some of them including miRNA-494 are experimentally confirmed to target *KIT* or *PDGFRA*. Deregulation of these miRNAs were associated with tumor progression and shorter disease-free survival, suggesting that GIST with low expression of miRNAs located at the 14q32.31 chromosomal loss might represent o group with higher risk of tumor progression[36].

POTENTIAL DIAGNOSTIC AND PROGNOSTIC BIOMARKERS

GISTs are considered among the best recognized tumors, regarding their specific phenotypic and molecular characteristics. The diagnosis relies on the specific morphology and the unique immunohistochemistry (*CD117*, *CD34* and/or *DOG1*). Although, despite the high specific value of these biomarkers, in many cases, the diagnosis may be difficult. Table 2 summarizes the studies concerning ncRNAs as new emerging novel biomarkers, highly specific to GISTs. First of all, Subramanian *et al*[41] founded 16 upregulated and 10 downregulated miRNAs specifically in GISTs. In this study, they compared 84 miRNAs (that met the filtering criteria) expression status of 27 mesenchymal tumors (including GISTs), 5 normal smooth muscle and 2 normal skeletal muscle. Remarkably, the miRNA expression patterns suggested that two of the mesenchymal tumors had been misdiagnosed and this was confirmed by reevaluation of the tumors using immunohistology and molecular analyses. These findings demonstrated that miRNA expression profiling is unique for each tumor type, suggesting the potential use of miRNAs as diagnostic biomarkers.

Table 1 Chromosomal loss of 14q and miRNA expression studies

Ref.	Samples	miRNAs studied	Results
Choi *et al*[38], 2010	20 GISTs (15 gastric, 5 intestinal)	73	38 miRNAs encoded at 14q region
Haller *et al*[40], 2010	12 GISTs for microarray analysis and then 49 GISTs for qRT-PCR analysis	miR-370; miR-134	Downregulated in GISTs with 14q loss
Kelly *et al*[39], 2013	73 GISTs 47 adult and 18 pediatric	667	74 downregulated miRNAs in GISTs with 14q loss

GISTs: Gastrointestinal stromal tumors.

Koelz *et al*[42] were the first who found significant depressed the 220/221 miRNAs compared to peripheral healthy tissue and blood samples. Niinuma *et al*[43], after the examination of 56 GISTs founded that, overexpression of miRNA-196a and *HOTAIR* was associated with high-risk grade, metastasis, and poor survival among GISTs. Yamamoto *et al*[44] later in 2013 published a clear correlation between fachin-1 overexpression and miRNA-133b downregulation in the progression of gastrointestinal stromal tumor, making fascin-1 as a useful potential biomarker to predict the aggressive behavior. Another two studies by Haller *et al*[40] and Gits *et al*[45] are coming to confirm the downregulation of these two miRNAs 220/221 specific in GIST. However, according to the findings of all the previously mentioned studies the 220/221 miRNAs may not have had any impact on routine diagnostics because KIT-positive and KIT-negative GIST exhibited a completely inverse expression pattern. One recent study by Gyvyte *et al*[46] 2017, the first one which used the next generation sequencing kit in order to reveal deregulated miRNAs in GISTs and their possible associations with oncogenes. They found 19 deregulated miRNAs, 13 of which were not previously reported. They also proposed miRNA-215-5p to be negatively correlated with the risk grade, while miRNA-509-3p to be associated with epithelioid and mixed histological subtypes. The same research team, one year later (2018)[47], found a significant correlation between a lincRNA H19 and GIST oncogene ETV1, and between H19 and miRNA-455-3p. A Polish study, by Kosela-Paterczyk *et al*[48], aimed to identify the miRNA expression profiles in four common soft tissue tumors. They also founded different miRNA signatures in serum samples in each soft tissue tumor, included GISTs. At the recent years, many studies came out regarding the lncRNAs and their task in GIST progression. A Chinese study by Hu *et al*[49], questioned for the first time about the role of lncRNA AOC4P in GIST development. They identified that AOC4P regulate the epithelial mesenchymal transition (EMT) related proteins, which is important step for the metastatic ability of the tumor cells. One year later Badalamenti *et al*[50], questioned about the role of H19 and MALAT1 in GISTs. They found high expression levels of both lncRNAs in tumor samples which could be associated with prognosis and clinical response to IM. Yan *et al*[51] in a latest study through a microarray analysis, compared 3 metastatic GISTs with 3 normal tissue and 3 low grade GISTs and found significant expression of certain lncRNAs, including lnc-DNAJC6-2 in high risk tumors.

THE ROLE OF NON-CODING RNAS IN IMATINIB RESISTANCE

Numerus studies (Table 3) have been released about the imatinib resistance GISTs and their potential prognostic biomarkers. Overexpression of miRNA-196a in GIST tissues was associated with high-risk grade, metastasis, and poor survival. Akçakaya *et al*[52] highlighted a novel functional role of miRNA-125a-5p on imatinib response. They experimentally showed that overexpression of miRNA-125a-5p suppressed *PTPN18* expression and furthermore this eventually increased the GIST cells viability upon imatinib treatment. Almost the same research team (Huang *et al*[53] 2018) evaluated phosphorylated FAK (pFAK) as a candidate target of PTPN18. They revealed a downstream regulation of pFAK and direct association with imatinib resistance. Fan *et al*[54] explored the role of miRNA-218 on imatinib resistance GIST cells and they found a clear correlation between the downregulation of miRNA-218 and imatinib resistance. They also proposed that, miRNA-218 overexpression can improve the sensitivity of GIST cells to imatinib mesylate, with PI3K/AKT signaling pathway

Table 2 Non-coding RNAs as potential prognostic biomarkers of gastrointestinal stromal tumors

Ref.	Compared groups	ncRNAs studied	Results and potential prognostic biomarkers
Subramanian *et al*[41], 2008	8 GISTs compared to 19 mesenchymal tumors	84 miRNAs	**16 upregulated miRNAs:** miRNA-10, miRNA-22, miRNA-29a, miRNA-29b, miRNA-29c, miRNA-30a-5p, miRNA-30e-5 miRNA-30c, miRNA-30d miRNA-99b miRNA-125a miRNA-140, miRNA-143, miRNA-145 miRNA-368 ABI-13268 let-7b, miRNA-1; **10 downregulated miRNAs:** miRNA-1 miR-92 miRNA-133a, miRNA-133b miRNA-200b miRNA-221, miRNA-222 miRNA-368, miRNA-376a ABI-13232
Haller *et al*[40], 2010	4 gastric PDGFRAmut, 4 gastric KITmut and 4 intestinal KITmut. 49 GISTs further analyzed by qRT-PCR	734 miRNAs	Downregulated miRNA-221 and miR-222 in in KIT-mutant GIST compared with KIT/PDGFRA wild type GIST
Koelz *et al*[42], 2011	54 GISTs compared to healthy blood samples	miRNAs-22/-222	Depressed miRNA-221 and 222 in kit positive tumor samples, whereas Kit-negative GISTs exhibited a completely inverse expression pattern
Niinuma *et al*[43], 2012	56 GISTs	939 miRNAs	Association of miR-196a and HOTAIR with high risk tumors, metastasis, and overall survival
Yamamoto *et al*[44], 2013	4 low grade *vs* 4 intermediate *vs* 11 high grade GISTs	904 miRNAs	Downregulation of miR-133b in high grade tumors and correlation with Fachin-1 overexpression
Gits *et al*[45], 2013	50 GISTs compared to 10 gastrointestinal leiomyosarcomas	725 miRNAs	Downregulated miR-17-92 and miRNAs 221/222 in tumor samples
Gyvyte *et al*[46], 2017	15 GISTs compared to 15 samples of adjacent tissue	1672 miRNAs	15 downregulated and 4 upregulated miRNAs; miRNA-215-5p negative correlation with the grade; miRNA-509-3p association with epithelioid and mixed subtypes
Gyvyte *et al*[48], 2018	15 gastric GISTs *vs* 15 adjacent tissue through next generation seq and then validation analysis of 22 more GISTs	7250 lincRNAs	6 upregulated lincRNAs, 3 downregulated lincRNAs; Strong correlation between expression of lincRNA H19 with both ETV1 and miR-455-3p
Hu *et al*[49], 2018	79 GISTs *vs* 79 paracancerous normal tissues	LncRNA AOC4P	Increased in GIST *vs* normal tissue, Higher expression in high risk *vs* low/medium risk. AOC4P regulate EMT thus increase the metastatic ability of the tumor
Yan *et al*[51], 2019	3 primary GISTs (A) *vs* 3 GISTs secondarily resistance to IM (B) *vs* 3 normal gastric tissue (C)	63,542 lncRNAs 27,134 miRNAs	2250 deregulated lncRNAs on group B *vs* group A; 2209 deregulated lncRNAs on group C *vs* group A; 922 deregulated lncRNAs on group C *vs* group B
Badalamenti *et al*[50], 2019	40 GISTs (25 localized disease *vs* 15 advanced disease)	H19, MALAT1	H19 and MALAT1 higher expression levels in advanced disease samples
Kosela-Paterczyk *et al*[48], 2020	31 high grade GISTs treated with IM, 16 high grade OS, 26 high grade SS, 8 high grade ES, 30 healthy controls	156 dysregulated miRNAs in sarcomas *vs* control group	10 microRNAs were commonly deregulated in SS, OS and GISTs; 99, 42, 36 and 24 differentiated controls from GISTs, ES, SS and OS, respectively

GISTs: Gastrointestinal stromal tumors; OS: Osteosarcoma; SS: Synovial sarcoma; ES: Ewing sarcoma.

possibly involved mechanism. Lee *et al*[55] revealed that HOTAIR is upregulated is GISTs and can promote GIST cell metastatic status *in vitro*. HOTAIR found to regulate promoter methylation of protocadherin 10 (PCDH10) and promote tumor invasion status. Bure *et al*[56] come to confirm the correlation of HOTAIR and tumor aggressiveness and propose specific methylation patterns caused by the upregulation

Table 3 Studies about the role of non-coding RNAs expression profile and imatinib resistance

Ref.	Compared groups and samples	NcRNAs studied	Results
Akçakaya *et al*[52], 2014	7 IM resistant *vs* 10 IM sensitive (profiling analysis) 10 IM resistant *vs* 14 IM sensitive (validation analysis)	903 miRNAs in profiling analysis (microarray) 10 miRs for validation analysis (RT-PCR)	27 overexpressed miRNAs and 17 underexpressed miRNAs in IM resistant group compared to IM sensitive. Mir-125a-5p as a key modulator to IM resistance
Huang *et al*[53], 2018	28 tumor samples (all patients received neoadjuvant IM)	miRNA-125a-5p RNU6B	Phosphorylation of FAK is regulated by PTPB18 and miR-125a-5p. Pfak plays crucial role in IM resistance
Fan *et al*[54], 2015	IM sensitive GIST cells (GIST882) *vs* IM resistance cell line (GIST430)	miRNA-218	MiR-218 is down-regulated in IM-resistant GIST430 cells; MiR-218 over-expression may improve the IM sensitivity through PI3K/AKT signaling pathway
Lee *et al*[55], 2016	9 low *vs* 1 intermediate *vs* 7 high risk tumors.	HOTAIR	HOTAIR higher expression in high risk GISTs. HOTAIR also found to regulate promoter methylation of PCDH10 through in vitro investigation of high-risk GIST cell lines
Bure *et al*[56], 2018	67 primary GIST samples subdivided according the tumor grade and the cell line.	HOTAIR	HOTAIR higher expression in high risk GISTs. Distinct methylation patterns through upregulation of HOTAIR during the different stages of carcinogenesis
Yan *et al*[51], 2019	3 primary GISTs (A) *vs* 3 GISTs secondarily resistance to IM (B) *vs* 3 normal gastric tissue (C)	63542 lncRNAs 27134 miRNAs	They found lnc-DNAJC6-2 to be associated with the HIF-1 pathway
Yan *et al*[58], 2019	IM sensitive cell lines (GIST-882) *vs* IM resistance cell lines (GIST-T1)	LncRNA CCDC26	LncRNA CCDC26 regulate IM resistance and interact with IGF-1R

GISTs: Gastrointestinal stromal tumors; IM: Imatinib.

of HOTAIR during the progression of carcinogenesis. Zhang *et al*[57] proposed Hsa-miRNA-28-5p and hsa-miRNA-125a-5p to be involved in the development and progression of GIST and therefore may be able to serve as prognostic markers for imatinib-response in GIST patients. Yan *et al*[58] In their study they found that lncRNA CCDC26 induced imatinib resistance and decreased imatinib induced apoptosis. These results introduced lncRNA CCDC26 to be a possible target to reverse IM resistance. The same author[51] also proposed lnc-DNAJC6-2 to be associated with the HIF-1 pathway. HIF-1 is responsive for the modulation of over 200 genes that are associated with proliferation, cycle arrest, apoptosis, and drug efflux. Therefore, investigating molecules that target the HIF-1 pathway may identify a novel treatment strategy.

There have been observations that miRNAs constantly export from cells and circulate in body fluids as a part of a lipoprotein complexes called exosomes, containing miRNAs and proteins[59]. Furthermore, to date, there is no study looking at the role of circulating miRNAs in GIST patients, which is essential for the potential clinical use.

GENE REGULATING NON-CODING RNAS AND THEIR ROLE IN GIST CARCINOGENESIS

miRNAs are thought to act as regulators in gene expression. Although *KIT* gene mutations and KIT protein overexpression are the main genetic characteristics of GISTs, little is known about the mechanism of KIT overexpression. It is essential to identify molecules that regulate c-KIT and other relative genes as they could be excellent candidates for future clinical trials on GIST treatment. Plenty of recent studies suggesting that miRNAs directly regulate KIT protein expression levels and inhibit cell proliferation in GISTs. Felli *et al*[60] reported, in 2005, the downregulation of KIT receptor by miRNA-221/miRNA-222 in erythroleukemic cells. MiRNA-221 and miRNA-222 are highly homologous miRNAs, whose upregulation has been recently described in several types of human tumors. Later studies have been proposed them as oncomirs, acting by targeting tumor suppressor genes such as PTEN, TIMP3 p57, $p27^{Kip1}$ and BIM[61]. MiRNA-221/222 overexpression induces cell proliferation through the activation of cell cycle and the Akt pathway and blocks TRAIL-induced apoptosis. Koelz *et al*[42] was the first to show that miRNA-221 and miRNA 222 act as regulators of Kit protein expression in GISTs and hence reveals a new aspect in the molecular pathogenesis of these tumors. They found a completely inverse expression among KIT positive and KIT negative tumors. Further studies came to correspond this by the

observation that miRNA-222 and miRNA -17/20a directly target KIT and ETV1 in GISTs. MiRNA-494 is proposed as a potential KIT targeting miRNA by Kim *et al*[62]. This study showed that miRNA-494 is a negative regulator of KIT in GISTs and an overexpressing miRNA-494 may be a promising approach to GIST treatment. Gits *et al*[45] published that miRNA-17, miRNA-20a directly target KIT. They also showed that overexpression of these two miRNAs induced apoptosis and significantly inhibited cell proliferation. Interestingly they did not found correlation of miRNA494 and KIT expression like Kim *et al*[62] did! Lu *et al*[63] founded at their study, that miRNA-152 induced cell apoptosis, prevents cell proliferation and migration by repressing cathepsin L, suggesting miRNA-152 an attractive anti-tumor agent. In a latest study, Badalamenti *et al*[50] founded that the expression levels of *MALAT1* lncRNA seem to affect the c-KIT mutational status. A recent Chinese study by Long *et al*[64], indicated that miRNA-374b inhibits apoptosis promotes viability of GIST cells by targeting *PTEN* gene through the PI3K/Akt signaling pathway. Another similar study[65] focused on the effects of neferine, an alkaloid derivative of lotus plant, in GIST development. They interestingly founded that neferine possibly upregulate miRNA-449a and then inactivate the PI3K/AKT and Notch pathways and by this mean suppress growth and migration of GIST cells. A latest paper came out from Chen *et al*[66]. Their results suggested that miR-4510 downregulation could promote GIST development, including growth, metastasis and invasion, through increasing *APOC2* expression. Needless to say that much more scientific effort is needed in order to clarify the exact role of non-coding RNAs in GIST carcinogenesis and their interaction with tumor related genes and the respectively molecular endocytic paths.

POTENTIAL ROLE OF NON-CODING RNAS IN GIST TREATMENT

The potential role of ncRNAs as treatment tools against cancer has been explored through many studies during the recent years. The main treatment strategies aim to inhibit cell proliferation by importing exogenous ncRNAs through viral vectors (adenoviral, lentiviral and rectoviral vectors), which are mainly tumor suppressor miRNAs[67]. A recent study by Tu *et al*[68] suggested miR-218 loaded nanoparticle as tumor suppressor miRNA in GIST. Another study by Durso *et al*[69] proposed modified miRNAs 221/222 as effective inhibitors of KIT. Nowadays it is generally accepted that miRNAs can act as oncogenes or tumor suppressor genes. For this reason, it seems reasonable to manipulate those molecules against the carcinogenetic process. For example, synthesized miRNAs mimics imports into the cells and enhance endogenous miRNA function (antagomirs)[70]. Another strategy is proposed for the inhibition of over-expressed oncogenic miRNAs (oncomirs), by the use of antisense oligonucleotides[71]. This strategy includes inhibition or replacement of miRNAs through anti-miRNA oligonucleotides, antagomirs, miRNA sponges and nanoparticles. Only a few of the investigated miRNAs are currently in phase 2 stage[72]. But it must be pointed out that, up to now, although they have been shown remarkable success in *in vitro* models, none of these particles have been tested in GIST clinical trials.

CONCLUSION

A huge amount of preclinical data introduces non-coding RNAs as a new weapon against cancer in biomedical sciences armamentarium, although many efforts need to be done in order to understand the role of epitranscriptomics in GISTs. Especially for GISTs, numerus studies identified association patterns among specific ncRNAs with subsequent phenotypic characteristics. NcRNAs related to the tumor progression, grade, site, chromosomal eliminations, and imatinib sensitivity could probably be of importance as diagnostic or prognostic tumor biomarkers. *In vitro* studies revealed some of the mechanisms of action of these molecules. The endocytic paths could be served as guidance for future targeted drugs, acting as interfering or enhancing molecules. In addition, published data concerning GISTs and ncRNAs is based mainly on *in vitro* cell lines and fresh frozen paraffin-embedded tumor tissue blocks, thus necessitating high quality, randomized, multicentric clinical studies at a large scale of patients.

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SYSTEMATIC REVIEWS

Exclusive cigar smoking in the United States and smoking-related diseases: A systematic review

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Abstract

BACKGROUND

Little information has been published on the risks of cigar smoking. Since 1990 cigar smoking has become more prevalent in the United States.

AIM

To summarise the evidence from the United States relating exclusive cigar smoking to risk of the major smoking-related diseases.

METHODS

Literature searches detected studies carried out in the United States which estimated the risk of lung cancer, chronic obstructive pulmonary disease (COPD), heart disease, stroke or overall circulatory disease in exclusive cigar smokers as compared to those who had never smoked any tobacco product. Papers were identified from reviews and detailed searches on MEDLINE. For each study, data were extracted onto a study database and a linked relative risk database. Relative risks and 95%CIs were extracted, or estimated, relating to current, former or ever exclusive cigar smokers, and meta-analysed using standard methods. Sensitivity analyses were conducted including or excluding results from studies that did not quite fit the full selection criteria (for example, a paper presenting combined results from five studies, where 86% of the population were in the United States).

RESULTS

The literature searches identified 17 relevant publications for lung cancer, four for COPD and 12 for heart disease, stroke and circulatory disease. These related to 11 studies for lung cancer, to four studies for COPD and to eight studies for heart

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disease, stroke or overall circulatory disease. As some studies provided results for more than one disease, the total number of studies considered was 13, with results from four of these used in sensitivity analyses. There was evidence of significant heterogeneity in some of the meta-analyses so the random-effects estimates are summarized below. As the results from the sensitivity analyses were generally very similar to those from the main analyses, and involved more data, only the sensitivity results are summarized below. For lung cancer, relative risks (95%CI) for current, former and ever smokers were respectively, 2.98 (2.08 to 4.26), 1.61 (1.23 to 2.09), and 2.22 (1.79 to 2.74) based on 6, 4 and 10 individual study estimates. For COPD, the corresponding estimates were 1.44 (1.16 to 1.77), 0.47 (0.02 to 9.88), and 0.86 (0.48 to 1.54) based on 4, 2 and 2 estimates. For ischaemic heart disease (IHD) the estimates were 1.11 (1.04 to 1.19), 1.26 (1.03 to 1.53) and 1.15 (1.08 to 1.23) based on 6, 3 and 4 estimates, while for stroke they were 1.02 (0.92 to 1.13), 1.08 (0.85 to 1.38), and 1.11 (0.95 to 1.31) based on 5, 3 and 4 estimates. For overall circulatory disease they were 1.10 (1.05 to 1.16), 1.11 (0.84 to 1.46), and 1.15 (1.06 to 1.26) based on 3, 3 and 4 estimates.

CONCLUSION
Exclusive cigar smoking is associated with an increased risk of lung cancer, and less so with COPD and IHD. The increases are lower than for cigarettes.

Key words: Tobacco products; Cigar smoking; Lung neoplasms; Pulmonary disease; Chronic obstructive; Heart diseases; Stroke; Circulatory disease; Systematic review; Meta-analysis

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Core tip: Thirteen studies in the United States presented evidence relating exclusive cigar smoking to risk of lung cancer, chronic obstructive pulmonary disease (COPD) and/or circulatory disease. Compared to never smokers, current exclusive cigar smoking increased risk of lung cancer about three-fold, COPD by about 40% and heart disease by about 10% but did not increase risk of stroke. These increases are much lower than those for cigarette smoking.

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INTRODUCTION

There is extensive evidence on the relationship of cigarette smoking to health endpoints but far less evidence relating to cigar smoking. While some studies have reported results relating to dual use of cigars and pipes[1-4] or to cigar smoking in those who may also smoke other tobacco products[5], the health effects of exclusive cigar smoking have been less often reported. Comparing disease risk in cigar smokers who have never smoked other tobacco products with that in never smokers of any tobacco product avoids the problems of residual confounding by other smoking habits and of possible differences in cigar smoking habits (such as depth of inhalation[6]) in those who have ever smoked other tobacco products.

Exclusive cigar smoking is much less common than cigarette smoking so the population studied must be large enough to include enough exclusive cigar smokers for a useful risk assessment to be made. For this reason we have restricted attention to studies in the United States, a country not only with a large population, but one where cigar smoking is relatively common compared with other countries[7,8]. We also restrict attention to the major smoking-related diseases.

MATERIALS AND METHODS

Study inclusion criteria

Published studies were included if they were carried out in the United States and reported the risk of lung cancer or chronic obstructive pulmonary disease (COPD) or heart disease, stroke and/or overall circulatory disease, comparing exclusive cigar smokers (current, former or ever cigar smokers who never smoked other tobacco products) with never smokers of any tobacco (or a closely-related comparison group). The results considered were for overall lung cancer rather than lung cancer subtypes, and related to overall risk measures rather than dose response indices, although dose response results by amount smoked were also identified.

Literature searches

Searching for results on cigar smoking was complicated by the MEDLINE search term "cigar smoking" being available only from the start of 2018. Before then the only search term to include cigar smoking was "tobacco products".

For lung cancer, the first step was to examine publications from a previous review relating lung cancer to various indices of smoking based on studies published during the 1900s[9]. Subsequently three different MEDLINE searches were conducted using terms such as ("cigar" or "cigars"), "United States" and "lung neoplasms". This was followed by a fourth search that attempted to retrieve relevant papers that had not yet been indexed with MeSH terms on MEDLINE, this search not being lung cancer specific. A fifth search used wholly non-MeSH search terms, with the final stage being to look for relevant results in papers identified as relevant in the searches for COPD and for heart disease, stroke and circulatory diseases.

For COPD, the process started with three different MEDLINE searches using the terms "COPD" or "pulmonary disease, chronic obstructive" to identify the disease. The fourth search used the term "Smoking" rather than "Cigar", while the fifth search used the MeSH terms "Smoking/mortality" or "Smoking/adverse effects". The next step was to review the results from the fourth lung cancer search, while the final step was to look at papers identified as relevant in the searches for lung cancer and for heart disease, stroke and circulatory diseases.

For heart disease, stroke and circulatory diseases, the process started with four different MEDLINE searches using the disease terms "Heart disease", "Stroke" or "Heart". The next step was again to review the results from the fourth lung cancer search, while the final step was to look at relevant papers from the lung cancer and COPD searches.

Searching ended when no new data was found and all the papers referenced by reviews had been examined. Full details of the searches are given in Supplementary material.

Sorting publications into studies and avoidance of overlap

The papers identified in the searches were reviewed for the studies they reported, and multiple publications reporting the same study were identified.

The source papers identified as providing relevant estimates were then considered for overlap of reporting. Where more than one of the source papers reported on the same study, the results may have been reported in different ways or for different lengths of follow-up, or have combined results from multiple studies.

Data recorded

For each paper identified as providing relevant results details were entered onto a study database and a linked relative risk (RR) database for the relevant disease.

The study database contained a record for each study describing the following aspects: A study name based on the published study name or on the name of the first author of the paper; study title; study design; sexes considered; age range and other details of the population studied; timing and length of follow-up; details of overlaps or links with other studies; number of cases; number of controls or subjects at risk; types of controls and matching factors used in case-control studies; and confounding variables considered.

The RR database holds the detailed results, typically containing multiple records for each study. Each record is linked to the relevant study via the study name, and holds details of a specific risk estimate. It records the type of estimate, its value and confidence interval, its source and other details such as the age range included in the estimate if this is different from the overall study age range. Some estimates were taken directly from the source paper. Others were derived using the details provided

in the paper.

Where no RR estimate was given or a RR estimate was given without a confidence interval, information on the sample size and the number of deaths was used to estimate these. Estimates for separate independent subsets of the population such as age groups were combined using simple meta-analysis. Non-independent RRs using a common comparison group (*e.g.*, never smokers) were combined using the Hamling method[10]. This method was used to combine RRs by number of cigars smoked per day and to combine RRs for former and current smokers to give an estimate for ever smokers. It was also used to estimate risk for overall circulatory disease when the study provided separate estimates for cerebrovascular disease and a broad definition of coronary heart disease, and to estimate overall stroke from separate risk estimates for ischaemic and haemorrhagic stroke. The International Classification of Disease codes used to define IHD, stroke and circulatory disease can be found at https://coder.aapc.com/icd-10-codes-range/110.

Each extract was carried out by one of the authors, the entered data and any additional calculations then being reported and checked by another of the authors, with problems discussed and amendments made until both agreed that the data entered were a true representation of the study data.

Dose-response data on risk by number of cigars smoked per day were also identified and are discussed below.

Statistical analysis

For each disease considered, fixed-effect and random-effects meta-analyses were conducted using the Fleiss and Gross method[11], with heterogeneity quantified by H, the ratio of the heterogeneity to its degrees of freedom, which is directly related to the I^2statistic[12] by the formula $I^2 = 100\ (H\text{-}1)/H$.

Whenever more than one paper provided equivalent results for a study, only one result was included in a meta-analysis. The selection of the result was based on four criteria: Prospective follow-up was given preference over cross-sectional analysis at baseline; the longest follow-up reported (for prospective studies); the widest age range reported; and finally the RR adjusted for the most confounding factors.

Some papers provided results for comparisons that did not exactly match our selection criteria. Where any were relevant to a meta-analysis, the analysis was performed excluding those results, and then including them in a sensitivity analysis.

The KAISER study[13] used a questionnaire that asked about the participant's history of cigarette smoking and their current pipe and cigar smoking. Ever cigarette smokers were excluded from their analyses. It was, therefore, possible to identify participants who had never smoked cigarettes and who did not, at baseline, smoke cigars or a pipe. This is not completely equivalent to our requirement for the comparison group to be never smokers of any tobacco product. Also, the participants categorised as current cigar smokers may have included former pipe smokers. However, the study was large (1546 current cigar smokers and 16228 never cigarette smokers) and had a long follow-up (25-26 years) so justified inclusion in sensitivity analyses.

For the MALHOT study a pooled analysis of data from five large prospective studies was reported[14]. Of these studies, two (the Netherlands Cohort Study and the Melbourne Collaborative Cohort Study) were conducted outside the United States, while the other three (the VITamins And Lifestyle study, the NIH-AARP Diet and Health study and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial) were conducted in the United States. However, the studies in the United States were larger and formed 85.7% of the total population reported, and the largest of the studies (NIH-AARP) followed up participants for a median of 15.5 years. The size of this pooled analysis, the large proportion of participants from the United States, the long follow-up in the largest study, and the lack of study-specific reports relating to cigar smoking for the studies pooled justified including the combined results in sensitivity analysis.

For the NHIS study, a cross-sectional analysis of baseline data[15] reports results for a definition of "Heart conditions" including angina, coronary heart disease, heart attack and other heart disease which is too broad for the results to be included in our main analysis of ischaemic heart disease (IHD). However, as the report considers data from four years' surveys of this large, repeated, nationally representative study, and as no other source was found that reported IHD for this study, it was decided to include its results in sensitivity analysis.

Also for the NHIS study, an analysis of prospective findings from six years' survey data with follow up for at least five years, the source publication[16] reports results for a broad definition of coronary heart disease which includes rheumatic fever, some hypertensive heart disease, IHD and some other heart disease. Though this definition

is too broad to be included in our analyses of IHD, the paper also reports results for cerebrovascular disease (stroke), and taken together, the definitions of coronary heart disease and cerebrovascular disease were very close to our ideal definition of circulatory disease. We therefore combined the results for coronary heart disease and for cerebrovascular disease, and included the resulting estimates in the sensitivity analyses for overall circulatory disease.

The NLMS study[17] reported too broad a definition of cardiovascular disease, an ideal definition of stroke and a definition of circulatory disease that included all the relevant disease categories except diseases of the veins and other diseases of the circulatory system. The reported results for stroke were included in the main meta-analyses and the results for circulatory disease were included in sensitivity analysis only.

RESULTS

Literature search

For lung cancer, 17 publications were identified that were relevant to the meta-analyses (including the sensitivity analyses), 13 from the previous review[9], three from additional searches, and one from reviews identified in the searches, as shown in Figure 1. Two of these[18,19] are by the same authors reporting the same study, the first giving overall study information and the second giving results by disease, so only the latter is cited in the analysis results. For COPD, four publications were identified (Figure 2), while for heart disease, stroke and circulatory disease 12 were found (Figure 3).

Studies

Table 1[13,14,17-31] (lung cancer), Table 2[13,17,23,27] (COPD), and Table 3[13,15-17,19,21,23,26-28,32,33] (heart disease, stroke and circulatory disease) present details on each study, including its name, the source publications, the study type, the years of follow-up, the study size, and the sexes and age groups considered. Results used in sensitivity analyses only are marked with an asterisk. Table 3 also includes details of the definition of heart disease, stroke and circulatory diseases.

For lung cancer, the 17 publications relate to 11 studies, though two (KAISER, MALHOT) are only used in sensitivity analyses. Eight of the studies are of prospective design and three case-controls. Except for NLMS, which considers both sexes, all provide results only for males. The studies vary widely in size, with three involving over 400000 people, and four less than 10000.

For COPD, the four publications concern separate studies, though again KAISER is only used in sensitivity analyses. All the studies are prospective, with all except NLMS considering only males. All four of these studies also provide results for lung cancer. The study size in KAISER is much lower than in the other three studies.

For heart disease, stroke and circulatory disease, the 12 publications concern eight studies: Six prospective, one case-control and one reported both as a cross-sectional analysis of baseline data and using prospective follow-up. KAISER (all results), NHIS (prospective results for circulatory disease and cross-sectional results for ischaemic heart disease) and NMLS (results for circulatory disease) were only used in the sensitivity analyses. Most results were for men only, the exceptions being those from NHIS and NLMS that were for the sexes combined. As for lung cancer, the studies varied widely in size.

Overall, as many studies provided data for more than one disease, there were 13 studies, of which two only provided results for the sensitivity analyses and two others had some results restricted to sensitivity analyses.

Meta-analyses

The individual study RR estimates used are given in Table 4[13-17,19,20,22,23,25,27-33], with the results of the meta-analyses conducted summarised in Table 5. Unless otherwise stated references to combined estimates are to random-effects estimates, with 95%CI given in parentheses.

Lung cancer

For current smokers there was highly significant heterogeneity ($P < 0.001$) between the five estimates, which ranged from 1.66 (1.18 to 2.34) for DORN to 5.10 (4.00 to 6.60) for CPS II. The overall estimate was 3.12 (2.11 to 4.62). Including the result from study

Table 1 Studies in meta-analysis of lung cancer and exclusive cigar smoking[1]

Study name[2]	Data source	Study type	Years of follow-up[3]	Sex	Age group	Study size[4]
BOUCOT	Boucot *et al*[20], 1972	P	10	M	45+	6027
CPS I	Hammond[21], 1966	P	4	M	35-84	440558[5]
	Hammond[22], 1972	P	6	M	35-84	
	Shanks *et al*[23], 1998	P	13	M	35+	
CPS II	Jacobs *et al*[24], 1999	P	12	M	30+	508576[6]
	Shapiro *et al*[25], 2000	P	12	M	30+	
DORN (US veterans study)	Dorn[26], 1959	P	2	M	30+	248046[7]
	Kahn[27], 1966	P	8	M	35-84	
	Rogot *et al*[28], 1980	P	16	M	31-84	
HAMMON	Hammond *et al*[18,19], 1958	P	3	M	50-69	187783
KAISER	Iribarren *et al*[13], 1999	P	25	M	30-85	17774
LEVIN	Levin[29], 1954	C	-	M	35+	2855
MALHOT	Malhotra *et al*[14], 2017	P	15	M	55-62	524440[8]
NLMS	Christensen *et al*[17], 2018	P	26	C	35-80	146529
SADOWS	Sadowsky *et al*[30], 1953	C	-	M	All	2605
WYNDE7	Higgins *et al*[31], 1988	C	-	M	20-80	6033[9]

[1]Compared with never smoking any tobacco product.

[2]Study name is an identifier assigned by the authors based on the published study name or the name of the first author of a source paper. This identifier is used in the meta-analysis output. Note that studies KAISER and MALHOT were included in sensitivity analysis only.

[3]Years of follow-up is relevant only to prospective studies.

[4]Study size: Total number of participants in the study, irrespective of smoking habits but taking account of sex.

[5]Size not given for second source, and a slightly different number of 442455 given for third source.

[6]Slightly different size of 508353 given for second source.

[7]Size taken from third source, other sources only gives person-years. The numbers include about 0.5% females.

[8]Includes 74770 from two non-United States studies.

[9]Size is for sexes combined. P: Prospective; C: Case control; M: Male; C: Combined male and female.

KAISER little affected the combined estimate, which became 2.98 (2.08 to 4.26).

The four results for former smokers showed no significant heterogeneity (at $P < 0.1$), and gave a somewhat lower estimate of 1.61 (1.23 to 2.09).

The nine results for ever smokers showed significant ($P < 0.05$) heterogeneity due to the high estimate from BOUCOT of 8.81. The rest of the estimates ranged from 1.02 to 3.01. The overall estimate was 2.11 (1.64 to 2.72). When the result from MALHOT was included, this became 2.22 (1.79 to 2.74).

COPD

None of the analyses showed significant heterogeneity, and there was very limited evidence of an association. Results were available from only four studies and for only two of these were results available for each of the exposures current, former and ever smokers (Table 4). This resulted in all the combined estimates being based on between two and four results. For current smokers the overall estimate was slightly raised at 1.42 (0.89 to 2.26) excluding KAISER and 1.44 (1.16 to 1.77) including KAISER, but no increase was seen for ever smokers 0.86 (0.48 to 1.54). For former smokers, the overall estimate of 0.47 had an extremely wide CI of 0.02 to 9.88, based on individual estimates of 0.05 (0.00 to 3.19) and 1.38 (0.42 to 4.51).

IHD, stroke and circulatory disease

As is evident from Table 5, overall estimates generally only slightly exceeded 1.00, though some of those for IHD and circulatory disease, but not stroke, were significantly raised (at $P < 0.05$). There was also evidence of heterogeneity in some of

Table 2 Studies in meta-analysis of chronic obstructive pulmonary disease and exclusive cigar smoking[1]

Study name[2]	Data source	Study type	Years of follow-up	Sex	Age group	Study size[3]
CPS I	Shanks *et al*[23], 1998	P	13	M	35+	442455
DORN (US veterans study)	Kahn[27], 1966	P	8	M	35-84	248046[4]
KAISER	Iribarren *et al*[13], 1999	P	25	M	30-85	17774
NLMS	Christensen *et al*[17], 2018	P	26	C	35-80	146529

[1]Compared with never smoking any tobacco product.

[2]Study name is an identifier assigned by the authors based on the published study name or the name of the first author of the source paper. This identifier is used in the meta-analysis output. Note that study KAISER was included in sensitivity analysis only.

[3]Study size: Total number of participants in the study, irrespective of smoking habits but taking account of sex.

[4]See Table 1 for study size. P: Prospective; M: Male; C: Combined male and female.

the meta-analyses presented. Generally, the results from the sensitivity analyses were similar to those from the main analyses, so only the former set of results, which involve more studies, are considered below.

For ischaemic heart disease, the estimates were somewhat higher for former than current smokers, being 1.11 (1.04 to 1.19) for current smokers, 1.26 (1.03 to 1.53) for former smokers, and 1.15 (1.08 to 1.23) for ever smokers.

For stroke, the estimates were all closer to 1.00, but again somewhat higher for former than current smokers, being 1.02 (0.92 to 1.13) for current smokers, 1.08 (0.85 to 1.38) for former smokers, and 1.11 (0.95 to 1.31) for ever smokers.

For overall circulatory disease, the three estimates were quite similar, being 1.10 (1.05 to 1.16) for current smokers, 1.11 (0.84 to 1.46) for former smokers and 1.15 (1.06 to 1.26) for ever smokers.

Dose-response data

Many studies did not provide data on risk by number of cigars smoked per day. Table 6[13,17,23,27,30,32,33] summarizes the limited data available from six studies, five of which provided data for ischaemic heart disease, four for lung cancer, and two for COPD. With the possible exception of the result for the SADOWS study, the data for lung cancer seemed consistent with an increasing risk with increasing amount smoked. The data for COPD and for ischaemic heart disease, however, did not consistently show any clear increase in risk with amount smoked.

DISCUSSION

The meta-analysis results show some increase in risk among exclusive cigar smokers for each disease studied, except for stroke where all the risk estimates were close to 1. For current smoking the overall estimates in the sensitivity analyses were 2.98 for lung cancer, 1.44 for COPD and 1.11 for ischaemic heart disease. These are much lower than those associated with cigarette smoking: For the United States, estimates for current cigarette smokers[34] are 11.68 for lung cancer and 4.56 for COPD; for ischaemic heart disease[34] the current cigarette smoker estimate for age 65 to 74 is 1.70, with estimates for younger ages being higher. Even for heavy cigar smokers, the RRs shown in Table 6 are still generally lower than the estimates for overall cigarette smoking. For former smoking the estimates of 1.61 for lung cancer, 0.47 for COPD (though based on only two widely differing estimates) and 1.26 for ischaemic heart disease are again much lower than those for cigarette smoking. Similar results were observed for ever smoking.

There are some limitations with the data available for our analyses. Several of the studies were conducted some time ago. The numbers of exclusive cigar smokers participating in the studies were often quite low. Very few studies have reported results for exclusive cigar smokers. For many of these studies, cigar smoking is not the primary focus of the study. This suggests that there may be reporting bias, in that other studies may have had relevant data but did not report a non-significant finding for the study's small number of cigar smokers.

Table 3 Studies on ischaemic heart disease, stroke and circulatory disease and exclusive cigar smoking[1]

Study name[2]	Data source	Study type	Years of follow-up[3]	Sex	Age group	Study size[4]	Disease data available and notes[5]
CPS I	Hammond[21], 1966	P	4	M	35-84	440558[6]	IHD: CHD (ICD-6 420); Stroke: -; Circ: -
	Shanks *et al*[23], 1998	P	13	M	35+		IHD: CHD; Stroke: CVD; Circ: -
CPS II	Jacobs *et al*[32], 1999	P	9	M	30+	508576	IHD: CHD; Stroke: -; Circ: -
DORN (US veterans study)	Dorn[26], 1959	P	2	M	30+	248046[6]	IHD: -; Stroke: -; Circ: Cardiovascular diseases (ICD-6 330-334, 400-468)
	Kahn[27], 1966	P	8	M	35-84		IHD: Arteriosclerotic (coronary) heart disease (ICD-6 420); Stroke: Cerebral vascular lesions (ICD-6 330-334); Circ: Total cardiovascular disease (ICD-6 330-334, 400-468)
	Rogot *et al*[28], 1980	P	16	M	31-84		IHD: CHD (ICD-6 420); Stroke: Stroke (ICD-6 330-334); Circ: Cardiovascular (ICD-6 330-334, 400-468)
HAMMON	Hammond *et al*[19], 1958	P	3	M	50-69	187783	IHD: Coronary artery disease; Stroke: Cerebral vascular diseases; Circ: Heart and circulatory diseases (ICD-6 330-334, 400-468)
KAISER	Iribarren *et al*[13], 1999	P	25	M	30-85	17774	IHD: CHD; Stroke: Ischaemic stroke and haemorrhagic stroke combined; Circ: -
KAUFMA	Kaufman *et al*[33], 1987	C	-	M	40-54	1506[7]	IHD: Non-fatal myocardial infarction; Stroke: -; Circ: -
NHIS	Inoue-Choi *et al*[16], 2019	P	17	C	18-95	71314	IHD: - ("Coronary heart disease" used too broad a definition); Stroke: Cardiovascular disease (ICD-10 I60-I69); Circ: Combined "Coronary heart disease" and Stroke; this excludes some hypertensive disease, diseases of arteries and diseases of veins and other circulatory disease – included in sensitivity analysis only
	Rostron *et al*[15], 2019[8]	X	-	C	35+	Not specified	IHD: Heart conditions; this includes angina, CHD, heart attack and other heart disease – included in sensitivity analysis only; Stroke: Stroke; Circ: -
NLMS	Christensen *et al*[17], 2018	P	26	C	35-80	146529	IHD: - ("Cardiovascular diseases" used too broad a definition); Stroke: Cerebrovascular disease (ICD-10 I60-I69); Circ: Circulatory disease (ICD-10 I00-I09, I20-I25, I26-I28, I29-I51, I60-I69, I70, I71, I72-I78); this definition excludes "Diseases of veins" and "Other circulatory diseases" (ICD-10 I80-I89) – included in sensitivity analysis only

[1]Compared with never smoking any tobacco product.

[2]Study name is an identifier assigned by the authors based on the published study name or the name of the first author of the source paper. This identifier is used in the meta-analysis output. Note that some study results were included in sensitivity analysis only: The results from study KAISER (each disease); study NHIS (the prospective result for circulatory disease and the cross-sectional result for IHD); and study NLMS (the result for circulatory disease).

[3]Years of follow-up is relevant only to prospective studies.

[4]Study size: Total number of participants in the study, irrespective of smoking habits but taking account of sex.

[5]The diseases considered were IHD, stroke and circ. The text gives, for each of these diseases, the disease definition as given in the paper together with additional notes where relevant (reason for rejection, method of derivation, problems with the result that mean it should be included in the sensitivity analysis only). The ICD codes relating to IHD, stroke and circ can be found at https://coder.aapc.com/icd-10-codes-range/110.

[6]See Table 1 for study size.

[7]Study limited to non-cigarette smokers.

[8]This is a cross-sectional analysis of baseline data: Results from the prospective study (above) were used in the meta-analysis when possible. IHD: Ischaemic heart disease; Circ: Stroke and circulatory disease P: Prospective; C: Case control; X: Cross-sectional; M: Male; C: Combined male and female; ICD: The International Classification of Disease.

There was a limited amount of dose-response data, and a lack of data on how risk varied by type of cigar smoked. No meta-analyses could be carried out by subgroups such as race and age and gender, as there was insufficient data. No study reported results for sex separately, so no analysis by sex could be done.

Nevertheless, the data provide fairly clear evidence that exclusive cigar smoking is associated with an increased risk of lung cancer, though less markedly than is the case for exclusive cigarette smoking. For COPD and ischaemic heart disease, the association is weaker, and is also less than that for cigarette smoking.

How do these results compare with previous estimates? It should be noted that no other review has provided meta-analysis estimates for exclusive cigar smokers in the United States, and that many of the previous reviews considered below were conducted many years ago.

The review of smoking and lung cancer[9] referred to under literature searches provided random effects meta-analysis estimates for lung cancer in current, former and ever exclusive cigar smokers of 4.67 (n = 15), 2.85 (n = 5) and 2.95 (n = 15) respectively, but these analyses were not restricted to studies in the United States. The risk estimates included in those analyses showed significant heterogeneity. A review by Wynder *et al*[35] considering the risk of lung cancer in pipe and cigar smokers noted that, in prospective studies in North America the mortality ratios were in the range 2 to 6. For retrospective studies, mostly conducted in Germany and Switzerland, "it appears that the risk of lung cancer is higher than that for such smokers in the United States". This review suggested that these differences stemmed from different patterns of inhalation in the two regions. A similar review by Higgins *et al*[31], also considering pipe and cigar smokers, again suggested that risk estimates from prospective studies in North America are lower than those from case control studies in Europe. Smoking and Tobacco Control Monograph No. 9[23], reviewing data from CPS-I, stated that "Lung cancer mortality ratios increase with increasing number of cigars smoked per day and with increasing depth of inhalation. When depth of inhalation and number of cigars per day are examined together, depth of inhalation is more powerful in predicting lung cancer risk than number of cigars smoked per day." The 1979 report by the Surgeon General[36] summarised the available evidence as "Several prospective epidemiological studies have demonstrated higher lung cancer mortality ratios for pipe and cigar smokers than for nonsmokers, but the risk of developing lung cancer for pipe and cigar smokers is less than for cigarette smokers".

Smoking and Tobacco Control Monograph No. 9[23] also reported estimates for COPD risk. It concluded that "The data taken as a whole support the conclusion that

Table 4 Individual estimates used in the meta-analyses[1]

Disease	Study name[2]	Source	Exposure	Relative risk (95%CI)
Lung cancer	BOUCOT	[20]	Ever	8.81 (0.45 to 170.58)
	CPSI	[23]	Current	3.30 (2.68 to 4.06)
		[22]	Ever	2.11 (1.45 to 3.07)
	CPSII	[25]	Current	5.10 (4.00 to 6.60)
		[25]	Former	1.60 (1.20 to 2.40)
		[25]	Ever	3.01 (2.42 to 3.74)
	DORN	[28]	Current	1.66 (1.18 to 2.34)
		[27]	Former	1.02 (0.41 to 2.54)
		[27]	Ever	1.49 (0.97 to 2.27)
	HAMMON	[19]	Ever	1.02 (0.42 to 2.51)
	KAISER	[13]	Current[3]	2.14 (1.12 to 4.11)
	LEVIN	[29]	Ever	1.41 (0.76 to 2.60)
	MALHOT	[14]	Ever[3]	2.73 (2.06 to 3.60)
	NLMS	[17]	Current	3.26 (1.86 to 5.71)
		[17]	Former	1.35 (0.70 to 2.61)
		[17]	Ever	2.04 (1.33 to 3.15)
	SADOWS	[30]	Ever	2.98 (1.06 to 8.33)
	WYNDE7	[31]	Current	3.15 (1.78 to 5.57)
		[31]	Former	2.46 (1.27 to 4.77)
		[31]	Ever	2.83 (1.78 to 4.51)
COPD	CPSI	[23]	Current	1.42 (0.96 to 2.03)
	DORN	[27]	Current	0.79 (0.31 to 2.03)
		[27]	Former	1.38 (0.42 to 4.51)
		[27]	Ever	0.94 (0.43 to 2.04)
	KAISER	[13]	Current[3]	1.45 (1.10 to 1.91)
	NLMS	[17]	Current	2.44 (0.98 to 6.05)
		[17]	Former	0.05 (0.00 to 3.19)
		[17]	Ever	0.77 (0.32 to 1.87)
IHD	CPSI	[23]	Current	1.05 (1.00 to 1.11)
	CPSII	[32]	Current	1.13 (0.96 to 1.34)
		[32]	Former	1.07 (0.93 to 1.24)
		[32]	Ever	1.09 (0.98 to 1.22)
	DORN	[28]	Current	1.12 (1.05 to 1.18)
		[27]	Former	1.41 (1.25 to 1.60)
		[27]	Ever	1.13 (1.05 to 1.22)
	HAMMON	[19]	Ever	1.28 (1.13 to 1.44)
	KAISER	[13]	Current[3]	1.27 (1.12 to 1.45)
	KAUFMA	[33]	Current	1.25 (0.58 to 2.67)
	NHIS	[15]	Current[3]	0.88 (0.61 to 1.27)
		[15]	Former[3]	1.33 (1.03 to 1.72)

		[15]	Ever[3]	1.15 (0.93 to 1.41)
Stroke	CPSI	[23]	Current	0.96 (0.87 to 1.06)
	DORN	[28]	Current	1.07 (0.95 to 1.21)
		[27]	Former	1.14 (0.85 to 1.53)
		[27]	Ever	1.09 (0.93 to 1.29)
	HAMMON	[19]	Ever	1.33 (1.04 to 1.70)
	KAISER	[13]	Current[3]	1.08 (0.88 to 1.33)
	NHIS	[16]	Current	1.60 (0.72 to 3.57)
		[16]	Former	0.56 (0.20 to 1.57)
		[16]	Ever	0.94 (0.50 to 1.77)
	NLMS	[17]	Current	0.50 (0.21 to 1.22)
		[17]	Former	1.08 (0.66 to 1.75)
		[17]	Ever	0.85 (0.55 to 1.30)
Circulatory disease	DORN	[28]	Current	1.10 (1.05 to 1.16)
		[27]	Former	1.37 (1.24 to 1.52)
		[27]	Ever	1.13 (1.06 to 1.20)
	HAMMON	[19]	Ever	1.27 (1.15 to 1.40)
	NHIS	[16]	Current[3]	1.34 (0.88 to 2.06)
		[16]	Former[3]	0.75 (0.52 to 1.10)
		[16]	Ever[3]	0.93 (0.70 to 1.24)
	NLMS	[17]	Current[3]	1.11 (0.87 to 1.42)
		[17]	Former[3]	1.13 (0.93 to 1.38)
		[17]	Ever[3]	1.12 (0.96 to 1.31)

[1]Compared with never smoking any tobacco product.

[2]Study name is an identifier assigned by the authors based on the published study name or the name of the first author of the source paper. This identifier is used in the meta-analysis output.

[3]These results were only included in the sensitivity analyses. COPD: Chronic obstructive pulmonary disease; IHD: Ischaemic heart disease.

cigar smoking can cause COPD in smokers who inhale deeply".

The same Monograph reviews coronary heart disease risk, concluding that "The studies of cigar smoking and coronary events present a pattern of slightly elevated rates among cigar smokers who smoke heavily or inhale deeply". The Surgeon General's 1983 report[37] states that "In general, the risk for coronary heart disease mortality of smoking pipes and cigars is substantially lower than the risk of smoking cigarettes. This is generally felt to be due to the tendency of pipe and cigar smokers not to inhale smoke into the lung".

For risk of stroke, the Surgeon General's 1983 report[37] cited results from the United States Veterans study[28] (which are included in this review), stating that "Mortality ratios for stroke were near unity for smokers of only cigars or pipes – 1.07 and 0.99, respectively." As noted for lung cancer, there may be differences in stroke risk estimates between studies in the United States and in Europe. Smoking and Tobacco Control Monograph No. 9[23] states "It is difficult to reconcile the results from the European studies and the CPS-I results. The CPS-I primary cigar data are primarily individuals who report that they do not inhale (78 percent), while inhalation information is not provided by the other studies. If inhalation rates are much higher in the European studies, this could explain some of the differences found in the RR of stroke between the two groups of studies."

Generally, these results reach conclusions quite similar to ours, and suggest that the conclusions we have drawn from our review of the evidence from the United States may not necessarily apply to cigar smoking in Europe.

In conclusion, we find that exclusive cigar smoking is associated with a moderate increase in risk of lung cancer, and a smaller increased risk of COPD and IHD, and

Table 5 Meta-analysis results for lung cancer, chronic obstructive pulmonary disease, ischaemic heart disease, stroke and circulatory disease for cigar smoking[1]

Disease	Exposure	Number of studies	Relative risk (95%CI) fixed effect	Relative risk (95%CI) random effects	Heterogeneity I^2([2])
Lung cancer	Current cigar smokers, excluding KAISER	5	3.36 (2.93 to 3.84)	3.12 (2.11 to 4.62)	85.22 ($P < 0.001$)
	Current cigar smokers, including KAISER	6	3.29 (2.88 to 3.76)	2.98 (2.08 to 4.26)	82.65 ($P < 0.001$)
	Former cigar smokers	4	1.61 (1.23 to 2.09)	1.61 (1.23 to 2.09)	0.00 (NS)
	Ever cigar smokers, excluding MALHOT	9	2.35 (2.04 to 2.71)	2.11 (1.64 to 2.72)	54.77 ($P < 0.05$)
	Ever cigar smokers, including MALHOT	10	2.43 (2.13 to 2.75)	2.22 (1.79 to 2.74)	51.51 ($P < 0.05$)
COPD	Current cigar smokers, excluding KAISER	3	1.42 (1.02 to 1.96)	1.42 (0.89 to 2.26)	29.95 (NS)
	Current cigar smokers, including KAISER	4	1.44 (1.16 to 1.77)	1.44 (1.16 to 1.77)	0.00 (NS)
	Former cigar smokers	2	1.06 (0.34 to 3.31)	0.47 (0.02 to 9.88)	58.19 (NS)
	Ever cigar smokers	2	0.86 (0.48 to 1.54)	0.86 (0.48 to 1.54)	0.00 (NS)
IHD	Current cigar smokers, excluding NHIS and KAISER	4	1.08 (1.04 to 1.13)	1.08 (1.04 to 1.13)	0.28 (NS)
	Current cigar smokers, including NHIS and KAISER	6	1.09 (1.06 to 1.14)	1.11 (1.04 to 1.19)	48.68 ($P < 0.1$)
	Former cigar smokers, excluding NHIS	2	1.25 (1.14 to 1.38)	1.23 (0.94 to 1.61)	87.72 ($P < 0.01$)
	Former cigar smokers, including NHIS	3	1.26 (1.16 to 1.38)	1.26 (1.03 to 1.53)	75.96 ($P < 0.05$)
	Ever cigar smokers, excluding NHIS	3	1.15 (1.09 to 1.21)	1.16 (1.06 to 1.26)	51.96 (NS)
	Ever cigar smokers, including NHIS	4	1.15 (1.09 to 1.21)	1.15 (1.08 to 1.23)	27.95 (NS)
Stroke	Current cigar smokers, excluding KAISER	4	1.00 (0.93 to 1.08)	1.01 (0.87 to 1.16)	46.09 (NS)
	Current cigar smokers, including KAISER	5	1.01 (0.94 to 1.09)	1.02 (0.92 to 1.13)	33.51 (NS)
	Former cigar smokers	3	1.08 (0.85 to 1.38)	1.08 (0.85 to 1.38)	0.00 (NS)
	Ever cigar smokers	4	1.12 (0.98 to 1.27)	1.11 (0.95 to 1.31)	21.80 (NS)
Circulatory disease	Current cigar smokers, excluding NHIS and NLMS	1	1.10 (1.05 to 1.16)	-	-
	Current cigar smokers, including NHIS and NLMS	3	1.10 (1.05 to 1.16)	1.10 (1.05 to 1.16)	0.00 (NS)
	Former cigar smokers, excluding NHIS and NLMS	1	1.37 (1.24 to 1.52)	-	-
	Former cigar smokers, including NHIS and NLMS	3	1.28 (1.17 to 1.39)	1.11 (0.84 to 1.46)	81.92 ($P < 0.01$)
	Ever cigar smokers, excluding NHIS and NLMS	2	1.17 (1.11 to 1.23)	1.19 (1.06 to 1.33)	72.63 ($P < 0.1$)
	Ever cigar smokers, including NHIS and NLMS	4	1.15 (1.10 to 1.21)	1.15 (1.06 to 1.26)	51.29 (NS)

[1]Compared with never smoking any tobacco product.

[2]Heterogeneity between the results included in the meta-analysis, given as the I^2 statistic together with its significance coded as: Not significant; $P < 0.1$; $P <$

0.05; $P < 0.01$; $P < 0.001$. The I^2 statistic is calculated as 100 × (H-df)/H, where H is the heterogeneity chi-squared statistic, df is the degrees of freedom, and if the resulting value is negative I^2 is taken to be 0. NS: Not significant; COPD: Chronic obstructive pulmonary disease; IHD: Ischaemic heart disease.

that these increases in risk are less than for cigarette smoking.

Table 6 Relative risks and 95%CIs by current amount smoked[1]

Study	Cigars per day	RR (95%CI) lung cancer	RR (95% CI) COPD	RR (95%CI) IHD	Adjustment factors
CPS I[23]					
	1-2	0.90 (0.54 to 1.66)	1.39 (0.74 to 2.38)	0.98 (0.91 to 1.07)	Age
	3-4	2.36 (1.49 to 3.54)	1.78 (0.89 to 3.18)	1.06 (0.96 to 1.16)	
	5+	3.40 (2.34 to 4.77)	1.03 (0.37 to 2.23)	1.14 (1.03 to 1.24)	
CPS II[32]					
Age 30-74 years	1			1.18 (0.76 to 1.82)	Age+[2]
	2-3			1.43 (1.03 to 1.99)	
	4+			1.33 (0.95 to 1.86)	
Age 75+ years	1			1.07 (0.64 to 1.78)	
	2-3			0.72 (0.45 to 1.16)	
	4+			1.03 (0.70 to 1.51)	
DORN[27][3]					
Age 55-64	< 5	0.64 (0.15 to 2.69)		0.84 (0.70 to 1.01)	None
	5+	3.74 (1.53 to 9.10)		0.98 (0.78 to 1.24)	
Age 65-74	< 5	1.32 (0.65 to 2.69)		1.07 (0.94 to 1.23)	
	5+	1.83 (0.78 to 4.27)		1.21 (1.02 to 1.45)	
KAISER[13]					
	< 5	1.57 (0.67 to 3.66)	1.30 (0.93 to 1.81)	1.20 (1.03 to 1.40)	Age+[4]
	5+	3.24 (1.01 to 10.40)	2.25 (1.39 to 3.65)	1.56 (1.21 to 2.01)	
KAUFMAN[33]					
	1-4			0.90 (0.30 to 2.70)	Age+[5]
	5+			1.70 (0.60 to 4.80)	
NLMS[17]					
	< 1	0.74 (0.08 to 7.26)			Age+[6]
	1+	4.18 (2.34 to 7.46)			
SADOW[30][7]					
	1	1.62			None
	2	4.51			
	3	4.38			
	4+	2.54			

[1]Relative to never smoking.

[2]Adjusted also for education, hypertension, BMI, exercise, alcohol, environmental tobacco smoke and vitamin C.

[3]Estimated from data provided.

[4]For lung cancer and chronic obstructive pulmonary disease adjusted also for race, body mass index (BMI), history of diabetes, alcohol and occupational exposure; for ischaemic heart disease adjusted also for race, BMI, history of diabetes, alcohol, education, systolic blood pressure and serum cholesterol.

[5]Adjusted for religion, education, ethnicity, personality, family history of myocardial infarction, doctor visits, geography, physical activity, BMI, and treatment for diabetes, cholesterol, hypertension and chest pain.

[6]Adjusted also for sex, race, education and survey year.

[7]95% CI not available, RRs estimated from data provided. RRs: Relative risks; COPD: Chronic obstructive pulmonary disease; IHD: Ischaemic heart disease.

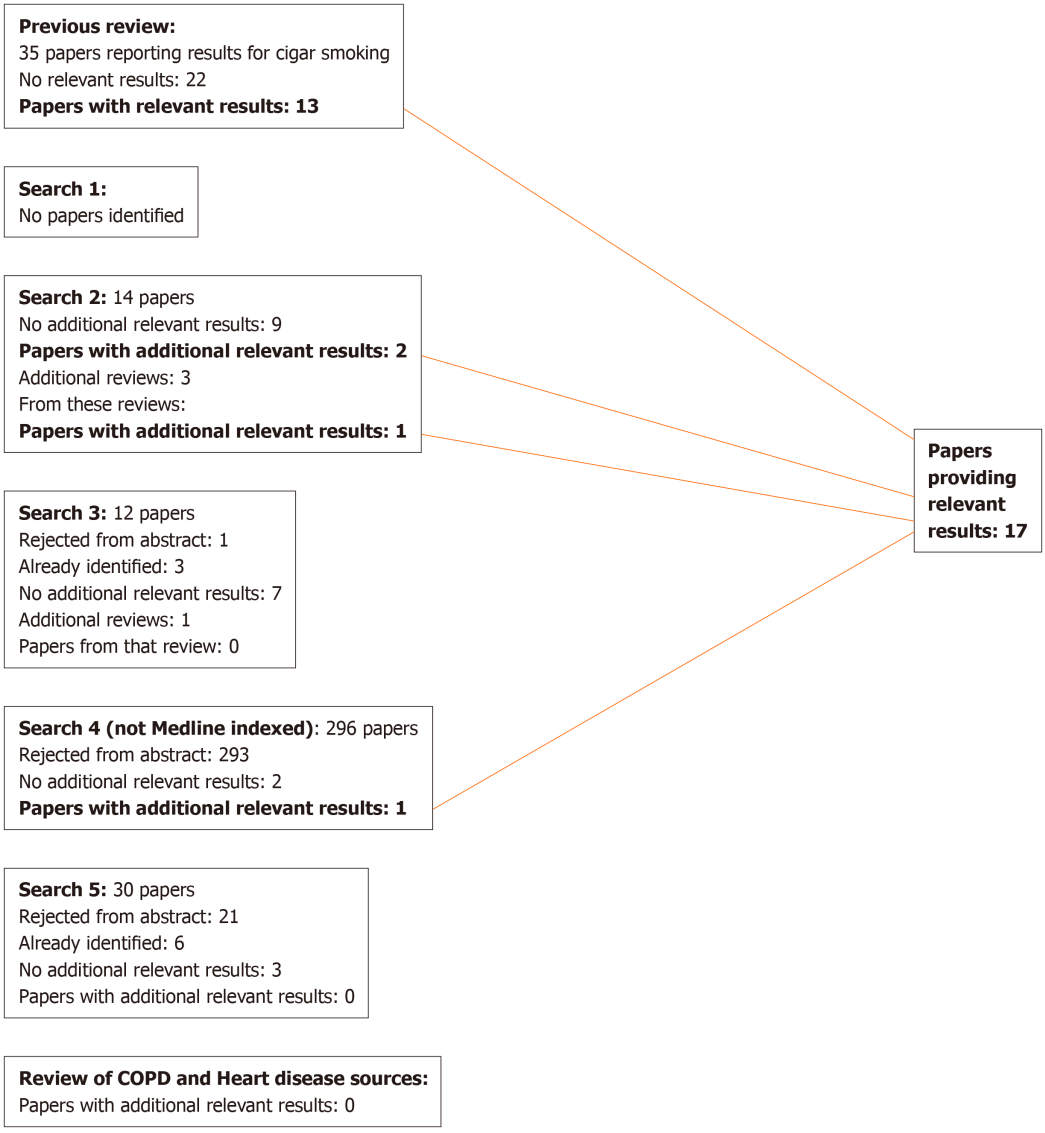


Figure 1 Lung cancer searches. COPD: Chronic obstructive pulmonary disease.

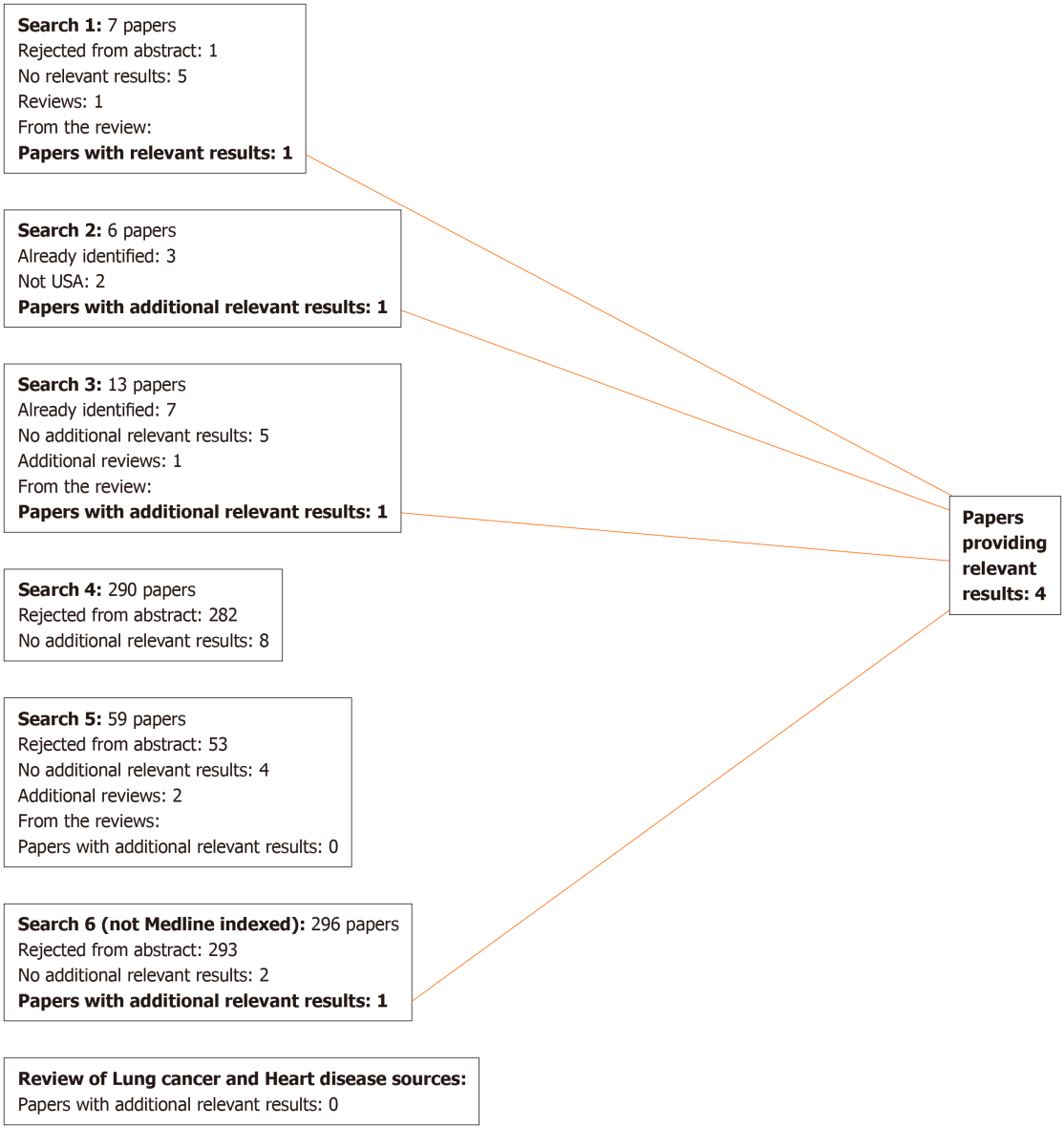


Figure 2 Chronic obstructive pulmonary disease searches.

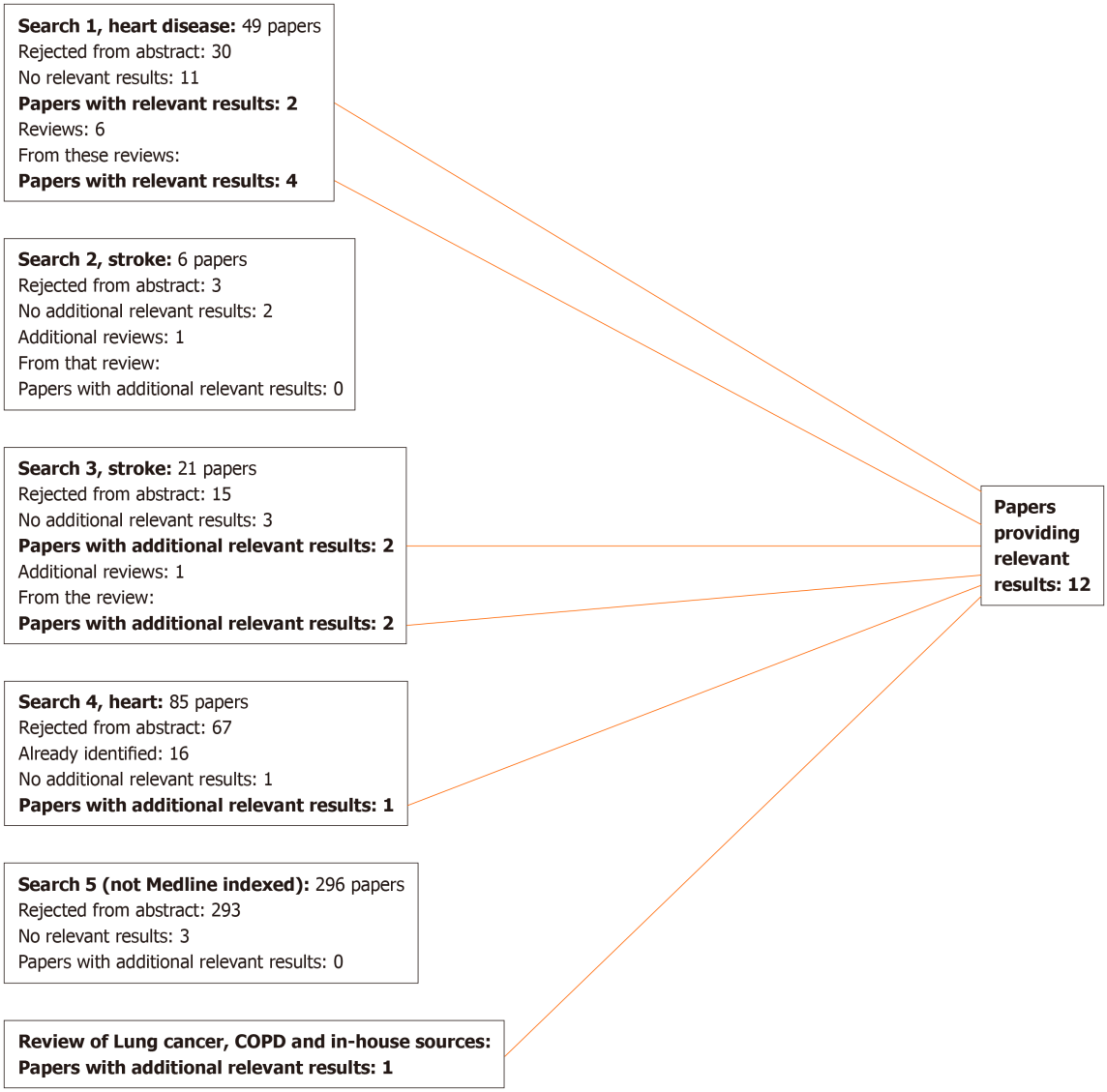


Figure 3 Heart disease, stroke and circulatory disease searches. COPD: Chronic obstructive pulmonary disease.

ARTICLE HIGHLIGHTS

Research background

Many reviews have studied the relationship of smoking to lung cancer, chronic obstructive pulmonary disease (COPD), heart disease and stroke, but the effects on these diseases of cigar smoking, particularly exclusive cigar smoking, have rarely been considered.

Research motivation

As the United States is a country with a large population and a relatively high percentage of cigar smokers, we felt that insight into the effects of exclusive cigar smoking could usefully be gained from studies conducted there.

Research objectives

To carry out a systematic review of the relationship of exclusive cigar smoking to the four main smoking-related diseases in studies conducted in the United States.

Research methods

Literature searches were conducted to identify studies in the United States that reported risk of lung cancer, COPD, heart-disease, stroke and/or overall circulatory disease comparing cigar smokers who had never smoked other tobacco products with those who had never smoked any tobacco. For each study identified as providing

relevant results, data were recorded on study characteristics and on the appropriate relative risks (RRs) and 95%CIs relating to overall current, former and ever exclusive cigar use, and, for current smokers, by daily cigar consumption. RRs for a given smoking group and disease were combined using fixed-effect and random-effects meta-analyses.

Research results

Data were available on lung cancer from 11 studies, on COPD from four studies and on heart disease, stroke and circulatory disease from 10 studies. As RRs tended to be heterogeneous, random-effects estimates are given below. For current smoking overall RR estimates were 2.98 (95%CI: 2.08 to 4.26, based on n = 6 estimates) for lung cancer, 1.44 (1.16 to 1.77, n = 4) for COPD, 1.11 (1.04 to 1.19, n = 6) for ischaemic heart disease, 1.02 (0.92 to 1.13, n = 5) for stroke and 1.10 (1.05 to 1.16, n = 3) for overall circulatory disease. These RRs are much lower than those reported for the United States for exclusive cigarette smokers; 11.68 for lung cancer, 4.56 for COPD and at least 1.70, depending on age, for ischaemic heart disease. Even for heavy cigar smoking, RRs are generally lower than for overall cigarette smoking. RRs for former and for ever smoking were also much lower than for cigarette smoking.

Research conclusions

Although our analyses were based on relatively few studies, some conducted some time ago, the results clearly show that exclusive cigar smoking is associated with an increased risk of lung cancer, though much less than is the case for exclusive cigarette smoking. For COPD and ischaemic heart disease the association is weaker, and also less than for cigarette smoking. No previous study has clarified the effects of exclusive cigarette smoking so clearly. Future research could extend results on exclusive cigar smoking to countries other than the United States, and compare risks of cigar smoking with those of using other nicotine products.

Research perspectives

While our results show that exclusive cigar smoking is associated with risks of smoking-related diseases that are much lower than those associated with cigarettes smoking, little of the evidence comes from studies conducted in this millenium. Further large prospective studies are needed to collect more up-to-date results, and to clarify how risk varies by type of cigar smoked.

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SYSTEMATIC REVIEWS

Hydatidosis and the duodenum: A systematic review of the literature

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Abstract

BACKGROUND

Injury to the duodenum (fistula formation, compression, or other complications) by a hydatid cyst (HC) is an exceptional complication.

AIM

To perform a systematic review of the literature on the fistulization of HC in the duodenum.

METHODS

Following PRISMA guidelines, a search for HC with duodenal involvement was carried out in the databases of PubMed, SCielo and EMBASE without time limits.

RESULTS

Fourteen patients were identified, seven men and seven women, with a mean age of 53.14 years (SD = 17.65, range: 28-78). Three out of the 14 (21%) had HC relapse. The most frequent clinical manifestations were abdominal pain and nausea and/or vomiting. Various imaging studies were performed in almost all cases, the most commonly used being abdominal computed tomography (10/14, 71%). A range of surgical techniques were reported, most frequently HC drainage (41%) and enucleations (16%). Ten of the 14 patients had no complications and one patient died. The follow-up period and recurrences could not be determined.

CONCLUSION

The most frequent symptoms were abdominal pain, nausea and vomiting. Computed tomography was the most used diagnostic imaging technique, and HC drainage and fistula closure *via* laparotomy was the most frequent treatment.

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However, all diagnostic and therapeutic options for HC fistulizing the duodenum had a low level of evidence.

Key words: Echinococcosis; Hydatidosis; Duodenum; Surgery; Review

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Core tip: Hydatidosis is a global zoonosis. The most frequent organs affected are the liver (50%-70%) and the lungs (20%-30%) The duodenum is an organ that is very rarely affected by hydatidosis, either primarily or secondarily. Possible forms of secondary duodenal involvement are compression, or, more frequently, fistulization from hydatid cysts located in neighboring organs. The hydatid cysts that most often fistulize the duodenum are those located in the liver (0.15% of instances), with cases also described from the pancreas and kidney. Its low prevalence and the few existing records mean that the choice of the best therapeutic management is a challenge. A systematic review let readers know all published cases and best management.

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INTRODUCTION

Hydatidosis is a zoonosis caused by the larval form of the genus *Equinococcus* spp, and *Equinococcus granulosus* (*E. granulosus*) causes the formation of hydatid cysts (HC). The life cycle of *E. granulosus* includes different herbivores (lambs, cows, pigs, deer) and carnivores (dogs, foxes, wolves); the dog is the most frequent definitive host[1]. The distribution of *E. granulosus* is associated with livestock exploitation and temperate climate zones such as southern Europe, Latin America, Australia and New Zealand, Africa, and the Middle East. A prevalence of HC of 2%-6% is estimated in the population in endemic areas[2].

The human being is an intermediate host, as it is infected orally with food contaminated by eggs from the feces of the definitive host. The eggs hatch in the intestine, releasing oncospheres that penetrate the intestinal wall and invade different sites *via* the bloodstream, the most frequent being the liver (50%-70%) and the lungs (20%-30%). The oncospheres evolve into HC, which may remain asymptomatic until their growth causes complications derived from the mass effect, depending on their location. Rupture of the HC can spread its contents throughout the body and trigger anaphylactic shock[3].

The duodenum is an organ that is very rarely affected by HC, either primarily or secondarily. Possible forms of secondary duodenal involvement are compression, or, more frequently, fistulization from HC located in neighboring organs[4]. The HC that most often fistulize the duodenum are those located in the liver (0.15% of instances), with cases also described from the pancreas and kidney. Its low prevalence and the few existing records mean that the choice of the best therapeutic management is a challenge[4].

Here we present a systematic literature review of cases of hydatidosis with duodenal involvement published in the scientific literature.

MATERIALS AND METHODS

Literature search

Following the guidelines of the PRISMA methodology for systematic reviews, a search was performed in the PubMed, SCielo and EMBASE databases[5], unlimited by language or time. The terms used for the search were: (duodenum) AND [(echinococcosis, hepatic) OR (echinococcosis) OR (echinococcosis, hepatic) OR (hepatic echinococcosis) OR (hepatic echinococcosis) OR (echinococcosis, hepatic

alveolar) hepa (alveolar) hepato) OR (alveolar echinococcosis, hepatic) OR (echinococcosis, hepatic alveolar) OR (hepatic alveolar echinococcosis) OR (hepatic alveolar echinococcosis) OR (hydatidosis, hepatic) OR (hepatic hydatidosis) OR (hepatic hydatidosis) OR (hepatic hydatidosis) hepa (alveolar echinococcus, hepatic) OR (echinococcus, hepatic alveolar) OR (hepatic alveolarechinococcus) OR (hydatid cyst, hepatic) OR (cyst, hepatic hydatid) OR (cysts, hepatic hydatid) OR (hepatic hydatid cyst) OR (hepatic hydatid cyst) OR (hepatic hydatid) OR (hydatid cysts, hepatic) OR (echinococcosis) OR (echinococcus infection) OR (echinococcus infections) OR (infection, echinococcus) OR (cystic echinococcosis) OR (cysticechinococcosis) OR (echinococcosis, cystic) OR (echinoco cystic) OR (hydatidosis) OR (hydatidosis) OR (cysts, hydatid) OR (cyst, hydatid) OR (hydatid cysts) OR (hydatid cyst) OR (hydatid disease) OR (hydatid diseases) OR (echinococcus granulosus infection) OR (echinococcus granulosus infections) OR (granulosus infection, echinococcus) OR (granulosus infections, echinococcus) OR (infection, echinococcusgranulosus) OR (infections, echinococcus granulosus)].

A case was defined as any patient diagnosed with primary duodenal hydatidosis or hydatidosis in any location with secondary duodenal involvement.

A total of 83 articles were obtained with the search strategy described, and all articles that included patients with the characteristics mentioned were selected. Case reports were not excluded. Fourteen articles were chosen, but only 12 could eventually be studied; the other two could not be accessed even though the corresponding publishers were contacted.

The available data on the patients were extracted and included in Tables 1 to 3, for later discussion. They were classified into three groups: Clinical data, including age, sex, main symptoms and signs and personal history; analytical and radiological data, including laboratory data and the main findings reported in the imaging and endoscopic tests performed; and intra and postoperative findings that include the surgeries performed and the complications detected in the postoperative course and follow-up.

RESULTS

The twelve articles selected were all case reports, describing 14 cases. The countries where the 14 patients were treated were: Spain (6), Tunisia (4), India (2), France (1), and Turkey (1)[4,6-16].

Clinical data

Seven patients were men and seven women, with a mean age of 53.14 ± 17.65 years (range: 28-78) (Table 1). Nine of the patients (64%) had abdominal pain (3/9 located in the right hypochondrium, 2/9 in the epigastrium and in the remaining 4/9 not specified). As for the rest of the clinical data, nine patients (64%) presented nausea and vomiting, two of them with hematemesis and one with hydatidemesis. Six patients (42%) had fever > 38ºC, three did not, and in five this information was not specified. Abdominal palpation was painful in ten cases (71%), and in seven patients masses were noted. Other symptoms and signs described included jaundice, melena, hydatidenteria, anorexia and weight loss. Three patients had previously undergone surgery for liver HC.

Analytical, radiological and endoscopic studies

Analytical values were only available in nine patients (Table 2). In six of these (66%) leukocytosis was observed, in three neutrophilia, and in one eosinophilia. Blood analysis was only reported in five patients: In one it was found to be normal, and the remaining four presented low levels of hemoglobin and hematocrit. Serological results of hydatidosis (Casoni test or others) were provided in only six cases (42%: Four positive and two negative); in the other eight, no serological studies were mentioned.

As for radiological studies, only abdominal X-ray was reported in six patients (42%) and thorax in three (21%); in one case both tests were discussed. No simple radiology information was available in six cases (the last six published). Abdominal radiography found masses in four patients, two of which presented calcification. The chest radiograph revealed an over-elevation of the right hemidiaphragm in one patient, the presence of intralesional gas in two, calcification of the wall in one. Abdominal ultrasound was reported in only four patients (28%), three of whom presented cystic lesions.

Computed tomography (CT) was performed in ten patients (71%) (in two of them

Table 1 Clinical data

Ref.	Sex	Age (yr)	Abdominal pain	Nausea and vomiting	Fever	Abdominal exploration	Past Medical history	Other signs and symptoms
Gilmas Y Mocoroa[16], 1963	Male	32	No	Yes (hematemesis)	38.5-39ºC	Six scars from previous surgeries. Slight collateral circulation. Soft splenomegaly with increased percussion. Epigastric pain on palpation	Dyspeptic vagal syndrome. Heartburn. Operated six times for hydatidosis	Melena. Daytime sleepiness and nocturnal delirium. Signs of hypoventilation and hypophonesis of right thorax
Perrotin *et al*[15], 1978	Male	37	Yes (RH)	Yes	38-39ºC	Guarding in the right hypochondrium. Right subcostal mass poorly limited and painful	NA	
Cosme *et al*[13], 1987	Male	55	Yes (E)	NA	38ºC	Painful abdominal mass (12 cm × 15 cm) located in the epigastric region	Pleural effusion, recurrent episodes of bronchitis	Asthenia, anorexia and weight loss of 3 kg
Robbana *et al*[12], 1991	Female	64	NA	Yes (hematemesis)	No	Tender mass in right hypochon-drium	NA	Dysuria, diarrhea
Noguera *et al*[11], 1993	Female	60	Yes	NA	NA	Large tender epigastric mass	NA	Weakness, anorexia and joint pain
Thomas *et al*[14], 1993	Male	31	Yes	Yes (hydatidemesis)	39ºC	Ill-defined mass in the LH. Non-tender mass in the left iliac fossa. Smooth and firm hepatomegaly (6 cm). Rectal examination: Soft cystic swelling in the rectovesical pouch	Fully excised intraabdominal cysts at age 9 and 21 years	Hydatidenteria
Diez Valladares *et al*[9], 1998	Female	68	Yes (E)	Yes	NA	A tender mass in the epigastrium and right hypochon-drium	NA	
Diez Valladares *et al*[9], 1998	Female	84	Yes	Yes	Yes	Tenderness and muscle guarding on the right side and a palpable mass	NA	
Patankar *et al*[10], 1998	Male	35	Yes (RH)	No	No	Non-tender hepatomegaly	NA	
Muinelo Lorenzo *et al*[8], 2012	Male	78	No	No	NA	NA	Osteoarthritis and benign prostatic hypertrophy. Laparoscopy cholecy-stectomy	
Daldoul *et al*[4], 2013	Female	28	Yes (RH)	Yes	NA	Tenderness in right hypochon-drium and right lumbar fossa with lumbar contact	Liver hydatid surgery and recurrent hydatid cysts 21 and 17 years previously	
Daldoul *et al*[4], 2013	Female	63	Yes (EH)	NA	38.6ºC	Abdominal involuntary guarding in the right hypochon-drium	NA	Anaphylactic reaction with diarrhea Chills and jaundice: Bilirubin: 10 mg/dl
Jarrar *et al*[7], 2015	Male	63	NA	Yes	NA	No palpable abdominal mass. Gastric distension	NA	Sensation of weight in RH
Akbulut *et al*[6], 2018	Female	46	No	Yes	No	Painful epigastric palpation	NA	Intra-abdominal aggressive fibromatosis

E: Epigastric; RH: Right hypochondrium; LH: Left hypochondrium; NA: Not available

using contrast). In all of them cystic lesions were discovered (two with calcification) a

Table 2 Analitical, radiological and endoscopic studies

Ref.	Leukocytosis	Red blood cell count	Hydatid serology	X-ray	Ultrasound	CT	Contrast studies	Endoscopy
Gilmas Y Mocoroa[16], 1963	16000 (84% neutrophils)	Erythrocytes: 3.286.000; Ht: 30% ESR: Katz Index 109	Casoni´s test: Positive	Chest: Right hemidia-phragm raised	NA	NA	Barium study: Stomach not filled due to huge cysts compressing the stomach body	NA
Perrotin *et al*[15], 1978	14300/μL	NA	Positive	Abdominal: No findings	NA	NA	Barium study: Heterogeneous opacity from the first portion of the duodenum, rounded cavity of 10 cm in the liver Cholangiography: Slightly dilated bile duct without signs of obstruction	NA
Cosme *et al*[13], 1987	12200/μL	Ht: 34%	NA	Abdominal: Supramesocolic mass of 15 cm deforming the stomach	Inconclusive	Cystic lesion with air-fluid level within the pancreas	Barium study: Fistula in the duodenum filling a pancreatic cyst with gas bubbles	Fistulous opening of 5 cm in diameter, in the duodenal bulb
Robbana *et al*[12], 1991	No	Hgb 11.1 g/dL	Positive	Abdominal: 10 cm right mass by L2-L3	14 cm cyst in right hypochon-drium	NA	X-ray with iodinated intravenous contrast: Right kidney delayed excretion and tumoral syndrome in lower lobe. Barium swallow test: Static stomach, megaduo-denum with fistulae to mass	Esophagitis type 1-2, chronic erosive gastritis. No access to duodenum
Noguera *et al*[11], 1993	NA	NA	NA	Chest and abdominal: Atypical gas bubble in the epigastric region with peripheral calcifications	NA	Cavity in the left hepatic lobe with partially calcified walls with communication with the duodenum. Another cystic multiloculated lesion in the peritoneal cavity lateral to the hepatic flexure of the colon	Iodinated oral contrast passing into the hepatic cavity confirmed the presence of a fistulous communication between the duodenal bulb and the cystic cavity	NA
Thomas *et al*[14], 1993	No	Hgb 11 g/dL	Casoni's test: Strongly positive	NA	Three cysts: Left and right hypochon-drium, pelvis	Multiple intra-abdominal hydatid cysts. One large cyst in the left hypochon-drium communicating with the stomach and the second part of the duodenum	NA	NA
Diez Valladares *et al*[9], 1998	No	Normal	Negative	Abdominal: Calcified circular line in the upper abdomen	NA	Calcified cystic mass in the left hepatic lobe and in continuity with the digestive tube	Barium swallow study: Cavitated mass communicating with the duodenum near the pylorus	Fistula in the pyloric region with features of an echinococcal cyst
Diez Valladares *et al*[9], 1998	18000/μL (85% neutrophils)	NA	NA	Abdominal: Calcified mass in right hypochon-drium	NA	Pneumoperitoneum and a liver cyst with an air fluid level	Barium swallow X-ray showed the presence of a cyst opening into de first duodenal knee	Cyst opening into duodenum
Patankar *et al*[10], 1998	NA	NA	NA	Chest: Crescentic gas shadow under the right dome of the diaphragm	NA	TC Dynamic contrast: Two cystic lesions in right liver lobe. Air in one of the cysts, tracking to the region of the first part of the duodenum. Another multiseptated cyst between urinary bladder and the rectum. CT oral contrast		NA

						showed a megaduodenum and a fistula to the mass		
Muinelo Lorenzo *et al*[8], 2012	NA	NA	NA	NA	NA	3.5 cm hepatic hydatic cyst in segment IV	NA	Fistulous communication with calcified liver mass
Daldoul *et al*[4], 2013	11300/μL (18% eosinophils)	NA	NA	NA	NA	One multilocular hydatic cyst in the posterior part of the lateral sector of the right lobe of the liver, extended into retroperitoneum (until right kidney). Second hydatid cyst in segments I and V of the liver compressing the duodenum with a distended stomach	Barium swallow X-ray: Opacification of the hydatid cavity through a duodenal fistula near the pylorus. Preoperative cholangiogram: Retrograde opacification of the cyst through the duodenal fistula	NA
Daldoul *et al*[4], 2013	13700/μl (92% neutrophils)	NA	NA	NA	Two multivesicular hydatid cysts in segments IV and VI of the liver (5 and 6 cm respectively)	NA	NA	NA
Jarrar *et al*[7], 2015	NA	NA	NA	NA	NA	Upper gastrointestinal stenosis due to a hydatid cyst located in segment VI of the liver attached to the duodenum compressing it extrinsically	NA	Gastric stasis due to extrinsic compression of the second portion of the duodenum
Akbulut *et al*[6], 2018	NA	NA	Negative (postoperative)	NA	NA	CT scan with contrast 100 mm x 80 mm lesion originated by the body of pancreas		NA

CT: Computed tomography; NA: Not available; HT: Hematocryte; HGB: Hemoglobin

The location of the HC causing the duodenal fistula, confirmed by imaging techniques, was the liver in 11 cases (78%), and kidney, pancreas and paraduodenal area in one case each.

Intraoperative findings, postoperative period and follow-up

Twelve patients presented fistula between the HC and the duodenum (Table 3). One patient presented a paraduodenal HC diagnosed incidentally during surgery for abdominal fibromatosis and another had large-scale duodenal compression due to a liver HC, without a true fistula.

Twelve patients (85%) received surgical treatment. Of the other two, one received only pharmacological medical treatment with antiparasitic drugs and in the other the treatment was not specified. The type of laparotomy was reported in seven patients: Three midline, two bilateral subcostal, and two right; in one, the type was not specified.

The techniques performed to treat HC in the twelve patients operated on were conservative in nine [partial cyst resection (3), enucleation (2), evacuation/drainage (2), Lagrot (1) and ablation (1)] and radical in three (all total cystectomies). The procedures performed on the duodenal fistula in the ten patients with this condition

Table 3 Intraoperative findings, postoperative period and follow-up

Ref.	Intraoperative findings	Surgical procedure	Postoperative period morbidity	Follow-up
Gilmas Y Mocoroa[16], 1963	Multiple adherences from past surgeries. Stomach filled with blood clots. Multiple cysts (> 10) across peritoneum, liver, and spleen; duodenal fistula connecting with 15 cm cavity in left hepatic lobe	Ablation of cysts. Gastrostomy. Pylorotomy. Suture of duodenal fistula with surgical drains placed in cavity	Evisceration with massive hemorrhage. Postoperative death	NA
Perrotin *et al*[15], 1978	Adherences are found in the right hepatic lobe. Visualization of liver mass with purulent liquid and food remains. Intraoperative cholangiography: Communication of 1-1.5 cm diameter between the cyst and the duodenum	Fistula closed, cyst drained with a gastric aspiration probe and placement of a cholecystostomy	NA	Day 3: Probe is removed The drainage of the cavity after being washed with lactic acid is removed after 18 d. Control cholangiography and duodenal transit are normal. Follow up in clinics
Cosme *et al*[13], 1987	Infected and multivesicular hydatid cyst in the head of the pancreas closely attached to and communicating with the duodenum	Partial removal of the cyst with two catheters inserted into the cavity	NA	8th week: Injection of contrast through the drainage tubes demonstrating progressive closure of the remaining cavity. Asymptomatic 4 mo after surgery
Robbana *et al*[12], 1991	Calcified hydatid cyst in anterior kidney area. Fistula connected the kidney mass to the duodenum	Evacuation, intralaminar pericystectomy, and reduction of fistula. Vagotomy	NA	Discharged on 17th postoperative day. 7-mo postoperative ultrasound and urography were normal
Noguera *et al*[11], 1993	NA	Enucleation	NA	NA
Thomas *et al*[14], 1993	NA	Medical treatment: Albendazole, Ciprofloxacin, Crystalline penicillin and Chloroquine + US guided aspiration	NA	NA
Diez Valladares *et al*[9], 1998	Segment IV of the liver a 5 cm diameter mass adherent to the pylorus	Total resection of the cyst, including a piece of the duodenal wall, a Heinecke Mikulicz pyloroplasty, cholecystectomy and truncal bilateral vagotomy	Postoperative course was uneventful	Discharged on the 7th day
Diez Valladares *et al*[9], 1998	15 cm multiloculated hydatid cyst in right hepatic lobe in contact with the duodenum. Two hydatid cysts in the greater omentum with purulent fluid	Total cystectomy with resection of the duodenal sinus, excision of the omental cyst. Closure of the bile fistula and bile drainage	NA	Discharged on 15th day
Patankar *et al*[10], 1998	NA	Enucleation	NA	NA
Muinelo Lorenzo *et al*[8], 2012	NA	NA	NA	NA
Daldoul *et al*[4], 2013	Single multilobular hydatid cyst in the posterior part of the lateral sector of the right lobe in close contact with the duodenum. Duodenal fistula affecting the posterior wall of the duodenal knee	Cholecystectomy. Large resection of the prominent cystic dome. Duodenostomy associated with gastrostomy and jejunostomy to treat duodenal fistula	NA	After 6 wk the patient was discharged
Daldoul *et al*[4], 2013	Two liver cysts: Segment V (5 cm) and in the underside of the segment IV (8 cm) in close contact with the first duodenum knee. Exploration of the cystic cavity: Wide communication with the first duodenum and a large fistula with the confluence of the hepatic biliary ducts	Resection of the dome of the 5 cm cyst, duodenal diverticulization and external drainage	NA	Discharged after 3 wk
Jarrar *et al*[7], 2015	Multivesicular hydatid cyst measuring 6 cm, at the right lateral sector, with extraparenchymal development, adhering to the duodenum. Exo-	Lagrot's procedure, a partial cystectomy and epiploplasty filling the residual cavity	Postoperative course was uneventful	NA

	vesiculation of 2 cm, communicating with the cyst, compressing the duodenal wall without fistula			
Akbulut *et al*[6], 2018	The diameter of the fibromatous mass was 120 mm × 100 mm, originated in the pancreatic body and creating adherences to adjacent tissues forming a conglomerate with the fourth portion of the duodenum, jejunal loops and prepyloric stomach antrum	The fourth portion of the duodenum, the jejunum, the distal pancreas and the spleen were removed en bloc. Anastomosis between the third part of the duodenum and the proximal jejunum	Postoperative course was uneventful	NA

NA: Not available; US: Ultrasound.

operated upon were: (1) Closure of the duodenal fistula in five patients, in one case associated with vagotomy, and in another with gastrostomy plus pylorotomy; (2) Partial duodenal resection plus pyloroplasty and bilateral truncated vagotomy (one patient); (3) Duodenostomy, gastrostomy and jejunostomy (one patient); (4) Duodenal diverticulization (one patient); (5) Not specified (one patient); and (6) Intracavitary drainage without action on the fistula (one patient).

Postoperative morbidity and mortality was not described in detail in the articles consulted. In three patients the postoperative period was reported to be uncomplicated, and one patient (7%) died due to hemoperitoneum following massive hemorrhage. In the five cases in which mean hospital admission was mentioned, it was 20.4 ± 11.72 d (range: 7-42). The follow-up was not described in detail, though it was usually very short.

DISCUSSION

Hydatidosis is a zoonosis caused by *Echinococcus spp* associated with livestock activity, predominantly in geographical areas with temperate climates such as Southern Europe, Latin America, Australia, New Zealand, Africa and the Middle East[2]. Primary or secondary duodenal involvement due to HC *via* fistulization or compression from other locations is extremely rare and the number of reported cases is very low[4,6-16]. The main objective of our study was to evaluate the clinical, diagnostic and therapeutic data of duodenal involvement recorded in the literature and to assess whether a diagnostic and therapeutic algorithm could be obtained.

The articles included do not describe any etiological factors or pathogenesis related to duodenal injury caused by HC. HC located in segments IVB, V and VI, and those located in the kidney or pancreas may affect the duodenum, but this is exceptional. Our (unprovable) opinion regarding this low frequency is that a series of conditions must exist for a fistula to appear: The cyst must be large, complicated, and long-standing, since the duodenal wall is sufficiently thick to allow compression without fistulization. If these conditions are met, after very prolonged inflammatory processes the fistula eventually forms.

The epidemiological data of the 14 patients studied do not indicate any differentiation according to sex (the ratio was 1:1) or age (the range was large). Duodenal injury caused by HC is mostly secondary. There was only one case of a paraduodenal cyst; in the cases reported the duodenum was secondarily affected by hepatic (11/13: 85%), renal (1/13) and pancreatic (1/13) HC[4,6-16]. In 90% of patients the injury is in the form of a fistula between the HC and the duodenum. The descriptions in the articles do not indicate which duodenal segment is the most affected. Three patients had previously undergone liver hydatidosis.

The most frequent clinical manifestations in the patients studied were abdominal pain, nausea and vomiting. The rest of the clinical data were very heterogeneous. Two cases presented hydatidemesis and/or hydatidenteria[14], but the condition may also be asymptomatic. Therefore, there is no typical clinical picture of HC affecting the duodenum.

The diagnosis of duodenal fistula due to HC is not always easy. Computed tomography was the most used diagnostic tool in the patients reviewed; it allows accurate location of the HC and its relationship with the digestive tract, but in our study only achieved preoperative diagnosis of fistula in four patients (40%). It should be noted that some reports do not specify whether oral contrast was used in the CT, and some of the older cases the CT techniques used are not comparable to the ones used today[4,6-16]. The use of various radiological tests with contrast, especially in the first cases, allowed the diagnosis of fistula, although barium contrast studies are not currently used. Upper gastrointestinal endoscopy was used in six patients with a diagnostic capacity of 66% (4/6)[4,6-16]. Therefore, we are unable to propose a definitive diagnostic algorithm, but the combination of CT with contrast and upper gastrointestinal endoscopy seems to be the most effective.

In the literature, there is no consensus on which treatment that should be applied. Surgery was performed in practically all patients (12/13), using a wide variety of procedures to treat both HC and duodenal fistula, adapting to intraoperative findings. Conservative techniques were used in 75% of HC, while in the duodenum the most frequent technique (50%) was the closure of the fistula associated with various types of intestinal bypass; partial duodenal resection was performed in only one case. The operative morbidity rate was 7% (1/14), and postoperative mortality 7% (1/14). We believe that the estimation of morbidity is excessively low, because in many articles it is not reported or it is simply stated that the postoperative period was uneventful; in addition, duodenal surgery usually presents a much higher complication rate than that reported in these patients. Furthermore, in the cases in which data on postoperative stays are given, these stays are much longer than one would expect in uncomplicated patients. The follow-up carried out in patients is not usually specified, so we cannot draw any conclusions about disease recurrence or long-term evolution.

Our intention in carrying out this systematic review was to provide a therapeutic algorithm for hydatidosis that affects the duodenum, but especially the surgical strategy to be performed. As we have summarized, no conclusion can be drawn from the literature. Our group thinks that radical surgery is the best approach in hydatidosis, but we also believe that surgical procdures should be tailored to every patient. Our non Evidence Based Medicine supported recommendation is: If the surgeon thinks that radical surgery is technically feasible, should be performed in the liver and duodenum. But if an unacceptable risk is to be assumed to carry it out, conservative procedures can be useful in the most bizarre cases. Duodenal surgery is not simple, nor is it free of complications, and techniques can range from a simple closure, duodenal exclusion to duodenectomies. The decision of which technique to perform in the duodenum will depend on the amount of circumference affected, the duodenal segment to be treated, and anatomical relationship with the Vater´s ampulla.

The following conclusions can be drawn from this systematic review. The reports of duodenal damage by HC are based on isolated clinical cases with very low scientific evidence; the most common clinical findings are abdominal pain, nausea and vomiting; the combination of computed tomography plus endoscopy seems to be the best diagnostic option; finally, the most frequently recommended treatment is surgery to treat the HC and to close the fistula.

ARTICLE HIGHLIGHTS

Research background

The duodenum is an organ that is very rarely affected by hydatid cysts, either primarily or secondarily.

Research motivation

Duodenal involvement by hydatidosis is an exceptional finding and this study aimed to characterize epidemiological, clinical and diagnostic features and options of treatments.

Research objectives

Our aim is to characterize epidemiological, clinical and pathological features and options of treatment in hydatidosis with duodenal involvement.

Research methods

We perform a system review including all patients with hydatidosis and duodenal involvement following PRISMA guidelines.

Research results

This review shows that the most frequent symptoms were abdominal pain, nausea and vomiting. Computed tomography was the most used diagnostic imaging technique, and hydatid cyst drainage and fistula closure *via* laparotomy was the most frequent treatment.

Research conclusions

Literature on hydatidosis with duodenal involvement is scarce, only case reports, so the evidence is low.

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META-ANALYSIS

Prevalence of anxiety among gestational diabetes mellitus patients: A systematic review and meta-analysis

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Abstract

BACKGROUND

A diagnosis of gestational diabetes mellitus (GDM) negatively influences maternal mental health. There is a lack of systematic review and meta-analysis on prevalence of anxiety among GDM women.

AIM

To pool data from existing literature to determine the pooled estimates for the prevalence of anxiety among women diagnosed with GDM.

METHODS

We searched multiple databases including MEDLINE, Cinahl, PubMed and Scopus to identify studies published up to 31 October 2019 with data on the prevalence of anxiety among women diagnosed with GDM. Data were extracted from published reports. Estimates were pooled using random-effects meta-analyses.

RESULTS

We reviewed 19 abstracts, retrieved 10 articles and included three studies

to the PRISMA 2009 Checklist.

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incorporating 12744 GDM women from three countries. The pooled prevalence of anxiety was 29.5% (95%CI: 6.9, 52.0) among GDM women.

CONCLUSION
Prevalence of anxiety among GDM women was high. We suggest that epidemiological studies on anxiety should be conducted urgently as it merits clinical attention. In addition, it is important to identify factors associated with anxiety among women diagnosed with GDM.

Key words: Prevalence; Anxiety; Gestational diabetes; Psychiatry; Meta-analysis; Systematic review

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Core tip: This is a systematic review and meta-analysis reporting the pooled prevalence of anxiety among gestational diabetes mellitus patients which stood at 29.5%.

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INTRODUCTION

The prevalence of gestational diabetes mellitus (GDM) has been increasing over the past decades[1,2]. Globally, GDM has been reported as a leading cause of morbidity and mortality among both the infants and their mother[3,4]. Mothers with GDM are at increased risk of getting pregnancy complications such as preterm delivery, preeclampsia, abnormal birth weight, metabolic and electrolyte disorders[5]. Studies also indicated that GDM may persist after postpartum and subsequently develop into overt diabetes mellitus, and it was estimated that the risk for developing diabetes mellitus after GDM increased linearly with the duration of follow-up ranged from 19.72% at 10 years. The estimated risks for type-2 diabetes mellitus ranged from 19.7% at 10 years to 39.0% at 30 years[6]. Neonates born to GDM mothers are at higher risk of suffering from adverse neonatal outcomes such as abnormal birth weight, congenital anomalies, hypoglycaemia and longer duration in neonatal intensive care unit for further investigation[7-9].

Previous studies showed that the prevalence of depression among mothers with GDM were ranging from 25.9% to 56.7%[10,11] and the prevalence of anxiety was within a range of 4.8% to 57.7%[12,13]. Anxiety is a normal reaction to stress which involves both psychological and physical reactions. It becomes clinically significant when the anxiety grows out of proportion to the situation and causes functional impairment. Anxiety disorders are among the most common mental illnesses, and are characterized by feelings of tension, worrying thoughts and physical changes such as increased blood pressure. People with anxiety disorders usually have recurring intrusive thoughts or concerns. They may avoid certain situations out of worry. They may also have physical symptoms such as sweating, trembling, dizziness or a rapid heartbeat[14]. With a remarkable increase in lifetime prevalence, anxiety has become a public health burden worldwide, causing increased use of mental health services and loss of productivity[15]. In particular, anxiety is a common psychiatric condition that affects up to one-fifth of the pregnant mothers[16] and is significantly associated with postpartum depression (odds ratio = 2.6, 95%CI: 2.0, 3.5) and reduced odds of breastfeeding (odds ratio = 0.63, 95%CI: 0.5, 0.7)[17]. Thus, anxiety and related mental conditions could pose negative effects on child development[18]. A high state of anxiety is found in 15.8% of pregnant women, while 12.5% of women suffer high trait anxiety[19]. Similarly, pregnant mothers with GDM were more anxious than pregnant women with other medical problems or healthy pregnant women[20].

There are multiple factors associated with anxiety during pregnancy, including current or past pregnancy-related complications, previous pregnant loss and personal history of mental illness[21]. Study have also shown that women with GDM experience

significantly worse quality of life[22]. However, findings from previous studies indicate that there is a lack of data on the epidemiology of antenatal anxiety among GDM patients. Therefore, we aimed to determine the pooled prevalence of anxiety among GDM patients by conducting a meta-analysis.

MATERIALS AND METHODS

This present study was registered in the Medical Research and Ethics Committee, Ministry of Health Malaysia (registration number: NMRR-20-117-52644), and conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses[23]. As this work only involved secondary data retrieval and analysis, no ethical approval was sought.

Literature search

Two investigators (Lee KW and Loh HC) independently searched MEDLINE, Cinahl, PubMed and Scopus databases for potential studies published in journals from inception to 31 October 2019. We used following search terms: (Anxiety OR anxiety symptom OR anxiety disorder OR generalized anxiety disorder OR panic disorder OR panic attack OR agoraphobia OR phobia OR specific phobia OR specific phobic disorder OR medication-induced anxiety disorder OR medical condition induced anxiety disorder OR social anxiety disorder) AND (prevalence) AND (gestational diabetes OR GDM OR gestational diabetes mellitus OR diabetes in pregnancy). The search strategies with the Boolean or phrase operators were shown in the Supplementary material 1. Studies in English, available in full-text and conducted among humans were searched. Then, we removed duplications using Endnote, after that we screened the title and abstracts for its suitability. Finally, articles with their full text were assessed for eligibility to be recruited into the quantitative analysis.

Inclusion criteria

Any studies that reported the prevalence or percentage for anxiety symptoms or anxiety disorders among GDM patients and fulfilled the inclusion criteria were analysed. The inclusion criteria were as follows: (1) Diagnosing or screening of anxiety was made according to Diagnostic and Statistical Manual of Mental Disorders/International Classification of Diseases diagnostic criteria or by any screening tools; (2) Participants in the study were diagnosed with GDM; and (3) Studies were published in English peer-reviewed journal from inception to 31 October 2019. Other related studies were also included through careful review of the reference lists of related review articles and reverse-forward citation tracking. Studies were excluded if they included only pregnant mothers with pre-existing diabetes mellitus, were of case-control design or examined anxiety prior to the diagnosis of GDM.

Study selection

All relevant articles identified through the above databases were imported into Endnote programme X5 version. Initially, we performed de-duplication. Two investigators independently screened each title and abstract for suitability based on the search strategies mentioned above. Then, full-text articles were assessed based on the inclusion criteria mentioned above. Any disagreements between the investigators were resolved through discussions before the final consensus for quantitative analysis was reached.

Data extraction

The following data were extracted from every study: The last name of the first author, year of publication, country, sample size, study design, recruitment duration, timing of GDM diagnosis, GDM diagnosis guidelines, assessment of anxiety guidelines, and timing of anxiety assessment. The outcomes measures included the numbers of GDM patients with anxiety symptoms or disorders and total number of GDM patients. Two investigators (Lee KW and Loh HC) individually extracted the data and assessed the study quality, with differences resolved through discussion with the third and fourth investigators (Ching SM and Hoo FK).

Quality assessment

The quality of the individual studies was determined using the checklist of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)[24].

The aim and use of STROBE were to assess the strengths and weaknesses of the studies reported in the medical literature. STROBE results also helps readers to know what was planned, done and found, as well as what is incomplete and inadequate in the reporting of the articles. The tool consists of 22 items to help assess the important components found in observational studies. In certain instances where the information provided was insufficient in order to make judgement for a certain item, that item was graded with a "0", rendering the item as having a high risk of bias. Each article's quality was graded as "good" if the STROBE score was ≥ 14; or graded as "poor" if the STROBE score was < 14. Two investigators (Devaraj NK and Maiza T) individually assessed the study quality, with differences resolved by discussion with the third and fourth investigators (Ching SM and Hoo FK). Studies were included in analysis regardless of STROBE score and grade.

Statistical analysis

A random-effects (DerSimonian and Laird Method) meta-analysis method was employed to pool the prevalence estimated from these related studies and was reported with a 95%CI. I^2 index was used to assess the studies heterogeneity (*i.e.* low is < 25%, moderate 25%–50%, and high > 50%) that indicated the total percent of discrepancy due to variation in the included studies[25]. For statistical analysis, Open Meta (Analyst) software was used, this software can be accessed and downloaded from http://www.cebm.brown.edu/openmeta/index.html[26]. Funnel plot was generated using The Jamovi project computer Software which can be retrieved from https://www.jamovi.org[27].

RESULTS

Description of included studies

Thirty manuscripts were identified in the initial screening as shown in Figure 1. After removal of duplicate articles (n = 11), a total of 19 studies were retrieved for further assessment. After screening for its suitability through title and abstract, 10 studies fulfilled both our inclusion and exclusion criteria. After careful evaluation of the 10 articles, only three studies were eligible for quantitative analysis in this study.

Characteristics of included studies

The main characteristics of the included studies are shown in Table 1. A total sample of 12744 women diagnosed with GDM was included in the analysis. The respondents were diagnosed using either American Diabetes Association or World Health Organization guidelines. These studies were conducted in Canada[13], Ireland[12] and Malaysia[28]. In terms of diagnosing or screening for anxiety, Beka *et al*[13] (2018) used the diagnostic criteria of the International Classification of Diseases- Ninth version (ICD-9) (prior to 2002) and the International Classification of Diseases- Tenth version (ICD-10) (2002 onward); while Egan *et al*[12] (2017) and Lee *et al*[28] (2019) used 21-item Depression Anxiety Stress Scale (DASS-21). For quality assessment, we assigned each study with an overall rating based on the tool derived from STROBE checklist. The overall quality of included studies appeared to be good.

Prevalence of anxiety

The overall pooled prevalence of anxiety was 29.5% (95%CI: 6.9, 52.0) (Figure 2). The pooled prevalence of anxiety using DASS-21 was higher than prevalence of anxiety using ICD-9/10 (42.4% *vs* 4.8%). Sensitivity analysis reveals that study by Beka *et al*[13] had substantial influences on the overall prevalence, which caused prevalence of anxiety to increase from 29.5% (95%CI: 6.9, 52.0) to 42.4% (95%CI: 13.2, 71.5). On the other hand, removal of studies by Egan *et al*[12], 2017 or Lee *et al*[28], 2019, it did not cause statistically significant changes to the overall prevalence of anxiety (Supplementary material 2). Indeed, funnel plot (Supplementary material 3) suggested that there was publication bias. Nevertheless, we did not exclude any studies from the meta-analysis in view there was only three studies available.

Quality assessment

We assigned the studies with an overall rating based on STROBE checklist. All three studies received an overall "Good" quality with a score of ≥ 14 over 22 (Supplementary materials 4-6). In summarizing the results, we concluded that all studies had methodological issues such as not describing any efforts to address

Table 1 Characteristics of the included studies

Ref.	Year	Country	Study setting	Diagnostic guidelines for GDM	Diagnostic or screening methods for anxiety	Mean age of GDM patients	Ethnicity among GDM	Number of GDM patients with anxiety	Total number of GDM patients	Prevalence of anxiety	Quality (score)
Beka *et al*[13]	2018	Canada	Population	ADA	ICD-9 (Prior to 2002) and ICD-10 (2002 onward)	32.1 ± 5.3	Aboriginal (6.9%); Caucasian (70.8%), Chinese (6.9%), South Asian (15.5%)	584	12140	4.8	Good (18)
Egan *et al*[12]	2017	Ireland	Hospital	N/A	21-item Depression Anxiety Stress Scale	33.6 ± 4.8	Caucasian (89.7); Non-Caucasian (9%)	45	78	57.7	Good (14)
Lee *et al*[28]	2019	Malaysia	Hospital	WHO	21-item Depression Anxiety Stress Scale	32.3 ± 4.9	Malay (82.3%); Non-Malay (17.7%)	147	526	27.9	Good (15)

Clinically significant anxiety symptoms/disorders in 2nd trimester. ADA: American Diabetes Association; WHO: World Health Organization; GDM: Gestational diabetes mellitus; ICD: International Classification of Diseases.

potential sources of bias, how the missing data were addressed, and lacking of sensitivity analysis.

DISCUSSION

Our systematic review and meta-analysis offer preliminary evidence regarding the prevalence of anxiety among GDM patients. The results indicated that the pooled prevalence of anxiety among GDM patients was 29.5%.

Several reasons may have contributed to the high heterogeneity (I^2 = 99.12%) in the pooled prevalence that was seen in our systematic review and meta-analysis. First, there are differences in terms of the methodological approach used in different studies for the detection of anxiety. The diagnostic method would identify specific anxiety disorders with more stringent criteria, while the screening method served as case identification. Diagnostic versus screening criteria used by different studies for the clinically significant anxiety symptoms were omitted. For instance, Beka *et al*[13] (2018) used ICD-9 and ICD-10 to diagnose anxiety disorder while Egan *et al*[12] (2017) and Lee *et al*[28] (2019) used DASS-21 for screening of anxiety symptoms. Unlike ICD, DASS-21 is a screening tool with 21 items which consists of three domains assessing depression, anxiety and stress[29]. DASS-21 English version has been translated and validated into Malay version by Musa *et al*[30]. DASS-21 has distinctive cut-off value for severity rating; anxiety is detected if anxiety domain score is ≥ 8 (Mild and above)[29], however it should be noted that clinically significant anxiety symptoms should be of moderate and above in its severity.

The vast disparity of anxiety prevalence between study population may be one of the reasons for the discrepancy. Study by Beka *et al*[13] (2018) was a population-based study, while studies by Egan *et al*[12] (2017) and Lee *et al*[28] (2019) were hospital-based

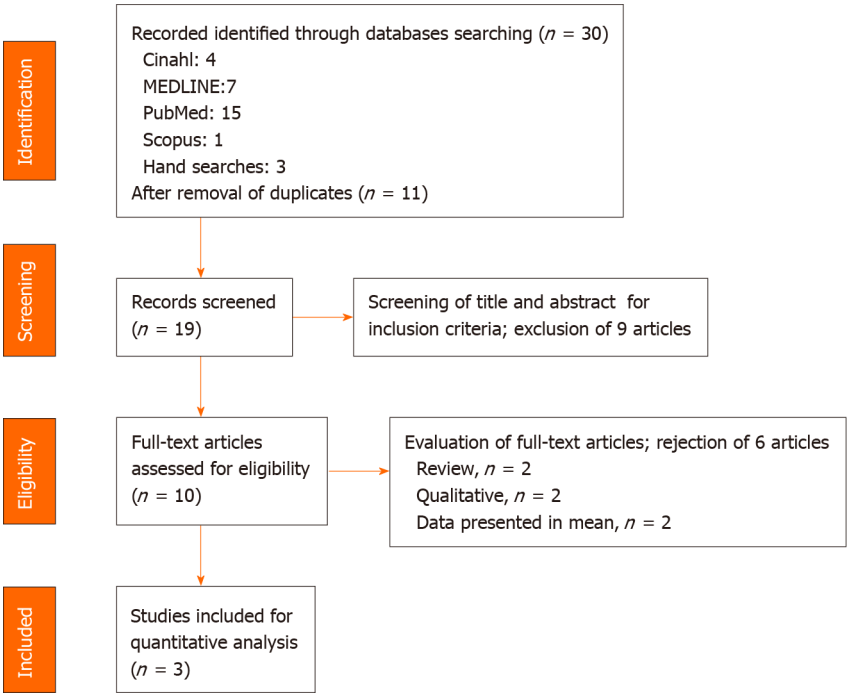


Figure 1 Preferred reporting items for systematic review and meta-analysis protocols flow diagram of the literature screening process.

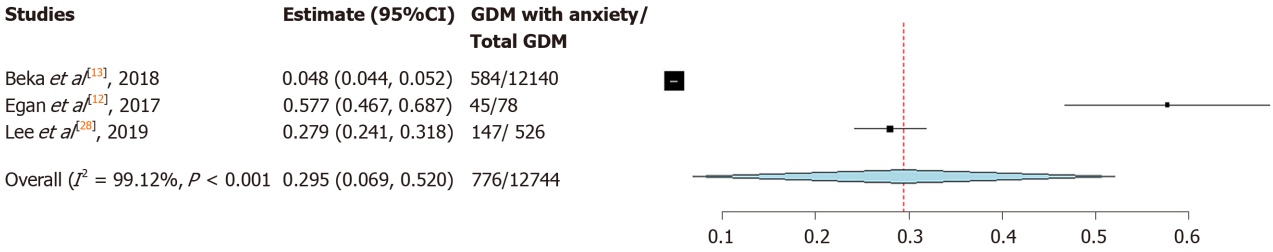


Figure 2 The forest plot of the pooled prevalence of anxiety among gestational diabetes mellitus patients. GDM: Gestational diabetes mellitus.

study. In the study by Beka *et al*[13] (2018), there was no sample size calculation, and the patients' medical information were obtained via health services databases. The weakness of health services databases is that it contains information about formal diagnosis and healthcare services provided for patients, yet it didn't provide results of mental health screening, therefore the prevalence of anxiety (4.8%) reported by Beka *et al*[13] (2018) may not reflect the prevalence of clinically significant anxiety faced by GDM patients. We noted that two studies which conducted in hospital (Egan *et al*[12], 2017 and Lee *et al*[28], 2019) had sample size calculation; these two studies achieved sufficient sample number. However, Egan *et al*[12] (2017) had a sample size of less than 100 for GDM patients. Hence, the prevalence of clinically significant anxiety symptoms in Egan *et al*[12] (2017) (57.7%) was higher compared to Lee *et al*[28] (2019) (27.9%). Sample size remains an important criteria when determining the prevalence of anxiety, as studies have shown the positive correlation between sample size and prevalence[31,32]. Hence, all these reasons might have contributed to the high heterogeneity in the prevalence of anxiety in our study.

More than half of pregnant women showed moderate anxiety during their pregnancy[33,34]. Anxiety during pregnancy could be due to worries about health and well-being of the babies and the mothers themselves. The worries also extend to the concern of parenting and the transition to maternal role after birth[28]. A meta-analysis reported that antenatal anxiety could increase the risk for adverse birth outcomes such as preterm delivery (relative risk = 1.50, 95%CI: 1.33, 1.70) and low birth weight (relative risk = 1.76, 95%CI: 1.32, 2.33)[35].

Around 11.5% of pregnant women in Asia are affected by GDM[36]. Recent meta-analysis reported that hyperglycaemia in pregnancy increases the risk for adverse outcomes such as caesarean section (OR = 1.59, 95%CI: 1.49, 1.70), large for gestational

age (OR = 2.11, 95%CI: 1.73, 2.58), macrosomia (OR = 2.06, 95%CI: 1.86, 2.28), neonatal hypoglycaemia (OR = 1.37, 95%CI: 1.20, 1.57), gestational hypertension (OR = 1.91, 95%CI: 1.49, 2.43) and pre-eclampsia (OR = 2.15, 95%CI: 1.45, 3.19)[5]. GDM patients are at higher risk for experiencing anxiety as compared to pregnant women without medical complications[20]. Similarly, the adverse birth outcomes could be exacerbated if women with GDM experiences anxiety during pregnancy.

Antenatal anxiety is an evolving field, and unlike depression, only a few studies have been conducted among GDM patients. However, studies have reported that antenatal anxiety is more prevalent than antenatal depression[28,34], and this study reports anxiety symptoms are prevalent in GDM patients. In order to promote the detection of antenatal anxiety, several screening tools have been recently recommended by National Institute for Health and Care Excellence, which include Generalized Anxiety Disorder scale, GAD-2[37]. GAD-2 can be used as an ultra-brief screening scale for antenatal anxiety. Even so, other screening scales are more commonly used in clinical setting as compared to GAD-2, such as DASS-21[29], Edinburgh Postnatal Depression Scale[38], Hospital Anxiety and Depression Scale – Anxiety subscale[39], State-Trait Anxiety Inventory[40], GAD-7[41], Brief Measure of Worry Severity[42], Cambridge Worry Scale[43] and Wijma Delivery Expectancy/Experience Questionnaire –Version A[44].

Impacts of anxiety after delivery period

Mental illness is a leading cause of maternal morbidity and even endangers maternal life especially in high -income countries[45]. Indirectly it also impacts new-born babies, causing perinatal morbidity and mortality as well as the impact on the long-term child development[46,47]. The National Institute for Health and Clinical Excellence (NICE) has emphasised that perinatal mental illness is one of the most important issues in women's health that need to be highlighted, especially in the postpartum period[48,49].

The prevalence of postpartum anxiety disorders varies. Reck *et al*[50] (2008) and Miller *et al*[51] (2006) found a comparable percentage of postpartum women having anxiety disorder, at 11.1 % and 10 % respectively. Matthey *et al*[52] documented that 16.2% of mothers were diagnosed with a pure anxiety disorder while Wenzel *et al*[53] (2005) noted a prevalence rate of 8.2% for generalized anxiety disorder.

There were many reviews confined to maternal depression in postpartum period but there were scarcity of data on anxiety disorder despite of the high health risks for both mother and child associated with postpartum disorders[50]. Maternal anxiety disorder is part of a broad spectrum condition comprising of mild to severe mental illnesses such as bipolar disorder and psychotic disorder. It is common that both anxiety and depression co-exist in postpartum women[51,54].

Socio-demographic factors and socioeconomically deprived status have important impacts on maternal mental illness. The most common risk factors include age of more than 35 year old, single parent, lower educational level and low -income family[47,55]. Women with greater socioeconomic deprivation are more likely to have maternal mental illness than those with lesser degree of socioeconomic deprivation[56,57]. An early recognition of women at risk and implementation of effective intervention are essential as preventive measures to treat maternal mental illness accordingly, aiming to reduce the complications related to maternal mental illness.

Strength and limitations

To date, this is the first systematic and meta-analysis on anxiety among patient with GDM. This study clearly indicates that anxiety is prevalent among GDM patients. The finding of this review is consistent with the previous literature pertaining to anxiety among pregnant women experiencing medical complications. However, there are several limitations. Strict inclusion/exclusion criteria and a paucity of literature on the topic of interest have resulted in the inclusion of only three papers. However, according to Valentine *et al*[58], 2010, the minimum number of studies needed to conduct a meta-analysis is two. On top of that, we did sensitivity analysis and funnel plot to show the publication bias. Nevertheless, due care is necessary when interpreting the results as at least 5 studies or more are needed to reasonably and consistently achieve powers from the random-effects meta-analyses that are greater than the studies that contribute to them[59]. Second, the pooled sample size is not large enough to reflect the anxiety prevalence in clinical setting, therefore limiting the generalizability of our study findings.

In conclusion, our study provides an estimation of the prevalence of anxiety among patients with GDM. Our study showed that the pooled prevalence was high at 29.5%. We recommend that more epidemiological studies on anxiety during pregnancy to be conducted in this particular population. In addition, it is important to identify factors

associated with anxiety during pregnancy so that early detection and intervention can be implemented to improve various obstetric and mental health outcomes.

ARTICLE HIGHLIGHTS

Research background

There is lack of systematic review and meta-analysis on prevalence of anxiety among GDM women.

Research motivation

The systematic review and meta-analysis reporting the pooled prevalence of anxiety among GDM patients is high (29.5%).

Research objectives

Authors aimed to pool data from existing literature to determine the pool estimates for the prevalence of anxiety among women diagnosed with GDM.

Research methods

Multiple databases including MEDLINE, Cinahl, PubMed and Scopus were searched to identify studies published up to 31 October 2019 with data on the prevalence of anxiety among women diagnosed with GDM.

Research results

Total 19 abstracts, retrieved 10 articles and included three studies incorporating 12744 GDM women from three countries were reviewed. The pooled prevalence of anxiety was 29.5% among GDM women.

Research conclusions

The results suggest that epidemiological studies on anxiety should be conducted urgently as it merits clinical attention. In addition, it is important to identify factors associated with anxiety among women diagnosed with GDM.

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MINIREVIEWS

Primary small cell neuroendocrine carcinoma of the right posterior tongue

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Abstract

Small cell neuroendocrine carcinoma (SNEC) is an extremely aggressive tumor and mainly occurs in the lung. Primary extra-pulmonary SNEC is rare. To date, only 11 primary SNECs occurring in the oral cavity have been reported in the English literature. We describe a case of primary SNEC of the right posterior tongue in a 46-year-old man. The patient had stage IVA disease and received adjuvant chemotherapy, followed by radical surgery and radiotherapy. He remained tumor-free for 20 mo before death due to gastrointestinal metastasis. The relevant literature on the 11 previously reported patients was reviewed, and the clinical features, histopathological characteristics, differential diagnosis and therapeutic strategies of this rare tumor were analyzed.

Key Words: Small cell carcinoma; Neuroendocrine carcinoma; Oral cavity; Head and neck; Extrapulmonary

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Core Tip: Primary extra-pulmonary small cell neuroendocrine carcinoma (SNEC) is extremely rare. There are only 11 primary SNECs occurring in the oral cavity reported in the English literature. This time, we describe a case of primary SNEC of the right posterior tongue in a 46-year-old man. The treatment of this patient is described in detail. The relevant literature of the 11 previously reported patients was reviewed, and the clinical features, histopathological characteristics, differential diagnosis and therapeutic strategies

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INTRODUCTION

Small cell neuroendocrine carcinoma (SNEC) is the most common type of pulmonary neoplasm and is an aggressive malignant tumor with a high tendency for regional and distant metastasis. Extra-pulmonary SNECs account for 2.5%-5% of all SNECs, of which head and neck SNECs account for 10%-15%[1]. The first case of SNEC in the head and neck was reported in 1963, and the larynx is the most common site, followed by the salivary glands and the sinonasal region[2]. Primary SNEC in the oral cavity is extremely rare and to date only 11 primary SNECs occurring in the oral cavity have been reported in the English literature[3-13].

The management of extra-pulmonary SNECs has not been standardized, but is extrapolated from pulmonary SNEC due to their similar clinicopathologic features[14,15]. In addition to chemotherapy as an effective therapeutic modality, radical surgery and radiation therapy may also play an important role depending on the primary site and clinical stage of the tumor[16]. The current report presents a rare case of primary SNEC arising in the right posterior tongue. The clinicopathologic characteristics of this rare tumor are discussed and the relevant literature is reviewed.

NEW CASE OF SCNC

A 46-year-old man, who had a 30-year history of smoking and alcohol consumption, presented to the oral and maxillofacial department in September 2013 with a painful mass which grew rapidly of the right posterior tongue during the previous 3 mo and a painful mass in the contralateral upper jugular area for 1 mo. Physical examination identified a hard polypoid mass of the right posterior tongue, measuring 2.5 cm × 2.5 cm, with obvious tenderness and low mobility (Figure 1). A painful mass with low mobility, measuring 2.0 cm × 3.0 cm, in the contralateral upper jugular area was also identified.

Computerized tomography (CT) scanning showed an ill-defined mass with heterogeneous enhancement in the right posterior tongue and contralateral cervical lymph node enlargement (Figure 2). A CT scan of the chest and abdomen and a positron emission tomography-CT scan revealed no abnormalities. Findings from laboratory examinations, including routine blood, blood biochemistry and urine analysis, were within normal limits. An incision biopsy under local anesthesia was performed. Microscopically, round and spindly small cells presented with ovoid or spindle shaped nuclei, fine granular chromatin, inconspicuous nucleoli and scant cytoplasm, forming broad nests, sheets or cord shapes (Figure 3). Immunohistochemically, these cells were positive for synaptophysin (referred to as syn), N-CAM (CD56), chromogranin A and cytokeratin AE1/AE3 (Figure 4). The proliferation index evaluated by Ki-67 labeling was 90% (Figure 5). The cells were negative for human melanoma black45 (referred to as HMB-45), cytokeratin 20 (referred to as CK20), vimentin, S100 protein, leucocyte common antigen, CD99, smooth muscle actin and thyroid transcription factor1.

A combined modality therapy was approved by multidisciplinary discussion. After two cycles of chemotherapy (cisplatin 75 mg/m^2, day 1 and etoposide 100 mg/m^2, day 1-3, 3 wk per cycle), the primary tumor and cervical lymph nodes partially decreased according to the Response Evaluation Criteria in Solid Tumors. Radical resection, including extensive resection of the primary tumor with contralateral radical and homolateral functional neck dissection and reconstruction of the tongue with a left free forearm flap was performed. The diagnosis of SCNC was confirmed by postoperative pathology. A 60 Gy dose of radiotherapy was administered after surgery. The patient remained tumor-free for 20 mo before his death due to gastrointestinal metastasis.



Figure 1 Clinical photograph showing the gross appearance of tumor in the right root of tongue.

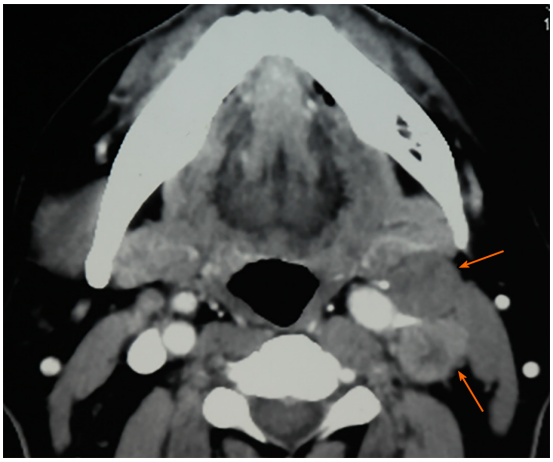


Figure 2 Preoperative computerized tomography scan showing contralateral cervical lymph nodes enlargement (orange arrows).

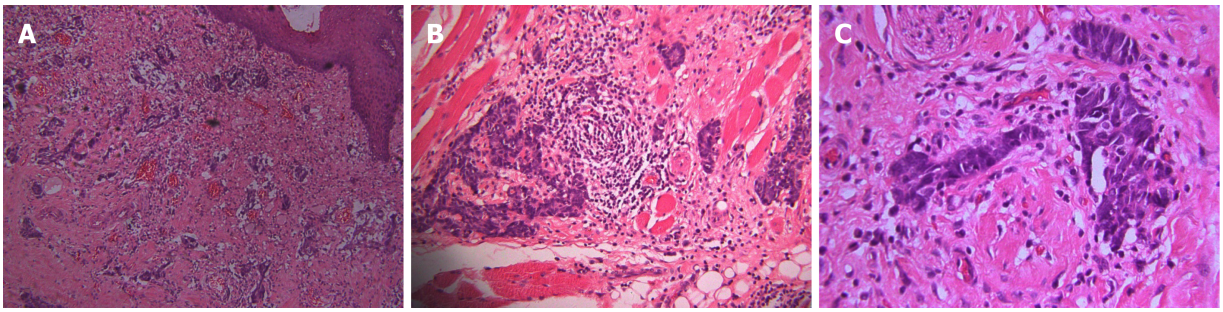


Figure 3 Microscopically, round and spindly small cells with ovoid- or spindle-shaped nuclei, fine granular chromatin, inconspicuous nucleoli and scant cytoplasm formed broad nests, sheets or cord shapes. A: The tumor cells were located in the fiber and striated muscle tissues below the mucous membrane, arranging in nest, sheet and cord shapes (× 100); B: The tumor cells were found to infiltrate the fiber and striated muscle tissues, secondary inflammatory reactions were also found in the interstitial fiber, vascular tissue and nerve tissue, and infiltrating lymphocytes and neutrophils were present (× 200); C: The nucleolus of the tumor cells were unsuspicious but heteromorphosis and nuclear mitosis were obvious (× 200).

DISCUSSION

According to the World Health Organization Blue Book 2017, neuroendocrine carcinomas (NECs) are now classified into three categories: Well-differentiated, moderately-differentiated and poorly-differentiated, which is additionally divided into two subtypes: SNEC and large cell NEC[17]. Primary NECs in the oral cavity have been subclassified into typical carcinoid, atypical carcinoid, large cell NEC and SNEC[18]. The synonyms for SNEC include "small cell carcinoma", "oat cell carcinoma" and "anaplastic small cell carcinoma"[19]. Primary SNEC occurring in the oral cavity is extremely rare and has a poor prognosis. Positron emission tomography-CT and CT

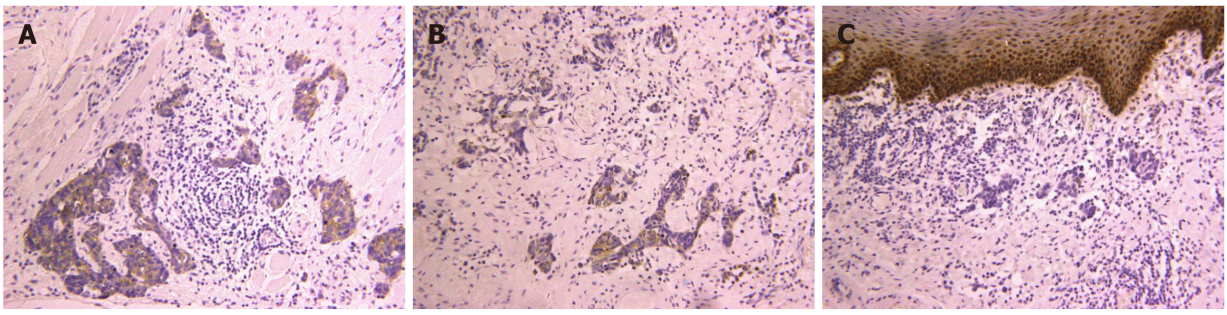


Figure 4 Immunohistochemical analysis showed positivity for (A) synaptophysin, (B) chromogranin A, and (C) cytokeratin AE1/AE3. Magnification: × 200.

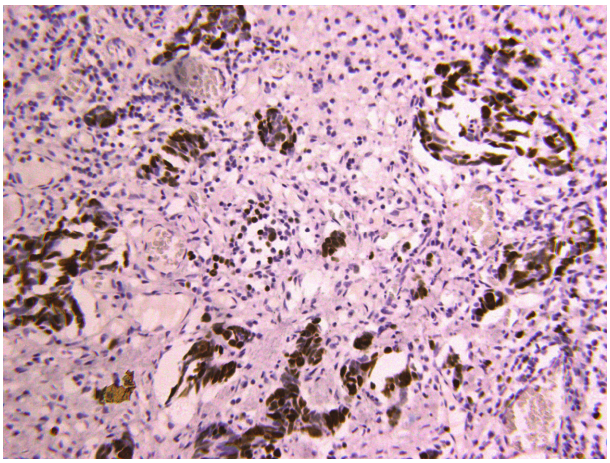


Figure 5 About 90% of the tumor cells showed Ki67+ nuclear staining, suggesting a high proliferation activity.

images confirmed that the current patient had a rare primary SNEC of the oral cavity, and not a metastatic SNEC arising from the lung.

The clinical characteristics of these rare cases reported in the English literature are shown in Table 1. Most of the patients were men (81.8%). The median age of the patients was 67.5 years (range: 40-83 years). Smoking and alcohol abuse, as the major risk factors, were also common among these patients. These results are similar to those of NEC in the head and neck[10]. The tongue was the most common subsite (63.6%). Minor salivary glands may be the prevailing origin of primary SNEC in the oral cavity[11]. Most of the patients had lymph node and distant metastasis with stage III, IVA, IVB and IVC tumors (72.7%), which are also similar to the results of previous studies on extra-pulmonary SNEC in the head and neck. In patients with positive cervical lymph nodes, the recurrence rate is high and distant metastasis frequently occurs. In addition, these patients have a poor median survival of 10.0 mo and a 2-year survival rate of 15.7%[16]. Therefore, a comprehensive examination is mandatory to exclude distant metastasis. The outcomes of the previously reported 11 patients were as follows: 3 died of tumor, 1 died from other causes, 6 showed no evidence of disease, and the status of 1 patient was unknown.

The definite diagnosis of SNEC depends on histopathology and immunohistochemistry analyses. Morphological examination showed extensive proliferation of round and spindly small cells with ovoid-to-spindle shaped nuclei, fine granular chromatin, inconspicuous nucleoli and scant cytoplasm, which could help in the differential diagnosis of typical and atypical carcinoids and large cell NEC[8]. These small cells formed broad nests, sheets or cord shapes. Necrosis is typically extensive and the mitotic count is high. The presence of electron-dense and neurosecretory granules is distinctive in SNEC[20]. The cells are distinctively positive for neuroendocrine markers including syn, N-CAM (CD56) and chromogranin A[17]. High Ki-67 labeling (90%) indicates high proliferative activity of tumor cells[8].

Immunohistochemical staining is crucial for the differential diagnosis of SNEC from other malignancies. Extensive mitotic index and necrosis can distinguish SNEC from typical and atypical carcinoids. In contrast to SNEC, large cell NEC contains cells with

Table 1 Clinical characteristics of the primary small cell neuroendocrine carcinomas in the oral cavity

Publication year	Age in yr	Sex	Smoking	Alcohol	Primary subsite	Disease stage	Therapy	RT dose in Gy	CM	Follow-up time in mo	RE	DM	Patient status[1]
1984	62	M	+	+	Tongue	IVA	S + RT + CM	Un	C+A+O	10	-	+	Died of DM
1995	76	M	+	+	Tongue	IVA	RT	72	-	2	-	-	Died of debility
2001	62	M	+	+	Mandible	IVA	-	-	-	3	-	+	Died of DM
2013	75	M	Un	Un	Tongue	III	S + RT	Un	-	Un	Un	Un	Un
2013	85	M	Un	Un	Cheek	III	CRT + S	40	CA	2.5	-	+	Died of DM
2013	59	M	Un	Un	Cheek	IVC	CM	Un	CI	16	-	+	Alive
2014	55	F	+	-	Tongue	IVA	S + CM	Un	Un	Un	-	-	Alive
2015	73	M	+	+	Lower gingiva	II	S + CM	-	CI + E	14	-	-	Alive
2015	54	M	Un	Un	Tongue	I	S + CM + RT	66	CI + E	Un	-	-	Alive
2015	40	F	Un	Un	Tongue	I	S + CM + CRT + CM	60	CI + E	52	-	-	Alive
2017	74	M	Un	Un	Tongue	IVC	CM	-	CA + E	9	-	-	Alive

[1]As of publication. C + A + O: Cytoxan + Adriamycin + Oncovin; CA: Carboplatin; CA + E: Carboplatin + Etoposide; CI: Cisplatin; CI + E: Cisplatin + Etoposide; CM: Chemotherapy; CRT: Chemoradiotherapy; CT: Computerized tomography; DM: Distant metastasis; M: Male; F: Female; RE: Recurrence; RT: Radiotherapy; S: Surgery; Un: Unknown.

a relatively large cytoplasm and vesicular chromatin and prominent nucleoli. Primary cutaneous high-grade NEC, which mainly develops due to ultraviolet light exposure, is termed Merkel cell carcinoma. Primary mucosal high-grade NEC, which typically develops because of heavy smoking and alcohol abuse, is called SNEC. In addition, CK20 is commonly positive in Merkel cell carcinoma, and is negative in SNEC[21]. HMB45 and S100 are special markers for malignant melanoma and malignant lymphoma. They can be used for detecting metastatic squamous cell carcinoma of the lung when thyroid transcription factor1 is positive[11]. Cytokeratin AE1/AE3 and P63 staining is helpful in identifying a basal cell carcinoma or a squamous cell-originated tumor[6,10,11].

Due to the paucity of primary SNECs in the oral cavity, the lack of definitive treatment recommendations is a challenge for clinicians. The appropriate treatment strategies for extra-pulmonary SNECs are extrapolated from their pulmonary counterparts[14]. A variety of therapeutic modalities, including surgery, chemotherapy, radiotherapy and chemoradiotherapy, are thought to be appropriate treatments[16,22]. Surgery could be curative when SNECs are limited within the primary sites. In addition, surgery is the main treatment of NECs at different body sites and has been

reported to significantly improve overall survival, over other single-modality treatments. To decrease local recurrence of SNECs, a wide excision is mandatory (up to 3 cm)[7]. In our case, the patient underwent extensive surgical excision of the primary tumor.

As SNEC is an extremely aggressive malignancy with high rates of local recurrence and distant metastasis, multimodal therapy is required. For patients without distant metastasis, chemotherapy can reduce tumor size and decrease the risk of distant metastasis. In early and locally advanced SNECs of the head and neck, chemoradiotherapy is also an effective treatment[16]. In patients with extensive SNEC, chemotherapy is recommended to prolong survival and improve prognosis[6,12]. The combination of cisplatin and etoposide is the first-line chemotherapy regimen for SNEC[23]. In recent research, chemotherapy in combination with first-line atezolizumab prolonged survival rate compared with chemotherapy alone in the treatment of extra-pulmonary SNECs with distant metastases[2].

Furthermore, a few novel therapeutic strategies for small cell carcinoma of the lung which are unsuccessful, including mammalian target of rapamycin inhibitors, breakpoint cluster region–Abelson tyrosine kinase inhibitors, epidermal growth factor receptor tyrosine kinase inhibitors, vascular endothelial growth factor inhibitors, deoxyribonucleic acid repair inhibitors, immunotherapies, and anti-delta-like protein 3 antibody-drug conjugates, have been introduced[24,25]. Of the 11 patients with primary SNEC in the oral cavity, 1 patient received radiotherapy only, due to his poor physical condition. Chemotherapy was the most common treatment in the majority of patients (75%). In the current case, the patient showed partial remission after two cycles of adjuvant chemotherapy. However, the patient died when gastrointestinal metastasis occurred 20 mo after treatment.

CONCLUSION

Primary SNEC occurring in the oral cavity represents a rare clinical entity, and is aggressive with a poor prognosis. The diagnosis of SNEC requires morphology and immunohistochemistry analyses. Due to its highly metastatic characteristics, a comprehensive clinical examination of the neck, chest, abdomen and bone is mandatory in SNEC patients. Multimodal therapy may be an effective treatment strategy for SNEC of the oral cavity. However, more effective treatments to improve the survival rate of patients with SNEC in the oral cavity are required.

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SYSTEMATIC REVIEWS

Treatment of *Helicobacter pylori* infection in children: A systematic review

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Abstract

BACKGROUND

Helicobacter pylori (H. pylori) infection is predominantly acquired in childhood. When indicated, the most accepted treatment for *H. pylori* eradication in this age group is first-line triple therapy. However, the increasing resistance to clarithromycin and nitroimidazoles has been associated with treatment failure, and thus, alternative treatment regimens have been proposed.

AIM

To perform a systematic review of randomized controlled trials on treatment regimens for *H. pylori* infection in children.

METHODS

We surveyed relevant articles published in English from 2010 to April 2020 in the PubMed and MEDLINE databases. Keywords included "*Helicobacter pylori*" /"children or childhood"/"treatment or eradication." The risk of bias was evaluated according to the Cochrane Handbook of Systematic Reviews for Interventions.

A PRISMA checklist was used to guide the development of the systematic review.

RESULTS

Among the 1144 records identified through the database, 20 articles were selected. Four studies compared the eradication rates of *H. pylori* infection between standard triple therapies, changing only the main antibiotic used. Seven studies evaluated the effectiveness of standard triple therapy with the addition of probiotics. One study investigated the relationship between the effectiveness in the eradication rates of standard triple therapy and vitamin E levels. Six studies analyzed the eradication rates of sequential therapy.

CONCLUSION

The findings suggest that although standard triple therapy is the most recommended regimen for children by the current guidelines, other therapeutic schemes have shown promising results and may also be recommended for clinical practice in the future.

Key Words: *Helicobacter pylori*; Children; Pediatric treatment; Standard triple therapy; Probiotics; Sequential therapy; Eradication therapies

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Core Tip: *Helicobacter pylori* (*H. pylori*) is a bacterium that infects more than 50% of the population worldwide. In the last several years, no significant changes in the treatment of infected children have been observed, mainly due to a lack of studies with satisfactory scientific evidence to support the indication of therapies in clinical practice. We performed a systematic review of randomized controlled trials on treatment regimens for *H. pylori* infection in children.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative spiral bacterium that colonizes the gastric mucosa of more than 50% of the population worldwide. The infection is acquired predominantly in childhood, and is more prevalent in developing countries where about 70% of children are infected until 15-years-old[1,2], whereas it is disappearing in developed countries. Once acquired, the bacterium is rarely eliminated without adequate antibiotic therapy and individuals remain infected throughout life[3]. Most infected individuals do not develop complications, but gastric colonization can progress to chronic gastritis, duodenal ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma, and bacterial eradication is associated with the prevention of such diseases[4]. *H. pylori* infection has also been implicated in the pathogenesis of extra-gastric diseases, including iron deficiency anemia and chronic immune thrombocytopenic purpura.

The mechanisms by which the infection progresses to the above-mentioned diseases are not completely understood and depend on the relationship between host genetics and factors regarding the environment and bacterial virulence.

Severe *H. pylori*-associated diseases are more common in adults than in children. This phenomenon can be explained, in part, by the differences in immune response between the two age groups, which seems to be a relevant factor influencing mucosal damage and clinical outcomes[5]. In general, *H. pylori* infection induces a T helper type 1 (Th1)-polarized response with high levels of interferon-γ that stimulate gastric inflammation and mucosal damage in adults. Moreover, Th17 cells and interleukin (IL)-17 levels are important in that process because they stimulate the recruitment and activation of neutrophils in the gastric mucosa, increasing the inflammatory environment against the bacterium. In contrast to the pro-inflammatory response

found in adults, children tend to exhibit an immune response pattern with a predominance of regulatory T cells that contribute to the persistence of the infection and milder clinical manifestations[6,7]. Children tend to have lower levels of Th1- and Th17-related cytokines as well as overexpression of IL-10 and transforming growth factor-β, resulting in a lower degree of polymorphonuclear cell activation in the acute phase of infection[8,9] and prominent mononuclear cell infiltration in chronic infection compared with adults[10].

H. pylori virulence factors also play an important role in the pathogenesis of infection in childhood. It is well documented that genes such as cytotoxin gene A (*cagA*) and vacuolating cytotoxin gene A (*vacA*) increase the risk of severe gastric diseases such as duodenal ulcer and gastric cancer[11].

H. pylori eradication prevents duodenal ulcer recurrence and prevention of gastric cancer. In addition, *H. pylori* eradication in children induces platelet and iron recovery in immune thrombocytopenic purpura and iron deficiency anemia, respectively.

Since eradication therapies show variable failure rates, retesting for *H. pylori* infection after an antimicrobial regimen is recommended to ensure that patients have been successfully treated[12]. There are various available therapeutic options aimed toward *H. pylori* eradication, and clarithromycin-based triple therapy, sequential therapy, bismuth-containing quadruple therapy or triple therapy, and hybrid therapy are the regimens most often used[13]. Moreover, various alternative approaches have been attempted in order to improve bacterial eradication such as susceptibility-guided therapies, probiotics, and vaccines[14-16]. In that context, a number of factors influence the choice of appropriate treatment, including antimicrobial susceptibility profile, economic factors, and importantly, individual characteristics such as previous exposure to antibiotics and age[17].

Performing *H. pylori* eradication in children demands specific precautions, of which avoiding regimens with unacceptable rates of adverse events (AEs) for this population is very important[18]. In this context, the number of treatment options usually available for children tends to be significantly lower compared to the range of treatments at hand for adults. Clarithromycin-based triple therapy is the most used therapeutic scheme for children, although other standard therapies have also been tried[19]. Moreover, to reduce the frequency and severity of side effects as well as to improve eradication rates, probiotics in association with standard therapy have been tested in children[20].

Therefore, we reviewed the randomized controlled trials (RCTs) of treatments for *H. pylori* eradication children.

MATERIALS AND METHODS

In this systematic review, the criteria recommended by the PRISMA checklist were used[21].

Types of studies

Prospective RCTs, published in peer-reviewed journals from 2010 to April 2020 and reporting the results of antibiotic therapy and/or supplementation with other drugs for the *H. pylori* eradication in infected children under 18-years-old, were included. There was no restriction regarding the therapeutic schemes used. Excluded studies were those including adults or lacking their complete or free full text. Only English language studies were included. The inclusion criteria are outlined in Table 1.

Types of participants

H. pylori-positive patients under 18-years-old diagnosed by any validated test accepted by the scientific community for *H. pylori* detection. Patients who had previous failed antibiotic therapies were included.

Types of intervention

Prospective RCTs evaluating *H. pylori* eradication rates in children.

Types of outcome measures

We collected outcomes of intention-to-treat (ITT), per protocol (PP), and simple percentages of *H. pylori* infection eradication.

Table 1 Inclusion criteria

Criteria	Description
Date range	January 2010 to April 2020
Language	Only English published articles
Location	No restriction of localization
Population	Children (< 18-years-old)
Type of study	Randomized controlled trials

Information sources

We surveyed the relevant articles published in English language from 2010 to April 2020 in the PubMed and MEDLINE databases. The term strategies used for the search at both databases were: ((*Helicobacter pylori* [and] children [and] treatment) [OR] eradication) and (*Helicobacter pylori* [and] childhood [and] (treatment [OR] eradication)).

Study selection

The eligibility of the articles was evaluated by two independent reviewers (Da Silva FAF and de Brito BB). Duplicate articles were excluded. The abstracts of the articles were evaluated and studies that were not prospective RCTs and/or did not evaluate *H. pylori* eradication rate in children were excluded. A third reviewer (de Melo FF) resolved disagreements between the two reviewers. To verify if the articles met all previously established criteria, each article was individually analyzed. To statistically evaluate the agreement between the reviewers, the Kappa coefficient (*K*) was calculated, which indicated a $K = 0.752$, considered as a substantially strong degree of agreement between the reviewers.

Data collection process

We developed a structured data extraction spreadsheet specifically for this review based on the criteria recommended by the Cochrane Handbook of Systematic Reviews for Interventions[42]. We independently reviewed the relevant study data and results of interest such as rates of eradication of *H. pylori* infection in childhood.

Data items

Information was extracted from each study including: General characteristics of the participants and studies, type of intervention, therapeutic regimen used, type of outcome measure, and positive and negative outcomes.

Risk of bias

To assess the validity of RCTs, two authors independently analyzed the risk of bias criteria recommended by the Cochrane Handbook of Systematic Reviews for Interventions[42]: Generation of the random sequence, concealment of allocation, blinding of participants and professionals, blinding of outcome evaluators, incomplete outcomes, and reporting of the selective outcome. Then the risk of bias was categorized as high, low, or uncertain.

RESULTS

Study selection

Of the 1144 articles reviewed (861 in NCBI and 283 in MEDLINE), 1118 were excluded using previously established inclusion criteria. Twenty-six articles were selected for complete analysis; however, eight were duplicate and thus were excluded. Finally, 2 studies were an additional reference list and 20 articles were included. Figure 1 shows the selection and distribution of articles according to the databases searched, from the first search to the application of all of the selection criteria.

Study characteristics

The characteristics of the 20 selected studies are summarized in Table 2. A total of 2261 children aged 22 mo to 18 years were included. Regarding the geographic distribution

Table 2 Summary of included studies

Ref.	Year	Country	Study design	Patients, *n*	Age range	Follow-up	Goal
Moubri *et al*[22]	2018	Algeria	RCT	272	5-16 yr	8-12 wk	To compare efficacy, side effects and influence of resistance of *H. pylori* strains between two different treatments in Algerian children.
Namkin *et al*[23]	2016	Iran	RCT	28	9-12 yr	4-8 wk	To evaluate the effect of *S. boulardii* supplementation on the eradication of *H. pylori* in children in the region.
Akcam *et al*[24]	2015	Turkey	RCT	61	7-18 yr	6 wk	To evaluate the effect of probiotics on eradication rates and side effects in association with standard triple therapy in *H. pylori*-positive children.
Tolone *et al*[25]	2012	Italy	RCT	68	4-11 yr	4 wk	To evaluate if addition of probiotics increases eradication rates and reduces side effects in children.
Farahmand *et al*[26]	2016	Iran	RCT	66	7-15 yr	4 wk	To compare the effect of ciprofloxacin and furazolidone on *H. pylori* eradication in combination with amoxicillin and omeprazole.
Ahmad *et al*[27]	2013	Iran	RCT	66	3-14 yr	4-8 wk	To evaluate the effect of probiotic supplementation with the combination of seven microorganisms on the treatment of *H. pylori* infection in childhood.
Bin *et al*[28]	2015	China	RCT	205	22 mo-16 yr	2 wk	To investigate the effects of *Saccharomyces boulardii* CNCM I-745 on eradication of *H. pylori* in children.
Kasiri *et al*[29]	2017	Iran	RCT	82	1-15 yr	4 wk	To compare the effect of amoxicillin and metronidazole in the triple therapy regimen to eradicate *H. pylori* infection in children aged 1 to 15 yr.
Iwańczak *et al*[30]	2016	Poland	RCT	69	5-17 yr	6-8 wk	To compare the efficacy of sequential therapy for 10 d with triple therapy for 7 d (PPI, amoxicillin and clarithromycin or PPI, amoxicillin and metronidazole) in children.
Tümgör *et al*[31]	2014	Turkey	RCT	90	10-17 yr	6 wk	To compare the treatment with lansoprazole, amoxicillin and clarithromycin (LAC) and with the combination of LAC + vitamin E (LACE) in Turkish children.
Ali Habib *et al*[32]	2013	India	RCT	18	12-15 yr	6 wk	To investigate which sequential or standard eradication regimen has the most effective improvement in the status of associated iron and iron deficiency in children.
Ustundag *et al*[33]	2017	Turkey	RCT	69	6-16 yr	4-6 wk	To evaluate the effects of the use of the symbiotic *Bifidobacterium lactis* B94 + inulin, together with standard triple therapy on the eradication rate, adherence, as well as in the symptoms of *H. pylori* infection in children.
Huang *et al*[34]	2013	China	RCT	360	3-16 yr	4 wk	To compare 10 d sequential therapy and standard triple therapy in Chinese children with *H. pylori* infection.
N Şirvan *et al*[35]	2017	Turkey	RCT	104	5-17 yr	4 wk	To evaluate the addition of symbiotics containing *Bifidobacterium lactis* to triple therapeutics in the rates of side effects, dyspeptic symptoms and *H. pylori* eradication in children.
Baysoy *et al*[36]	2013	Turkey	RCT	61	4-18 yr	6-8 wk	To compare ornidazole-based sequential therapy with standard triple therapy for the eradication of *H. pylori* in children.
Esmaeili-Dooki *et al*[37]	2015	Iran	RCT	64	2-15 yr	4-6 wk	To evaluate the effect of the classic triple therapy and azithromycin eradication regimen against *H. pylori* in children.
Laving *et al*[38]	2013	Kenya	RCT	71	2-15 yr	2-6 wk	To determine the effectiveness of a new 10 d sequential therapy compared to the standard 10 d triple therapy for the treatment of *H. pylori* infection in children.
Prieto-Jimenez *et al*[39]	2011	United States of America	RCT	110	3-11 yr	6 wk	To evaluate the efficacy of sequential quadruple eradication therapy for 10 d and observe the iron levels of positive *H. pylori* children.
Nguyen *et al*[40]	2012	Vietnam	RCT	232	3–15 yr	4 wk	To investigate the role of antibiotic resistance, drug dosage, and administration frequency in treatment of *H. pylori* infection in Vietnamese children.
Bontems *et al*[41]	2011	Belgium, France and Italy	RCT	165	2,7 a 17 yr	8 wk	To compare sequential *vs* tailored triple therapy regimens on *H. pylori* eradication rate and to assess the effect of antimicrobial susceptibility in children.

RCTs: Randomized controlled trials. *H. pylori*: *Helicobacter pylori*; PPI: Proton pump inhibitor.

of the studies, 25% of the articles were from Iran; 25% from Turkey; 10% from China;

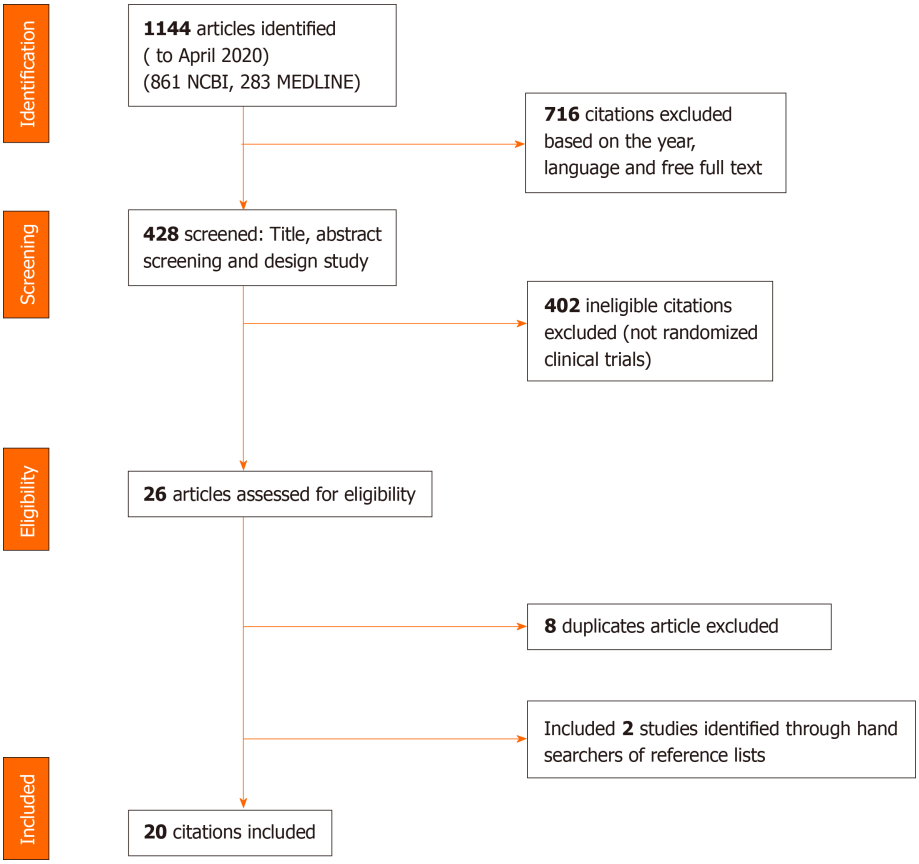


Figure 1 Summary of the study selection process.

and 40% from Algeria, Italy, Poland, India, Kenya, United States, Belgium, and France. The studies had a follow-up average of 6 wk. In addition, the articles evaluated conventional eradication therapies, probiotics, or sequential therapies as well as the eradication rates of *H. pylori* infection in childhood.

Results of individual studies

All patients were *H. pylori*-positive children diagnosed by validated methods. Randomization methods and loss of follow-up were highlighted for analysis of the risk of bias. All articles were classified according to the criteria of the Oxford Center for Evidence-based Medicine - Levels of Evidence[43] (Table 3). Standard triple therapy was the main therapeutic scheme evaluated. The studies compared conventional triple therapy alone to triple therapy containing probiotics and to sequential therapies (Table 4).

Synthesis of results

Table 5 summarizes the positive and negative outcomes of the studies.

Risk of bias assessment

Using the Cochrane risk of bias tool[44], seven RCTs[23,26,27,34,37-39] had a low risk of bias for the following criteria: Generation of the random sequence, allocation concealment, blinding of participants and professionals, and blinding of outcome evaluators. Only the work by Akcam *et al*[24] was classified as high risk of bias for generating the random sequence. All other RCTs had an uncertain or low rating for all of the criteria mentioned above. In general, we observed some risks of biases through deviations from the intended interventions, represented by the concealment of how the drugs were distributed and how the researchers made recommendations to the participants. In addition, interaction with a healthcare professional can improve symptoms and treatment adherence, becoming a possible bias for all the analyzed results.

Table 3 Criteria analyzed individually in the studies

Ref.	Specific disease	Diagnosis	Groups	Randomization	Loss of follow-up	Level of evidence
Moubri *et al*[22]	No	Biopsy, RUT, culture, HpSA and ^{13}C-UBT/(Biopsy, RUT, culture, HpSA and ^{13}C-UBT)	2	The method chosen was to classify closed envelopes, which were randomly assigned to treatment	12 patients	1b
Namkin *et al*[23]	No	HpSA/(HpSA)	2	Limited information on the method used	4 patients	2b
Akcam *et al*[24]	No	Biopsy, histology, RUT and ^{13}C-UBT/(^{13}C-UBT)	2	Admission order	5 patients	1b
Tolone *et al*[25]	No	Biopsy, histology, RUT and ^{14}C-UBT/(^{13}C-UBT)	2	No reported	No loss	2b
Farahmand *et al*[26]	No	Biopsy, RUT and HpSA/(HpSA)	2	Table of aleatory numbers	No loss	1b
Ahmad *et al*[27]	No	RUT, histology, and HpSA/(HpSA)	2	Limited information on the method used	No loss	1b
Bin *et al*[28]	No	Immunoglobulin G Antibody, histology and ^{13}C-UBT/(^{13}C-UBT)	2	No reported	14	1b
Kasiri *et al*[29]	Dyspepsia, epigastric pain and GB	Biopsy, histology and RUT/(HpSA)	2	Limited information on the method used	3	1b
Iwańczak *et al*[30]	Dyspepsia and gastric and/or duodenal ulcer	Histology and/or culture/(Biopsy and culture)	3	No reported	No loss	1b
Tümgör *et al*[31]	Dyspepsia.	^{14}C-UBT and histology/(^{13}C-UBT)	2	No reported	2	2b
Ali Habib *et al*[32]	No	Immunoglobulin G Antibody and ^{13}C-UBT/(^{13}C-UBT)	2	No reported	2	2b
Ustundag *et al*[33]	Gastrointestinal symptoms, severe regurgitation, gastrointestinal bleeding, unexplained weight loss or chronic diarrhea	Histology/(^{14}C-UBT)	2	Double-blind randomization list	5	2b
Huang *et al*[34]	No	RUT, HpSA, histology and culture/(HpSA)	3	Limited information on the method used	42	1b
N Şirvan *et al*[35]	Chronic diseases, abdominal pain and dyspepsia	Histology/(HpSA)	2	Limited information on the method used	No loss	2b
Baysoy *et al*[36]	No	Histology, RUT, ^{13}C-UBT/(HpSA or Biopsy and histology)	2	Limited information on the method used	8	1b
Esmaeili-Dooki *et al*[37]	No	Biopsy, histology and HpSA/(^{13}C-UBT)	2	Table of aleatory numbers	No loss	1b
Laving *et al*[38]	No	Histology and HpSA/(HpSA)	2	Computer-generated random numbers	33	1b
Prieto-Jimenez *et al*[39]	No	^{13}C-UBT and urine antibody	6	Computer-generated random numbers	20	1b
Nguyen *et al*[40]	No	Biopsy, RUT, histology and culture	3	No reported	10	1b
Bontems *et al*[41]	Abdominal pain, nausea, vomiting, heartburn and anemia	Biopsy, histology and culture/(HpSA)	2	No reported	15	1b

The levels of evidence were based in the Oxford Centre for Evidence-based Medicine – Levels of Evidence[43]. All diagnostic methods in brackets were used after treatment to verify *Helicobacter pylori* (*H. pylori*) eradication. RUT: Rapid urease test; HpSA: *H. pylori* monoclonal stool antigen test; ^{13}C-UBT: ^{13}C-urea breath test; ^{14}C-UBT: ^{14}C-urea breath test; GB: Gastrointestinal bleeding.

Table 4 Summary of therapeutic schemes used in included studies

Ref.	Treatment schemes
Moubri *et al*[22]	Group A - Amoxicillin-based triple therapy: (1) Children > 30 kg: Omeprazole 40 mg/d, amoxicillin 50 mg/kg/d with a maximum of 2 g/d and clarithromycin 15 mg/kg/d for 7 d. All given in two daily doses; and (2) Children < 30 kg: omeprazole 2 × 10 mg/d b.i.d., amoxicillin 50 mg/kg/d b.i.d. with a maximum of 2 g/d, and clarithromycin 15 mg/kg/d b.i.d. for 7 d. Group B - Metronidazole-based triple therapy: (1) Children > 30 kg: Omeprazole 2 × 20 mg/d, amoxicillin 50 mg/kg/d b.i.d. with a maximum of 2 g/d and metronidazole 40 mg/kg/d b.i.d. for 10 d; and (2) Children < 30 kg: omeprazole 2 × 10 mg/d, amoxicillin 50 mg/kg/d b.i.d. with a maximum of 2 g/d and metronidazole 40 mg/kg/d b.i.d., for 10 d, with a maximum of 1.5 g/d in children above 30 kg and 1 g/d in children below 30 kg body weight.
Namkin *et al*[23]	Probiotic group: Yomogi® 250 mg lyophilizate - 1 capsule daily for 30 d. Placebo group: placebo capsule 250 mg - lactose and powdered wheat starch - 1 capsule daily for 30 d.
Akcam *et al*[24]	Standard triple therapy group: Omeprazole 1 mg/kg, before breakfast, amoxicillin 50 mg/kg b.i.d. after meals, clarithromycin 15 mg/kg b.i.d. after meals for 7 d. Standard triple therapy group + probiotics: Omeprazole 1 mg/kg, before breakfast + amoxicillin 50 mg/kg b.i.d. after meals, clarithromycin 15 mg/kg b.i.d. after meals, PROBINUL® 5 g once a day for 7 d.
Tolone *et al*[25]	Standard triple therapy group - lansoprazole 30 mg, before breakfast, amoxicillin 50 mg/kg/d b.i.d., clarithromycin 15 mg/kg/d b.i.d. for 14 d. Standard triple therapy group + probiotics: Lansoprazol 30 mg, before breakfast + amoxicillin 50 mg/kg/d b.i.d., clarithromycin 15 mg/kg/d b.i.d., Maflor® plus 1 capsule, b.i.d. for 14 d.
Farahmand *et al*[26]	Group A - Ciprofloxacin-based triple therapy: Ciprofloxacin 30 mg/kg/d b.i.d. and amoxicillin 50 mg/kg/d b.i.d. for 1 wk. Omeprazole 1 mg/kg/d b.i.d for 4 wk. Group B - Furazolidone-based triple therapy: furazolidone 6 mg/kg/d single-dose over and amoxicillin 50 mg/kg/d b.i.d. for 1 wk. omeprazole 1 mg/kg/d b.i.d. for 4 wk.
Ahmad *et al*[27]	Group A - Triple therapy + placebo: Amoxicillin 50 mg/kg/d b.i.d., as syrup or capsule, and furazolidone 6 mg/kg/d b.i.d. as syrup or tablet for 1 wk. Omeprazole 1 mg/kg/d plus placebo for 4 wk. Group B - Triple therapy + probiotics: Amoxicillin 50 mg/kg/d b.i.d., as syrup or capsule, and furazolidone 6 mg/kg/d b.i.d. as syrup or tablet, for 1 wk. Omeprazole 1 mg/kg/d plus placebo for 1 sachet/d for 4 wk.
Bin *et al*[28]	Group A - Triple therapy + *Saccharomyces boulardii:* Amoxicillin, omeprazole, clarithromycin and 2 sachet *S. boulardii* 250 mg per day, for 2 wk. Allergic to penicillin: metronidazole + omeprazole, clarithromycin and *S. boulardii* (dosages not reported). Group B - Only triple therapy: Amoxicillin, omeprazole, clarithromycin. Allergic to penicillin: metronidazole + omeprazole, clarithromycin (dosages not reported).
Kasiri *et al*[29]	Group A - Amoxicillin-based triple therapy: Omeprazole 1–2 mg/kg b.i.d. for 1 mo. Amoxicillin 50 mg/kg and clarithromycin 15 mg/kg b.i.d. for 2 wk. Group B - Metronidazole-based triple therapy: Omeprazole 1 mg/kg b.i.d. divided into two doses for 1 mo. Metronidazole 15 mg/kg and clarithromycin 15 mg/kg b.i.d. for 2 wk.
Iwańczak *et al*[30]	Group I - Amoxicillin-based triple therapy: Omeprazole, amoxicillin and clarithromycin for 7 d (dosages not reported). Group II - Metronidazole-based triple therapy: Omeprazole, amoxicillin and metronidazole for 7 d (dosages not reported). Group III - Sequential therapy: omeprazole 1 mg/kg of body weight/d, max 20 mg b.i.d. and amoxicillin 50 mg/kg of body weight/d, max 1000 mg/24 h for 5 d, b.i.d. followed by 5 d treatment with omeprazole 1 mg/kg of body weight/d, max 20 mg/24 h twice daily, clarithromycin 15 mg/kg of body weight/d, max 500 mg/24 h twice daily and metronidazole 20 mg/kg of body weight/d, max 500 mg/24 h twice daily.
Tümgör *et al*[31]	Group A - Clarithromycin-based triple therapy: Lansoprazole 1 mg/kg/d, amoxicillin 50 mg/kg/d, and clarithromycin 14 mg/kg/d, all medications b.i.d. for 14 d. Group B - Clarithromycin-based triple therapy + vitamin E: Lansoprazole 1 mg/kg/d b.i.d., amoxicillin 50 mg/kg/d b.i.d., and clarithromycin 14 mg/kg/d b.i.d., vitamin E 200 IU/d for 14 d.
Ali Habib *et al*[32]	Group A - Standard therapy: Rabeprazole 20 mg, clarithromycin 250 mg, and amoxicillin 500 mg each administered orally twice daily for 10 d. Group B - Sequential therapy: Rabeprazole 20 mg and amoxicillin 500 mg for 5 d, followed by rabeprazole 20 mg clarithromycin 250 mg and tinidazole 500 mg for another 5 d, twice daily.
Ustundag *et al*[33]	Group A - Standard triple therapy: Amoxicillin 50 mg/kg/d, clarithromycin 15 mg/kg/d twice daily for 14 d and omeprazole 1 mg/kg/d once daily for 1 mo. Group B - Standard triple therapy + symbiotic: Amoxicillin 50 mg/kg/d and clarithromycin 15 mg/kg/d twice daily for 14 d. Omeprazole 1 mg/kg/d once daily for 1 mo and Maflor® a single dose for 14 d concurrently.
Huang *et al*[34]	Group A – 1 d sequential therapy: Omeprazole 0.8–1.0 mg/kg/d, amoxicillin 30 mg/kg/d for the first 5 d, followed by omeprazole 0.8–1.0 mg/kg/d, clarithromycin 20 mg/kg/d, and metronidazole 20 mg/kg/d for the remaining 5 d.

	Group B – 7 d triple standard therapy: Omeprazole 0.8–1.0 mg/kg/d, amoxicillin 30 mg/kg/d, and clarithromycin 20 mg/kg/d.
	Group C – 10 d triple standard therapy: Omeprazole 0.8–1.0 mg/kg/d, amoxicillin 30 mg/kg/d, and clarithromycin 20 mg/kg/d.
N Şirvan *et al*[35]	Group 1 - Standard triple therapy + symbiotic: Amoxicillin 50 mg/kg/d b.i.d. for 7 d, clarithromycin 15 mg/kg/d b.i.d. for 14 d, and lansoprazole 1 mg/kg/d - single dose, in the morning for 14 d. Maflor® sachet - single dose for 14 d.
	Group 2 - Standard triple therapy: Amoxicillin 50 mg/kg/d b.i.d. for 14 d, clarithromycin 15 mg/kg/d b.i.d. for 14 d, and lansoprazole 1 mg/kg/d - single dose, in the morning for 14 d.
Baysoy *et al*[36]	Group A - Standard triple therapy: Amoxicillin 50 mg/kg/d, clarithromycin 15 mg/kg/d, and lansoprazole 1 mg/kg/d for 14 d.
	Group B - Sequential therapy group: Amoxicillin 50 mg/kg/d and lansoprazole 1 mg/kg/d for the first 5 d and clarithromycin 15 mg/kg/d, ornidazole 30 mg/kg/d and lansoprazole 1 mg/kg/d for another 5 d.
Esmaeili-Dooki *et al*[37]	Group 1 - Clarithromycin-based triple therapy: Clarithromycin 7.5 mg/kg/d b.i.d. and amoxicillin 50 mg/kg/d every b.i.d. for 10 d, and omeprazole 1 mg/kg/d b.i.d. for 2 wk.
	Group 2 - Amoxicillin-based triple therapy: Azithromycin 10 mg/kg/d once a day, before meal, for 6 d along with amoxicillin and omeprazole.
Laving *et al*[38]	Conventional therapy group: Omeprazole plus 1 mg/kg/d, amoxicillin plus 50 mg/kg/d and clarithromycin 15 mg/kg/d for 10 d.
	10 d sequential therapy group: Omeprazole plus 1 mg/kg/d, amoxicillin plus 50 mg/kg/d for 5 d followed by omeprazole plus 1 mg/kg/d, clarithromycin 15 mg/kg/d, and tinidazole 20 mg/kg/d for the next 5 d.
Prieto-Jimenez *et al*[39]	Group I - Quadruple sequential eradication and placebo: Lansoprazole, once per day before breakfast, plus an oral solution containing amoxicillin 50 mg/kg/d for 5 d followed by lansoprazole, once per day, plus an oral solution containing clarithromycin 15 mg/kg/d and an oral solution containing tinidazole 20 mg/kg/d for another 5 d. 6 wk of a placebo matched to iron supplementation.
	Group II - Quadruple sequential eradication and iron: Lansoprazole, once per day before breakfast, plus an oral solution containing amoxicillin 50 mg/kg/d for 5 d followed by lansoprazole, once per day, plus an oral solution containing clarithromycin 15 mg/kg/d and an oral solution containing tinidazole 20 mg/kg/d for another 5 d. 6 wk of iron supplementation.
	Group III: Iron and placebo: A 10 d course of a placebo matched to *H pylori* sequential eradication therapy plus 6 wk of iron supplementation.
	Group IV: Placebo alone: A 10 d course of placebo matched to *H. pylori* sequential eradication therapy plus 6 wk of a placebo matched to iron supplementation.
Nguyen *et al*[40]	Group A: Lansoprazole, amoxicillin and clarithromycin: (1) Children weighing 13–22 kg: lansoprazole 15 mg once daily, amoxicillin 500 mg twice daily and clarithromycin 250 mg once daily; and (2) Children weighing 23–45 kg: lansoprazole 15 mg, amoxicillin 750 mg and clarithromycin 250 mg, all given twice daily.
	Group B: Lansoprazole, amoxicillin and metronidazole: (1) Children weighing 13-22 kg: lansoprazole 15 mg once daily, amoxicillin 500 mg and metronidazole 250 mg twice daily; and (2) Children weighing 23–45 kg: Lansoprazole 15 mg, amoxicillin 750 mg and metronidazole 500 mg, all given twice daily.
Bontems *et al*[41]	Group A: 10 d sequential treatment: 5 d therapy with a combination of omeprazole (10 mg b.i.d. below 30 kg body weight or 20 mg b.i.d. above 30 kg) and amoxicillin 25 mg/kg b.i.d.—maximum 2 g/d), followed by 5 d of omeprazole, clarithromycin (7.5 mg/kg b.i.d.—maximum 1 g/d), and metronidazole (10 mg/kg b.i.d.—maximum 1.5 g/d).
	Group B: 7 d triple therapy: 7 d treatment tailored comprising omeprazole and amoxicillin with clarithromycin in cases of *H. pylori* strains susceptible to clarithromycin or with clarithromycin in cases of H. pylori strains susceptible to metronidazole and resistant to clarithromycin.

H. pylori: Helicobacter pylori.

DISCUSSION

In pediatric clinical practice, *H. pylori* infection is common, especially in developing countries and certain populations such as ethnic minorities and migrant communities living in developed countries. In this systematic review, standard triple therapies, given for 7, 10, or 14 d were compared with sequential, third-line, and quadruple therapies for *H. pylori* eradication. In addition, some studies evaluated the efficacy of probiotics as adjuvant therapy for triple therapy.

Currently, triple therapies recommended by the main guidelines for *H. pylori* eradication include a proton pump inhibitor (PPI) or ranitidine, amoxicillin, and either clarithromycin or metronidazole, considered the first-line regimen, and bismuth, administered for 7, 10, or 14 d[45]. The desirable target of anti-*H. pylori* treatment regimens is to reach an eradication rate of at least 90% in the per-protocol analysis whereas antibiotic use eradication rate below 80% is considered unacceptable[46]. However, few studies have achieved this goal. The low efficacy of triple therapy observed in diverse geographic areas has been attributed to the rising resistance of *H. pylori* strains to clarithromycin and metronidazole, poor compliance, duration of treatment, and inadequate dosage and number of daily doses. The growing *H. pylori* resistance is due to the previous exposition of children to these antimicrobials that are

Table 5 Synthesis of results from included studies

Ref.	Adverse events	Deaths	Eradication rates	Conclusion
Moubri *et al*[22]	Mild and moderate symptoms, mainly gastrointestinal.	No	(1) ITT: Group A - Amoxicillin - based triple therapy: 68%; Group B - Metronidazole - based triple therapy: 80%; and (2) PP: Group A - Amoxicillin - based triple therapy: 71%; Group B - Metronidazole - based triple therapy: 88%.	The group B eradication rates were higher than Group A rates. The differences were only significant for PP ($P < 0.03$).
Namkin *et al*[23]	Loss of appetite.	No	Probiotic group: 0.40 ± 0.32 to 0.21 ± 0.27 average HpSA title; $P = 0.005$. Placebo group: 0.24 ± 0.2 to 0.24 ± 0.27 average HpSA title; $P = 0.89$.	There was no significant difference between the two groups in relation to the eradication rate of *H. pylori* infection ($P = 0.16$), however the decrease in the concentration of HpSA was significantly greater in the group treated with probiotics ($P = 0.005$ *vs* $P = 0.89$).
Akcam *et al*[24]	Abdominal pain, nausea, vomiting, constipation, belching, changes in taste, poor appetite and diarrhea.	No	Standard triple therapy group: 68.9%; Standard triple therapy group + probiotics: 66.6%; $P = 0.78$.	There was no statistically significant difference between the two groups.
Tolone *et al*[25]	Epigastric pain, nausea, vomiting, diarrhea and constipation.	No	Group A standard therapy group: 76.4%; Group B standard therapy + probiotics: 88.2%; $P = 0.1$.	There was no significant difference in the rate of *H. pylori* eradication between group A and group B, however, the side effects were significantly greater ($P < 0.05$) in group A than in group B.
Farahmand *et al*[26]	Vomiting, abdominal pain, iron deficiency, anemia and gastrointestinal bleeding.	No	Group A - Ciprofloxacin-based triple therapy: 87.9%; Group B - Furazolidone-based triple therapy: 60.6%; $P = 0.011$.	This study concludes that triple therapy consisting of ciprofloxacin, amoxicillin and omeprazole is highly valuable as *H. pylori* is not resistant to the antimicrobials.
Ahmad *et al*[27]	Diarrhea, nausea, vomiting and abdominal swelling.	No	Group A - Triple therapy + placebo: 69.69%; Group B - Triple therapy + probiotics: 90.09%; $P = 0.04$.	The group treated with the combination of probiotic and standard therapeutic regimen showed a more significant eradication rate. Supplementation with probiotics has a positive effect on *H. pylori* and decreases adverse events and effectiveness.
Bin *et al*[28]	Diarrhea.	11	Group A - Triple therapy + *S. boulardii*: 71.4%; Group B - Only triple therapy: 61.9%; $P = 0.51$	The probiotic prevented diarrhea associated with triple *H. pylori* eradication therapy. In addition, when diarrhea developed, it was less severe and of shorter duration in the *S. boulardii* group. The probiotic increased the adherence to *H. pylori* eradication therapy, which may be related to a small increase in *H. pylori* eradication by 10 percent.
Kasiri *et al*[29]	Intolerance to clarithromycin.	No	Group A - Amoxicillin-based triple therapy: 87.2%; Group B - Metronidazole-based triple therapy: 92.5%; $P = 0.43$.	There was no significant difference in the complete recovery and eradication of *H. pylori* between the two regimens. Both therapeutic regimens were considered to be effective, since both have similar rates of eradication, recovery and side effects.
Iwańczak *et al*[30]	-	-	Group I - Amoxicillin-based triple therapy: 78.2%; Group II Metronidazole-based triple therapy: 78.2%; Group III - Sequential therapy: 91.3%; $P > 0.5$.	For strains susceptible to clarithromycin: treatment with amoxicillin-based triple therapy was the most effective. For regions with clarithromycin resistance greater than 20%, quadruple therapy or therapy based on the susceptibility of the strains is recommended.
Tümgör *et al*[31]	Nausea, headache, vomiting, abdominal pain and diarrhea.	-	Group A - Clarithromycin-based triple therapy: 46.6%; Group B - Clarithromycin-based triple therapy + vitamin E: 64.4%; $P = 0.13$.	Although, Group B showed a higher rate of eradication, no statistically significant difference was observed between the two groups.
Ali Habib *et al*[32]	-	No	Group A - Standard therapy: 55.6%; Group B - Sequential therapy: 57.1%; $P = 0.949$.	The rates of *H. pylori* eradication were not significantly different in sequential *vs* standard therapy. In addition, serum ferritin was not significantly different between the two therapies and in the same therapy group before and after treatment.

Ustundag *et al*[33]	Abdominal pain and nausea.	No	ITT: Group A - Standard triple therapy: 58.8%; Group B - Standard triple therapy + symbiotic: 77.1%; $P = 0.16$. PP: Group A: 64.5%; Group B: 81.8%; $P = 0.19$.	The results of the study demonstrated that the addition of Bifidobacterium lactis B94 (5×10^9 CFU/dose) plus inulin once daily to standard triple therapy did not show superiority in eradication rates compared to standard triple therapy administered alone.
Huang *et al*[34]	Nausea, vomiting and diarrhea.	-	(1) ITT: Group A – 10-d sequential therapy: 81.4%; Group B – 7 d triple standard therapy: 61.9%; Group C – 10 d triple standard therapy: 67.7%; $P < 0.05$; and (2) PP: Group A – 10 d sequential therapy: 89.7%; Group B – 7 d triple standard therapy: 70.8%; Group C – 10 d triple standard therapy: 77.8%; $P < 0.05$.	The 10 d sequential regimen was significantly more effective than the 7 or 10 d triple regimens in eradicating Chinese children. In addition, the adverse events between the three groups were also similar, with no statistical differences $P > 0.05$.
N Şirvan *et al*[35]	Abdominal pain, nausea and diarrhea.	-	Group 1 - Standard triple therapy + symbiotic: 88%; Group 2 - Standard triple therapy: 72%; $P = 0.046$.	The rate of eradication was statistically higher in group I. The addition of probiotics to triple therapy is effective in eradicating *H. pylori* infection in children and is generally useful in reducing or eliminating dyspeptic symptoms, such as abdominal pain, diarrhea and vomiting.
Baysoy *et al*[36]	Metallic taste sensation, abdominal pain, diarrhea, vomiting, rash, itching.	-	(1) ITT: Group A- Standard triple therapy: 46.0%; Group B - Sequential therapy group: 40.9%; and (2) PP: Group A - Standard triple therapy: 54.2%; Group B - Sequential therapy group: 48.6%.	Sequential ornidazole therapy did not show superiority compared to standard triple treatment in children with *H. pylori* infection.
Esmaeili-Dooki *et al*[37]	-	-	(1) ITT: Group 1 - Clarithromycin-based triple therapy: 62.5%; Group 2 - Amoxicillin-based triple therapy: 56.2%; $P = 0.4$; and (2) PP: Group 1 - Clarithromycin-based triple therapy: 69%; Group 1 - Amoxicillin-based triple therapy: 61.9%; $P = 0.431$.	The therapeutic response was observed in more than half of the patients treated with triple therapy of the *H. Pylori* eradication regimen, including azithromycin or clarithromycin, and there was no significant difference between the two treatment groups.
Laving *et al*[38]	-	-	Conventional therapy Group: 48.8%; 10 d sequential therapy Group: 84.6%; $P = 0.02$.	The sequential treatment had a significantly higher rate of *H. pylori* eradication than conventional treatment.
Prieto-Jimenez *et al*[39]	Abdominal pain, nausea, diarrhea, rash.	No	(1) ITT: Group I - Quadruple sequential eradication and placebo: 44.8%; Group II - Quadruple sequential eradication and iron: 43.7%; Group III: Iron and placebo: 17.4%; Group IV: Placebo alone: 7.7%; $P < 0.001$; and (2) PP: Group I - Quadruple sequential eradication and placebo: 56.5%; Group II - Quadruple sequential eradication and iron: 50%; Group III: Iron and placebo: 20%; Group IV: Placebo alone: 10.5%; $P < 0.001$.	A sequential quadruple regimen eradicated *H. pylori* in only half of asymptomatic children who received this treatment. Eradication rates did not differ between patients who received iron supplementation and those who received placebo.
Nguyen *et al*[40]	No reported.	No	(1) Group A: Antibiotic sensitive - 79% using high medication dosages, $P = 0.278$; 75% using lansoprazole twice daily, $P = 0.096$. Antibiotic resistant - 67.5% using high medication dosages, $P = 0.006$; 69.2% using lansoprazole twice daily, $P = 0.004$; and (2) Group B: Antibiotic sensitive - 85.2% using high medication dosages, $P = 0.278$; 87.5% using lansoprazole Twice daily, $P = 0.096$. Antibiotic resistant - 45.3% using high medication dosages, $P = 0.096$; 50.0% using lansoprazole Twice daily, $P = 0.004$.	The prevalence of resistance to clarithromycin, metronidazole, and amoxicillin was 50.9%, 65.3% and 0.5%, respectively. The two treatment regimens used did not successfully eradicate *H. pylori* in Vietnamese children, mainly because of the unexpectedly high prevalence of antibiotic resistance.
Bontems *et al*[41]	Abdominal pain, diarrhea, nausea and vomiting.		(1) ITT: Group A – 10 d sequential treatment: 81.9%; Group B: 7 d triple therapy: 71.9%; and (2) PP: Group B – 10 d sequential treatment: 88.3%, Group B: 7 d triple therapy: 80.8%.	The sequential treatment is greatly effective for eradicating *H. pylori* in children except in clarithromycin-resistant strains. Sequential treatment can be used as a first-line therapy, but only in areas with a low clarithromycin resistance rate.

ITT: Intention-to-treat; PP: Per protocol; *H. pylori: Helicobacter pylori*.

overused to treat upper and lower respiratory diseases that are very common in childhood. Eradication rates of standard triple therapies are often below 80% in various regions of the world[47]. In this study, extremely low eradication rates were observed in China, India, Kenya, and Turkey. The frequency of *H. pylori*-resistant strains was 16.4%, 75.2%, and 0.06% to clarithromycin, metronidazole, and amoxicillin,

respectively, in children from the southeast regions of China[48]. The resistance to clarithromycin in Turkish children ranges from 9.5% to 27%[49]. No data are available on clarithromycin resistance in Indian and Kenyan children. Concerning resistance to metronidazole, no data are available on those countries.

Standard triple therapy

A trial comparing amoxicillin-based with metronidazole-based triple therapy, by ITT analysis, found that the latter showed a significantly higher eradication rate (68% *vs* 80%, respectively)[22]. The 7 and 10 d triple therapy failed to eradicate *H. pylori* infection in most of the studies[22,26,27,30,34,37-39]. In some of them, the eradication rates were less than 60%.

Esmaeili-Dooki *et al*[37] compared a triple therapy consisting of azithromycin once daily plus amoxicillin and omeprazole given twice daily for 6 d with clarithromycin in association with amoxicillin and omeprazole twice daily for 10 d. Based on the ITT analysis, the eradication rates in the azithromycin and clarithromycin groups were 56.2% and 62.5%, respectively (P = 0.40)[37]. AEs were 15.6% in the omeprazole, clarithromycin, and amoxicillin group and 3.1% in the omeprazole, azithromycin, and amoxicillin group (P = 0.19). Per protocol, the eradication rate was 61.9% in the azithromycin group and 69% in the clarithromycin group (P = 0.431).

High resistance of *H. pylori* to clarithromycin and metronidazole, poor compliance, and a short duration of treatment may explain these findings, in part. European Society for Paediatric Gastroenterology, Hepatology and Nutrition and North American Society for Paediatric Gastroenterology, and Hepatology and Nutrition guidelines recommend when antimicrobials susceptibility profiles are either unknown or *H. pylori* is susceptible to clarithromycin or metronidazole, a high-dose triple therapy with PPI, amoxicillin and triple for 14 d or bismuth-base quadruple therapy. In this review, the effectiveness of a triple therapy for 14 d was evaluated in eight studies. Eradication rate superior to 80% was observed in two of them[29,40]. Kasiri *et al*[29] did not observe a significant difference in the eradication rate between 14 d amoxicillin-based (87.2%) and for metronidazole-based triple therapy (92.5%) in Iranian children.

A study from Vietnam, triple therapy consisting of lansoprazole amoxicillin and either clarithromycin or metronidazole (LAM) therapy given once or twice daily for 14 d were compared[40]. Eradication success was associated with the strain susceptibility to clarithromycin (78.2% *vs* 29.3%, P = 0.0001). PPI and clarithromycin given twice daily was superior to once-daily dosage for resistant strains (50.0% *vs* 14.7%, P = 0.004) and tended to be effective so also for sensitive strains (87.5% *vs* 65.2%, P = 0.051). The differences were less pronounced with LAM when PPI was given twice daily in comparison with PPI once a day (69.2% *vs* 50.0%, P = 0.096). The reported resistance to clarithromycin, metronidazole, and amoxicillin was 50.9%, 65.3%, and 0.5%, respectively. The authors found that resistance to clarithromycin was an important cause for treatment failure[40]. Higher doses of PPI improve the success of eradication rate of clarithromycin and amoxicillin based-therapy[46]. Moreover, younger children need a higher PPI dose per kg of bodyweight compared to adolescents and adults to obtain sufficient acid suppression[46].

Eradication rate with triple therapy for 14 d ranged from 46% to 76.4% in five studies[24,25,31,33,36].

One study evaluated the efficacy of a third-line therapy. Farahmand *et al*[26] compared a regimen consisting of ciprofloxacin, amoxicillin, and omeprazole, third-line therapy, with the standard triple therapy, amoxicillin, and omeprazole twice a day plus furazolidone once a day. Both regimens given for 1 wk reported that the eradication rate was significantly higher (P = 0.011) in the group treated with ciprofloxacin (87.9%) than in that receiving furazolidone (60.6%)[32].

Standard triple therapy and probiotics

Probiotics have been proposed as an adjuvant to triple therapy to improve the efficacy and diminish AEs in both children and adults. Diarrhea, nausea, and vomiting are the most frequent side effects of eradication therapy and are an important cause of poor compliance and treatment failure.

Several studies did not show an increase in the eradication rate when triple therapy was supplemented with probiotics[23,24,25,33], by contrast, an increase in the *H. pylori* eradication rate was observed by others[27,28,35]. Although the beneficial effects of probiotics depend on the strains of the microorganisms selected, more robust studies and meta-analyses on this issue should be performed to clarify these discrepant results[50]. Of note, some studies demonstrated a statistically significant decrease in adverse gastrointestinal effects during the treatment[25,27,28,35]. This result was also

observed in a meta-analysis using multiple strains to eradicate *H. pylori* and prevent AEs, in children and adults[51]. Another meta-analysis observed that the addition of *Lactobacillus, Bifidobacterium*, and *Saccharomyces* to standard triple therapy improved medication tolerance and patient compliance due to the decrease in side effects, both in children and adults[52]. In the studies included in this review, commercial probiotics were used; however, the use in clinical practice is not economically accessible for many countries with high prevalence rates of *H. pylori*.

Standard triple therapy and antioxidant vitamins

Tümgör *et al*[31] evaluated the use of vitamin E with clarithromycin-based triple therapy in children and found no statistically significant difference between its eradication rate and triple therapy alone. Although the addition of antioxidant vitamins in the eradication treatments has been evaluated[53,54], no statistically significant differences were observed. It is important to highlight that the available studies on this therapeutic alternative have a small sample size and to moderate methodological design[53].

Sequential therapy

Sequential therapy has been related to high *H. pylori* eradication rates[47], and some articles included here corroborated this, showing success rates of 91.3%, 81.4%, and 84.6%, whereas the effectiveness of standard triple therapy ranged from 48.8% to 78.2%[30,34,38]. However, two studies did not observe a statistically significant increase in eradication rates when this regimen was used[32,36]. A meta-analysis that evaluated sequential therapy compared to triple therapy in 13 RCTs also found a higher rate of *H. pylori* eradication in children when using sequential therapy, in accordance with the results of the articles analyzed in this review[55]. Although it is a therapeutic regimen with encouraging rates of *H. pylori* eradication, more robust studies are necessary to prove its effectiveness and safety to substitute triple therapy in high clarithromycin and/or nitroimidazole resistance settings[56].

Study limitations

This study had some limitations due to the small number of available studies evaluating each therapeutic regimen in children. The quality of included studies, the great diversity of treatment regimens, and the duration of treatment, doses, and administration frequency of the drugs as well as lack of antimicrobial susceptibility tests limit the comparison of the results. Most studies had an uncertain degree of bias for concealing the processes of allocation of patients as well as the blinding of participants, professionals, and outcome evaluators.

In summary, the eradication rate associated with current treatments is not satisfactory in many geographical areas. Unfortunately, many of the published works on *H. pylori* eradication in children have weaknesses in their methods and do not meet the ideal scientific criteria to be indicated in practice. It has to be emphasized that, because *H. pylori* is disappearing in developed, studies investigating *H. pylori* treatment are scarce in adults and children. Otherwise, a number of countries that have a high prevalence of *H. pylori* infection face difficulties in conducting research with a good level of evidence due to the lack of structural and financial supports. Finally, the studies were limited to a few countries (n = 10), and good results observed in some studies may not work well in other geographical areas.

CONCLUSION

In conclusion, although some studies support the use of new therapeutic regimens in the treatment of *H. pylori* infection in children, more methodologically reliable prospective studies evaluating the most promising new therapeutic regimens are needed to assess the applicability of these treatments in pediatric clinical practice.

ARTICLE HIGHLIGHTS

Research background

Helicobacter pylori (*H. pylori*) is a gram-negative microaerophilic bacterium that infects the gastric epithelium and whose acquisition occurs mainly during childhood. In the

last several years, no significant changes in the treatment of infected children have been observed, mainly due to the lack of studies with satisfactory scientific evidence to support the indication of new therapies in clinical practice. This systematic review evaluated the eradication rates of *H. pylori* infection using various therapeutic regimens and their positive and negative outcomes in pediatric patients.

Research motivation

Standard triple therapy for the eradication of *H. pylori* infection has been used as first-line treatment in children worldwide. However, the effectiveness of standard triple therapy in eradicating *H. pylori* is decreasing in various geographical areas as a consequence of increasing bacterial resistance to clarithromycin and nitroimidazoles.

Research objectives

To compare the eradication rates of *H. pylori* infection in childhood in controlled, randomized, and prospective studies evaluating different therapeutic schemes during the last 10 years.

Research methods

We systematically reviewed in PubMed and MEDLINE relevant publications from 2010 to April 2020. Twenty studies were shortlisted. This systematic review uses guidance from the PRISMA checklist.

Research results

The results were quite heterogeneous. Standard triple therapy is still the most used regimen and its eradication rates vary according to the *H. pylori* susceptibility profiles in different world regions. The addition of probiotics to therapeutic schemes shows discrepant results in eradication rate, but decrease the incidence of side effects and increases the treatment adherence. Sequential therapy has been associated with higher eradication rates than triple therapies and is a promising therapeutic regimen for this population.

Research conclusions

Currently, standard triple therapy is the most recommended *H. pylori* eradication regimen for children worldwide. However, other therapeutic schemes have shown promising results in controlled trials and in a near future may be included in the guidelines recommendations.

Research perspectives

There are still few studies with satisfactory evidence levels evaluating the eradication of *H. pylori* infection in children, mainly due to the difficulties to conduct controlled clinical trials as well as to the low availability of sources for research in many developing countries where the prevalence of *H. pylori* infection remain elevated. Well-designed studies evaluating treatments for *H. pylori* eradication in children are needed to further evaluate new therapeutic options in pediatric clinical practice in high bacterial resistance settings.

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META-ANALYSIS

Importance of reporting quality: An assessment of the COVID-19 meta-analysis laboratory hematology literature

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Abstract

BACKGROUND

Meta-analysis, a form of quantitative review, is an attempt to combine data from multiple independent studies to improve statistical power. Because of the complexity of process involved in study selection, data analysis, and evaluation of bias and heterogeneity, checklists have been prepared by the Institutes of Medicine (IOM), Preferred Reporting Items for Systemic Reviews and Meta-analyses (PRISMA), and Meta-analyses of Observational Studies in Epidemiology (MOOSE) to standardize the reporting quality of a meta-analysis.

AIM

To use these checklists to assess the reporting quality of the coronavirus disease-2019 (COVID-19) meta-analysis literature relevant to laboratory hematology.

METHODS

After a search of the literature 19 studies were selected for analysis, including 10 studies appearing in the preprint literature (studies that can be identified by database search but have not yet completed peer review).

RESULTS

The average IOM (76% of required elements completed), PRISMA (75% of required elements completed), and MOOSE (60% of required elements completed) scores enumerated demonstrated a reporting quality inferior to that of earlier reports of pathology and medicine meta-analyses. There was no statistically significant difference in performance between accepted/ published and preprint studies. Comparison of the results of PRISMA and MOOSE studies demonstrated a weak positive correlation (Pearson's correlation coefficient = 0.39).

CONCLUSION

The most common deficits in the studies included sensitivity analysis, assessment for bias, and details of the search strategy. Although the COVID-19 laboratory hematology meta-analysis literature can be a helpful source of information,

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readers should be aware of these reporting quality deficits.

Key Words: COVID-19; Meta-analysis; Reporting quality

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Core Tip: The Institutes of Medicine, Preferred Reporting Items for Systemic Reviews and Meta-analyses, and Meta-analyses of Observational Studies in Epidemiology checklists were created to standardize the reporting quality of a meta-analysis. The purpose of this study was to use these checklists to assess the reporting quality of the coronavirus disease-2019 meta-analysis literature relevant to laboratory hematology.

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INTRODUCTION

Meta-analysis, the examination of data from multiple independent studies of the same subject, is a useful form of quantitative review that can provide improved statistical power compared to studies with smaller numbers of subjects and demonstrate the presence or lack of consensus regarding a specific scientific question[1]. In recent years, the number of published meta-analyses has increased, particularly in the realm of clinical medicine, and they have become important sources of information for practitioners, especially in areas where information is rapidly evolving.

In pathology and laboratory medicine, meta-analyses are published less frequently compared to other areas of clinical medicine. Kinzler and Zhang, in their survey of the meta-analysis literature in pathology journals compared to medicine journals, note a significantly larger percentage of publication space dedicated to meta-analyses in medicine journals[1]. This is despite the proven high quality of meta-analyses in both journal categories, as evidenced by similar adjusted citation ratios (which they defined as article's citation count divided by the mean citations for the meta-analysis, review, and original research articles published in the same journal the same year)[1].

Because meta-analyses are an important source of information for clinicians and others, it is essential that they are formatted to easily allow the reader to assess their strengths and weaknesses. Several checklists have been established by national and international committees, including the Institutes of Medicine (IOM), Preferred Reporting Items for Systemic Reviews and Meta-analyses (PRISMA), and Meta-analyses of Observational Studies in Epidemiology (MOOSE)[2-4]. A recent survey by Liu *et al*[5] using the PRISMA criteria noted that the reporting quality for a sampling of medicine meta-analyses was higher than that of pathology meta-analyses. The overall reporting quality for laboratory hematology-focused meta-analyses was not specifically addressed[5].

The coronavirus disease-2019 (COVID-19) pandemic, which originated in the city of Wuhan in the Hubei Province of China in December 2019 quickly spread to Europe and then to North America[6,7]. In an effort to study the disease and improve the world health community's response, over 30000 papers have been added to the medical literature since December 2019, based on a search of the PubMed database for the keyword "COVID-19" conducted on July 16, 2020. In a situation such as this, it is essential for the practicing clinician to have access to reliable studies with good statistical power, hence the need for meta-analyses with high reporting quality. Laboratory hematology is an essential component of the medical response to COVID-19 since several biomarkers of infection derived from the complete blood count (CBC) and coagulation testing are of proven utility in assessing prognosis and likely outcome[8-10]. As in all quickly evolving fields, a large fraction of the accessible medical COVID medical literature appears in the form of preprint publications. These are manuscripts that are indexed in services such as Google Scholar, but have not yet completed the peer-review process. The purpose of this study is two-fold; to assess the reporting quality of COVID-19 meta-analyses focused on laboratory hematology and to compare the reporting quality of published studies of COVID-19 to the preprint

literature.

MATERIALS AND METHODS

Study selection

The study selection processes is summarized in Figure 1. A search was conducted in PubMed and Google Scholar using the search terms "COVID-19" OR "COVID", "SARS-CoV-2", OR "coronavirus" AND "meta-analysis", which yielded 34 entries in PubMed and 3080 in Google Scholar (total = 3114 studies). Initial screening for letters to the editor, editorials, and non-meta-analysis reviews removed 3029 publications, with 85 entries remaining for further consideration. After removal of 27 duplicate entries, 58 publications remained. The full text of the remaining 58 studies were examined for content, and 39 studies that fell out of scope for further consideration were removed, leaving 19 studies for the analysis.

Checklists

The studies were separated into published studies (n = 9, Table 1)[11-19] and manuscripts appearing in the preprint literature (n = 10, Table 1)[20-28]. For the purposes of this study, preprint literature refers to manuscripts discoverable in the Google Scholar database which have been submitted for publication and are assigned an identifier through a service such as doi.org or preprints.org but have not completed the peer-review process.

The studies were then evaluated using the IOM, PRISMA, and MOOSE criteria. The IOM has compiled a list of 5 required elements that serve as recommended standards for meta-analysis (Table 2)[2]. The PRISMA group compiled a list of 27 checklist items to facilitate the assessment of the reporting quality of meta-analyses[3]. The MOOSE criteria consist of a 34-point checklist categorized under 5 divisions[4]. The criteria were evaluated for each study, and a numeric score was assigned based on the sum total of positive results for each element of the IOM, PRISMA and MOOSE checklists.

Statistics

The mean PRISMA and MOOSE scores for the accepted/published and preprint studies were compared using the student 2-tail t-test, with significance defined as $P < 0.05$. The PRISMA and MOOSE scores were compared using Pearson's correlation coefficient. All statistics were calculated using Excel (Microsoft, Redmond, WA, United States).

RESULTS

Qualitative aspects of the identified studies

Qualitative features of the studies are summarized in Table 1. Most cases (17 of 19, 89%) were from Chinese patient populations. For the remaining 2 studies, the national origin of the patient populations was not defined, but given the affiliations of the authors, the patient cohorts were also likely from China. The number of patients in each study was highly variable, ranging from 50 to 59254. The hematology data reported in the studies was heterogeneous. The most common evaluated tests were white blood cell count (15 studies), absolute lymphocyte count (15 studies), and platelet count (10 studies).

Because of the limited number of reporting elements in the IOM checklist (Table 2), a comparison with the PRISMA (Table 3) and MOOSE (Table 4) checklists was not performed. The mean IOM score was 3.8/5 (76%) for all studies. The average scores for preprint (4.0/5, 80%) and accepted/ published (3.5, 70%) studies was similar, and there was no statistically significant difference between the two groups ($P > 0.05$). Reviewing the IOM required elements, the most common deficiencies were in explaining why a pooled estimate might be useful to decision makers and lack of sensitivity analysis.

Due to the larger number of reporting elements in the PRISMA and MOOSE checklists a more robust comparison could be performed. The average PRISMA score for all studies was 20.3/27 (75%) (median = 22/27, 81%).The average scores of the accepted/published (mean = 20.4/27, 76% median = 21.5/27, 80%) and preprint (mean = 20.2/27, 75%, median = 22/27, 81%) groups were similar (student t-test, $P > 0.05$). The most common elements which were lacking were checklist numbers 15 (methods:

Table 1 Articles considered in the analysis

Ref.	Country[1]	No. of patients	Evaluated hematologic parameters
Published studies			
Borges *et al*[11]	Multinational, predominantly China	59254	WBC, ANC, ALC, PLT, D-Dimer
Cao *et al*[12]	China	46959	WBC, ALC
Fu *et al*[13]	Not stated, likely all China	3600	WBC, ALC, PLT, D-dimer
Henry *et al*[14]	China, Singapore	2984	WBC, ANC, ALC, MONO, EOS, HGB, PT, PTT, D-dimer
Lagunes-Rangel[15]	China	828	Estimate of N/L ratio
Li *et al*[16]	China	1995	WBC
Lippi *et al*[17]	China, Singapore	1099	PLT
Rodriguez-Morales *et al*[18]	China, Australia	2874	WBC, ALC, HGB
Zhu *et al*[19]	China	3062	WBC, ALC, D-dimer
Preprint studies			
Arabi *et al*[20]	China	50	WBC
Ebrahami *et al*[21]	China	2217	WBC, ANC, ALC, HGB, PLT, PT, PTT, D-Dimer
Han *et al*[22]	China	1208	ALC, ANC, PLT, PT, PTT, D-Dimer
Heydari *et al*[23]	China, S. Korea	49504	WBC, ANC, ALC, D-dimer
Ma *et al*[24]	China	53000	ALC, PLT, D-dimer
Nasiri *et al*[25]	China, Germany	4679	WBC, ANC, ALC, HGB, PLT
Pormohammad *et al*[26]	China	52251	WBC, ALC, ANC, PLT, HGB
Xu *et al*[27]	China	4062	WBC, ANC,ALC, PLT, D-dimer
Zhang *et al*[28]	Not stated, likely all China	275	WBC, ALC

[1]Country of origin of patient population. WBC: White blood cell count; ANC: Absolute neutrophil count; ALC: Absolute lymphocyte count; PLT: Platelet count; MONO: Absolute monocyte count; EOS: Absolute eosinophil count; HGB: Hemoglobin; PT: Prothrombin time; PTT: Partial thromboplastin time; N/L: Neutrophil-to-lymphocyte.

Table 2 Institutes of Medicine recommended standards for meta-analysis

Required element	Papers meeting this standard (total number and percentage)	Published/ accepted papers meeting this standard (total number and percentage)	Preprint papers meeting this standard (total number and percentage)
Explain why a pooled estimate might be useful to decision makers	9/19 (47%)	5/9 (56%)	4/10 (40%)
Use expert methodologists to develop, execute, and peer review the meta-analyses	15/19 (79%)	7/9 (78%)	8/10 (80%)
Address heterogeneity among study effects	18/19 (95%)	8/9 (89%)	10/10 (100%)
Accompany all estimates with measures of statistical uncertainty	19/19 (100%)	9/9 (100%)	10/10 (100%)
Assess the sensitivity of conclusions to changes in the protocol, assumptions, and study selection (sensitivity analysis)	12/19 (63%)	5/9 (56%)	7/10 (70%)

risk of bias across studies), 16 (methods: additional analyses), 22 (results: risk of bias across studies), and 23 (results: risk of bias across studies). The average MOOSE scores for all studies was 19.9/34, 60% (median = 20/34, 60%).The average scores of the accepted/published (mean = 20.6, 61% median = 21/34, 62%) and preprint (mean = 19.1, 56% median = 19, 56%) groups were similar (student *t*-test, $P > 0.05$). The most common elements which were lacking were II.A [Qualifications of searchers (*e.g.*,

Table 3 Preferred Reporting Items for Systemic Reviews and Meta-analyses checklist

Item number	Element	Papers meeting this standard (total number and percentage)	Published/ accepted papers meeting this standard (total number and percentage)	Preprint papers meeting this standard (total number and percentage)
1	Title	19/19 (100%)	8/9 (89%)	10/10 (100%)
2	Structured summary	18/19 (95%)	8/9 (89%)	10/10 (100%)
Introduction				
3	Rationale	16/19 (84%)	8/9 (80%)	8/10 (89%)
4	Objectives	17/19 (89%)	9/9 (90%)	8/10 (89%)
Methods				
5	Protocol/Registration	16/19 (84%)	8/9 (89%)	8/10 (78%)
6	Eligibility criteria	17/19 (89%)	8/9 (89%)	9/10 (89%)
7	Information sources	18/19 (95%)	9/9 (100%)	9/10 (89%)
8	Search	18/19 (95%)	9/9 (100%)	9/10 (89%)
9	Study selection	19/19 (100%)	9/9 (100%)	10/10 (100%)
10	Data collection process	18/19 (95%)	8/9 (89%)	10/10 (100%)
11	Data items	17/19 (89%)	8/9 (89%)	9/10 (89%)
12	Risk of bias in individual studies	10/19 (53%)	4/9 (44%)	6/10 (56%)
13	Summary measures	15/19 (79%)	6/9 (66%)	8/10 (78%)
14	Synthesis of results	16/19 (84%)	7/9 (78%)	9/10 (89%)
15	Risk of bias across studies	2/19 (11%)	2/9 (22%)	0/10 (0)
16	Additional analyses	2/19 (11%)	1/9 (11%)	1/10 (10%)
Results				
17	Study selection	19/19 (100%)	9/9 (100%)	10/10 (100%)
18	Study characteristics	19/19 (100%)	9/9 (100%)	10/10 (100%)
19	Risk of bias within studies	12/19 (63%)	5/9 (56%)	7/10 (70%)
20	Results of individual studies	11/19 (58%)	5/9 (56%)	6/10 (60%)
21	Synthesis of results	16/19 (84%)	8/9 (89%)	8/10 (80%)
22	Risk of bias across studies	9/19 (47%)	5/9 (56%)	4/10 (40%)
23	Additional analysis	0/19 (0)	0/9 (0)	0/10 (0)
Discussion				
24	Summary of evidence	19/19 (100%)	9/9 (100%)	10/10 (100%)
25	Limitations	16/19 (84%)	7/9 (78%)	9/10 (90%)
26	Conclusions	19/19 (100%)	9/9(100%)	10/10 (100%)
Funding				
27	Funding	7/19 (37%)	3/9 (33%)	4/10 (40%)

librarians and investigators)], II.H (Method of addressing articles published in languages other than English, II.I (Method of handling abstracts and unpublished studies) and II.J (Description of any contact with authors).

To determine the degree to which the PRISMA and MOOSE scores correlated, analysis using Pearson's correlation coefficient was performed. The resulting coefficient, 0.39, suggests a weak positive correlation.

Table 4 Meta-analyses of Observational Studies in Epidemiology criteria checklist

Checklist Number	Papers meeting this standard (total number and percentage)	Published/ accepted papers meeting this standard (total number and percentage)	Preprint papers meeting this standard (total number and percentage)
I. Reporting of background			
A. Problem definition	10/19 (53%)	7/9 (78%)	3/10 (30%)
B. Hypothesis statement	2/19 (11%)	1/9 (11%)	1/10 (10%)
C. Description of study outcome(s)	19/19 (100%)	10/9 (100%)	9/10 (90%)
D. Type of exposure or intervention used	18/19 (95%)	9/9 (100%)	8/10 (80%)
E. Type of study designs used	18/19 (95%)	9/9 (100%)	9/10 (90%)
F. Study population	18/19 (95%)	9/9 (100%)	9/10 (90%)
II. Reporting of search strategy			
A. Qualifications of searchers	0/19 (0)	0/9 (0)	0/10 (0)
B. Search strategy	17/19 (89%)	9/9 (100%)	8/10 (80%)
C. Effort to include all available studies	10/19 (53%)	7/9 (78%)	3/10 (30%)
D. Databases and registries searched	17/19 (89%)	7/9 (78%)	10/10 (100%)
E. Search software used	8/19 (42%)	4/9 (44%)	4/10 (40%)
F. Use of hand searching	2/19 (11%)	1/9 (11%)	1/10 (10%)
G. List of citations located and those excluded	10/19 (53%)	5/9 (56%)	5/10 (50%)
H. Method of addressing articles published in languages other than English	0/19 (0)	0/9 (0)	0/10 (0)
I. Method of handling abstracts and unpublished studies	0/19 (0)	0/9 (0)	0/10 (0)
J. Description of any contact with authors	0/19 (0)	0/9 (0)	0/10 (0)
III. Reporting of methods			
A. Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	8/19 (42%)	4/9 (44%)	4/10 (40%)
B. Rationale for the selection and coding of data	13/19 (68%)	7/9 (78%)	6/10 (60%)
C. Documentation of how data were classified and coded	12/19 (63%)	8/9 (89%)	4/10 (40%)
D. Assessment of confounding	1/19 (5%)	0/9 (0)	1/10 (10%)
E. Assessment of study quality	16/19 (84%)	7/9 (78%)	9/10 (90%)
F. Assessment of heterogeneity	18/19 (95%)	8/9 (89%)	10/10 (100%)
G. Description of statistical methods	19/19 (100%)	9/9 (100%)	10/10 (100%)
H. Provision of appropriate tables and graphics	18/19 (95%)	9/9 (100%)	9/10 (90%)
IV. Reporting of results			
A. Graphic summarizing individual study estimates and overall estimate	19/19 (100%)	9/9 (100%)	10/10 (100%)
B. Table giving descriptive information for each study included	16/19 (84%)	7/9 (78%)	9/10 (90%)
C. Results of sensitivity testing (*e.g*, subgroup analysis)	12/19 (63%)	7/9 (78%)	5/10 (50%)
D. Indication of statistical uncertainty of findings	17/19 (89%)	8/9 (89%)	9/10 (90%)
E. Reporting of discussion should include			
1. Quantitative assessment of bias (*e.g*, publication bias)	11/19 (58%)	4/9 (44%)	7/10 (70%)

2. Justification for exclusion (eg, exclusion of non–English-language citations)	3/19 (16%)	1/9 (11%)	2/10 (20%)
3. Assessment of quality of included studies	12/19 (63%)	4/9 (44%)	8/10 (80%)
V. Reporting of conclusions			
A. Consideration of alternative explanations for observed results	1/19 (11%)	0/9 (0)	1/10 (10%)
B. Generalization of the conclusions (*i.e*, appropriate for the data presented and within the domain of the literature review)	19/19 (100%)	9/9 (100%)	10/10 (100%)
C. Guidelines for future research	8/19 (42%)	6/9 (66%)	2/10 (20%)

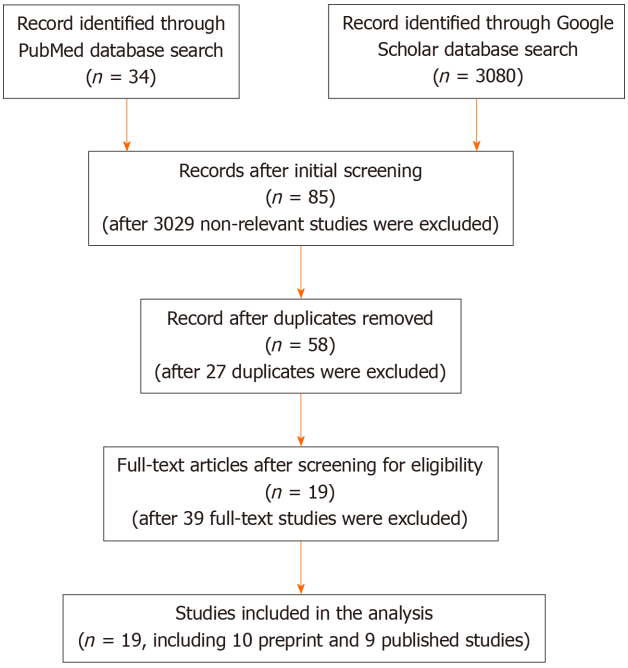


Figure 1 Study selection flow diagram.

DISCUSSION

The use of meta-analysis in the COVID-19 literature

Narrative, nonquantitative review papers have existed in the medical literature for many years and are an important source for succinctly reported and up-to-date information for clinicians and others interested in patient care and other issues. In recognition of the importance of the evidence-based approach to the dissemination of medical information, authors added increasingly rigorous approaches to their publications to provide quantitative information, minimize bias, identify knowledge gaps in the regarding a subject, and provide guidance for further growth of the area of study. This trend resulted in the development of the meta-analysis[29].

Meta-analysis is a modification and attempted improvement of more traditional forms of review publication Meta-analysis attempts to move beyond the narrative review process by adding numeric data synthesized from previously published data[30]. By combining data from more than one study, there is an obvious improvement in statistical power. Meta-analysis has been widely employed in the behavioral science and clinical medicine literatures but has been underutilized in the pathology and laboratory medicine literature. Kinzler and Zhang published a study comparing the use of meta-analysis in the diagnostic pathology literature compared to the clinical medicine literature and noted that meta-analyses comprised < 1% of diagnostic pathology articles compared to 4%-6% of the clinical medicine literature[1]. Despite their relatively low numbers, meta-analyses in the diagnostic pathology literature were highly cited, with a citation rate similar to that of meta-analyses appearing in the clinical medicine literature[1]. This finding is also noted in the current study: although numerous studies have been published addressing the laboratory

hematologic aspects of COVID-19, the number of meta-analyses is low and comprises < 1% of the published literature in this area.

To be successful, the meta-analysis must address several elements[29]: (1) The question must be stated unambiguously; (2) A search of the medical literature must be performed in a comprehensive way; (3) The articles identified by the search must be screened; (4) The appropriate data must be extracted from the selected papers; (5) An assessment of the quality of the information is performed, by a review of the contents of the manuscripts and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) criteria[30]; (6) Determine whether the data in each publication are heterogeneous; (7) Determine summary effect size as odds ratio and generate graphical depictions of data, for example as a forest plot; (8) Assess for publication bias using funnel plot or some other mechanism; and (9) Conduct subset analysis to look for subsets of groups that capture the summary effect.

Purpose of reporting quality analysis and its limits

Because of the complexity of design and execution of meta-analyses, there are numerous opportunities to introduce biases and other errors that may significantly alter the outcome. To make the reporting of data and statistical analysis in meta-analyses transparent to the reader and to clearly advertise the limits of the data used in the study, 3 checklist systems have been promulgated to list the major elements that researchers should use to structure their work.

The first of these systems, the IOM checklist, was created by a committee by the United States Institutes of Medicine. This is a relatively simple 5-point checklist that broadly addresses the reporting of the planning and execution of meta-analyses[2]. The Institutes of Medicine, along with a large number of journals and other publishers, later endorsed the PRISMA statement, which addresses these issues in a more granular fashion[3]. Anther checklist, the MOOSE guidelines, may also be applied to evaluate reporting quality of systematic reviews including meta-analyses[4]. In the reported literature, PRISMA guidelines are utilized more frequently than MOOSE guidelines. In a survey of the medical literature by Fleming *et al*[31], the vast majority of publications used PRISMA guidelines, compared to MOOSE guidelines, which were cited in only 17% of reviews. Fleming *et al*[31] note that although there is a high degree of overlap between the MOOSE and PRISMA checklists, MOOSE provides more advice about features such as the search strategy and interpretation of the results of the review, both of which may introduce bias if not adequately addressed[31,32].

In the current study the most common deficiencies were (1) lack of an articulated rationale for why a pooled analysis is necessary; (2) lack of detail of how to address the use of data that has not been peer reviewed; (3) a lack of sensitivity analysis; and (4) a lack of assessment of studies for bias. Although the rationale for why a meta-analysis is performed is generally obvious (*e.g.*, improved statistical power, identification of a consensus/lack of consensus regarding a specific clinical question) it is not explicitly articulated in a significant number of studies included in this survey. The lack of transparency about the use of non-English language literature and preprint and other non-peer reviewed materials may be problematic, in particular in COVID-19 studies. Sensitivity analysis is a fundamental element of meta-analysis and provides an estimate of the appropriateness of the assumptions made by the analysis[29]. Bias can be introduced into a study in many ways, most commonly by publication bias, in which the medical literature has an underrepresentation of studies with negative findings[29].

The overall reporting of quality in the pathology literature appears to lag behind that for clinical medicine[5]. Liu *et al*[5] compared the reporting quality of a group of diagnostic pathology meta-analyses to a group published in clinical medicine journals using the PRISMA checklist, and found a higher average PRISMA score for the medicine studies that was statistically significant ($P < 0.01$). The average PRISMA score for the COVID-19 meta-analyses in the current study (20.3/27, 75% of items addressed) is below that for both groups analyzed by Liu *et al*[5]. This reflects a significant weakness in the COVID-19 meta-analysis laboratory hematology literature, since the potential strengths of the meta-analysis approach as a force multiplier for evidence-based medicine requires good reporting quality[5].

It is important to note the assessment of reporting quality is not synonymous with assessment of methodological quality of a meta-analysis. The purpose of reporting quality guidelines is to provide an appropriate framework to the authors of meta-analyses and other systematic reviews so that their data and statistical analysis is reported in an unambiguous way. The assessment of methodological quality is a separate exercise and can only proceed if the data can be unambiguously extracted from the publication. The methodological assessment of systematic reviews is addressed by other guidelines such as QUADAS and QUADAS-2[33]. Due to the

apparent suboptimal average reporting quality of COVID-19 laboratory hematology meta-analyses literature, the ability of the reader to assess methodological quality is limited in many cases.

Preprint literature and its reporting quality

In academic publishing, a preprint is the version of a manuscript that has been submitted for publication but has not yet finished the peer review process. In recent years, publishers and others have electronically posted preprint manuscripts to rapidly disseminate scientific knowledge. In addition, studies that have been uploaded to dedicated servers but not submitted for peer review are also included in the category of preprints. Preprints are particularly useful in fields such as COVID-19, which are rapidly evolving and are of intense clinical and scientific interest.

Since preprints are widely accessible, it would be important for readers to be aware of their quality compared to studies published in the peer review literature. Although it would be assumed that the reporting quality of the peer review process would be higher than the comparable preprint literature since the purpose of peer review is to permit scrutiny of one's work by experts[34], there have apparently been no studies in the peer review literature that directly compare the reporting quality of clinical studies in the preprint and published literature. A single study in the preprint literature (Carneiro *et al*[35]) has attempted to address this question. The authors compared a sample of studies identified in the bioRXIV preprint server with studies identified in a Medline (PubMed interface) search. They also compared a group of preprint studies with their final versions. Carneiro *et al*[35] identified a small increase in quality in the published studies compared to the preprint group.

In the current study, using the PRISMA and MOOSE criteria, a significant difference was not identified comparing the preprint and published studies in the COVID-19 meta-analysis literature. Taken together, these findings suggest that the peer review process itself does not guarantee an improvement in quality, and authors should take the initiative to conform to reporting quality norms.

CONCLUSION

This study represents an attempt to assess the overall reporting quality of the laboratory hematology COVID-19 meta-analysis literature. Using the IOM, PRISMA, and MOOSE, guidelines, there were consistent deficits in the reporting of bias and sensitivity. The results for the preprint and published literature were similar and suggest that the preprint literature on this subject is not decidedly inferior to the published literature. Because of the suboptimal reporting quality, it is important for clinicians and others to carefully assess the individual studies used in a given meta-analysis for evidence of bias or other methodological flaws that have not been reported by the authors. Although there is a positive correlation between the PRISMA and MOOSE guidelines, it is relatively weak. This implies that authors of meta-analyses should consider using both systems to increase the strength of the reporting quality of their studies.

ARTICLE HIGHLIGHTS

Research background

Meta-analyses, which are underutilized in pathology and laboratory medicine, combine the data from multiple studies to produce a publication with increased statistical power. It is important for readers of meta-analyses to have the information in these studies reported in a transparent fashion. Hence the Institutes of Medicine (IOM), Preferred Reporting Items for Systemic Reviews and Meta-analyses (PRISMA), and Meta-analyses of Observational Studies in Epidemiology (MOOSE) checklists have been promulgated to standardize the reporting of meta-analyses.

Research motivation

Several parameters evaluated by the hematology laboratory have been identified as potential biomarkers of prognosis and outcome in the coronavirus disease 2019 (COVID-19). The data from many of these studies have been pooled and published as meta-analyses. Many of these studies have been identified in the preprint literature (studies that have not yet completed peer review). The reporting quality of this body

of work is unknown.

Research objectives

The purposes of this study were to 1) evaluate the reporting quality of laboratory hematology-focused COVID-19 meta-analyses using the IOM, PRISMA, and MOOSE checklists and 2) compare the reporting quality of published vs. preprint studies.

Research methods

Based on a search of the literature, 19 studies were selected for analysis (9 published studies and 10 preprint studies). The reporting quality of the studies was evaluated using the IOM, PRISMA, and MOOSE checklists.

Research results

The reporting quality of the published and preprint studies was similar, and was inferior in quality to that described in similar studies on reporting quality of meta-analyses published in the pathology and medicine literature.

Research conclusions

Readers of COVID-19 laboratory hematology meta-analyses should be cognizant of their reporting quality problems, and critically evaluate them before using their information for patient care.

Research perspectives

The issue of reporting quality is of critical importance, and the assessment of reporting quality has been underreported in the medical literature. Studies similar to this one will emphasize that the use of the IOM, PRISMA, and MOOSE checklists is a simple strategy to optimize the overall quality of meta-analyses.

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META-ANALYSIS

Prevalence, awareness and control of hypertension in Malaysia from 1980-2018: A systematic review and meta-analysis

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Abstract

BACKGROUND

Hypertension is a common public health problem worldwide and is a well-known risk factor for increased risk of cardiovascular diseases, contributing to high morbidity and mortality. However, there has been no systematic review and meta-analysis of a multiethnic population such as that of Malaysia.

AIM

To determine the trend in prevalence, awareness and control rate of hypertension in Malaysia.

METHODS

Systematic searches were conducted in six databases (PubMed, Scopus, Ovid, CINAHL, Malaysian Medical Repository and Malaysia Citation Index) for articles

Conflict-of-interest statement: The authors declare that they have no competing interests.

PRISMA 2009 Checklist statement: This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

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published between 1980 and 2018. Two authors reviewed the studies and performed quality assessment and data extraction independently. Pooled estimates of hypertension prevalence, awareness and control rate were calculated using the DerSimonian-Laird random-effects model. Subgroup and sensitivity analyses were performed.

RESULTS

We included 56 studies involving a total of 241796 participants. The overall pooled prevalence of hypertension aged ≥ 18 years was 29.7%. The prevalence of hypertension was the lowest in the 1980s (16.2%, 95% confidence interval (CI): 13.4, 19.0), increasing up to 36.8% (95%CI: 6.1, 67.5) in the 1990s, then decreasing to 28.7% (95%CI: 21.7, 35.8) in the 2000s and 26.8% (95%CI: 21.3, 32.3) in the 2010s. The prevalence of awareness was 51.4% (95%CI: 46.6, 56.3), while 33.3% (95%CI: 28.4, 38.2) of those on treatment had achieved control of their blood pressure.

CONCLUSION

In Malaysia, three in ten adults aged ≥ 18 years have hypertension, while four in ten adults aged ≥ 30 years have hypertension. Five out of ten people are aware of their hypertension status and only one-third of those under treatment achieved control of their hypertension. Concerted efforts by policymakers and healthcare professionals to improve awareness and control of hypertension should be of high priority.

Key Words: Prevalence; Awareness; Control; Hypertension; Blood pressure; Malaysia; Systematic review; Meta-analysis

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Core Tip: This is a systematic review and meta-analysis reporting that the pooled prevalence of hypertension in people aged ≥ 18 years in Malaysia was 29.7%. The pooled prevalence of awareness towards hypertension was 51.4 % and blood pressure control rate was 33.3%.

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INTRODUCTION

Hypertension is a common public health problem over the past several decades[1,2]. It is one of the major risk factors for cardiovascular diseases such as stroke, heart failure and ischemic heart disease[3]. In Malaysia, trend analyses have been conducted based on the National Health and Morbidity Surveys, which are nationwide studies that have been performed every 10 years since 1986. The analyses have also shown the trend of hypertension in different genders and ethnicities[4-7]. Besides the National Health and Morbidity Surveys, several other studies have examined the prevalence, awareness and control of hypertension that differed from that of the National Health and Morbidity Surveys[8,9]. The prevalence in different settings and in different age groups may present a slightly different picture along with their accompanying set of problems.

The present systematic review aimed to determine the pooled prevalence of hypertension, awareness and control of hypertension in Malaysia from 1980 to 2018 based on a nationwide survey and other important studies that reported the prevalence of hypertension in different settings and specific groups. Heterogeneities among cross-sectional studies and the national survey may conceivably cause different rates of prevalence. Hence, there is a need for a systematic review and meta-analysis to estimate the pooled prevalence of hypertension in Malaysia. We are not only reporting

the trend of hypertension prevalence in Malaysia from 1980 to 2018 but are also providing some important insights into the awareness and control of hypertension among Malaysians.

MATERIALS AND METHODS

Protocol registration

The present review was registered with PROSPERO (2018: CRD42018075369) and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[10].

Data sources

We searched six databases (PubMed, Ovid, Scopus, CINAHL, Malaysian Medical Repository and Malaysia Citation Index) for the relevant articles and included all relevant citations published before February 28, 2018.

Search strategy

The literature search used the following search terms: (prevalence) and (awareness) and (control) and (hypertension or high blood pressure) and (Malaysia), and a combination of expanded MeSH terms and free-text searches were used for the final search up to February 28, 2018: (Prevalence OR Aware* OR Unaware* OR Undiagnosed OR Control* OR Uncontrol* OR "Achieved target") AND (Hypertension OR "Blood pressure" OR "Systolic blood pressure" OR "Diastolic blood pressure" OR "Raised blood pressure" OR "High blood pressure") AND (Malaysia OR Perlis OR Kedah OR Penang OR "Pulau Pinang" OR Perak OR Johor OR Selangor OR Pahang OR Malacca OR Melaka OR Terengganu OR Kelantan OR "Negeri Sembilan" OR Sabah OR Sarawak OR "Wilayah Persekutuan" OR Putrajaya OR "Kuala Lumpur" OR "Labuan").

Eligibility criteria

Any studies, reports or articles published between January 1, 1980 and February 28, 2018 and fulfilling the following criteria were included in the analysis: (1) Included the general population aged ≥ 18 years; (2) Studied the prevalence of hypertension diagnosed using digital automated or mercury sphygmomanometers; (3) Studied either the prevalence, awareness and control of blood pressure (BP) or hypertension individually or any combination of the three; and (4) In English only. We excluded intervention studies, case studies, pharmacogenetic studies, case series, qualitative data, comments or letters, audits, narrative reviews, conference proceeding, opinion pieces, methodological, editorials, animal studies or any other publications lacking primary data and/or explicit method descriptions. When several publications were derived from the same dataset or cohorts, the article that provided the most updated data was selected. We identified other pertinent studies through reverse–forward citation tracking of relevant articles.

Selection process

We imported relevant articles identified through the databases into EndNote X5 and removed duplicate publications. Two authors (Chow ZY and Tan CH) screened the titles and abstracts independently to search for eligible articles based on the inclusion criteria. If there were discrepancies on including studies, discussions were held and resolved by the senior authors (Ching SM, Hoo FK, Devaraj NK, Salim HS and Cheong AT) for final consensus before the full text of each relevant article was reviewed.

Data collection

One author (Ramachandran V) recorded data from the selected studies into the extraction form using Excel, while the second author (Ching SM) verified the accuracy and completeness of the extracted data.

Data items

The following characteristics of the selected studies were extracted: First author, year of publication, study setting, study design, location of study, geographical area (rural or urban), BP apparatus, sample size and cases with hypertension. The outcome measures extracted were the prevalence of hypertension in terms of the difference of proportion/percent of hypertension, awareness and control in the total patients

examined.

Summary measures

Hypertension was defined as BP ≥ 140/90 mmHg based on guidelines from the Fifth Joint National Committee. Awareness was defined as knowing one's own hypertension status or having been diagnosed with hypertension previously. Control was defined as achieving a target BP of < 140/90 mmHg[11].

Risk of assessment based on the critical appraisal checklist

Each article underwent quality assessment by two authors (Soo MJ and Lee KW) using a modified critical appraisal checklist (Supplementary material Appendix 1)[8]. The checklist consisted of 11 items that assessed the components in observational studies. Whenever the information provided was insufficient to assist in the evaluation of a certain item, the two authors agreed to grade that item as "0" indicating absence of the item, "0.5" indicated that the information was incomplete, and "1" indicated that the item was presented clearly. The quality of each article was graded as high if it scored ≥ 7/11, or low if it scored < 7/11[8]. The results of the quality assessment are shown in supplementary material Appendix 2.

Data synthesis

The results from the meta-analysis summarized the data narrative and statistically used a tabulated format. Heterogeneity between studies for the pooled estimates was examined using I^2 (I^2 < 25%, low; I^2 = 25%-30%, moderate; I^2 > 50%, high), indicating the percent of total discrepancy due to variation in the studies[12].

Statistical analysis

We used OpenMeta[Analyst] for data analysis (http://www.cebm.brown.edu/openmeta/index.html)[13]. The prevalence estimated from individual studies was pooled using random-effects (DerSimonian-Laird method) meta-analysis and was reported with 95% confidence interval (CI). Subgroup analysis was performed to examine the prevalence, awareness and control of hypertension by age group, sex, ethnicity, study setting, study design, geographical origin and BP tools.

Additional analysis

Publication bias was assessed by sensitivity analysis using the leave-one-out analysis. We also performed sensitivity analyses for the prevalence of hypertension by discarding low-quality studies, removing outlier subpopulations (point estimates > ± 3 standard deviations)[14] or removing smaller subpopulations (size < 100) or large sample sizes (size > 5000)[15], where these publication biases are known to have effects on the estimated prevalence. The effect size of interest was the proportion of individuals with hypertension. The secular trend of prevalence was estimated by calculating the point estimates for four separate decades.

RESULTS

Description of included studies

We identified 1493 manuscripts in the initial search (Figure 1). After removal of duplicate records (n = 251), 1242 studies were retrieved for further assessment. After careful evaluation of the inclusion/exclusion criteria, 52 studies fulfilled our criteria, and this together with another four studies identified from cross-referencing, a total of 56 studies were included in our meta-analysis.

Characteristics of the included studies

The main characteristics of the included studies are shown in Table 1[16-71] and supplementary material Appendix 3 in encompassing the prevalence, awareness and control of hypertension of the included studies. A total sample size of 241796 respondents from Malaysia was included in the analysis. Overall, the ethnicity distribution was 56.5% Malay, 24.2% Chinese, 9.7% Indian and 9.5% other ethnicities. Fifty-one studies were conducted in the community setting, four were in a hospital setting and one was in a primary care clinic. Quality assessment using a modified critical appraisal checklist showed that the majority of the studies (52/56) were of good quality with only four having poor quality.

Table 1 Prevalence, awareness and control of hypertension in Malaysia

Ref.	Sample size, *n*	Prevalence of hypertension, %	Prevalence of awareness, %	Prevalence of control, %	Quality of article
Abdul-Razak *et al*[16], 2016	11288	47.9	52.5	15.9	High
Jinam *et al*[17], 2008	213	40.4	NA	NA	High
Akter *et al*[18], 2010	219	35.6	NA	NA	High
Amiri *et al*[19], 2014	1096	39.3	NA	NA	High
Amplavanar *et al*[20], 2010	3765	34.2	NA	NA	High
Aniza *et al*[21], 2015	1107	24.0	NA	NA	High
Aniza *et al*[22], 2016	1489	39.5	NA	NA	High
Annamalai *et al*[23], 2011	903	26.9	39.5	15.7	High
Azuwani *et al*[24], 2013	138	42.0	NA	NA	High
Tan *et al*[25], 2008	109	33.0	NA	NA	High
Chang *et al*[26], 2012	260	13.9	NA	NA	High
Cheah *et al*[27], 2015	218	7.3	NA	NA	High
Chee *et al*[28], 2002	968	1.8	NA	NA	High
Chin *et al*[29], 2009	1417	52.4	NA	NA	High
Chow *et al*[30], 2013	11324	47.0	48.3	12.8	High
Chua *et al*[31], 2017	482	25.5	NA	NA	High
Gan *et al*[32], 1993	648	16.2	NA	NA	High
Ghazi *et al*[33], 2017	410	10.0	41.5	NA	Low
Goh *et al*[34], 2013	1621	22.1	NA	NA	High
Hasnah *et al*[35], 2012	125	34.4	NA	NA	High
Hazmi *et al*[36], 2015	308	14.3	NA	NA	High
Ministry of Health Malaysia[37], 2015	23845	30.3	NA	NA	High
Jamal *et al*[38], 2015	106527	46.4	NA	NA	High
Khan *et al*[39], 2008	240	58.3	48.6	51.4	High
Latiffah *et al*[40], 2008	73	39.7	70.8	40.9	High
Latiffah *et al*[41], 2008	92	51.1	NA	NA	High
Lee *et al*[42], 2010	226	12.8	NA	NA	Low
Lian *et al*[43], 2015	223	25.1	NA	NA	High
Liao *et al*[44], 2010	206	19.9	NA	NA	High
Lim *et al*[45], 1991	368	30.4	NA	NA	High
Loh *et al*[46], 2013	1961	54.9	NA	NA	High
Ministry of Health Malaysia[47], 2010	2572	25.7	NA	NA	High
Mohamed *et al*[48], 2005	4117	33.0	NA	NA	High
Nazri *et al*[49], 2008	148	13.5	NA	NA	High
Nazri *et al*[50], 2008	348	12.6	NA	NA	High
Moy *et al*[51], 2010	380	20.3	NA	NA	High
Narayan *et al*[52], 2007	431	33.0	NA	NA	High
Narayan *et al*[53], 2007	479	33.6	28.0	27.5	High
Nasarudin *et al*[54], 2016	535	21.1	NA	NA	Low

Nawawi *et al*[55], 2002	608	30.3	NA	NA	High
Ng *et al*[56], 1995	427	21.1	NA	NA	Low
Noor Hassim *et al*[57], 2016	5505	25.2	NA	NA	High
Ong *et al*[58], 2010	205	36.1	81.1	33.8	High
Ong *et al*[59], 2013	40400	30.1	NA	NA	High
Raihan *et al*[60], 2013	251	14.3	NA	NA	High
Rampal *et al*[61], 2008	454	34.4	64.1	25.0	High
Rashid *et al*[62], 2011	418	54.6	51.8	NA	High
Rasiah *et al*[63], 2015	6690	37.4	NA	NA	High
Shahar *et al*[64], 2011	71	32.4	NA	NA	High
Samsudin *et al*[65], 2016	1414	42.4	NA	NA	High
Sherina *et al*[66], 2011	202	12.4	NA	NA	High
Shomad *et al*[67], 2016	56	4.00	NA	NA	High
Sidik *et al*[68], 2004	223	22.0	NA	NA	High
Teh *et al*[69], 2014	3406	35.8	NA	NA	Low
Yusoff *et al*[70], 2010	289	30.1	NA	NA	High
Zainuddin *et al*[71], 201	298	37.3	NA	NA	High

NA: Not available.

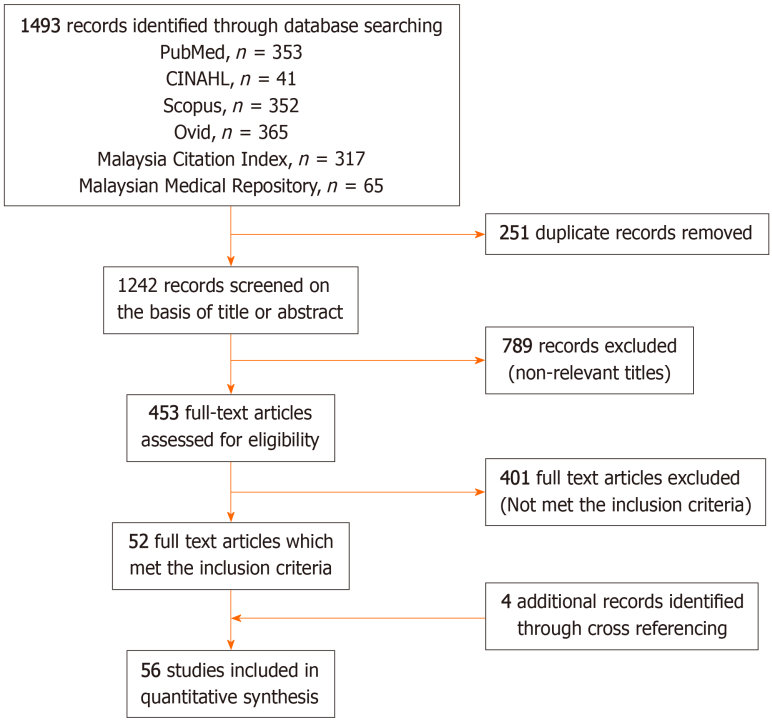


Figure 1 Preferred reporting items for systematic review and meta-analysis flow diagram of the literature screening process. Numbers indicate the article count retained at each step of the process.

Prevalence of hypertension: Overall and subgroup analysis

Table 2 shows the prevalence of hypertension in Malaysia is 29.7% (95%CI: 26.1, 33.3) (Figure 2). The prevalence of hypertension for those aged ≥ 30 years was 40.0% (Figure 3). The pooled prevalence of hypertension increased with age as the prevalence was 8.6% in adults aged 18–29 years as compared to 42.8% in adults aged ≥ 60 years (Figure 4).

Table 2 Pooled prevalence and 95% confidence interval of hypertension and its subgroup analysis

Variable	Number of studies, *n*	Prevalence, %	95%CI	*P* value	*I*², %	Figure
Overall prevalence						
Malaysia	56	29.7	26.1, 33.3	< 0.001	99.7	2
30 and above	16	40.0	35.3, 44.8	< 0.001	98.9	3
Mean age, yr						4
18–29	4	8.6	5.30, 11.90	0.03	66.9	
30–39	3	13.5	2.9, 29.9	< 0.001	98.7	
40–49	17	27.9	24.9, 30.9	< 0.001	96.5	
50–59	4	39.4	29.3, 49.4	< 0.001	99.7	
60 and above	6	42.8	30.2, 55.3	< 0.001	95.8	
Gender						
Male	26	31.4	26.5, 36.2	< 0.001	98.1	5A
Female	26	27.8	20.7, 34.9	< 0.001	99.5	5B
Ethnicity						
Malay	17	37.3	32.9, 41.7	< 0.001	98.9	6A
Chinese	8	36.4	31.6, 41.2	< 0.001	96.6	6B
Indian	10	34.8	31.2, 38.4	< 0.001	81.5	6C
Other	9	32.9	25.8, 40.0	< 0.001	98.0	6D
Study setting						7
Community	51	30.2	26.4, 34.1	< 0.001	99.7	
Health care setting	5	24.3	16.6, 32.0	< 0.001	97.5	
Geographical origin						
Rural	19	35.6	29.9, 41.4	< 0.001	98.77	8A
Urban	21	25.4	20.4, 30.4	< 0.001	98.43	8B
Study decade						9
1980-1989	1	16.2	13.4, 19.0	NA	NA	
1990-1999	2	36.8	6.1, 67.5	< 0.001	99.42	
2000-2009	19	28.7	21.7, 35.8	< 0.001	99.9	
2010-2018	9	26.8	21.3, 32.2	< 0.001	98.7	
Blood pressure measurement tools						10
Mercury sphygmomanometer	15	33.2	26.4, 40.0	< 0.001	98.8	
Digital blood pressure device	16	30.8	25.5, 36.0	< 0.001	99.8	

CI: Confidence interval.

Among adults aged ≥ 18 years, the prevalence of hypertension was higher in men compared to women (31.4%, 95%CI: 26.5, 36.2 *vs* 27.8%, 95%CI: 20.7, 34.9) (Figure 5A and 5B). The prevalence of hypertension was highest among Malays (37.3%, 95%CI: 32.9, 41.7), followed by Chinese (36.4%, 95%CI: 31.6, 41.2) and Indians (34.8%, 95%CI: 31.2, 38.4) (Figure 6A-D). The prevalence of hypertension was 24.3% in healthcare setting as compared to 30.2% in community setting (Figure 7). The prevalence of hypertension in rural areas was 35.6% as compared to 25.4% in urban areas (Figure 8A and 8B).

The prevalence of hypertension was 16.2% in the first decade (1980–1989), 36.8% in the second decade (1990–1999), 28.7% in the third decade (2000–2009) and 26.8% in the fourth decade (2010–2018) (Figure 9).

The prevalence of hypertension in studies that used mercury sphygmomanometers

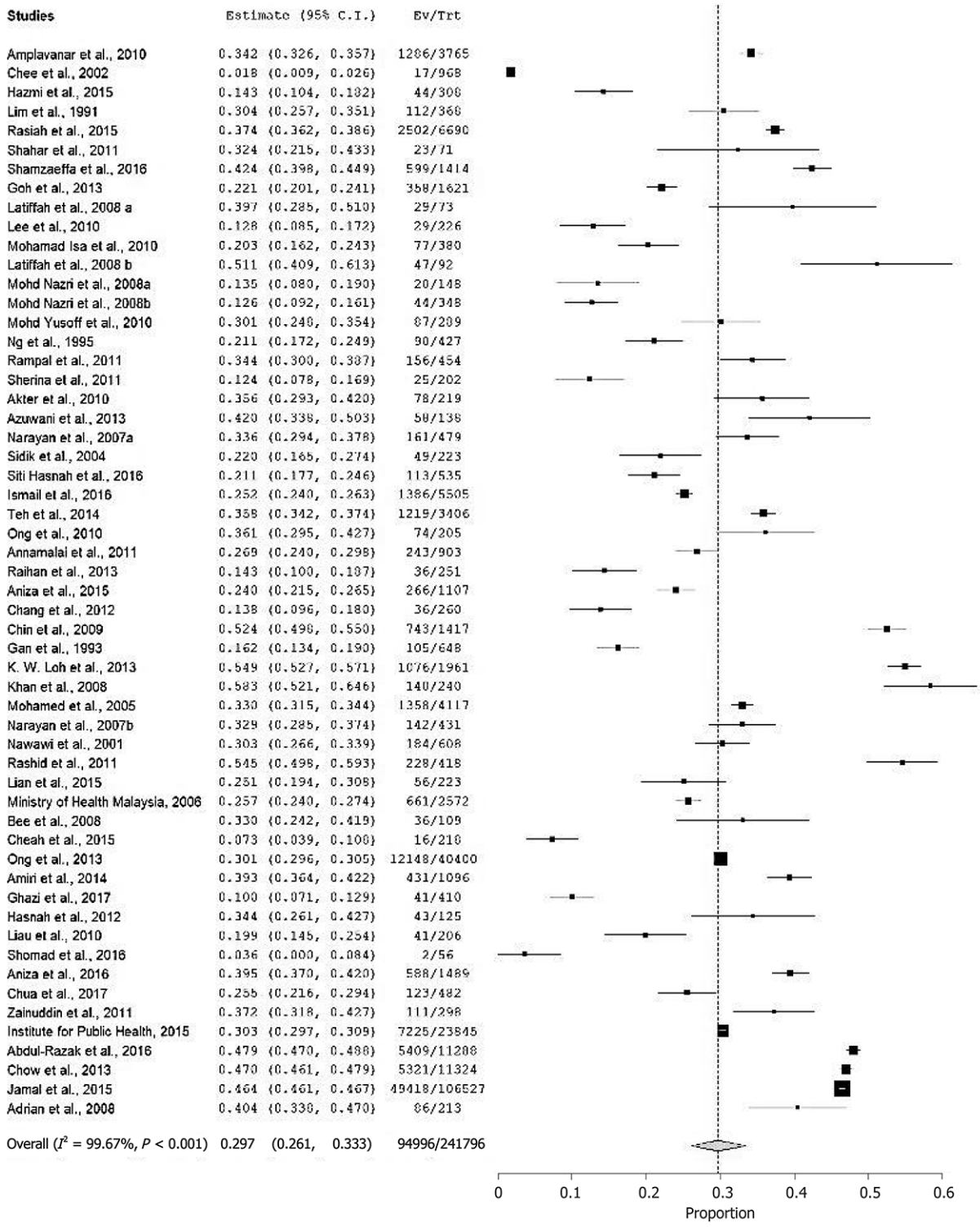


Figure 2 Pooled prevalence of hypertension.

was 33.2% (95%CI: 26.4, 40.0) as compared to 30.8% (95%CI: 25.5, 36.0) in those studies that used a digital BP device (Figure 10). Sensitivity analysis showed that all studies affected the pooled prevalence of hypertension causing it to vary from 25.6% to 30.2%. Therefore, we did not eliminate any studies from the analysis.

Prevalence of awareness towards hypertension

The overall prevalence of awareness towards hypertension in Malaysia was 51.4% (95%CI: 46.6, 56.3) (Table 3 and Figure 11). The prevalence of awareness towards hypertension among male hypertensive patients was 67.8% (Figure 12A), whereas it was 62.7% (Figure 12B) among female hypertensive patients[52,56]. Hypertension awareness among the Malays was 45.4% (Figure 13A), while that among non-Malay

Table 3 Pooled awareness, pooled control and subgroup analyses

Variable	Number of studies, *n*	Prevalence, %	95%CI	*P* value	I^2, %	Figure
Overall awareness	10	51.4	46.6, 56.3	< 0.001	93.4	11
Gender						
Male	3	67.8	42.4, 93.1	< 0.001	97.7	12A
Female	3	62.7	52.8, 72.6	0.046	67.6	12B
Ethnicity						
Malay	3	45.4	29.8, 61.0	< 0.001	96.7	13A
Non-Malay	3	47.9	38.9, 56.8	< 0.001	90.4	13B
Geographical origin						
Rural	4	45.3	34.9, 55.8	< 0.001	93.3	14
Urban	1	54.1	52.2, 56.0	NA	NA	
Overall control	8	33.3	28.4, 38.2	< 0.001	85.2	15
Gender						
Male	3	37.1	26.0, 48.2	< 0.001	59.1	16A
Female	3	30.4	16.0, 44.8	< 0.001	80.7	16B
Ethnicity						
Malay	2	29.3	27.2, 31.5	0.68	0	17A
Non-Malay	3	35.6	29.9, 41.3	0.210	35.9	17B
Geographical origin						
Rural	3	34.1	15.5, 52.7	< 0.001	94.7	18
Urban	1	36.5	33.6, 39.3	NA	NA	

CI: Confidence interval; NA: Not available.

was 47.9% (Figure 13B). The prevalence of awareness towards hypertension among hypertensive patients living in rural areas was 45.3% (Figure 14) as compared to 54.1% in urban areas (Table 3).

Prevalence of control rate in hypertension

The control rate of hypertension was indicated in Table 3. Among the patients who were aware they were hypertensive, 33.3% (95%CI: 28.4, 38.2) achieved control of their BP (Figure 15). Our analysis found that men had slightly better control than women (37.1% *vs* 30.4%) (Figure 16A and 16B). We also found that 29.3% of Malays had control of their BP (Figure 17A), while that of non-Malays was 35.6% (Figure 17B). Urbanites had higher hypertension control than those living in rural areas (36.5% *vs* 34.1%) (Figure 18) (Table 3).

Sensitivity analyses

Visual inspection of the funnel plot of the result of overall prevalence of hypertension showed an asymmetrical plot, suggesting some degree of publication bias (Figure 19). The main analysis for the prevalence of hypertension was rerun by removing one subpopulation at a time. The pooled estimates did not vary much from the original analysis during each removal. The removal of five low-quality studies or smaller subpopulations (size < 100) also did not affect the original estimate of hypertension rates (Table 4).

DISCUSSION

To the best of our knowledge, this systematic review is the first in Malaysia to describe the prevalence and its trends over four decades for hypertension awareness and

Table 4 Sensitivity analysis

Variable	Number of studies, *n*	Total sample size, *n*	Total hypertensive, *n*	Prevalence, %	95%CI	*P* value	I^2, %
Overall prevalence	56	241796	94996	29.7	26.1, 33.3	< 0.001	99.7
Upon removal of studies with poor quality studies	51	237181	93473	28.5	26.0, 31.2	< 0.001	99.4
Upon removal of studies with nonrandom sampling	19	131904	59786	29.7	26.2, 33.4	< 0.001	98.6
Upon removal of studies with sample size < 100	52	131904	59786	28.0	25.6, 30.6	< 0.001	99.3
Upon removal of studies with large sample size > 500	47	28335	8943	28.4	23.3, 33.4	< 0.001	99.2
Upon removal of poor-quality studies, nonrandom studies and studies with sample size < 100	12	46618	16031	26.3	21.6, 31.6	< 0.001	99.2

CI: Confidence interval.

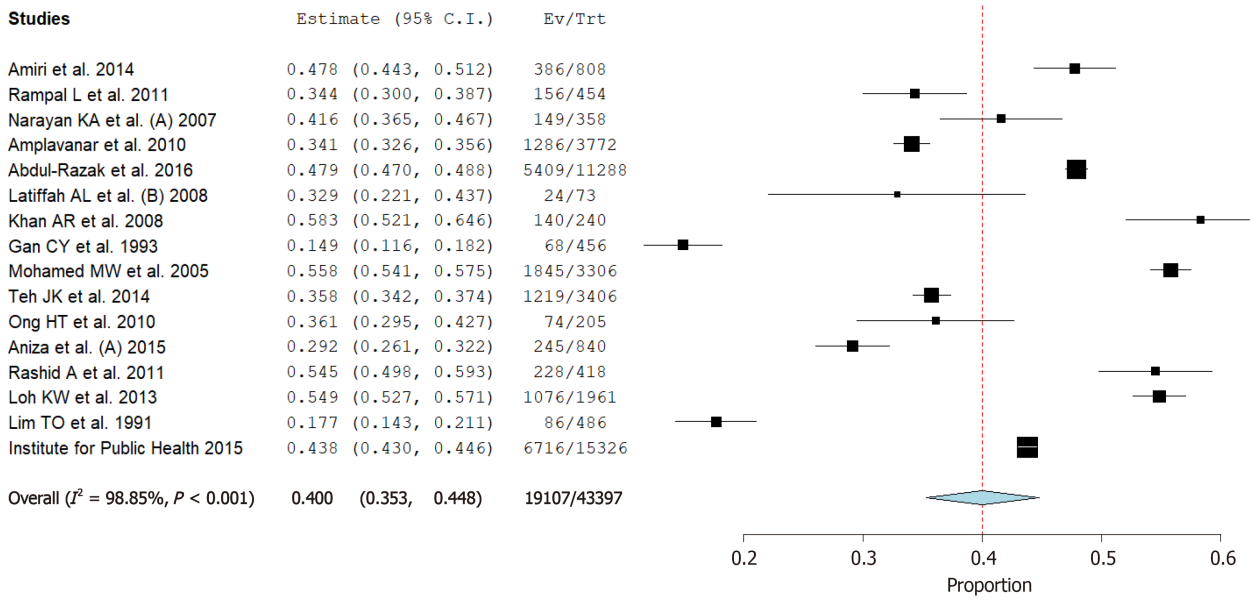


Figure 3 Pooled prevalence of hypertension in adults aged ≥ 30 years.

control. In addition, due to the fact that Malaysia is a multiethnic country, its variation in the prevalence, awareness and control of hypertension is crucial for us to examine in order to plan our policy in managing hypertension on a nationwide scale.

Prevalence

The overall pooled prevalence of hypertension in Malaysia was 29.7%. The overall prevalence of hypertension in Malaysia was within the range of worldwide hypertension prevalence (20%–50%), as described in a systematic review by Kearney *et al*[72]. Malaysia has a higher prevalence of hypertension as compared to Thailand (24.7%), Singapore (23.5%) and China (25.2%)[73-75]. A review showed that this prevalence is as high as that in developed countries despite Malaysia being a developing country[76]. In fact, the prevalence of hypertension in Malaysia is higher than that of the United States by 0.7%[77].

Trend of hypertension

We noticed a low prevalence of hypertension in the 1980s. This could be due to only having one study that reported the prevalence of hypertension in the 1980s. Furthermore, that study involved the Kadazan and Bajau ethnic groups, which are

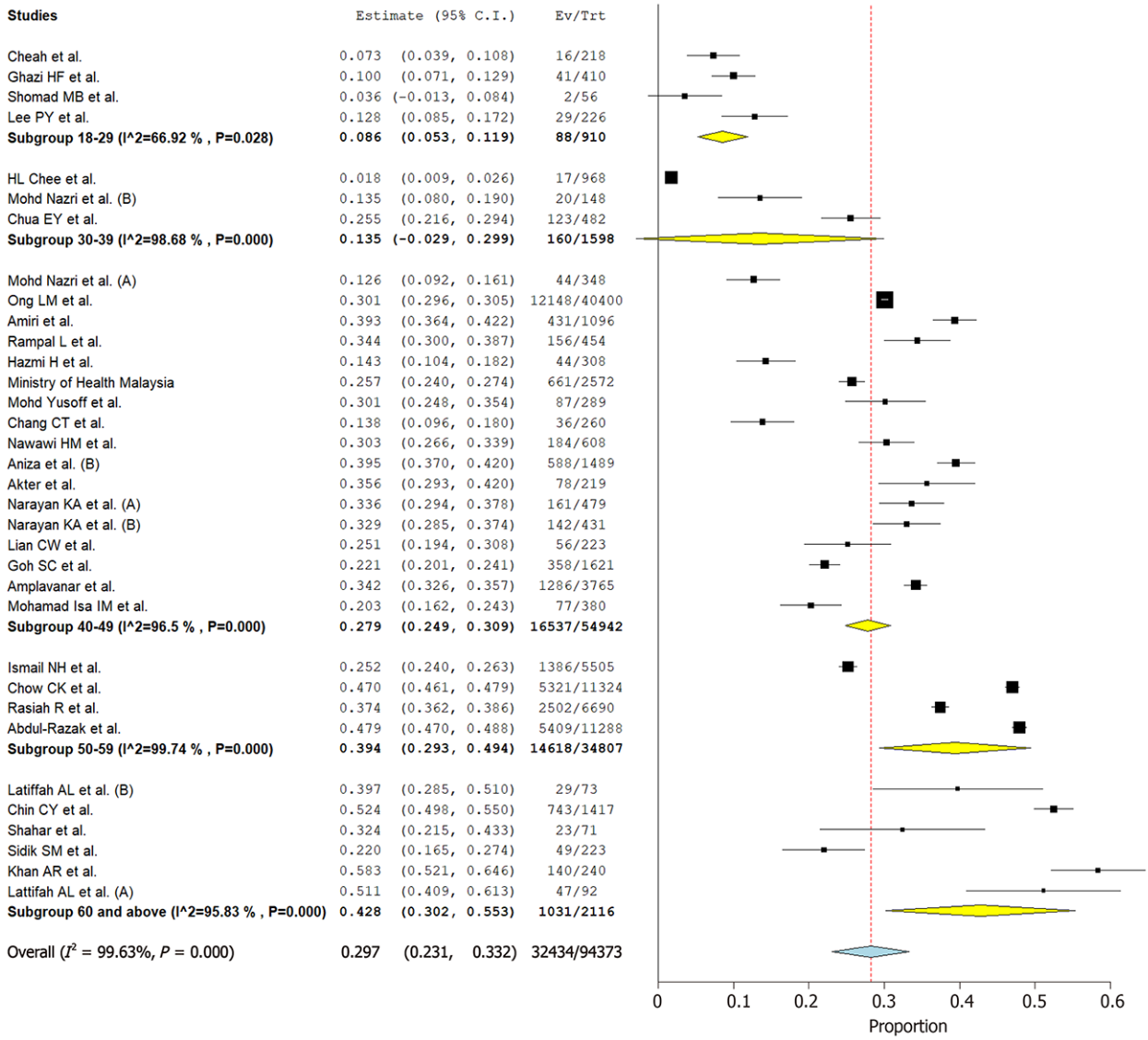


Figure 4 Pooled prevalence of hypertension subanalysis by age groups.

minority groups in a rural part of Sabah[32]. Hence, it is not surprising that the prevalence was so low. The possible explanations include the fact that the study was not only limited to a rural population, but it was also the era before urbanization whereby unhealthy lifestyles were not practiced commonly reflected strongly by a low prevalence of diabetes of less than 5% in the years 1980-1985 in South East Asia[78]. Otherwise, we noticed a spike in hypertension prevalence from the 1980s to the 1990s (36.8%). Then, it decreased to 28.7% in the 2000s and further decreased to 26.8% in the 2000s. A possible reason for the increased prevalence in the 1990s could be due to the fact that among the 30 studies that specified their study period, only two studies were conducted in the 1990s. One study, which reported hypertension prevalence of 21.1%, involved three rural communities in Bagan Datoh involving a wide variation of citizens from different age groups[56], whereas the other study involved three semi-rural areas in Kuala Langat where the study respondents were from the older age groups (range, 55–95 years; mean age, 65.4 years)[29]. This significantly increases the overall pooled prevalence of hypertension if we only take these two studies with their extreme ends of prevalence into account. In comparison to the trend of prevalence of hypertension in other countries, United States was one of the countries with a consistent prevalence of hypertension of around 29% according to the United States' National Health and Nutrition Examination Survey[77].

Age and hypertension

Epidemiological studies have shown that the prevalence of hypertension increases with age, which is consistent with our review. Importantly, we also found that

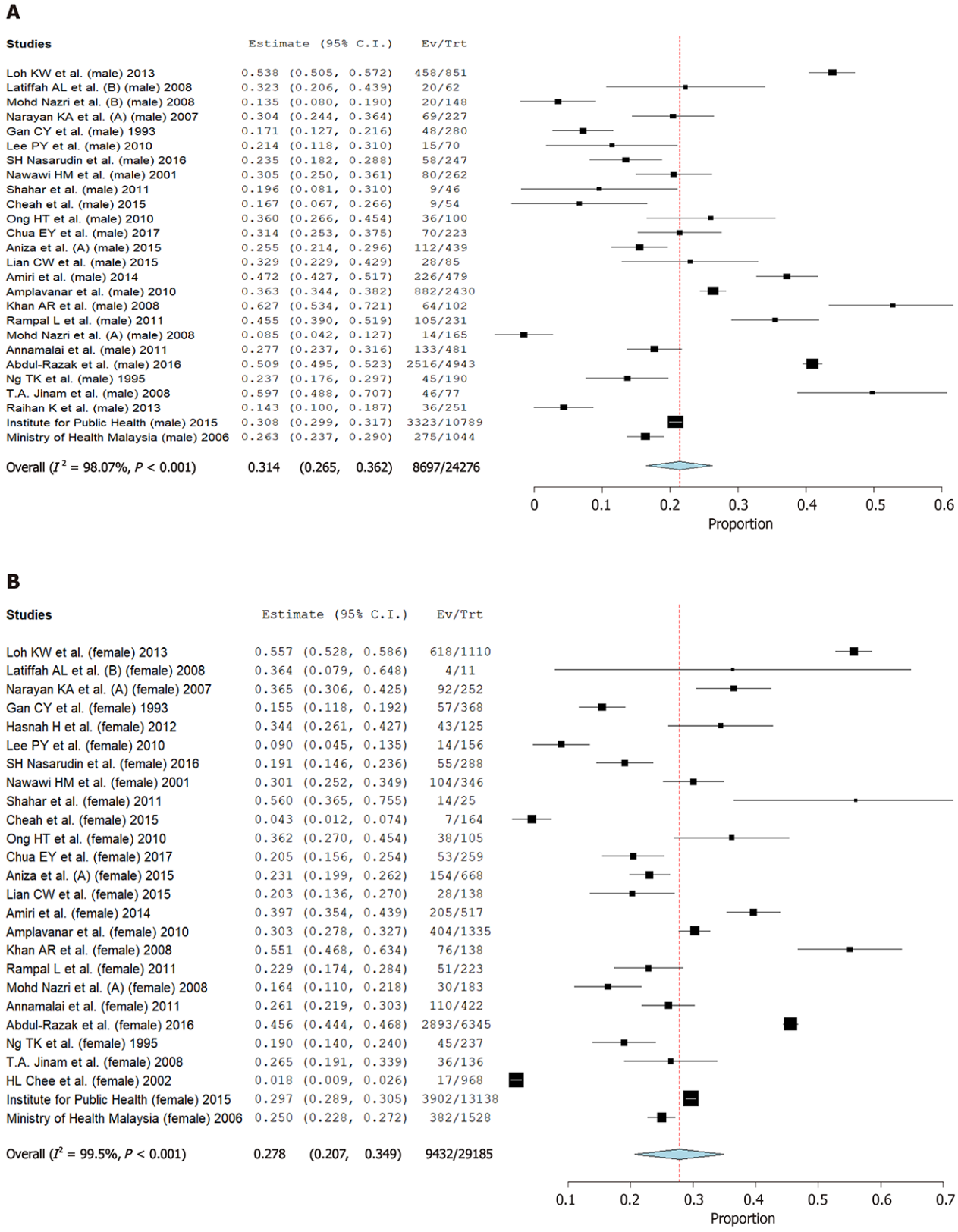


Figure 5 Pooled prevalence of hypertension in male and female adults. A: In male adults; B: In female adults.

hypertension prevalence was doubled in those aged 40–49 years (27.9%) from those aged 30–39 years (13.5%). Comparing our results to that of a developed country, we also found a similar doubling phenomenon in hypertension prevalence, but it only happened in the older age group, which was 63.1% in those aged ≥ 60 years, rising from 33.2% from those aged 40–59 years[77]. It is expected that aging is closely related to increased rates of hypertension because of the arterial structure alteration and ongoing calcification that leads to increased arterial stiffness[79]. However, when focusing on the

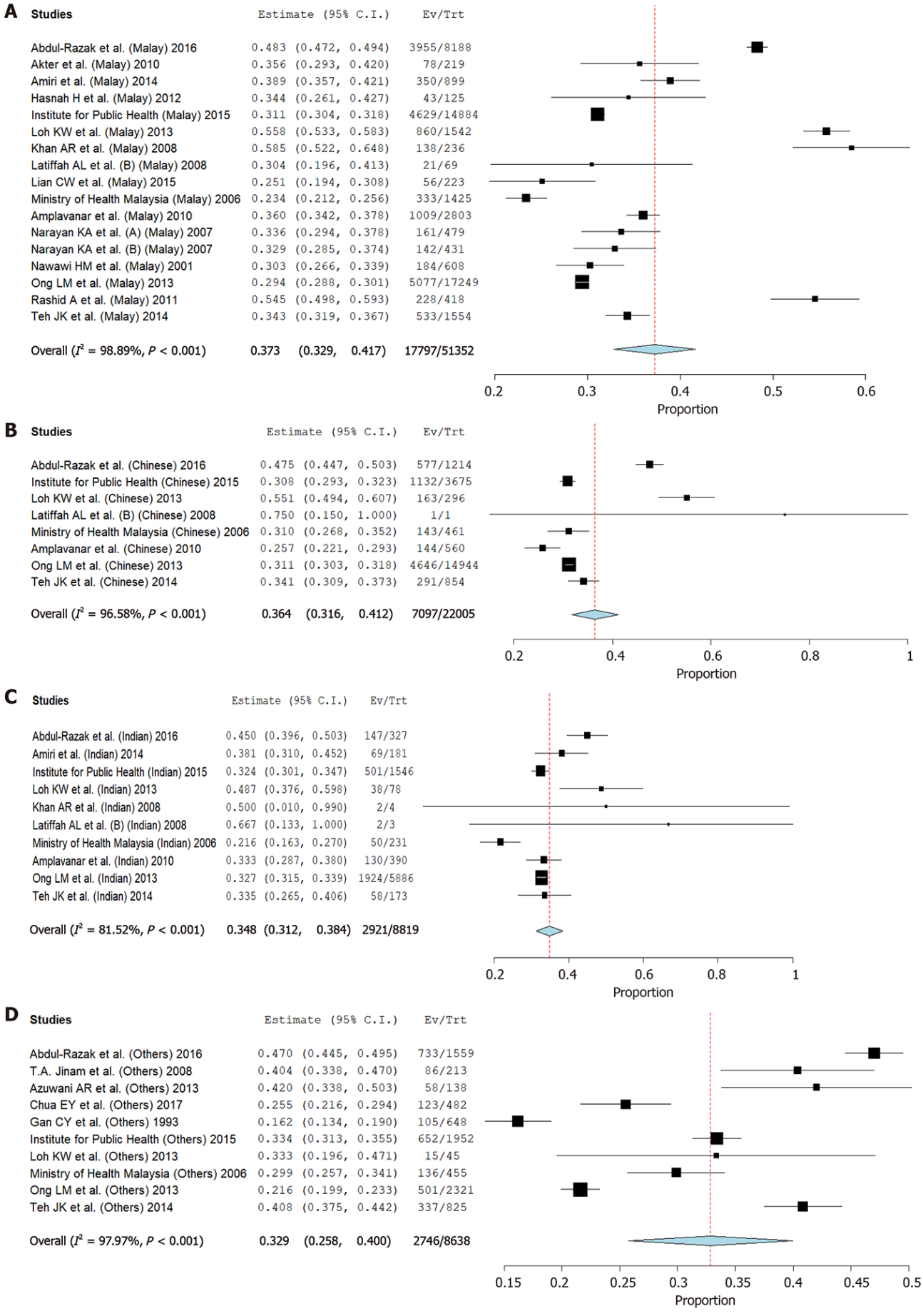


Figure 6 Pooled prevalence of hypertension in Malay, Chinese, Indian and other ethnicity. A: In Malay; B: In Chinese; C: In Indian; D: In other

ethnicity.

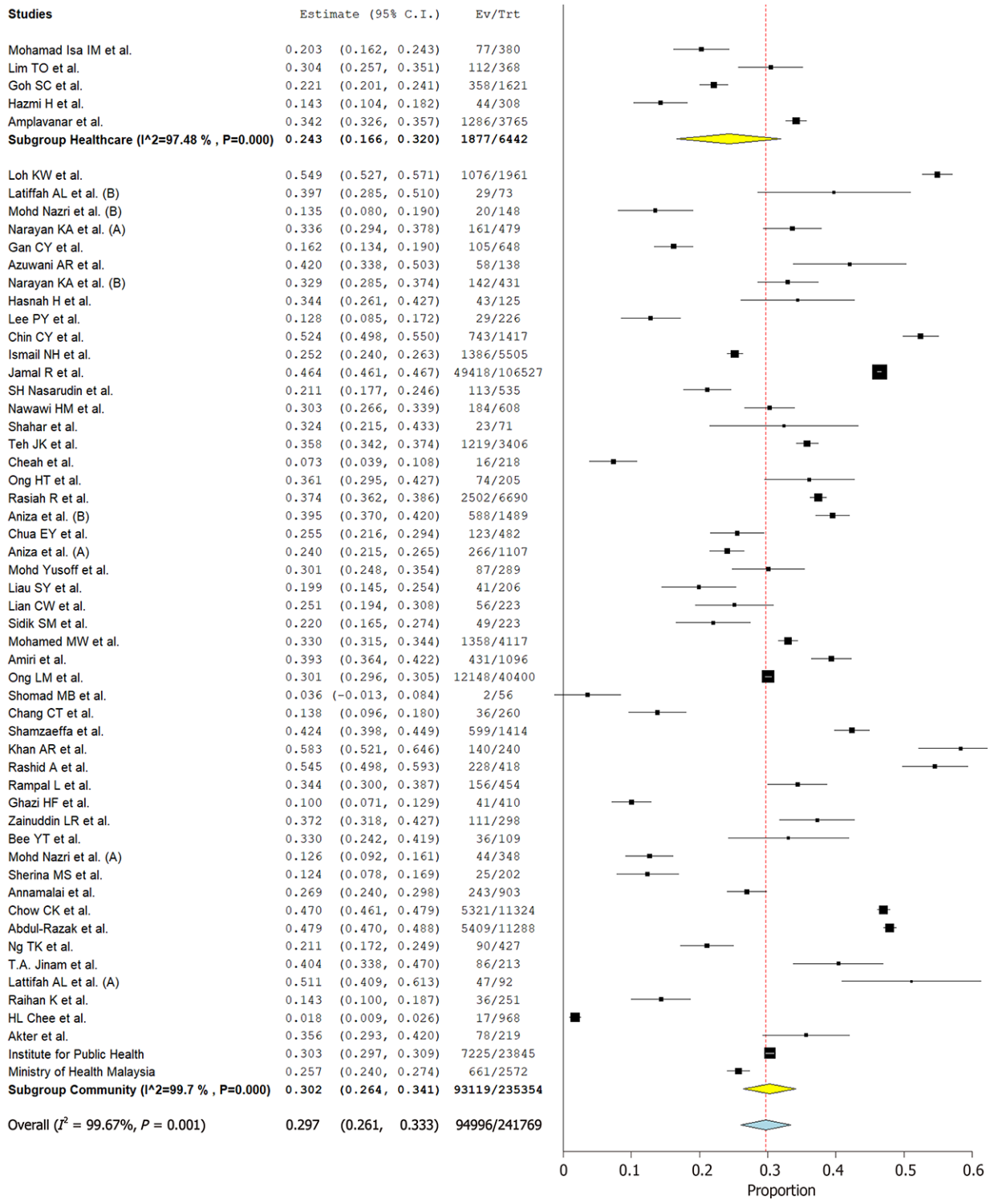


Figure 7 Prevalence of hypertension subanalysis by study setting.

older population aged ≥ 60 years, the prevalence of hypertension in this age group in Malaysia is the lowest among Asian countries such as Singapore (73.9%), Korea (68.7%), India and Bangladesh (65%), Taiwan (60.4%), Thailand (51.5%) and China (48.8%)[80-85]. However, this could be due to the fact that studies in Malaysia have defined the elderly as people aged ≥ 60 years old compared to the other studies above, which defined the elderly as people aged ≥ 65 years[80,81,83,85]. In Malaysia, the prevalence of hypertension was 8.6% among those aged 18–29 years and 13.5% among

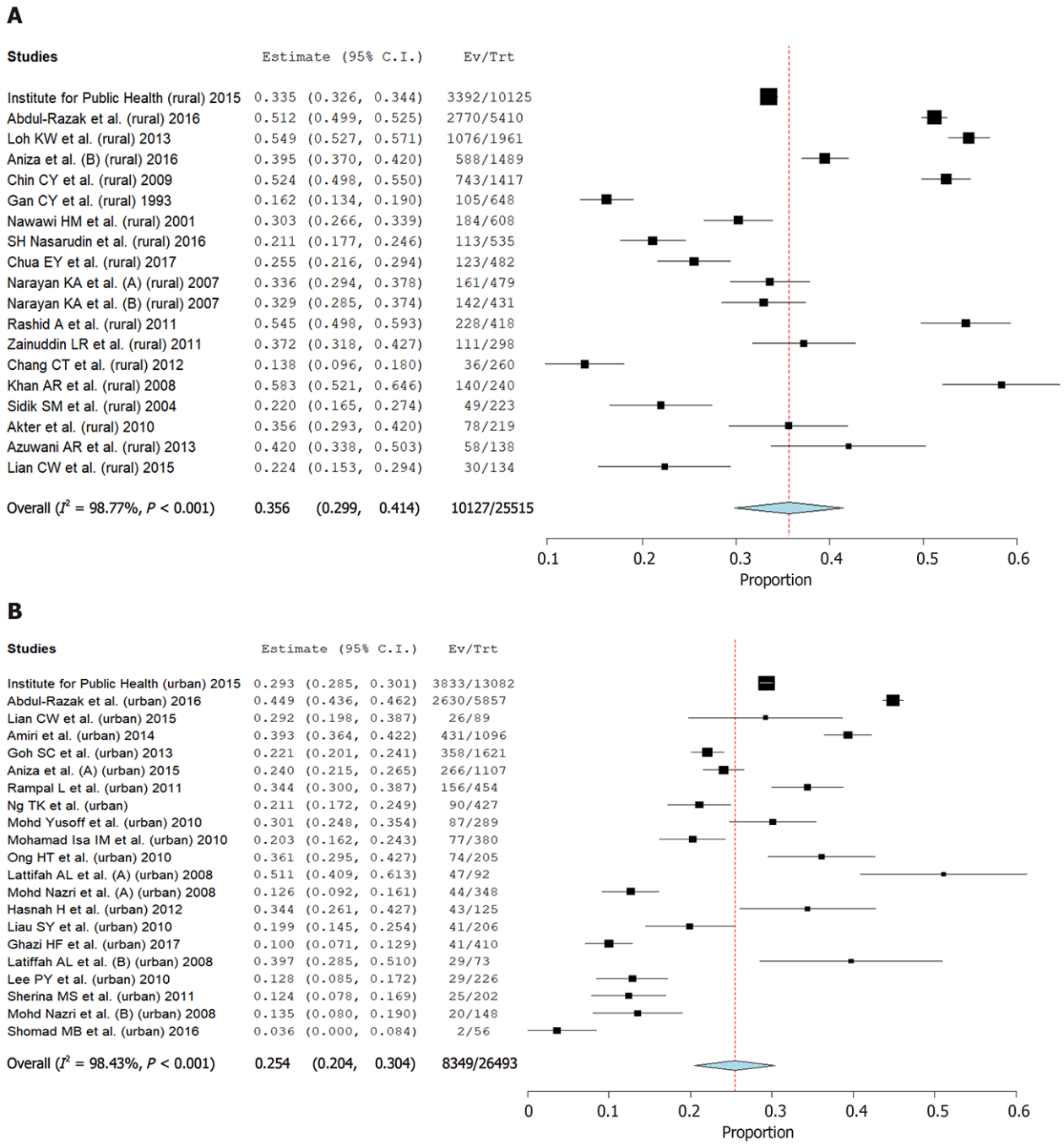


Figure 8 Pooled prevalence of hypertension in rural and urban area. A: In rural areas; B: In urban areas.

those aged 30–39 years. The prevalence rates are fairly similar to China (18–29 year age group, 9.6%; 30–39 year age group, 13.1%)[86] but lower compared to India (18–29 year age group, 13%; 30–39 year age group, 23%)[87].

Gender and hypertension

We found that the prevalence of hypertension was higher in men compared to women. This finding is similar to that of the National Health and Nutrition Examination Survey in the United States[88], which reported that regardless of race and ethnicity, men in the 20–40 year age group had higher prevalence of hypertension than women[88]. The sex differences in hypertension are due to both biological and behavioral factors[89]. Biologically, the female sex hormone, estrogen, serves as a protective factor against hypertension and other cardiovascular-related diseases in women[90,91]. Unhealthy lifestyle such as smoking was more prevalent among men compared to women[90-93]. Because smoking is a risk factor for hypertension[94,95], it is not surprising that the prevalence of hypertension is higher in men.

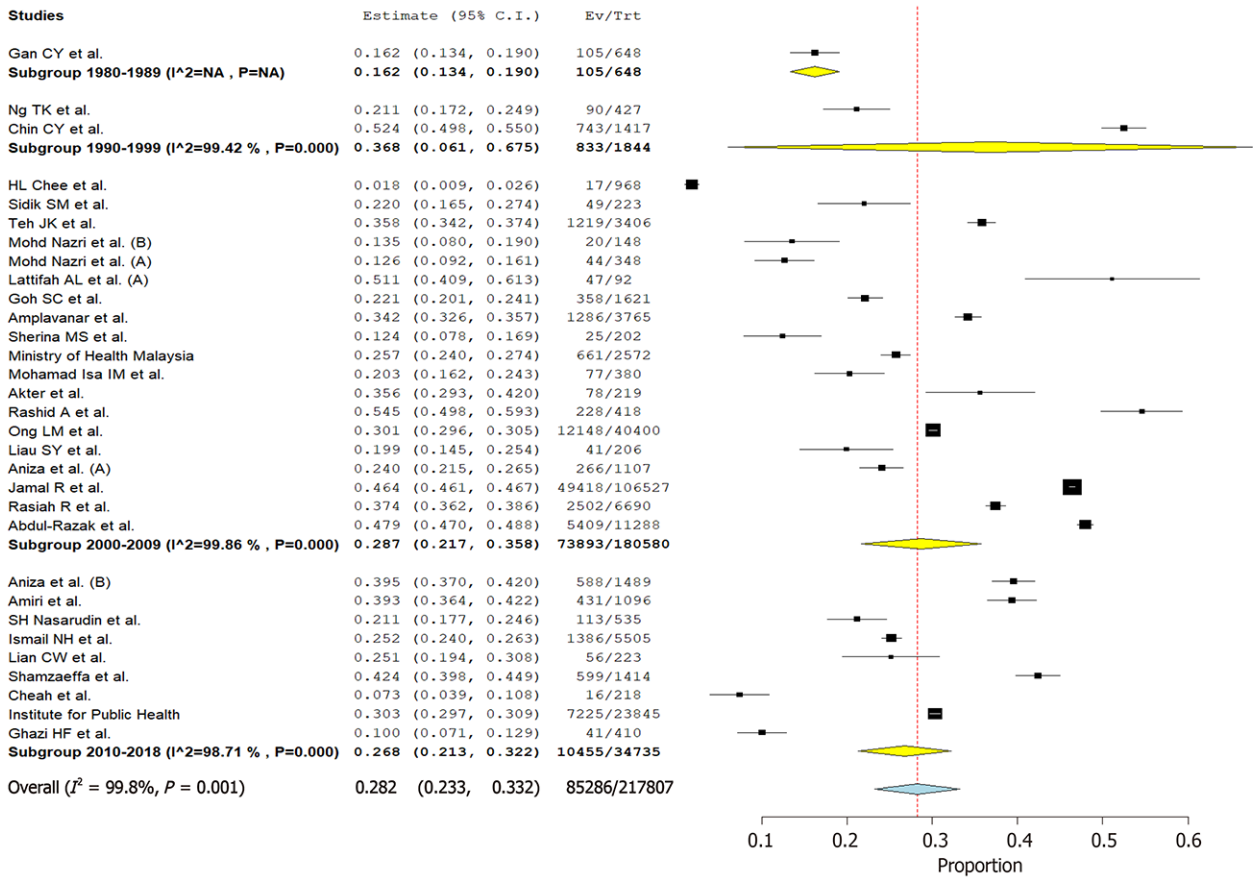


Figure 9 Overall pooled prevalence of hypertension subgroup by study decade.

Awareness

In our review, 51.4% (95%CI: 46.6, 56.3) of the included sample was aware of their hypertension status. This finding is lower than the rates reported in United States (63%)[96], Singapore (69.7%)[80] and Korea (91.7%)[81]. Even though, the awareness of hypertension in Malaysia is higher than that in India (25.1%)[83] and Indonesia (35.8%)[73], this finding is still worrying as it indicates one out of two adults remain undetected or untreated for their hypertension. Therefore, various nationwide blood pressure screening campaign is urgently needed. Indeed May Measurement Month was a good move as it was a nationwide blood pressure screening program that was conducted in conjunction with World Hypertension Day under the tutelage of the International Society of Hypertension[94].

Regarding the higher prevalence of awareness towards hypertension in Malaysia as compared to India[83] and Indonesia[73], the possible explanation could be due to the fact that one of the studies was conducted in a residential home with a higher caregiver to resident ratio and frequent supervision. This explained why the residents' awareness of hypertension was high[58]. On the other hand, another study involved university staff with high education levels, and therefore the awareness of hypertension will certainly be high[61]. In terms of ethnicity, only one study examined the ethnic differences of hypertension awareness[16], while the two other studies involved only Malay ethnicity as the study population[62] and Malay villagers in rural communities, respectively[70]. Comparison of geographical origin yielded similar results, where only one study examined the difference in awareness[16] while the three other studies all focused on awareness among the rural communities rather than examining the geographical difference of hypertensive awareness[46,52,62]. It will be right to assume that there will be much bias and higher heterogeneity, and therefore pooled analyses were not done for these subgroups.

Control

Hypertension control in Malaysia was 33.3%, which is much lower than that of developed countries such as the United States (53%)[17]. Conversely, it is higher than that of nearby countries such as China (13.8%)[73,84], Hong Kong (25.8%)[95] and the

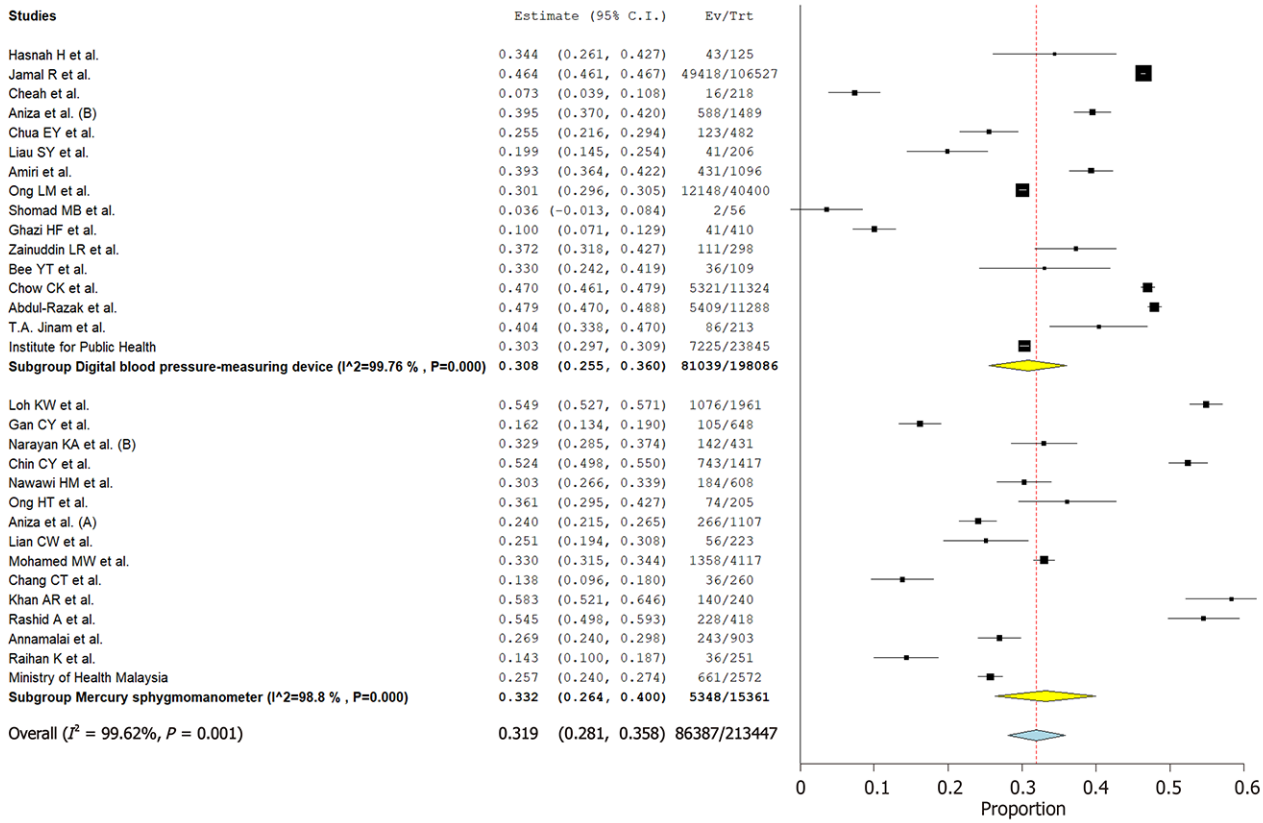


Figure 10 Pooled prevalence of hypertension subanalysis by measurement tools.

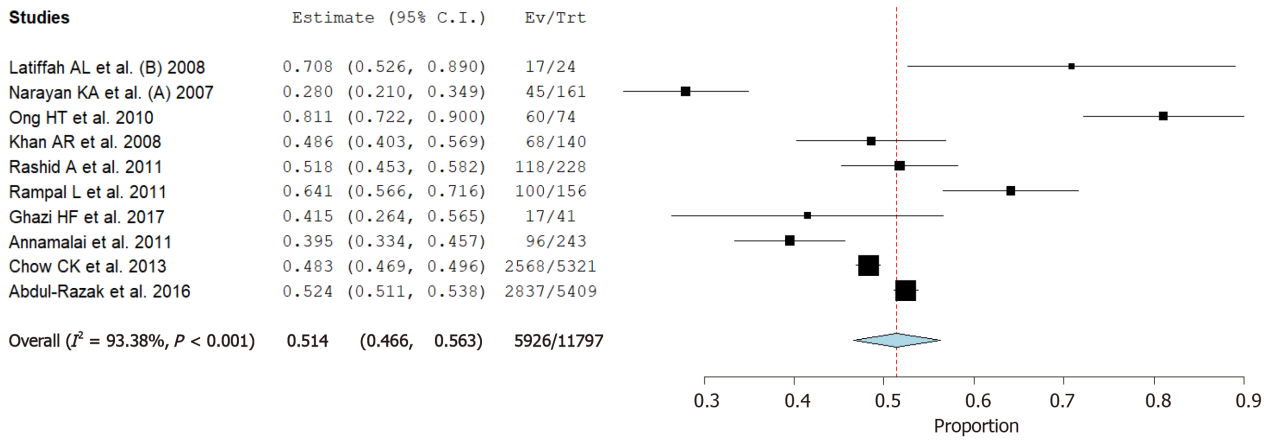


Figure 11 Pooled prevalence of awareness in hypertension.

Philippines (27.0%)[97]. This could be due to the fact that Malaysia has been improving its quality of healthcare facilities, building more clinics and hospitals and more of the latest drugs are available in these healthcare facilities[98]. We found that men achieved better BP control than women. This is surprising because the literature reported that women are always more likely to have better health-seeking behavior and expected to have better blood pressure control[99]. Urban dwellers had better BP control, which correlates with a study in Southern China that reported similar results[100]. This may be due to limited access to healthcare facilities in rural areas despite the number of rural clinics increasing throughout the past four decades in Malaysia[98]. It seems likely that a poorer health awareness among those living in rural areas or with lower socioeconomic profiles remains an important barrier to visiting healthcare facilities and thereby receiving proper treatment.

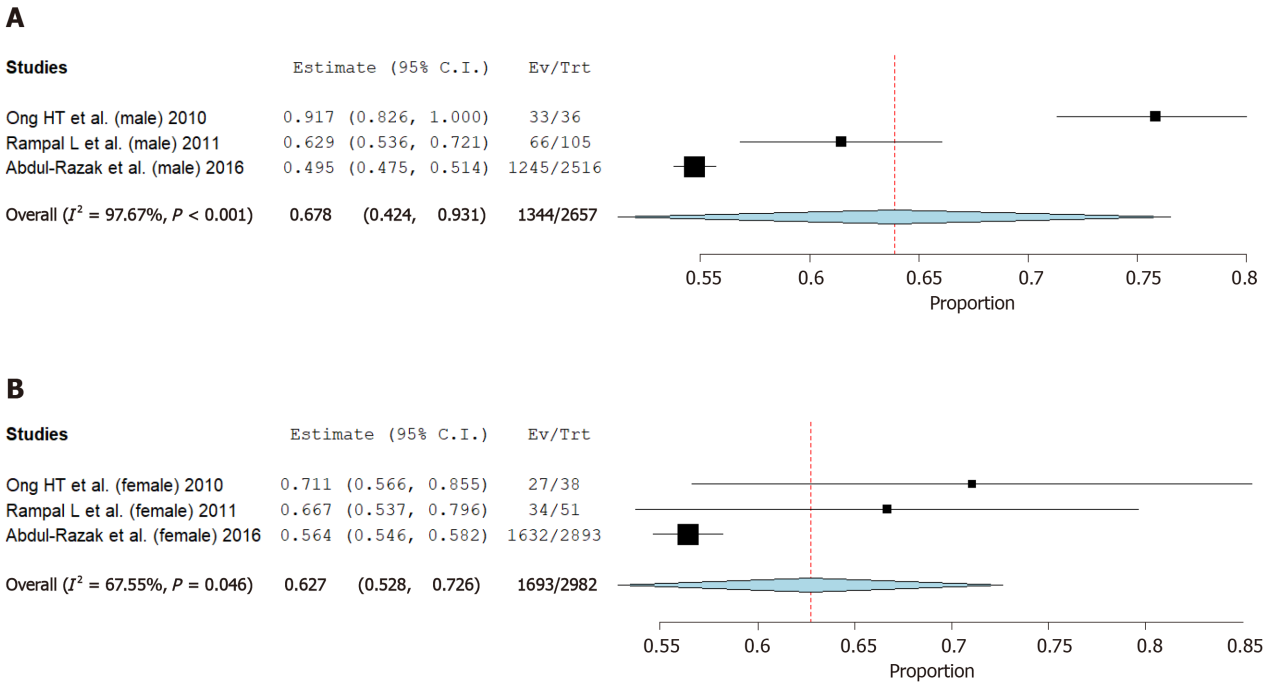


Figure 12 Pooled prevalence of awareness in hypertension in male and female. A: In male; B: In female.

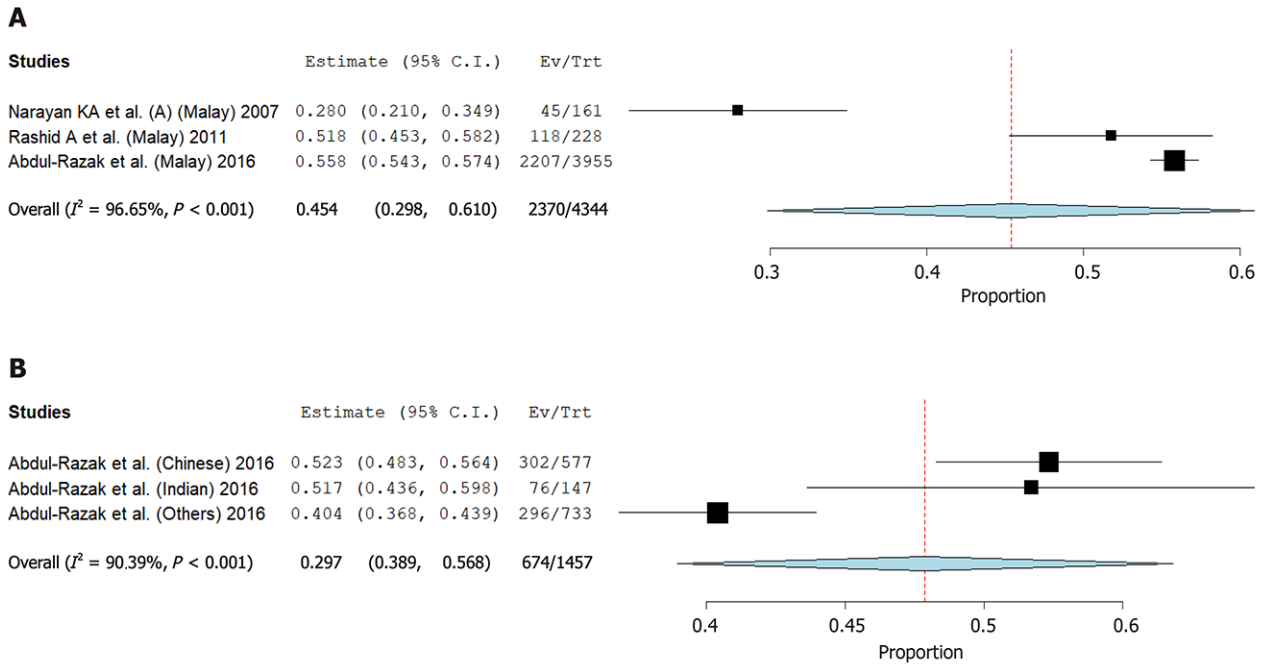


Figure 13 Pooled prevalence of awareness in hypertension in Malay and non-Malay. A: In Malay; B: In non-Malay.

Strengths and limitations

The strength of this review was the large sample size summarizing prevalence of hypertension in Malaysia across four decades. Furthermore, this is interesting to analyze the prevalence of hypertension according to different subgroups especially when Malaysia is known to have different races with corresponding different cultures and lifestyle. The accompanying underlying problems were different from each other, and it has been addressed in this systematic review.

However, there are several limitations. First, we found that many studies did not report data of prevalence, awareness and control of hypertension in subgroups of gender, ethnicity and geographical origin, whereby these factors could further help

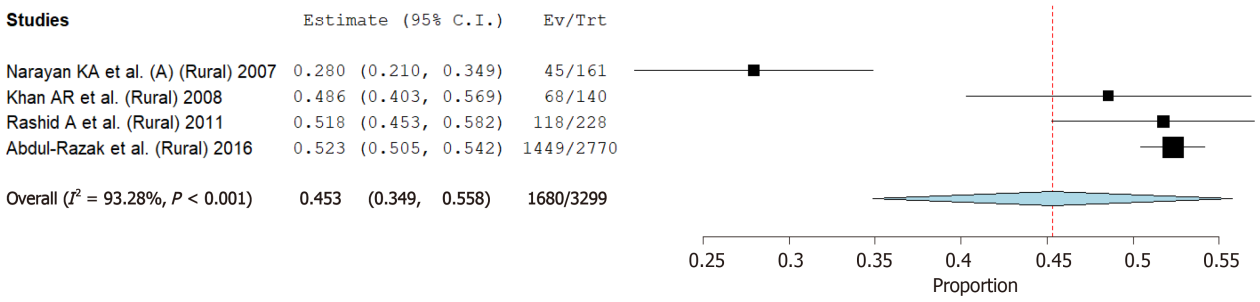


Figure 14 Pooled prevalence of awareness in hypertension in rural areas.

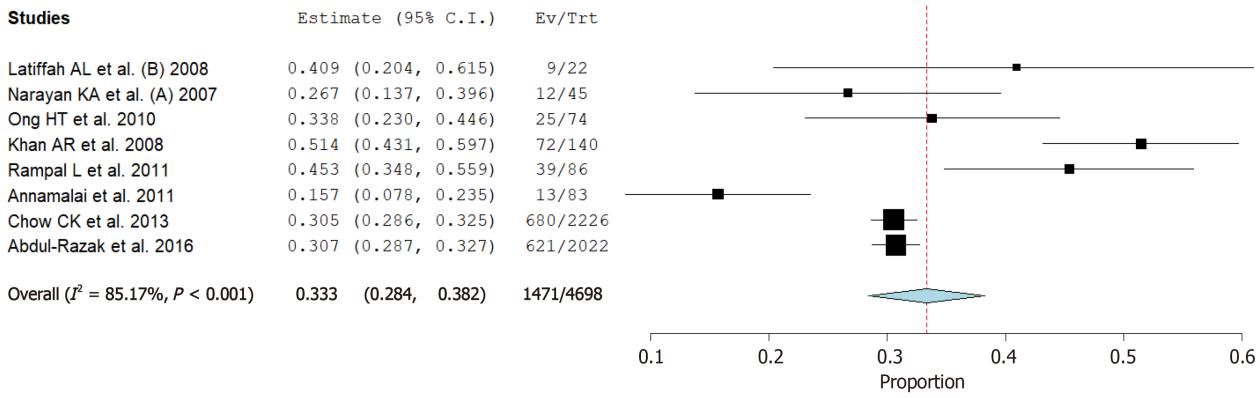


Figure 15 Pooled prevalence of control in hypertension.

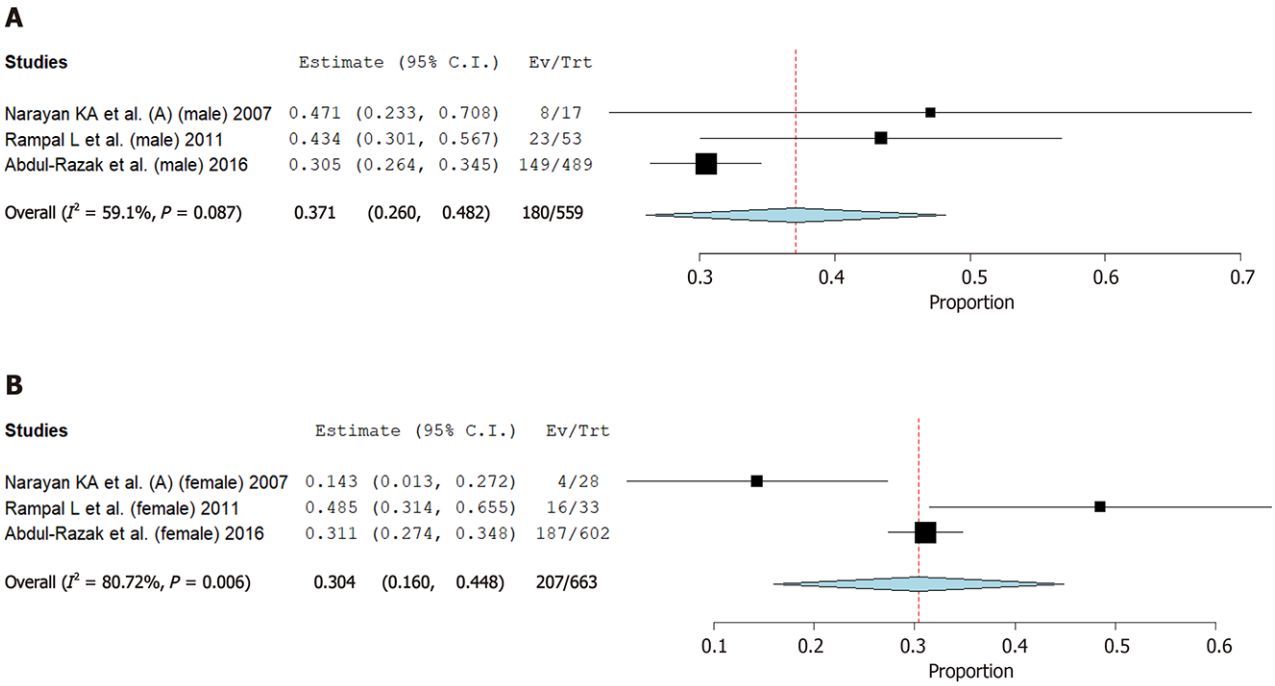


Figure 16 Pooled prevalence of control in hypertension in male and female. A: In male; B: In female.

health care policy makers to configure hypertension screening and awareness campaigns according to these subgroups in regards to hypertension prevalence, poor awareness and lack of control. Secondly, we adopted strict inclusion and exclusion criteria and therefore unpublished data or grey literature were not included in the

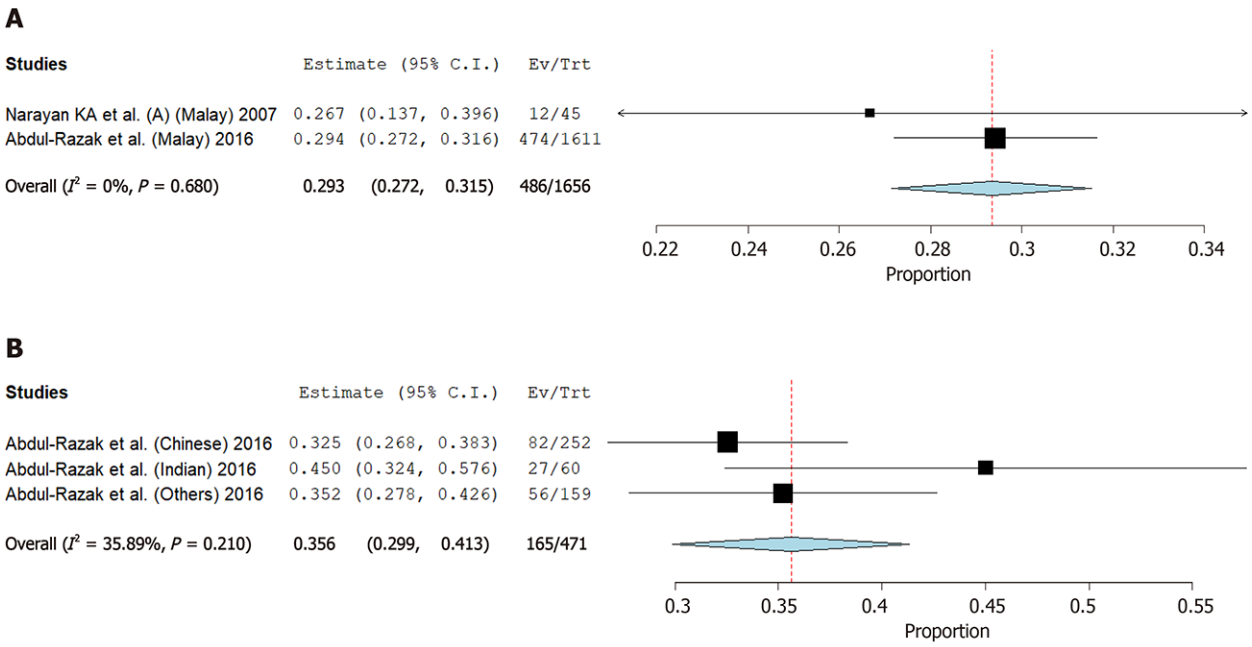


Figure 17 Pooled prevalence of control in hypertension in Malay and non-Malay. A: In Malay; B: In non-Malay.

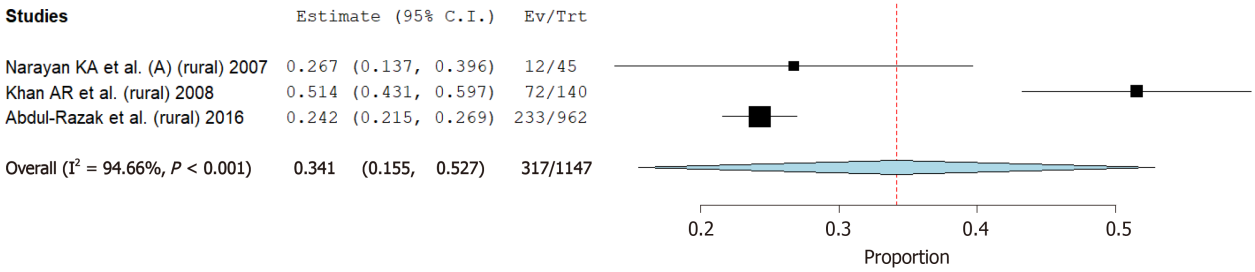


Figure 18 Pooled prevalence of control in hypertension in rural areas.

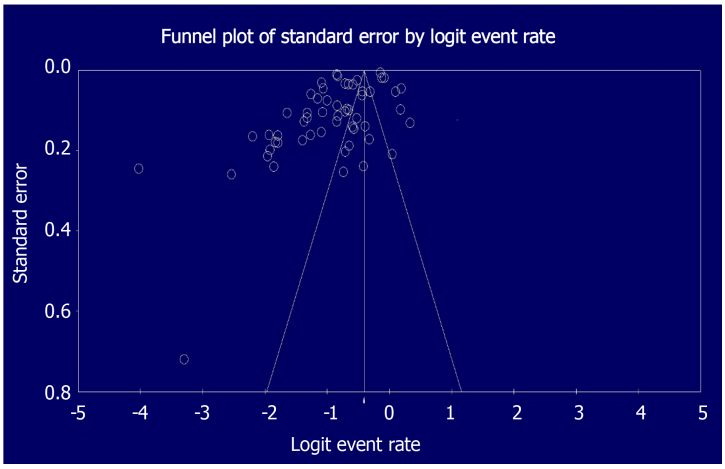


Figure 19 Publication bias assessed using funnel plot.

study. However, based on the sensitivity analysis, prevalence of hypertension after removal of these studies with poor quality, nonrandom sampling and/or extreme sample size were not changed as compared to overall pooled prevalence of hypertension. Thirdly, the estimates for the earlier time periods were based on fewer studies when compared to that for latter periods, which may have caused a paucity of

literature on the topic of interest.

Suggestion for future research

Future studies on the prevalence of hypertension can address some of the issues noted in this research. The prevalence of hypertension according to gender, ethnicity and geographical origin should be studied in more detail. Nonrandom sampling method should be avoided because it would lead to bias in the conducted study. Besides that, future studies should also emphasize on adequate or larger sample size, which is more representative of a population.

CONCLUSION

One-third of Malaysian adults are hypertensive. The prevalence of hypertension is higher in people who reside in rural areas than in those who stay in urban areas. Slightly more than half of the adults are aware of their hypertension status and one-third of these patients achieved target BP control. In view of these findings, urgent steps for improving health promotion and health education need to be undertaken on a larger scale. Although our review shows a decreasing trend of hypertension prevalence throughout the past four decades, hypertension awareness and BP control among Malaysians have yet to improve significantly.

ARTICLE HIGHLIGHTS

Research background

Hypertension is a common public health problem worldwide.

Research motivation

Future studies on the prevalence of hypertension can address some of issues noted in this research. The prevalence of hypertension according to gender, ethnicity and geographical origin should be studied in more detail.

Research objectives

This systematic review aimed to determine the trend in prevalence, awareness and control rate of hypertension in Malaysia.

Research methods

A systematic search was conducted in six databases for articles published between 1980 and 2018. Authors reviewed the studies and performed quality assessment and data extraction independently.

Research results

The overall pooled prevalence of hypertension in Malaysia was 29.7%. The overall prevalence of awareness was 51.4%, and 33.3% of those on treatment had achieved control of their blood pressure.

Research conclusions

In Malaysia, three in ten adults aged ≥ 18 years have hypertension, while four in ten adults aged ≥ 30 years have hypertension. Five out of ten people are aware of their hypertension status and only one-third of those under treatment achieved control of their hypertension.

Research perspectives

Concerted efforts by policymakers and healthcare professionals to improve awareness and control of hypertension should be of high priority.

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LETTER TO THE EDITOR

Integrating contextual variables in meta-analyses

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Abstract

Meta-analysis is an important statistical tool, and it is often used to solve clinical problems. However inevitably when conducting a meta-analysis, the included studies often have heterogeneity. This paper suggests the inclusion of relevant background data or contextual variables into the model. The contextual variables are those variables not explicitly measured in the studies included in a meta-analysis; thus, these must be very well-described and justified as parameters for analyses.

Key Words: Covariates; Moderator; Meta-analysis; Contextual; Subgroup analysis; Meta regression

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Core Tip: This letter call for the use of contextual variables, that are typically not in use for covariate analyses. Contextual variables are introduced and defined as variables not immediately/directly measured by the original studies in the meta-analysis but rather can be estimated knowing the background of each study. For example in a meta analysis of clinical trails one might want to adjust for studies from high income vs low income countries or studies that were funded vs independent.

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TO THE EDITOR

Meta-analysis is a statistical procedure for combining data from several studies about a specific problem[1]. When performing meta-analyses, it is important to determine the

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level of homogeneity between studies[2]. When effect size is consistent between the studies, meta-analysis approach can be used to identify this common effect by combining all of the studies[1,2]. However, problems are not always simple and often researchers face the issue of heterogeneity in meta-analysis and therefore employ moderator's analysis techniques. A moderator is an independent variable that conditions the relations between two others variables[1,3]. Because effect sizes are the relationship between two variables, any variable that predicts the effect sizes is a moderator. When performing a moderator analysis, the moderator data type determines the type of analysis used. For categorical moderators (*e.g.*, males *vs* females) usually a subgroup analysis is performed. For continuous moderators (*e.g.*, age) usually a meta-regression is performed, it must be note here that continuous moderators can be either integer or decimal[1,3]. Meta-analysis researchers often extract these moderator variables from the studies being reviewed. Available moderator variables might be limited to basic demographics or irrelevant to the core analysis at hand.

The purpose of this paper is to define and describe "contextual variables" and provide reasons for integrating them in meta-analysis studies. Brief section describes a worked example of contextual variables in a moderator analysis. The strengths and limitations of incorporating a contextual variable in moderator analyses are discussed as well as approaches to routinely define and include these variables in future meta-analyses.

A contextual variable is defined as a variable that was not explicitly measured in the studies included in a meta-analysis but rather is inferred or computed for the included studies. Contextual variables are variables that characterize the study setting, methodology or conduct. By adding a contextual variable meta-analysis researcher are encouraged to incorporate other relevant background data into the model. This extends meta-regression to a higher level. These variables would include things like the population sampled, the response rate, the geographical setting, the design, sponsorship *etc*. An example of contextual variable is to include an analysis of therapeutic trials by source of funding (industry, independent, mixed) which has repeatedly shown the presence of publication bias.

To illustrate this, a recent meta-analysis of ours was undertaken to examine the effect of Ramadan intermitting fasting on metabolic syndrome components[4]. The MetS components analyzed were: Waist circumference (WC), systolic blood pressure (SBP), fasting glucose (FG), triglycerides (TG), and high-density lipoprotein (HDL) cholesterol. We identified 85 studies (4326 participants in total) that were conducted in 23 countries between 1982 and 2019. For illustration purposes, we will use WC. Ramadan fasting induced effect sizes for WC was small (K = 24, N = 1557) Hedge's g = -0.312, 95%CI: -0.387 to -0.236). To better understand the effects of Ramadan fasting on WC we performed moderator analysis for age and sex (proportion of male subjects) in the form of meta-regression. These simple demographic data (age and sex) were available for all of the included 24 studies. Meta-regression results revealed that sex was significant in explaining variation in WC (β = -0.20, P = 0.03), but age was not significant moderator.

As researchers we were also interested in examining the effect of fasting time/day in explaining changes in WC. Unfortunately, the included studies did not include this variable in their results section. However, by having some information about the study *e.g.* location and year of data collection we were able to use compute the contextual variable of fasting time/day (in minutes) which was defined according to Ramadan as time between sunrise and sunset, see https://www.sunrise-and-sunset.com/. Other plausible examples, would be metrological data such as mean temperature or relative humidity which can be obtained easily from historical weather platform available online.

Based on our experience of including contextual variables, this paper proposes the following three conditions to be included in models. First, ensuring that the data available are obtained during the same period of the original included study time. For example, for Ramadan fasting study that was included from Bahrain, 2000 it was crucial that sunrise and sunset were calculated for the same timeframe. Second, Gather data for all of the included studies from the same reliable/trustworthy source. For example, the calculated data on daytime length for a study from Bahrain, 2000 and study from the United Kingdom, 2018 are obtained from https://www.sunrise-and-sunset.com. Third, authors need to give detailed descriptions on how contextual variables were computed, summarized and analyzed.

The inclusion of contextual has high potentials in improving the methodology of meta-analysis and the benefits of including contextual variables can be summarized as follow: (1) Increase generalisability of results, as it would be important to demonstrate

that effect size is consistent among variables of interest; (2) Facilitate the identification of iatrogenic effects in subgroups; (3) Modelling improvement, by including meaningful variables that would give scientifically interpretable results; and (4) Have practical and research implications by generating future hypotheses for practice or further studies.

There are few limitations of including contextual variables which include the following: (1) Instability of models due to collinearity *e.g.*, fasting time and country of origin results might yield same results; (2) Difficulty in obtaining some variables with high level of precision; and (3) Interpreting the results might be challenging.

In conclusion, this paper propose that contextual variables are encouraged in meta-analyses to further understand and interpret the data and enrich study findings and inform future studies. These variables must meet a minimum set of conditions before inclusion.

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ABOUT COVER

Editorial board member of *World Journal of Meta-Analysis*, Dr. Huang is Distinguished Professor at the First Affiliated Hospital, Zhejiang Chinese Medical University in Zhejiang, China. Having received his Bachelor's degree from Zhejiang University in 1999, Dr. Huang undertook postgraduate training first at Zhejiang University, receiving his Master's degree in 2005, and then at Zhejiang Chinese Medical University, receiving his PhD in 2012. He rose to Chief Physician in the Gastroenterology Division of the First Affiliated Hospital, Zhejiang Chinese Medical University in 2016 and has held the position since. His ongoing research interests involve the application of evidence-based medicine in digestive diseases. Currently, he serves as Commissioner of the Spleen and Stomach Disease Branch of the China Association of Chinese Medicine. (L-Editor: Filipodia)

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EVIDENCE REVIEW

Gastrointestinal and hepatic manifestations of COVID-19 infection: Lessons for practitioners

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Abstract

Originating from Wuhan in China, coronavirus disease 2019 (COVID-19) spread globally within months and was declared a pandemic by World Health Organization in March 2020, making it one of the biggest healthcare calamities of our time. As more data on COVID-19 infection became available, what was initially thought to be a simple respiratory illness was found to be more complex. Many extra-pulmonary manifestations are now frequently reported for COVID-19 in available literature, most commonly gastrointestinal and hepatopancreato-biliary manifestations. Due to early scarcity of data, extra pulmonary manifestations were initially overlooked and may have contributed to nosocomial spread of the infection. Practitioners, especially gastroenterologists, who frequently encounter patients with these symptoms, need to be aware of them. This can not only help minimize the nosocomial spread, ensure safety of provider but also help conserve already stretched-thin healthcare resources. A tremendous amount of COVID-19 related literature is getting added to the growing pool every day, making it difficult for providers to follow. The aim of our review is to summarize the available evidence for gastrointestinal and hepatopancreatobiliary manifestations of COVID-19. We here briefly discussed the possible pathophysiologic mechanism for these manifestations and summarized the recommendations put forward by multiple gastrointestinal societies regarding safe and effective clinical practice during the ongoing pandemic.

Key Words: COVID-19; Severe acute respiratory syndrome; Coronavirus; Angiotensin converting enzyme; Gastrointestinal; Liver

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Core Tip: This review is to summarize the available evidence for extrapulmonary manifestations of coronavirus disease 2019 infection, particularly emphasizing on gastrointestinal and hepatopancreatobiliary manifestations. Here we briefly discussed the possible pathophysiologic mechanism for the disease, and summarized the recommendations of multiple gastrointestinal societies regarding safe and effective clinical practice during the ongoing pandemic. Lastly, we've touched briefly upon management of chronic liver disease and transplant patients.

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INTRODUCTION

The pathogen responsible for a mysterious cause of pneumonia, linked to a seafood market in Wuhan, China, first reported in early December 2019, was later identified as a novel coronavirus by the Chinese Center for Disease Control (CDC)[1,2]. Based on phylogenetics, the Coronavirus Study Group of the International Committee on Taxonomy of Viruses named it the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the disease was later named coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO)[3-5]. Within months, the disease spread across the globe like wildfire infecting many and was declared a pandemic by the WHO on March 11, 2020[6].

This is not the first time that a CoV has caused a global health crisis. Just within the last two decades, the SARS- and MERS-CoVs had been responsible for two major viral outbreaks, causing the death of 774 and 838, respectively[7-9]. This number in comparison to the ongoing pandemic appears to be miniscule. Within a period of nine months, the SARS-CoV-2 has managed to infect more than 28 million people and has nearly claimed a million lives[10]. The ongoing pandemic has left the global economies shattered and health care systems exhausted. The current pandemic has reached a level not seen since the Spanish flu of 1918 and will go down the history books as the worst healthcare crisis experienced by mankind in the 21st century.

Early in the pandemic, COVID-19 was thought be an exclusively respiratory illness. However, as the number of cases grew and reports of numerous extra pulmonary and atypical presentations emerged, it became apparent that COVID-19, is much more than a simple case of viral pneumonia.

Typical symptoms of COVID-19 include fever, cough, dyspnea, fatigue which are accompanied by radiographic evidence of pneumonia[11]. Additionally, a wide range of extra-pulmonary symptoms, including gastrointestinal (GI) and hepatic dysfunction are frequently reported. The presence of viral RNA in fecal samples from COVID-19 patients also raise concern for fecal-oral transmission in addition to the already established droplet and airborne routes[11].

GI symptoms of COVID-19 may not only precede the typical respiratory manifestations but can sometimes be the only presenting symptom[12]. Interestingly, the first reported case of COVID-19 in the US developed GI symptoms such as vomiting and diarrhea prior to the onset of respiratory symptoms[11]. The predominant focus on the respiratory aspect of the disease can allow these atypical presenters to slip through the cracks. This can potentially result in significant exposure and increased risk of transmission in unsuspecting healthcare workers (HCWs). Wang *et al*[13]'s study had particularly highlighted this concern. They reported isolated GI symptoms in around 10% of cases and mentioned of one patient who was overlooked and admitted under a surgical specialty. This single patient was inadvertently responsible for infecting more than 10 HCWs[13].

Thus, it is important for clinicians, especially gastroenterologists to be aware of these atypical and often missed presentations of COVID-19. This will not only serve to mitigate the spread but also help reduce clinician's anxiety during this pandemic.

VIROLOGY OF SARS-COV-2

Before moving on to these extra-pulmonary manifestations of COVID-19, a brief overview of SARS-CoV-2 would better serve the ensuing discussion. Ranging from 60 to 140 nm in diameter, coronaviruses are one of the largest, single-stranded RNA viruses known. Around 39 different types of coronaviruses have been identified since they were first discovered[11,14-16].

Most of them are zoonotic and their natural hosts include mammals and birds[17-20]. They are responsible for a multitude of diseases involving respiratory, enteric and other organ systems in their hosts[21,22]. Majority of coronaviruses that are capable of infecting humans have been thought to originate from an inter-specie crossover event between animals and humans[2].

SARS-CoV-2 is the seventh and the newest member of human coronaviruses, which also include OC43-, 229E-, NL63-, HKU1-, SARS- and MERS -CoVs[23,24]. The former four are endemic and have been responsible for common seasonal upper respiratory tract illnesses, while the latter two and the SARS-CoV-2, cause severe pneumonia outbreaks which can lead to multi organ failure and death[25].

Like most viruses, there are five key steps in the life cycle of coronaviruses. These include attachment, penetration, biosynthesis, maturation and release (Figure 1)[26]. Attachment and penetration are crucial steps which rely on receptor recognition and subsequent downstream processes allowing virus to gain entry into host cell (Figure 2)[27]. The structural transmembrane glycoprotein called Spike (S) is important for this process. Functionally it consists of two subunits, S1 and S2, which after a host protease dependent activation, allows the virus to bind to its specific receptor and the host-virus membrane fusion to take place[26]. In the case of SARS-CoV-2, the target receptor is the Angiotensin- converting enzyme 2 (ACE2) receptor and the host protease required for S glycoprotein activation is identified as a transmembrane serine protease called TMPRSS2[28-33].

The degree to which the virus specific receptor is expressed on a host cell determines viral tropism[34]. The high expression of SARS-CoV-2's target receptor, the ACE2 in lungs and distal airway makes them an easy target for SARS-CoV-2 and explain the predominant respiratory nature of the disease[35]. However, this ACE2 receptor is not exclusive to pulmonary tissues and is widely expressed across a broad range of human tissues. They are abundant throughout the glandular epithelial cells of the GI tract, cholangiocytes, proximal renal tubules among many others[12,36-38]. A lot of these cell types also have high co-expression of TMPRSS2, which makes them a target for SARS-CoV-2[39,40].

Another important concept is the affinity of a virus for its specific receptor. This not only is an important determinant of its successful replication, but also demonstrates a correlation with transmissibility and disease severity. The evidence for this comes from an observation made during the 2002-03 SARS pandemic. Compared to the earlier 2002 phase, a lower pathogenicity and transmissibility was seen with the 2003 re-emergence phase which also co-related with a lower viral affinity for its receptor[27]. The role of host proteases such as the TMPRSS2 is also important in this regard. Not only can they facilitate viral penetrance but can also enhance it. This can allow the virus to infect cells with relatively low ACE2 expression, which would otherwise not be possible[30]. The binding affinity of SAR-CoV-2 is 10 to 20-fold greater than that of SARS-CoV[41]. This can not only explain its higher transmissibility but also the involvement of multiple organ systems in COVID-19.

PATHOPHYSIOLOGY OF COVID-19

The innate immune system consists of epithelial cells, dendritic cells and macrophages[35]. These cells not only serve as the first line of defense, but by the process of antigen presentation also activate the adaptive arm of immune system. Effectors cells of adaptive immunity are CD4 and the CD8 T-Cells. By activating B-Cells, the CD4 T-cells also activate humoral immunity, while CD8 T-cells directly kill the virus infected cell[26]. This is body's natural immune response to any foreign pathogen. However, in a subset of COVID-19 patients, overactivation of this normal immune response can occur. This results in severe manifestations of the disease including acute respiratory distress syndrome and multi-organ failure. Higher levels of various pro-inflammatory cytokine, including IL-6, IL-10, granulocyte-colony stimulating factor, monocyte chemoattractant protein 1, macrophage inflammatory protein 1 alpha and tumor necrosis factor (TNF) - alpha is often seen in such patients.

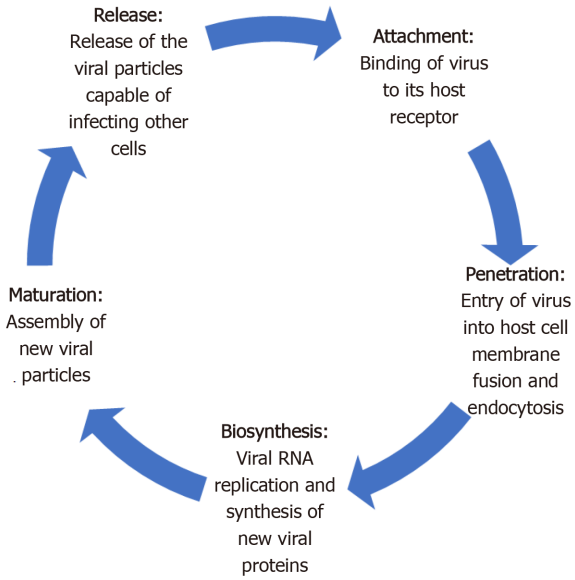


Figure 1 Five steps of severe acute respiratory syndrome coronavirus-2 life cycle.

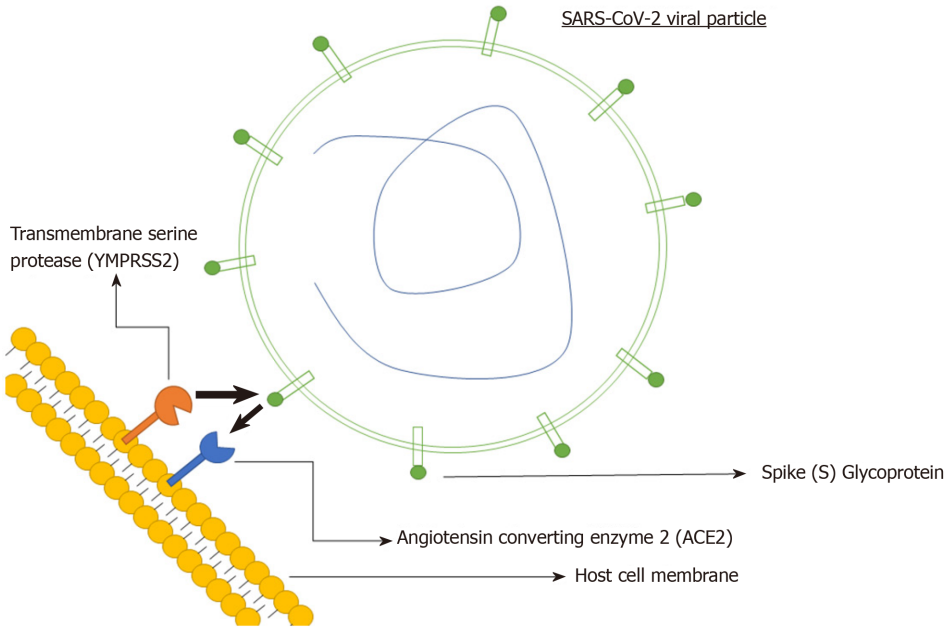


Figure 2 Entry of viral particle in the host cell. SARS-Cov-2: Severe acute respiratory syndrome coronavirus-2.

This phenomenon is also called 'cytokine storm'[42-44]. The levels of these pro-inflammatory cytokines correlates with disease severity[26]. Thus an aberrant immune response is the underlying mechanism for severe COVID-19.

MORBIDITY AND MORTALITY OF COVID-19

Case fatality rate (CFR), which is a crude way to assess mortality has demonstrated tremendous regional and demographic variability for COVID-19[45]. While a mild disease phenotype is reported for the majority (80%), epidemiological studies have indicated a higher mortality in the elderly (> 60 years)[46].

Initial studies from China estimated a CFR between 1.36% and 33% and reported majority (80%) of deaths in the elderly[42,47-50]. The official numbers later released by the Chinese government reported it at 3.17%[51]. The US CDC not only reported variable

CFRs across different age groups but also found a positive correlation between older age and rates of hospitalization[46]. CFRs ranged from 0 in < 19 years to up to 10%-27% in > 85 years[46]. Similarly, the rates of hospitalization also ranged between 2%-3% in <19 years to greater than 31% in > 85 years[46]. The worldwide CFR for COVID-19 for all age groups currently stands at approximately 3% but can range from as high as 12% in Italy to < 0.1% in Singapore[45].

Some authors have attributed the observed regional differences in mortality to their respective demographic characteristics. For example, the abnormally high CFR reported from Italy was thought to be related to its relatively older population[26]. While the morbidity and mortality of COVID-19 is almost certainly higher in older age groups, it is important to be aware that severe disease can occur in any age group[46].

Compared to its predecessors, the SARS-CoV (approximately 10%) and MERS-CoV (34%-37%), the mortality rate for COVID-19 appears to be substantially low[45,52,53]. But longer incubation period and sustained viral shedding during pre-symptomatic phase have enabled a much more efficient spread for SARS-CoV-2[53,54]. The estimated reproduction number for SARS-CoV-2 ranges from 2.24 to 3.58, which is higher than that of its predecessor[55,56]. The mean incubation period for SARS-CoV-2 is 5.2 days (95%CI: 4.1-7.0)[57], but the incubation period can generally range from 1 to 14 d[16,56,58].

TRANSMISSION

A direct person-to-person transmission by respiratory droplets is the predominant route of transmission for COVID-19[59]. This can occur during coughing, sneezing, or normal breathing. Droplets generated *via* these routes can travel up to six feet and can remain suspended in air for up to three hours[60]. Aerosol transmission during aerosol generating procedures, such as endotracheal intubation and nebulization can also occur[61]. Indirect transmission *via* fomites is another potential mode of transmission. Viral particles can remain viable on different surfaces (plastic, steel *etc*.) for up to a few days[62]. Viral particles have also been detected in samples from toilet bowl and sink used by COVID-19 patients[63]. A cluster of COVID-19 cases associated with a shopping mall in Wenzhou, China was thought to be linked to a common restroom[64].

During the SARS outbreak of 2003, the SARS-CoV particles were detected in the GI biopsy and stool specimens, indicating potential for fecal-oral transmission and GI troposim[65]. Based on shared homology, the same was speculated for SARS-CoV-2, which was afterwards confirmed by the presence of fecal viral RNA and nucleocapsid proteins in the cytoplasm of GI epithelial cells on autopsy[66,67]. Around 36% to 53% of stool samples can test positive for SARS-CoV-2 viral RNA[38]. The stool sample from the first US case of COVID-19 also tested positive for the viral RNA[68].

Patients with GI symptoms can have a 70% higher chance of testing positive on the nasopharyngeal swabs, suggesting a higher overall viral burden[69]. Stool samples can turn positive around 2-5 d after a positive respiratory specimen and can remain positive despite a negative respiratory specimen in up to 23% to 82% of cases[38]. The reason for this can be a longer survival or persistent viral replication in the GI environment[38,68].

A study from China reported positive stool samples in more than half (53%) of the cases, which can remain positive up to 12 d. Around 23% of these samples can continue to test positive despite a negative respiratory test[67]. Another study reported 53% of stool samples testing positive on RT-PCR. But reported lower fecal viral load compared to respiratory samples[70]. Zhang *et al*[71] also demonstrated presence of viral particles in oral and anal swabs. While it can be argued that positive RT-PCR does not necessarily mean a live virus capable of causing infections, Wang *et al*[72] actually found live SARS-CoV-2 virus in stool samples.

In summary, droplet and aerosol routes are still responsible for bulk of transmission but fecal-oral transmission can be another possibility. Gastroenterologists who can encounter patients with GI symptoms in their practice should be aware of the risk of such transmissions and adopt necessary precautions.

GI SYMPTOMS OF COVID-19

GI symptoms such as diarrhea, anorexia, nausea, vomiting and abdominal pain are commonly reported in COVID-19[25,73]. The retrospective study by Guan *et al*[48] which included 1099 COVID-19 cases from 552 centers across China, reported nausea, vomiting in 5% and diarrhea in 3.8% cases. Another study reported at least one GI

symptom in 32.5% of cases. These included anorexia (56.7%), diarrhea (37.8%), nausea (16.5%), abdominal pain (10.4%) and vomiting (7.9%)[39]. A cross-sectional study found GI symptoms in more than half (50.5%) of the cases on initial presentation. The severity of these symptoms correlated with the overall disease severity and significant liver function abnormalities[74]. The retrospective study by Luo *et al*[75] which included 1141 cases found at least one GI symptom in 16% of cases on initial presentation. These included anorexia (98%), nausea, vomiting (66%), diarrhea (37%) and abdominal pain (25%). Jin *et al*[76]'s study which was the first COVID-19 study outside Wuhan reported the prevalence of GI symptoms at 11.4%. Diarrhea, at 8.14% was the most common of these symptoms[76].

A single-center case series from Flushing, New York, including 892 cases, reported GI symptoms in a quarter. Diarrhea (19.8%) was the most common, followed by nausea (16.6%), anorexia (11.8%), vomiting (10.2%) and abdominal pain (7.8%)[77]. Another retrospective study from New York which included 1059 patients, reported diarrhea in 22% of cases. Nausea, vomiting and abdominal pain were reported in 16%, 9% and 7%, respectively[78]. A multicenter, retrospective cohort study, from Massachusetts reported at least one GI symptom in up to two-thirds (61.3%) of cases. These symptoms included anorexia (34.8%), diarrhea (33.7%) and nausea (26.4%). Around 20% experienced isolated GI symptoms, while 14% developed these symptoms prior to the onset of other disease manifestations[79]. An outpatient questionnaire based study by Sierpiński *et al*[80] which included 1942 cases with mild to moderate disease reported at least one GI symptom in 53.6%. These included Anorexia (47%) and diarrhea (24%).

A meta-analysis of 60 studies including 4243 cases from 6 countries, found pooled prevalence of GI symptoms at 17.6%. Anorexia (26.8%) was most common, followed by diarrhea (12.5%), nausea/vomiting (10.2%) and abdominal pain/discomfort (9.2%)[3].

GI symptoms are also seen in pediatric cases. A retrospective study including 20 pediatric cases from Wuhan, reported GI symptoms, such as diarrhea and vomiting in 15% and 10% of cases, respectively[81].

In addition to GI symptoms, gross and microscopic changes of the GI tract are also seen on autopsy of COVID-19 patients. Segmental dilation and stenosis of small intestine, along with mucosal shedding and necrosis is reported on autopsy, while inflammatory infiltrates and interstitial edema, consistent with a colitis/enteritis like picture is seen on histology[38,67]. Macroscopic abnormalities of GI tract can also be evident on imaging. Lui *et al*[82] reported bowel wall thickening involving any portion of the bowel, as well as ileus, ascites, pneumatosis intestinalis and pneumoperitoneum on abdominal imaging.

We have summarized the most frequently reported GI symptoms across different studies in Tables 1-4.

PROPOSED MECHANISM FOR GI SYMPTOMS

There are several proposed mechanisms for the GI manifestations of COVID-19 (Figure 3). Subjective and nonspecific symptoms such as anorexia and nausea are difficult to accurately characterize and can be over-reported. Objective symptoms, such as diarrhea and vomiting are speculated to be either a result of direct viral attack or due to an adverse drug reaction. An overwhelming systemic inflammatory response commonly seen in severe cases can also produce these symptoms.

The structural similarities and inter-specie immunologic cross-reactivity between animal and human coronaviruses have indicated a zoonotic origin for most human coronaviruses[54]. Some of these inherently have the ability to cause gastroenteritis in their animal hosts[18,20,54]. Some of the endemic human coronaviruses, such as OC43 and 229E have antigenic similarities to these animal coronaviruses[54,138,141]. It is possible that the recombination events resulting in these inter-specie barrier crossover can allow some of them to retain their enteric infectivity[54]. This can be a possible scenario in SARS-CoV-2, which *via* the ACE2 receptor can infect and exert a direct cytopathic effect on enterocytes. Fecal leukocytes and occult blood which can be seen in other cases of viral diarrhea is reported in 1.7% and 5.2% of fecal samples, respectively[38]. Increased inflammatory infiltrates and interstitial edema of the GI tract indicating active inflammation is also observed on histology[67]. The presence of an infectious SARS-CoV-2 virion in stool, and persistent fecal viral RNA shedding which in some cases, can outlast respiratory shedding points towards viral replication in GI tract[67,72]. Together, these findings can support a direct viral attack on enterocytes resulting in

Table 1 Prevalence of common gastrointestinal symptoms reported by coronavirus disease 2019 patients in studies from Wuhan, China, *n* (%)

Ref.	Number of patients	Diarrhea	Nausea	Vomiting	Anorexia	Abdominal pain
Huang *et al*[42]	41	1 (3)	NA	NA	NA	NA
Yang *et al*[58]	52	NA	NA	2 (4)	NA	NA
Chen *et al*[1]	99	2 (2)	1(1)		NA	NA
Ping *et al*[83]	9	1 (11.1)	1(11.1)	1(11.1)	6 (66.7)	NA
Wang *et al*[13]	138	14 (10.1)	14 (10.1)		55 (40)	3 (2.2)
Luo *et al*[75]	1141	68 (6)	134 (11.7)	119 (10.4)	NA	45 (3.9)
Zhou *et al*[84]	191	9 (4.7)	7 (3.7)		NA	NA
Zhang *et al*[85]	140	18 (12.9)	24 (17.3)	7 (5)	NA	8 (5.8)
Chen *et al*[86]	274	77 (28.1)	24 (8.8)	16 (5.8)	NA	19 (6.9)
Xu *et al*[87]	1324	28 (2.1)	NA	NA	56 (4.2)	NA
Shi *et al*[88]	645	29 (4.5)	NA	NA	NA	NA
Han *et al*[89]	108	15 (14)	NA	NA	NA	NA
Fang *et al*[90]	305	146 (49.5)	59 (29.4)	3 (2)	101 (50.2)	12 (6)
Xu *et al*[91]	355	130 (36.6)	NA	NA	NA	NA
Ma *et al*[92]	81	6 (7.41)	NA	NA	NA	NA
Liu *et al*[93]	238	14 (9.2)	2 (1.3)	3 (2)	14 (9.2)	1 (0.7)
Huang *et al*[94]	36	3 (8.3)	NA	NA	NA	NA
Mao *et al*[95]	214	41 (19.2)	NA	NA	NA	10 (4.7)
Liu *et al*[96]	109	12 (11)	NA	NA	NA	NA
Shu *et al*[97]	545	49 (8.9)	0 (0)		NA	NA

NA: Not applicable.

cellular dysfunction and increased permeability which in turn can lead to malabsorption, culminating in diarrhea[73]. It is also a possibility that viral involvement of upper GI glandular epithelium produces nausea and vomiting in a similar way.

Adverse drug reactions can be another possible explanation. Some of the antivirals, antibiotics and immunomodulators, commonly prescribed to COVID-19 patients are known to cause diarrhea. Compared to earlier studies, a higher prevalence of GI symptoms was reported in later studies. This temporal variation also happens to coincide with a more widespread use of these medications[38]. Hypoxia induced necrosis and cellular injury resulting in enterocyte dysfunction is another possibility[38].

HEPATOBILIARY MANIFESTATIONS OF COVID-19

Hepatic dysfunction, as evidenced by abnormal laboratory tests is frequently seen in COVID-19. Histological changes including lobular and portal inflammation have also been reported[142]. Most cases of COVID-19 associated hepatic dysfunction are mild and transient, but in a small subset of patients a higher degree of dysfunction and hepatic enzyme derangements can be seen[143].

One of the earliest studies from Wuhan, reported aspartate transaminase (AST) elevation in 37% of cases[42]. This number was even higher in critically ill [62% *vs* 25% intensive care unit (ICU) *vs* non-ICU cases, respectively][42]. Another study from Wuhan, reported liver function test (LFT) abnormalities in 43% of cases. AST elevations were more common than alanine aminotransferase (ALT) (35% *vs* 28%), while total bilirubin was elevated in only 18%[1]. Though most cases had only mild to moderate degree of LFT derangements, a case of severe liver injury with profound ALT and AST elevation was also reported[1]. Guan *et al*[48]'s analysis of data reported to

Table 2 Prevalence of common gastrointestinal symptoms reported by coronavirus disease 2019 patients in Chinese studies outside Wuhan, *n* (%)

Ref.	Number of patients	Diarrhea	Nausea	Vomiting	Anorexia	Abdominal pain
Ai *et al*[98]	102	15 (14.3)	9 (8.8)	2 (2)	NA	3 (2.9)
Chen *et al*[99]	9	2 (22)	0 (0)		NA	0 (0)
Zhao *et al*[100]	77	1 (1.3)	6 (7.8)	NA	NA	NA
Chang *et al*[101]	13	1 (7.7)	NA	NA	NA	NA
Zhao *et al*[102]	75	7 (9.3)	NA	NA	NA	1 (1.3)
Yang *et al*[103]	55	2 (3.6)	NA	NA	NA	NA
Li *et al*[104]	83	7 (8.4)	NA	NA	NA	7 (8.4)
Qi *et al*[105]	267	10 (3.7)	6 (2.2)		46 (17.2)	NA
Xu *et al*[106]	90	5(5.6)	2 (2.2)	5 (5.6)	NA	NA
Xiao *et al*[67]	73	26 (35.6)	NA	NA	NA	NA
Lin *et al*[107]	95	23 (24.2)	17 (17.9)	4 (4.2)	17 (17.8)	2 (2.1)
Wen *et al*[108]	417	29 (7)	NA	NA	NA	NA
Xu *et al*[109]	45	0 (0)	NA	NA	NA	NA
Yan *et al*[110]	168	12 (7.1)	9 (5.4)	7 (4.2)	14 (8.3)	7 (4.2)
Wang *et al*[111]	18	3 (16.7)	1 (5.6)		NA	NA
Chen *et al*[112]	291	25 (8.6)	17 (5.8)		NA	1 (0.3)
Liu *et al*[113]	620	53 (8.5)	NA	NA	NA	NA
Fan *et al*[114]	55	6 (10.9)	NA	4 (7.3)	NA	NA
Yao *et al*[115]	40	3 (7.5)	3 (7.5)	NA	NA	NA
Tian *et al*[116]	37	8 (25.8)	NA	NA	NA	NA
Song *et al*[117]	51	5 (10)	3 (6)	9 (18)	NA	NA
Lu *et al*[118]	265	17 (6.4)	6 (2.3)		NA	NA
Fu *et al*[119]	52	7 (13.5)	1 (1.9)	NA	NA	NA
Fu *et al*[120]	36	3 (8.3)	NA	NA	NA	NA
Xu *et al*[121]	62	3 (4.8)	NA	NA	NA	NA
Jin *et al*[76]	651	56 (8.6)	28 (4.3)		NA	NA
Qian *et al*[122]	91	21 (23.1)	11 (12.1)	6 (6.6)	23 (25.3)	NA
Chen *et al*[123]	175	35 (20)	NA	7 (4)	NA	5 (3)
Kuang *et al*[124]	944	21 (2.7)	NA	NA	NA	NA
Hu *et al*[125]	24	2 (8.3)	NA	NA	NA	NA
Guan *et al*[48]	1099	42 (3.8)	55 (5)		NA	NA

NA: Not applicable.

the Chinese National Health Commission found AST, ALT and total bilirubin elevation in 22.2%, 21.3% and 10.5% of cases, respectively. LFT derangements were more commonly seen in severe cases (AST 39.4% *vs* 18.2%: ALT 28.1% *vs* 19.8% and T. Bili 13.3% *vs* 9.9%; Severe *vs* non-severe, respectively)[48]. Data from 56 COVID-19 patients admitted to the People's Liberation Army Hospital in Beijing reported LFT abnormalities in 28.6% of cases[144].

A study from New York, reported mild (2-5 × ULN) AST and ALT elevation in 13.8% and 11.5% cases on admission. Severe (> 5 × ULN) AST and ALT elevations were seen in 2.8% and 1.9%. 4.3% had abnormal bilirubin (> 1.2 mg/dL), 11.9% had abnormal alkaline phosphatase and 24% had an INR > 1.13[77]. LFT abnormalities, especially AST elevations were associated with severe disease and higher mortality[77].

Table 3 Prevalence of common gastrointestinal symptoms reported by coronavirus disease 2019 patients in studies from United States, *n* (%)

Ref.	Number of patients	Diarrhea	Nausea	Vomiting	Anorexia	Abdominal pain
Cholankeril *et al*[126]	207	22 (10.8)	22 (10.8)		NA	14 (7.1)
Hajifathalian *et al*[78]	1059	234 (22.1)	168 (15.3)	91 (8.6)	240 (22.7)	72 (6.8)
Kujawski *et al*[127]	12	4 (33.3)	3 (25)	NA	NA	2 (16.7)
Ferm *et al*[77]	892	177 (19.8)	148 (16.6)	91 (10.2)	105 (11.8)	70 (7.8)
Redd *et al*[79]	318	107 (33.7)	84 (26.4)	49 (15.4)	110 (34.8)	46 (14.5)

NA: Not applicable.

Table 4 Prevalence of common gastrointestinal symptoms reported by coronavirus disease 2019 patients in studies from across the world

Ref.	Location	Number of patients	Diarrhea	Nausea	Vomiting	Anorexia	Abdominal pain
COVID-19 National Emergency response Center[128]	South Korea	28	2 (7)	NA	NA	NA	1 (4)
Young *et al*[129]	Singapore	18	3 (17)	NA	NA	NA	NA
Pung *et al*[130]	Singapore	17	4 (23.5)	1 (5.9)		NA	NA
Tabata *et al*[131]	Diamond Princess Cruise Ship	104	8 (9.6)	NA	NA	NA	NA
Kluytmans *et al*[132]	Netherlands	86	16 (18.6)	NA	NA	NA	5 (5.8)
Wölfel *et al*[133]	Germany	9	2 (22)	NA	NA	NA	NA
Dreher *et al*[134]	Germany	50	8 (16)	1 (2)	2 (4)	NA	NA
Gritti *et al*[135]	Italy	21	5 (23.8)	NA	NA	NA	NA
Spiteri *et al*[136]	Europe	38	1 (3.2)	NA	NA	NA	NA
COVID-19 National Incident Room Surveillance Team[137]	Australia	295	48 (16.3)	34 (11.5)		NA	6 (1)
Sierpiński *et al*[80]	Poland	1942	470 (24.2)	NA	NA	NA	NA

NA: Not applicable; COVID-19: Coronavirus disease 2019.

The strength of this study was that it looked at the prevalence of LFT derangements on admission. This can help mitigate the effects of confounders such as medications and other factors.

Like adult cases, pediatric cases have also demonstrated hepatic dysfunction. A study from Wuhan reported ALT elevation in a quarter of pediatric cases[81].

In summary, liver dysfunction seen in COVID-19 is mostly transient and mild with a hepatocellular, rather than cholestatic pattern[145]. AST elevations are also more common than ALT, which is in contrasts to the typical pattern of viral induced liver injury[77]. Liver injury is more commonly seen in severe cases. Except for a few, most studies have not described prevalence of pre-existing liver diseases which can be a potential confounder[145].

We have summarized studies which have reported hepatic dysfunction in Table 5.

PROPOSED MECHANISM FOR HEPATIC DYSFUNCTION IN COVID-19

There are many possible explanations for COVID-19 associated hepatic dysfunction. SARS-CoV-2 can directly infect hepatocytes and cholangiocytes, which also harbor the virus specific ACE2 receptors (Figure 4)[145]. Expression profiling have indicated a 20-fold higher expression of ACE2 in cholangiocytes, compared to hepatocytes[145]. In

Table 5 Prevalence of hepatic abnormalities reported in different studies, *n* (%)

Ref.	Location	Number of patients	AST elevation	ALT elevation	T. Bili elevation
Huang *et al*[42]	Wuhan, China	41	15 (37)	NA	NA
Chen *et al*[1]	Wuhan, China	99	35 (35)	28 (28)	18 (18)
Zhou *et al*[84]	Wuhan, China	191	NA	59 (31)	NA
Chen *et al*[86]	Wuhan, China	274	84 (31)	60 (27)	NA
Xu *et al*[91]	Wuhan, China	355	102 (28.7)	91 (25.6)	66 (18.6)
Huang *et al*[94]	Wuhan, China	36	18 (58)	4 (13.3)	4 (12.9)
Shu *et al*[97]	Wuhan, China	545	35 (10.1)	41 (7.5)	189 (34.7)
Cai *et al*[146]	Shenzhen City, Guangdong, China	298	25 (8.4)	39 (13.1)	24 (8.1)
Ai *et al*[98]	Xiangyang, China	102	26 (25.5)	20 (19.6)	NA
Zhao *et al*[102]	Hefei, Anhui, China	75	14 (18.7)	15 (20)	12 (16)
Zhao *et al*[100]	Beijing, China	77	20 (26)	26 (33.8)	NA
Qi *et al*[105]	Chongqing, China	267	19 (7.1)	20 (7.5)	6 (2.2)
Lin *et al*[107]	Zhuhai, Guangdong, China	95	4 (4.2)	5 (5.3)	22 (23.2)
Xu *et al*[109]	Multiple locations in China (All outside Hubei province)	45	NA	NA	NA
Yan *et al*[110]	Hainan Province, China	168	18 (17.3)	9 (8)	7 (6.4)
Wang *et al*[111]	Zhengzhou, Henan, China	18	Abnormal LFTs 4 (25)		NA
Chen *et al*[112]	Changsha and Loudi, Hunan, China	291	44 (15.1)	30 (10.3)	27(9.3)
Yao *et al*[115]	Xi'an, Shaanxi, China	40	16 (40)	21 (52.5)	10 (25)
Tian *et al*[116]	Liaocheng, Shandong, China	37	4 (10.8)	2 (5.4)	13 (35.1)
Fu *et al*[120]	Kunming, Yunnan, China	36	4 (11.1)	4 (11.1)	11 (30.56)
Xu *et al*[121]	Zhejiang, China	62	10 (16.1)	NA	NA
Qian *et al*[122]	Multiple sites, Zhejiang, China	91	9 (9.9)	7 (7.7)	NA
Chen *et al*[123]	Multiple sites, Wenzhou, Zhejiang, China	175	19 (11)	23 (13)	NA
Guan *et al*[48]	Multiple sites across China	1099	168 (22.2)	158 (21.3)	76 (10.5)
Ferm *et al*[77]	New York, United States	892	Borderline elevation (1-2 × ULN) 40% mild elevation (2-5 × ULN) 13.8% moderate to severe elevation (> 5 × ULN) 2.8%	Borderline elevation (1-2 × ULN) 26.5% mild elevation (2-5 × ULN) 11.5% moderate to severe (> 5 × ULN) 1.9%	4.3%
Tabata *et al*[131]	Diamond Princess Cruise, Japan	104	18 (17.3)	17 (16.3)	NA

AST: Aspartate transaminase; ALT: Alanine aminotransferase; NA: Not applicable.

addition to maintaining normal function of biliary system, cholangiocytes also play an important role in liver regeneration and immune response. Direct involvement of these cells can cause liver injury as manifested by impaired hepatic transaminases[145]. Liver biopsy on autopsy of COVID-19 patients show micro vesicular steatosis as well as lobular and portal inflammation[142]. Although non-specific, these findings can represent direct hepatic injury from SARS-CoV-2 infection. However, no viral inclusions have been detected to date in hepatic or biliary tissue and the pattern of LFT derangement seen is mostly hepatocellular rather than cholestatic, which is expected in biliary and cholangiocyte involvement[145]. This indicates that the liver involvement in COVID-19 is not a likely consequence of direct liver injury.

Indirect liver injury from overwhelming release of inflammatory cytokines (cytokine storm) or ischemia is a more likely explanation[144]. AST elevation is more commonly seen than ALT which is in contrast to the pattern of liver injury typically seen in viral hepatitis[147]. Preferential AST elevations are more commonly seen with toxic and ischemic liver injury which involves zone 3 hepatocytes[147]. A higher prevalence of LFT abnormalities in severe cases of COVID-19 also supports this finding[77]. Overwhelming release of inflammatory cytokines such TNF-α, interferon-λ and various interleukins such IL-2, IL-6, IL-7, IL-8 can lead to global tissue damage and multiorgan dysfunction including the liver[145].

Liver injury seen in COVID-19 can also be drug induced. A wide range of medications can cause hepatotoxicity. These can range from commonly taken OTC acetaminophen to antiviral drugs currently under investigation for COVID-19. A summary of these drugs will be mentioned in Table 6.

Due to high worldwide prevalence of chronic liver disease (CLD), it is possible that hepatic dysfunction seen with COVID-19 is a consequence of pre-existing liver disease exacerbation[143]. Patients with CLD can have interruptions in their ongoing treatment resulting in exacerbations. Corticosteroids and immunomodulators which are increasingly being used to treat COVID-19 can also exacerbate underlying viral hepatitis. Singh *et al*[148] did a comparative study of patients with and without pre-existing CLD. Their study, which included 2780 patients, reported similar elevations in ALT, AST, GGT, Alkaline Phosphatase and total bilirubin in both groups, suggesting COVID-19 associated hepatic injury as a more likely reason for LFT derangements[148].

CHRONIC LIVER DISEASE AND COVID-19

Data on the effects of COVID-19 on pre-existing liver disease, at present is limited. Most studies have not specified the prevalence of chronic liver disease in their COVID-19 cases. The few studies that have, report it between 1.25% to 11%[145]. 2.1% of 1099 COVID-19 cases included in Guan *et al*[48]'s study had pre-existing chronic hepatitis B which was associated with worse outcomes. In Singh *et al*[148]'s study, 9% of COVID-19 patients also had pre-existing liver disease. At 42%, nonalcoholic steatohepatitis was the most common liver condition, while cirrhosis was present in 1.8% of cases. The authors also compared laboratory and clinical characteristics of patients with pre-existing liver disease to those without. The results demonstrated a significantly higher mortality rate with chronic liver disease (RR 3.0, 95%CI: 1.5-6.0; $P = 0.001$) and cirrhosis (RR 4.6, 95%CI: 2.6-8.3, $P < 0.001$), irrespective of the cause[148]. However, liver function derangements from baseline were seen in both groups and the overall prevalence of hepatic dysfunction during the disease course did not appear to be different either[148].

Another study found a significantly higher risk of disease progression, LFT derangement and prolonger viral shedding in patients with co-existing nonalcoholic fatty liver disease[149]. Impaired hepatic immune system in these patients was speculated to be a reason[149]. A case series from Northern Italy did not find any COVID-19 related complications in the majority of Autoimmune liver disease patients[150]. Another Italian study did not find any difference in disease course between patients with autoimmune hepatitis (AIH) on immunosuppressive therapy and who are those not[151].

To address the current scarcity of data for CLD patients, two international registries have been created. These are the Surveillance Epidemiology of Coronavirus (COVID-19) Under Research Exclusion (SECURE-Cirrhosis) and European association for the study of liver (EASL) supported COVID-HEP registries. According to the combined weekly update (released on August 25th, 2020) a 31% mortality rate is seen in CLD patients with cirrhosis compared to 7% in those without. In liver transplant recipients, the mortality rate is 18%[152].

Table 6 Commonly administered pharmacotherapy for coronavirus disease 2019 and their gastrointestinal adverse effects[162]

Drug	Proposed mechanism in COVID-19	GI side effects	Hepatic side effects	Pancreatic side effects
Remdesivir	Inhibitor of viral RNA-dependent RNA polymerase.	Nausea, Vomiting, gastroparesis, GI bleeding	Increased LFTs	NA
Chloroquine and Hydroxychloroquine	Disruption of endosome-mediated viral entry and transport.	Nausea, vomiting, abdominal cramping	NA	NA
Lopinavir/Ritonavir	Protease inhibitor. Disrupts viral replication.	Diarrhea, nausea, vomiting	LFT elevation, hepatitis, worsening of underlying CLD	Pancreatitis
Ribavirin	Nucleoside analog inhibitor of viral RNA synthesis.	Nausea	NA	NA
Nitazoxanide	Increase production of type I IFNs enhancing antiviral activity.	Abdominal pain, nausea, diarrhea, acid reflux	NA	NA
Nelfinavir	HIV-1 protease inhibitor. Action against SARS-CoV-2 unclear.	Diarrhea, nausea, increased flatulence	LFT elevation	NA
INF-α	Enhances cell mediated immune response against the virus.	NA	Auto-immune hepatitis	NA
Barcitinib	JAK inhibitor. Downregulation of hyper-inflammatory state seen in severe disease.	Nausea	LFT elevation	NA
Tocilizumab	IL-6 inhibitor. Downregulation of hyper-inflammatory state seen in severe disease.	Gastrointestinal perforation in patients on high dose steroids or history of diverticulitis	LFT elevation	NA

LFT: Liver function test; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; IFN: Interferon; HIV: Human immunodeficiency viruses; CLD: Chronic liver disease; GI: Gastrointestinal; NA: Not applicable.

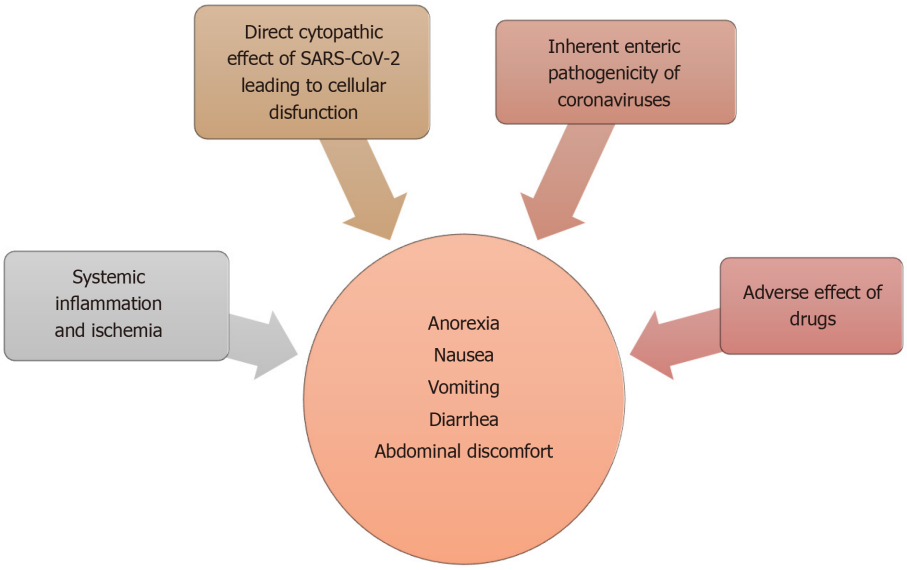


Figure 3 Summary of proposed mechanisms for gastrointestinal manifestations of coronavirus disease 2019. SARS-Cov-2: Severe acute respiratory syndrome coronavirus-2.

To summarize, an overall higher COVID-19 related mortality is seen with pre-existing liver diseases, especially cirrhosis. However, autoimmune hepatitis appears to be an exception to this rule.

PANCREATIC MANIFESTATION OF COVID-19

Although not as common as the other extra-pulmonary organs, pancreatic

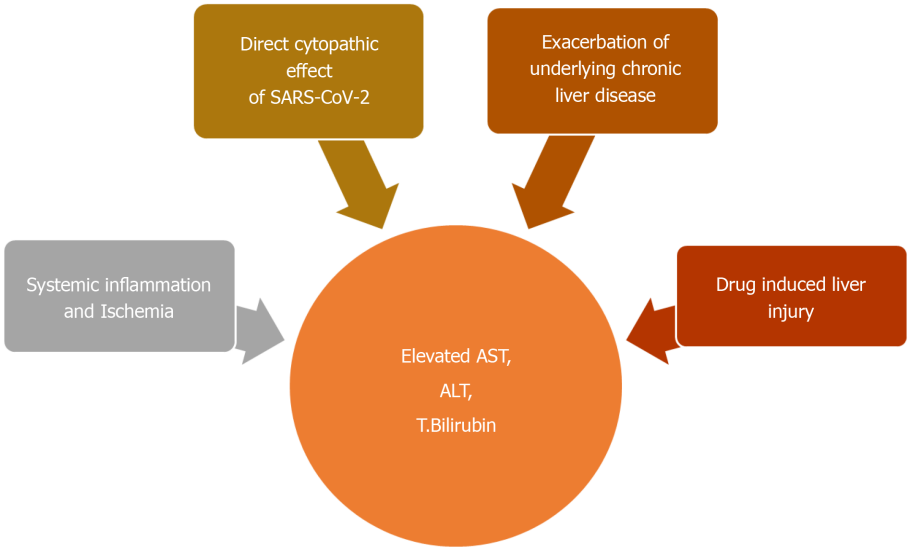


Figure 4 Summary of proposed mechanisms for Hepatobiliary dysfunction seen in coronavirus disease 2019. AST: Aspartate transaminase; ALT: Alanine aminotransferase.

involvement can also be seen in COVID-19. A few cases of possible COVID-19 induced pancreatitis have been reported[153,154]. A Chinese and an Italian study also found some form of pancreatic injury in 17% and 8.5% of cases, respectively[155,156].

PROPOSED MECHANISM FOR PANCREATIC INJURY

ACE2 receptor is also found in pancreas, especially its islet cells[157]. Pancreatic islet cell injury and ensuing, acute diabetes was previously seen with SARS infection[157]. The SARS-CoV virus was detected in pancreatic cells, indicating direct viral attack[158]. Like its predecessor, SARS-CoV-2 can also involve the pancreas[159]. The two-thirds of cases reported by Wang *et al*[155] developed hyperglycemia, which can be a consequence of endocrine pancreas involvement. Pancreatic injury can also be a part of the multi organ failure seen in severe COVID-19[155,156,160]. An English study from Liverpool reported 5 COVID-19 related cases of metabolic-pancreatitis. All these patients were overweight or obese and had hypertriglyceridemia and glucose intolerance. Transient metabolic distress induced by COVID-19 was hypothesized to cause pancreatitis in these cases[161]. Dehydration and drug induced pancreatic injury are other possibilities (Figure 5)[156].

COVID-19 PHARMACOTHERAPY AS A POTENTIAL DRIVER OF GI, HEPATIC AND PANCREATIC MANIFESTATIONS

Many of the commonly encountered extra-pulmonary manifestations of COVID-19 can be an adverse reaction to frequently prescribed drugs. We will summarize some of the well-known GI and hepatic side effects of these commonly administered drugs (Table 6)[162].

MANAGEMENT OF GI SYMPTOMS

GI symptoms associated with COVID-19 are mostly mild to moderate in severity. Diarrhea seen in these patients is typically low volume and does not cause severe dehydration[74]. Management of these patients should involve adequate hydration and correction of electrolyte imbalances. Anti-emetics can also be administered for nausea and vomiting, provided there are no contraindications. Anorexia is a nonspecific symptom and is likely due to severe inflammation. Enteral nutrition should be encouraged whenever possible. A dietician consultation can also be beneficial for these

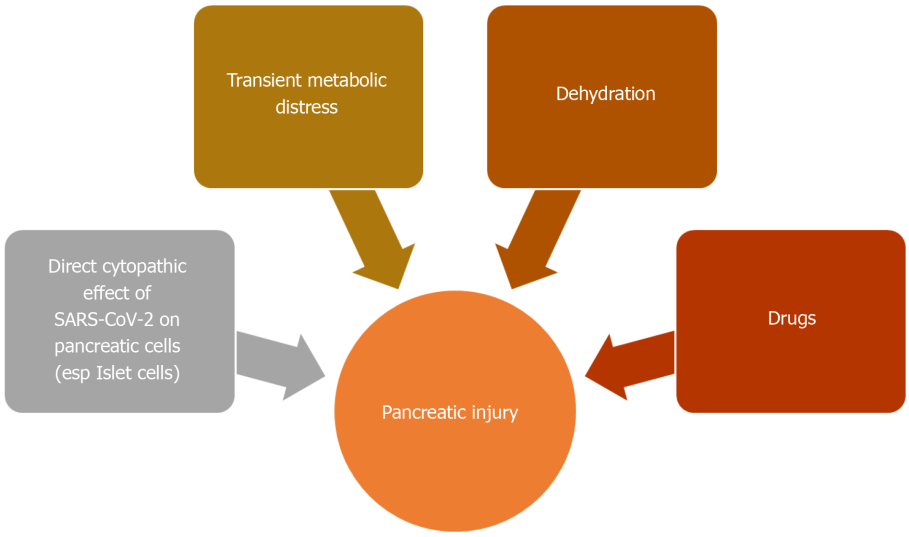


Figure 5 Summary of proposed mechanisms for pancreatic injury seen in coronavirus disease 2019.

patients.

MANAGEMENT OF LIVER INJURY IN COVID-19

COVID-19 associated liver injury is mostly transient and is likely indirect, which could be related to hypoxia, systemic inflammatory response, and drugs. Therefore, management of liver injury in COVID-19 should revolve around correction or removal of underlying etiology. Correction of hypoxia *via* oxygen supplementation or mechanical ventilation, renal replacement therapy for cytokine storm and restoration of effective intravascular volume in cases of shock can often correct liver injury[145]. Prompt identification and discontinuation or dose reduction of drugs responsible for drug induced liver injury is also important. Hepato-protective anti-inflammatory drugs such as L-ornithine-L-aspartate can also be used as adjuvant treatment in severe cases[145].

MANAGEMENT OF CHRONIC LIVER DISEASE DURING PANDEMIC

Significantly higher COVID-19 associated mortality is seen in cirrhotics[163]. Data from international registries have also pointed towards a higher mortality in these patients[152]. Therefore cirrhotics and CLD patients warrant special attention and for this reason three liver associations have provided recommendations for management of CLD during the pandemic (Table 7)[164].

The EASL[165], the American Association for the Study of Liver Diseases (AASLD)[166] and three European referral centers have also made recommendation for the management of AIH which are summarized in Table 8[167].

At present, the data on the safety of corticosteroids or other immunosuppressive medications is limited. Given the incomplete understanding of the effect of immune-modulating therapy on COVID-19, as well as the effect of COVID□19 on immunosuppressed patients, the AASLD and EASL have provided guidance on the use of these drugs which is summarized in Table 9[168].

MANAGEMENT OF CHRONIC GI DISORDERS DURING PANDEMIC

Management of chronic GI disorders such as inflammatory bowel diseases (IBD) which require close follow ups and often require immunosuppressive medications is also challenging. Despite its limitations, virtual platforms, such as telehealth offer a safe and convenient alternative to in-person visits and can be an effective platform for triaging. This can minimize potential exposure to COVID-19 and can determine need for in-person visits and urgent interventions. In situations where no alternative to in-

Table 7 Selected American Association for the Study of Liver Diseases, Asian Pacific Association for the Study of the Liver, and European Association for the Study of the Liver recommendations for liver disease management during the coronavirus disease 2019 pandemic[164]

	Selected recommendations
To limit nosocomial spread	(1) Limit in-person visits by effective triaging; (2) Use of virtual platforms such as telehealth to provide alternatives for in-person visits; (3) Symptom and exposure screening prior to entering the healthcare facility to identify at-risk individuals; (4) Minimize staffing to essential staff only; (5) Decrease frequency of laboratory and imaging monitoring; (6) Ensure adherence to recommended PPE by HCW and patients; (7) Ensure adequate social distancing at the healthcare facility (Remodel if necessary); (8) Postpone and delay nonurgent or elective procedures (refer to Table 12); and (9) Minimize research activities including clinical trials wherever possible.
Management of CLD patients with COVID-19	(1) Early hospital admission is recommended for these patients; (2) Prioritization of COVID-19 testing for cirrhotics, CLD patients on immunosuppressive therapy and those with acute decompensation; (3) Frequent LFT monitoring is recommended; (4) Consider early enrollment in clinical trial when possible; (5) Include non-COVID-19 etiologies in differentials for liver dysfunction; (6) Pay special attention to COVID-19 patients with NAFLD, which is often associated with severe COVID-19; (7) Consider hepatitis B surface antigen screening; (8) Monitor for drug induced liver injury; (9) 2-3 g/d of acetaminophen is generally safe and can be used in these patients. NSAIDs can also be used as needed but limit their use whenever possible; (10) Consider HBV prophylaxis prior to initiating immunosuppressive medications, especially IL-6; and (11) Hold Remdesivir in patients with decompensated liver disease and ALT > 5 × ULN.
Management of chronic viral hepatitis (HCV and HBV)	(1) Continuing treatment for chronic HCV and HBV despite COVID-19 status is recommended; (2) Can hold initiating treatment for HBV in the absence of flare; and (3) Treatment for HCV and HBV in the uninfected should continue according to established guidelines.
Management of HCC	(1) Based on the risk and benefit, a delay in surveillance of up to 2 mo is acceptable for high risk individuals; (2) Continuation of HCC treatment per guidelines is recommended, however can be postponed if necessary; (3) For COVID-19 patients, can consider postponing elective transplant and resection surgery and withholding immunotherapy; and (4) Early inpatient admission is advised for HCC patients.
Management of pre- and post- transplant recipients	(1) Screening of both, the donor and recipient for COVID-19 is recommended; (2) Donors testing positive for COVID-19 should be deferred; (3) CMS has classified transplant surgeries as Tier 3b. As such, these procedures should not be postponed or delayed; (4) Patients with poor short-term prognosis should be prioritized; (5) Low threshold for admitting transplant listed COVID-19 patients to the hospital is recommended; (6) For post-transplant patients, immunosuppressive dose reductions can be considered in moderate COVID-19 cases. For mild COVID-19 cases no immunosuppressive dose reduction is advised; and (7) Vaccination against pneumonia and influenza is recommended in post-transplant recipients.

AASLD: American Association for the Study of Liver Diseases; APASL: Asian Pacific Association for the Study of the Liver; EASL: European Association for the Study of the Liver; PPE: Personal protective equipment; HCWs: Healthcare workers; CLD: Chronic liver disease; COVID-19: Coronavirus disease 2019; LFT: Liver function test; NAFLD: Nonalcoholic fatty liver disease; NSAIDs: Non-steroidal anti-inflammatory drugs; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; CMS: Centers for Medicare & Medicaid Services.

person evaluation exists, precautions including social distancing, proper hand hygiene, wearing of facemasks at all times and symptoms and exposure screening prior to entry in the healthcare facility is recommended[169].

Another problem gastroenterologists face during the pandemic is to determine whether to continue chronic immunosuppressive treatments that are often required for these conditions. Many of these medications carry an increased risk of infections which range between 0.5% to 30%[169]. Societies like American Gastroenterological Association (AGA), European Crohn's and Colitis Organization and International Organization for the Study of IBD have provided recommendations regarding their use during the pandemic which will be summarized in Table 10[169].

Table 8 Expert guidance for management of autoimmune liver disease during coronavirus disease 2019 pandemic[168]

	Selected recommendations from EASL, AASLD for management of auto-immune hepatitis
	(1) In-person visits should be minimized as much as possible. Switch to virtual platforms whenever possible; and (2) COVID-19 testing is recommended in cases of acute decompensation and liver failure.
Low risk of complications (stable patient on chronic immunosuppressive treatment)	(1) Frequent patient-provider communications for close monitoring; (2) Use of virtual platforms should be preferred whenever possible; and (3) Ensure adequate drug supply and refills to reduce running out of medications.
Moderate risk of complications (symptomatic disease in the absence of cirrhosis, compensated cirrhosis)	(1) Can empirically treat *via* virtual healthcare platform whenever possible; and (2) Avoiding liver biopsy whenever possible is also recommended.
High risk of complications (acute flare, decompensated cirrhosis)	(1) Minimize invasive procedures wherever possible; (2) In COVID-19 patients, dose reduction of antimetabolites is recommended if lymphopenia develops; and (3) In case of infection corticosteroids should be tapered as quickly as possible.

EASL: European Association for the Study of the Liver; AASLD: American Association for the Study of Liver Diseases; COVID-19: Coronavirus disease 2019.

Table 9 Expert guidance on immunosuppression for liver disease in the setting of coronavirus disease 2019[168]

	Selected recommendations from AASLD and EASL on use of immunosuppressive therapies in CLD patients
	(1) Initiating corticosteroids and immunosuppressive therapy should be preceded; and (2) by careful risk vs benefit assessment.
Patients uninfected by COVID-19 on immunosuppressive therapy	Dosage reductions or adjustment is not advised.
Patients infected with COVID-19 on immunosuppressive therapy	(1) Corticosteroid dose reductions after specialist consultation (consider tapering to avoid adrenal insufficiency); and (2) Dose reductions in azathioprine, cyclosporine, mycophenolate is recommended in severe COVID-19 (especially with accompanying lymphopenia).
Patients requiring initiation of immunosuppressive therapy	Initiating treatment is recommended in these patients regardless of COVID-19 status.

AASLD: American Association for the Study of Liver Diseases; EASL: European Association for the Study of the Liver; CLD: Chronic liver disease; COVID-19: Coronavirus disease 2019.

Table 10 Recommendations on use of immunosuppressive therapies in inflammatory bowel diseases[169]

	Asymptomatic infection	Mild to moderate infection	Severe or Critical infection
Corticosteroids	(1) Dose reduction of Prednisone to < 20 mg/d; and (2) Consider switching to budesonide.	(1) Discontinue; and (2) Taper in chronic users.	
Immunomodulators (thiopurines, methotrexate)	(1) Consider risks *vs* benefits; and (2) Can resume after 14 d in asymptomatic patients or when test negative.		
Biologicals	(1) Consider risks *vs* benefits; (2) Can resume after 14 d in asymptomatic patients or when test negative; (3) Can decrease infusion frequencies for infliximab and vedolizumab; and (4) Tofacitinib and JAK inhibitors should be discontinued.		

GENERAL RECOMMENDATION FOR GASTROENTEROLOGISTS AND HEPATOLOGISTS

The ongoing pandemic has put an immense strain on healthcare systems and created and overwhelming demand for critical supplies. Judicious use of available resources and ensuring safety of HCWs who are constantly exposed, with consequently higher rates of infections is essential for optimum functioning of healthcare machinery during the pandemic[170,171]. The US CDC has issued interim guidelines for infection prevention and control which is summarized in Table 11[172].

Table 11 Interim infection prevention and control guidelines by Centers for Disease Control and Prevention[172]

Interim Infection Prevention and Control guidelines by CDC
Encourage and promote Telehealth visits over in-person visits whenever possible.
COVID-19 symptom screening for everyone entering a healthcare facility.
Limiting entry points to a healthcare facility, ensuring effective screening for all.
Encourage hand hygiene and source control measures for everyone, especially HCWs.
Encourage physical distancing and limiting the number of visitors and personnel at any given time.
Implementing use of PPEs (*e.g.* N95/PAPR) for HCWs in areas with moderate to substantial community spread.
To mitigate spread from asymptomatic carriers; depending on the availability, a targeted SARS-CoV-2 testing for all patients can be considered.
To reduce exposures for HCWs the use of Engineering controls should be optimized.

CDC: Centers for Disease Control and Prevention; PPE: Personal protective equipment; HCWs: Healthcare workers; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2.

RECOMMENDATIONS FOR ENDOSCOPIES

To mitigate the spread and to conserve limited resources, Joint Gastroenterology Societies, the Centers for Medicare and Medicaid Services, the US surgeon General and American College of Surgeons have all proposed delaying non-urgent and elective procedures[173-176]. AGA recommends a review of any planned procedures by trained medical personnel to determine urgency of a procedure[171]. AGA and AASLD have also provided an outline for triaging[166,171]. Though this approach is quite reasonable; the ensuing diagnostic and therapeutic delays is another challenge that needs to be addressed. Therefore, gradual, and stepwise resumption of procedures, while, of course ensuring patient and provider safety is the need of the day. Canadian Association of Gastroenterology has recently laid a framework for resumption of nonurgent endoscopic procedures[177]. Stepwise increment and expansion of endoscopic services with a minimum of 2 wk' time in between is recommended. Factors related to local risk of transmission, infection dynamics and success of mitigation measures in the community must considered before this[177]. Adequate availability of healthcare resources including personal protective equipment (PPE), staff and equipment, as well as the ability to implement recommended social distancing must be taken into consideration as well[177]. Lastly, triaging which is a powerful tool for efficient healthcare delivery should be vigilantly implemented at every level. Evidence based approach to triaging endoscopic procedures are summarized in Table 12.

When a procedure needs to be performed, proper personal protection equipment must be worn by the providers. Recommendations for PPEs during GI procedures are summarized in Table 13[171].

CONCLUSION

The disease caused by the novel coronavirus is more of a systemic condition than an isolated respiratory illness. GI and hepatic manifestations of COVID-19 are frequently reported and anorexia, nausea, vomiting, diarrhea are few of the commonly seen GI symptoms in COVID-19. Hepatic function derangement is also common, mostly mild, and transient and pancreatic injury is also reported. These symptoms can be a result of a direct infection or an indirect consequence of associated systemic inflammation or hypoxia from respiratory failure. Side effects of drugs currently being used to treat COVID-19 could also be a possible explanation for these manifestations. Fecal oral transmission in the setting of these symptoms poses a considerable risk of exposure for gastroenterologists who deal with these patients. The limited availability of PPE only makes matters worse. Effective and safe delivery of care while conserving these critical supplies is of utmost importance. For this purpose, various gastroenterology societies have also put forward their recommendations. With no end to the pandemic in sight, it is important for HCWs and gastroenterologists to be aware of and adherent to these guidelines.

Table 12 Procedures that can be delayed during the ongoing pandemic

Procedures that can be delayed	Procedures that cannot be delayed
Diagnostic procedures for mild/stable dysphagia[177].	Endoscopies for upper GI bleeding (Blatchford score > 1)[166,171,177].
Diagnostic procedures for suspected GI malignancy can be delayed for up to a few months[171].	Upper endoscopies for foreign body with severe/progressive dysphagia[177].
Routine surveillance and screening colonoscopies are non-urgent and can be postponed[171].	Lower endoscopies for acute obstruction requiring decompression[177].
Procedures for asymptomatic (with normal LFTs) gallstones and biliary strictures can be postponed[177].	ERCP for acute cholangitis or symptomatic common bile duct stone[166,177].
Follow up Colonoscopies for positive FIT test can be delayed for up to 7 to 9 mo without any adverse impact on outcomes[178].	Follow up band ligation for recent variceal bleeding[166,177].
	Placement of percutaneous endoscopic gastrostomy or jejunostomy tubes[177].
	Procedures that can significantly impact medical decisions should be performed. These can include endoscopies for evaluation of high likelihood GI malignancies, resection of high-grade dysplasia or histologically proven neoplasia and investigation for IBD flares[171,177].
	Colonoscopies for lower GI bleeding can be delayed up to 72 h without any change in outcome[178].
	Liver biopsies to diagnose autoimmune hepatitis and transplant rejection in liver transplant patients[166].
	Transplant surgeries are categorized Tier 3b by CMS and should be performed[175].
	Endoscopic drainage of infected pancreatic fluid collections and necrosectomies[177].

GI: Gastrointestinal; LFT: Liver function test; ERCP: Endoscopic retrograde cholangiopancreatography; IBD: Inflammatory bowel disease; CMS: Centers for Medicare & Medicaid Services.

Table 13 Recommended personal protective equipment during gastrointestinal procedures[171]

	Recommendations for PPEs during GI procedures
Masks	Regardless of patient's COVID-19 status, NIOSH approved N95/PAPR should be used by staff for any GI procedure.
Gloves	Regardless of patient's COVID-19 status, gloves should be worn by providers during any GI procedure. Double gloves are preferred over single.
Negative Pressure Rooms	GI procedures for confirmed COVID-19 patients should be performed in negative pressure rooms when available.
Disinfection	Standard endoscope disinfectants can reduce microorganisms by 99.99% and should be sufficient for SARS-CoV-2.

NIOSH: National Institute for Occupational Safety and Health; COVID-19: Coronavirus disease 2019; GI: Gastrointestinal; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2.

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EVIDENCE REVIEW

Current trend in the diagnosis and management of malignant pheochromocytoma: Clinical and prognostic factors

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Abstract

Pheochromocytomas are tumors arising from the chromaffin cell of the adrenal gland and paragangliomas as tumors from extra-adrenal sympathetic chromaffin cells. The combined yearly incidence of pheochromocytoma and paraganglioma (PPGL) is approximately 0.8 per 100000 person/year. Malignant pheochromocytoma is defined only by the presence of metastasis, as there is no confirmatory histology or biomarkers. The most common metastatic sites of these chromaffin tumors are the lymph node, bone, lungs, and liver. This review focuses on relevant clinical and immunohistological factors that are predictive of malignant PPGL or metastasis and determinants of prognosis. Findings showed that the risk of malignant PPGL, along with disease survival, is closely associated with age, primary tumor size, gender, synchronous metastasis, and absence of surgical excision. Other essential biomarkers or immunohistology investigated were galectin-3, COX-2, nm-23, microRNA-210, ERBB-2 overexpression and succinate dehydrogenase subunit mutation, which were predictive of malignancy as well as disease prognosis. Curative resection is possible but most metastatic diseases are amenable to radiopharmaceuticals and chemotherapy due to late presentation. Other therapeutic options, like molecular-targeted therapy, are still undergoing clinical trials.

Key Words: Chromaffin; Malignancy; Metastatic; Paraganglioma; Pheochromocytoma; Prognosis

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Core Tip: The diagnosis of malignant pheochromocytoma/paraganglioma (PPGL) is challenging. To date, confirmatory histology and biomarkers are still lacking. Data from recent studies have shown various biomarkers and genetic mutations that are predictive of malignancy, metastasis and disease prognosis of PPGL, but primary tumor size, male sex and synchronous metastasis have revealed consistent association with malignant PPGL and its prognostication.

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INTRODUCTION

The World Health Organization (WHO) in 2004 defined pheochromocytomas as tumors arising from the chromaffin cell of the adrenal gland and paragangliomas as tumors from extra-adrenal sympathetic chromaffin cells or parasympathetic nonchromaffin cells of the head and neck[1]. Pheochromocytoma and paraganglioma (PPGL) are histologically similar, with both secreting catecholamine (norepinephrine), but pheochromocytoma may also secrete epinephrine[2]. The combined yearly incidence of PPGL is approximately 0.8 per 100000 person/year[3]. Although the majority of PPGL are benign, about 10% to 31% are metastatic[3,4]. According to the WHO, malignant pheochromocytoma is defined only by the presence of metastasis[4].

Despite PPGL being mostly sporadic, nearly 40% are linked to somatic and/or germline mutations in at least 20 known susceptibility genes associated with the pathogenesis of these tumors[5]. PPGL can also occur with hereditary syndromes and genetic testing in patients may be required[5].

Genetically, PPGL with associated succinate dehydrogenase subunit B (SDHB) germline mutations may require a stringent follow-up, as the risk of malignancy is almost 40% and these tumors display aggressive behaviors[6]. Biochemical studies using plasma-free metanephrines or 24-h urine collection for fractionated metanephrines can rule-out PPGL in most instances.

Currently, no diagnostic molecular or histological marker exists for malignancy and most patients are diagnosed with malignant PPGL after they have developed unresectable disease and metastatic disease[7]. The most common metastatic sites of these chromaffin tumors are the lymph node, bone, lungs, and liver[8]. The 5-year survival without metastasis has been reported to be approximately 89.3%[8]. When metastasis is detected, the prognosis is usually poor, with a 5-year survival rate of only 60%[9]. Most of these cases then resort to systemic chemotherapy or radiopharmaceutical agents, such as metaiodobenzylguanidine. These therapies lead mostly to partial radiographic response, disease stabilization, and symptomatic control in about 40% of cases[7,9].

This review has focused on relevant clinical and immuno-histological factors that are predictive of malignant PPGL or metastasis. The review also highlights essential biomarkers that are relevant to prognostication of malignant PPGL. A succinct overview of the current standard of care is provided in the Discussion.

METHODOLOGY

An extensive literature search was conducted from 2000 to 2020 using the PubMed database and search engine. The keyword utilized was "clinical and prognostic factors of malignant pheochromocytoma". There were a total of 74 articles. The abstracts and full texts of 36 publications on malignant PPGL were reviewed after extraction of duplicate text.

After perusal of abstracts and full texts, 11 publications investigating the clinical parameters and biomarkers on the risk of malignancy, metastasis and prognosis were selected for qualitative and quantitative synthesis as projected in Table 1. All articles

Table 1 Clinical factors and biomarkers assessing the risk of malignancy and prognosis of pheochromocytoma and paraganglioma

Ref.	Study duration and type	Study population	Study protocol/objective	Study outcome
Choi *et al*[10], 2015	1997-2013, retrospective	345 pts	Prognostic factors associated with survival for PPGL	Poor survival: Older age and synchronous metastasis
De Wailly *et al*[11], 2012	1993-2009, retrospective	53 pts	New immunohistologic elements correlating to malignant PPGL	Risk of malignancy/recurrence: Tumor necrosis, Ki-67 index > 4% and pS100
Hamidi *et al*[12], 2017	1960-2016, retrospective	272 pts	Epidemiology and survival outcome of malignant PPGL	MOS: 24.6 years, DSS: 33.7 years; poor survival: Older age at diagnosis, male sex, synchronous metastasis, larger tumor size, elevated dopamine
Mei *et al*[13], 2019	1973-2013, retrospective	1014 pts	Survival pattern of malignant PPGL	Survival advantage: Younger age, female sex, surgical resection and origin from of aortic/carotid bodies
Ruff *et al*[14], 2019	Prospective	35 pts	Serum miR-210 levels as a biomarker of malignancy	Lower serum miR-210 expression level and larger primary tumor size strongly correlated with malignant disease
Zelinka *et al*[15], 2011	Retrospective	41 pts	Biochemical and clinical outcome of malignant PPGL	Metastatic pheochromocytoma presented at a significantly younger age, with larger tumor, tumor necrosis and more frequent secreted norepinephrine
Hamidi *et al*[16], 2017	Systemic review and meta-analysis of 20 studies	1338 pts	Baseline characteristics and outcome of patients with malignant PPGL	5-year and 10-year mortality rates of 37% (95%CI: 24%-51%) and 29% (95%CI: 17%-42%), respectively; male sex and synchronous metastases were associated with higher mortality
Saffar *et al*[17], 2011	1986-2006, retrospective	51 pts	Galectin-3, COX-2, and nm-23 to discriminate benign disease from malignancy	Negative nm-23, along with positive galectin-3 or COX-2 were predictive of malignancy, with a sensitivity of 100%
Suh *et al*[18], 2017	1988-2013, retrospective	176 pts	Variations in genomic expressions and mutations of malignant PPGL	mRNA expression of malignant PPGL was up-regulated in five pathways; disease-free survival rates were closely related the presence or absence of mutations
Szalat *et al*[19], 2011	1980-2008, retrospective	16 pts	Predictive features of malignancy in PPGL	High levels of chromogranin A at the time of diagnosis were associated with malignancy; 10-year survival rate after initial diagnosis was 50% and 25% after metastasis
Zhong *et al*[20], 2017	2002-2014, retrospective	414 pts	Clinical significance of biomarker nomogram in detecting malignancy and rate of metastasis	Tumor size, tumor location, vascular invasion, ERBB-2 overexpression and SDHB mutation were independent predictors of malignancy and metastasis

DSS: Disease-specific survival; MOS: Median overall survival; PPGL: Pheochromocytoma and paraganglioma; SDHB: Succinate dehydrogenase subunit B.

on benign pheochromocytoma exclusively were excluded from the study. All case reports and case series with less than 10 subjects were not eligible for inclusion.

ELIGIBILITY

A total of 11 articles met the desired objective for further qualitative analysis. All studies with research methodology emphasizing clinical and immuno-histological factors that are predictive of malignant PPGL or metastasis were selected (Figure 1).

The selected articles were reviewed for study type and duration, study population, study protocol/objectives, clinical parameters, biomarker, and study outcome. The retrieved information was analyzed and reflected in the main text of the Results and Discussion.

The term "pheochromocytoma and paraganglioma" was used concurrently and abbreviated as PPGL.

EVIDENCE SYNTHESIS

A total of 11 studies[10-20] were synthesized (1 systematic review, 1 meta-analysis, 1 prospective study, and 9 retrospective studies) to include 3755 patients with malignant

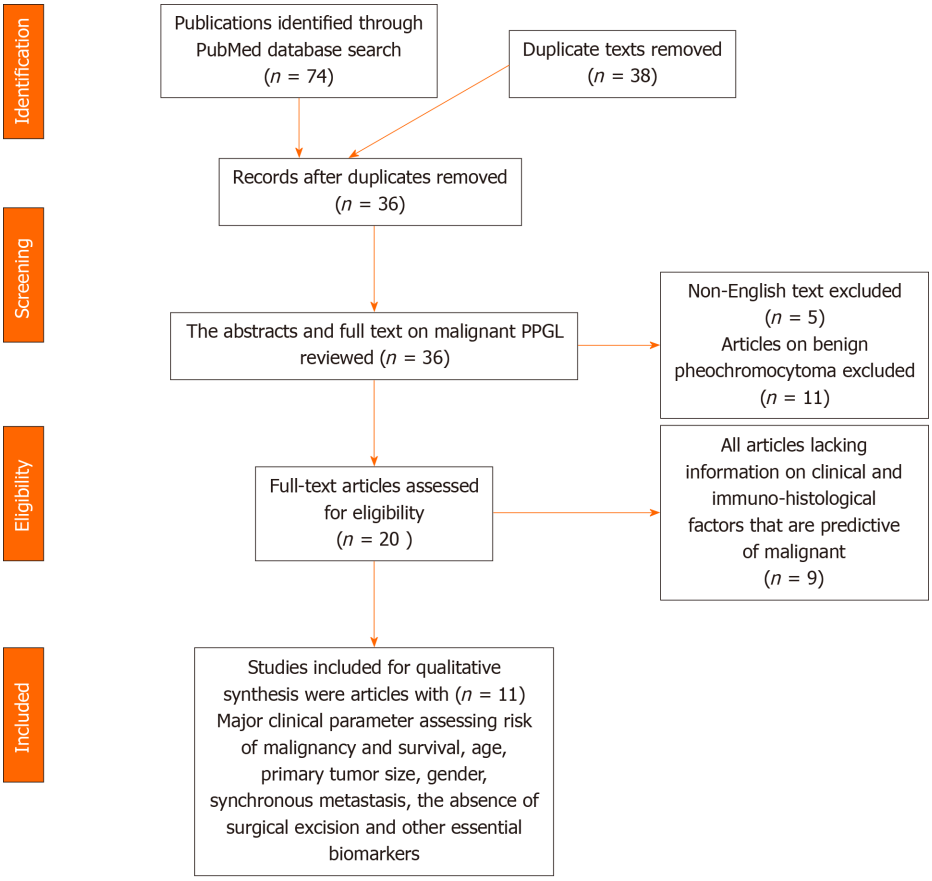


Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-analyses flow chart outlining the selection of articles for qualitative analysis.

PPGL. The various objectives outlined essential clinical factors and immuno-histological factors that are predictive of malignant PPGL or metastasis. The major clinical parameters assessed for risk of malignancy and survival were age, primary tumor size, gender, synchronous metastasis, and absence of surgical excision. Other essential biomarkers/immunohistology features investigated were galectin-3, COX-2, nm-23, microRNA-210 (miR-210), and ERBB-2 overexpression, and SDHB mutation.

A retrospective study by Choi *et al*[10] of 345 patients treated for PPGL evaluating prognostic factors associated with survival for PPGL showed that the median survival was 7.2 years, with a 5-year survival rate of 75.4%. Univariate analysis revealed that older age (> 45 years), larger tumor size (> 6 cm), synchronous metastasis and lack of surgical excision correlated with poor survival. Further multivariate analysis showed that older age and synchronous metastasis were associated with poor overall survival.

De Wailly *et al*[11], in a review of 53 patients operated for PPGL, assessed for new immunohistological elements correlating to malignant pheochromocytoma and the findings indicated that the size and weight of pheochromocytoma were directly related to the Pheochromocytoma of Adrenal gland Scaled Score and malignancy[11]. The presence of tumor necrosis, Ki-67 index > 4%, and absence of pS100 indicated the need for close follow-up, as there was a high risk of malignancy or recurrence.

A retrospective review of 272 patients with malignant PPGL by Hamidi *et al*[12] assessing survivor outcome and predictors of shorter survivors displayed that the median overall survival was 24.6 years and disease-specific survival was 33.7 years. Older age at diagnosis, male sex, synchronous metastasis, larger tumor size, elevated dopamine, and not undergoing primary tumor resection were associated with shorter survival.

Review of the Surveillance, Epidemiology, and End Results program and Cancer Genome Atlas (commonly known as TCGA) database (data from 1973 to 2013) with 1014 patients to analyze epidemiology and survival pattern of malignant PPGL found that younger age, female sex, surgical resection, and origin from of aortic/carotid bodies conferred remarkable survival advantage[13]. Opposingly, distant metastasis was associated with a worse prognosis. ATRX was the most common oncogenic mutation in the metastatic group, while SHDB was higher among males.

A prospective study by Ruff *et al*[14] of 35 patients investigating serum miR-210 levels as a biomarker of malignancy revealed that the most common germline mutation involved SDHB[14]. Univariate analysis showed that lower serum miR-210 expression level and larger primary tumor size strongly correlated with malignant disease.

Zelinka *et al*[15] reported from a retrospective review investigating biochemical and clinical outcome of 41 cases of malignant pheochromocytoma to 108 cases of benign disease that patients with metastatic pheochromocytoma presented at a significantly younger age, with larger tumor, and more often secreted norepinephrine compared to benign pheochromocytomas.

The histological marker of potential malignancy of tumor necrosis was more consistent with metastatic pheochromocytoma. The median period to the development of metastasis from initial diagnosis was 3.6 years.

Results from a systematic review and meta-analysis of 20 retrospective studies of 1338 patients with malignant PPGL by Hamidi *et al*[16] showed that 40.4% of patients had synchronous metastasis. The 5-year and 10-year mortality rates were 37% (95% confidence interval (CI): 24%-51%) and 29% (95%CI: 17%-42%), respectively. Male sex and synchronous metastases were associated with higher mortality.

Retrospective data from Saffar *et al*[17] involving 55 cases of pheochromocytoma determining the concurrent expression of galectin-3, COX-2, and nm-23 to discriminate benign disease from malignancy showed that negativity for nm-23 along with positivity for galectin-3 or COX-2 were predictive of malignancy, with a sensitivity of 100%. Meanwhile, positivity of nm-23 along with dual negativity of COX-2 and galectin-3 showed a benign disease, with a 100% sensitivity.

A multigenomic analysis of data collected from 1988 to 2013 involving 176 patients by Suh *et al*[18] assessing the variations in genomic expressions and mutations of malignant PPGL with TCGA found that mRNA expression of malignant PPGL was up-regulated in five pathways, namely cell cycle (*BUB1*, *BUB1B*, *CCNB2*, *CDC2*, *ESPL1*), gap junction (*CDC2*, *PRKCB1*), calcium signaling (*CCNB2*, *CDC2*, *PRKCB1*), regulation of actin cytoskeleton (*DIAPH3*, *FGF18*, *IQGAP3*), and phosphatidylinositol (*PRKCB1*, *TTK*). Disease-free survival rates were closely related to the presence or absence of mutations, such as *RP11-798G7.5*, *HERC2*, *SETD2*, *TGDS*, *TRHDE*, *FKBP9*, and *BMS1*.

Data from Szalat *et al*[19] in a retrospective study of 16 patients from 1980 to 2008 identifying the predictive features of malignancy in PPGL displayed that high levels of chromogranin A at the time of diagnosis was associated with malignancy, metastasis and poor prognosis. The 10-year survival rate after initial diagnosis was 50% and 25% after metastasis is found.

A retrospective study by Zhong *et al*[20] randomly assigned 414 patients with PPGL to a training or validation group investigating the clinical significance of biomarker nomogram in detecting malignancy and rate of metastasis. The overall rate of metastasis was 10.6%. Univariate analysis revealed that primary tumor size, tumor location, vascular invasion, capsular invasion, ERBB-2 overexpression, and SDHB mutation were remarkably associated with malignancy[20]. Multivariate logistic regression analysis showed that tumor size, tumor location, vascular invasion, ERBB-2 overexpression, and SDHB mutation were independent predictors of malignancy and metastasis. Further analysis revealed that biomarker-based nomogram was useful for assessing the risk of metastasis compared to nomogram without biomarkers (ERBB-2 overexpression and SDHB mutation).

DIAGNOSIS

It has been proven that measurement of metanephrine is superior to other routine catecholamines and metabolites, like vanillylmandelic acid; therefore, the measurement of 24-h plasma metanephrine or fractionated urinary metanephrine is acceptable[21].

The role of imaging is to evaluate primary tumor, assess multifocality, and identify metastasis. Computed tomography scan and magnetic resonance imaging (commonly known as MRI) are ideal for primary tumors, with a sensitivity of 98%-100% for adrenal lesions[21]. MRI is more sensitive for extra-adrenal paraganglioma, with sensitivities of 93% *vs* 90% respectively.

Metaiodobenzylguanidine scintigraphy (I^{131}/I^{123}MIBG) has been used for decades to image neuroendocrine tumors because the uptake represents adrenergic innervation and catecholamine excretion. In patients with large tumors, inherited tumors or multifocal tumors, conventional imaging along with I^{123}MIBG or 18F-

fluorodeoxyglucose positron emission tomography can be used to detect metastases[22].

The American Joint Committee on Cancer has recently developed a staging criterion for malignant PPGL using the tumor-node-metastasis classification. The classification was based on certain parameters that allow the size of the primary tumor to reliably predict the presence of malignancy and shorter survival[23]. Patients with primary PPGL lager than 5 cm have a shorter overall survival than patients with smaller tumors[23]. Therefore, a pheochromocytoma lesser than 5 cm is stage as T_1. Tumors that are larger than 5 cm are staged T_2. Pheochromocytomas that invade surrounding structures, like the liver or kidney, requiring extensive surgery are staged T_3.

TREATMENT

Treatment for malignant PPGL is palliative; therefore, a shared decision-making is required for the patient's quality-of-life. Concerns are focused on the tumor growth, tumor hypersecretion, or the intervention itself. Bony metastasis to the spine is usually painful, with additional risk of spinal cord compression. Therefore, treatment of pain with nonsteroidal anti-inflammatory, analgesics, bisphosphonates, and radiotherapy are all viable options.

Patients with malignant PPGL may already present with cardiovascular complications from excess catecholamine, like acute adrenergic cardiomyopathy, arrhythmia, myocardial infarction, stroke, and sudden death[22,24]. For patients who may require surgery, preoperative control of blood pressure and adrenergic outflow is crucial to prevent adverse events. The first pharmaceutical agent of choice is an alpha-blocker preferably, either noncompetitive (phenoxybenzamine) or competitive (doxazosin, prazosin, and terazosin)[25,26]. Beta-blockers may be needed to prevent or ameliorate reflex tachycardia or orthostatic hypotension associated with the alpha-blocker. If the pressure is not controlled by an alpha- or beta-blocker, calcium channel inhibitor or metyrosine are alternatives[26]. Liberal salt and fluid intake are encouraged to promote intravascular expansion.

The only definitive treatment for PPGL is surgical resection. Though surgery may not be curative in most scenarios, it offers long-term disease remission in some patients. Patients with malignant PPGL and isolated metastasis can have a better result with surgery. In patients with incurable disease, resection can reduce the catecholamine hypersecretion and avoid complication of tumoral compression on vital organs[21,22]. Accurate preoperative imaging is crucial to assess the vasculature of the tumor. The tumor should be dissected without capsular infraction. It is advised that the veins be ligated first, to prevent excess catecholamine release into circulation. If regional lymph nodes are assessed preoperative or intraoperatively, a regional lymphadenectomy should be performed[21,22].

I^{123} MIBG is frequently being investigated as a treatment option for metastatic PPGL. It is recommended as the first-line treatment in patients with slow-growing I^{123} MIBG-positive metastasis. Treatment can be administered as both fractionated low dose and high dose; both are shown to have considerable efficacy[27]. A systematic review and meta-analysis of 17 studies involving 243 patients with malignant PPGL evaluating the effect of I^{131} MIBG therapy on tumor volume in patients with malignant PPGL showed that the complete response was 3%, partial response was 27% and stable disease was 52%[28]. Separate analysis showed better hormonal response among paraganglioma than pheochromocytoma.

Chemotherapy may have a role in the management of malignant PPGL. The best recommended chemotherapeutic protocol includes cyclophosphamide, vincristine, and dacarbazine (CVD) administered as (cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, and dacarbazine 600 mg/m^2 on day 1 and dacarbazine 600 mg/m^2 on day 2). A systematic review and meta-analysis of four studies, by Niemeijer *et al*[29], using chemotherapy with CVD involving a pool of 50 patients with PPGL revealed varying response of malignant PPGL to CVD[29]. The pooled percentages were 4% for complete response (95%CI: 1%-15%), 37% for partial response (95%CI: 25%-51%) and 14% for stable disease (95%CI: 7%-27%). Sub analysis of two studies also showed varying response of catecholamine excess, with complete response in 14% (95%CI: 6%-30%), partial response in 40% (95%CI: 25%-57%), and stable hormonal response in 20% (95%CI: 10%-36%)[29].

Molecular-targeted therapies, like receptor tyrosine kinase inhibitors, mTORC1 inhibitors, HIF-2α antagonist and SSTR2 analogues, are still investigational[3,7]. Receptor tyrosine kinase inhibitors like unitinib, cabozantinib, axitinib and pazopanib are considered to have potential therapeutic benefits and still part of ongoing clinical

trials[7,9,30].

CONCLUSION

The diagnosis of malignant PPGL is challenging. To date, confirmatory histology and biomarkers are still lacking. The presence of metastasis is still being considered as a reference for malignancy. Data from recent studies have shown various biomarkers and genetic mutations that are predictive of malignancy, metastasis and disease prognosis of PPGL. Biomarkers like galectin-3, COX-2, nm-23, miR-210, ERBB-2 overexpression and SDHB mutation have shown correlation to malignant PPGL and disease prognosis. However, primary tumor size, male sex and synchronous metastasis have revealed consistent association with malignancy PPGL and prognostication.

Most malignant PPGL may not be amenable or curable with surgical excision. As such, radiopharmaceuticals and chemotherapy are alternative treatment options. Other therapeutic options, like molecular-targeted therapy, are still undergoing clinical trials.

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REVIEW

Implications of COVID-19 for inflammatory bowel disease: Opportunities and challenges amidst the pandemic

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Abstract

Since the beginning of the coronavirus disease 2019 (COVID-19) pandemic various measures have been taken to mitigate the effects of the global health crisis in this unprecedented time. According to the World Health Organization, more than 5 million people have been infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and with more than 300000 deaths attributed to COVID-19 worldwide. There is emerging evidence that SARS-CoV-2 utilizes angiotensin-converting enzyme 2 receptors to enter human cells which are found in abundance in the alveoli and intestines. In addition, the infection is noted to be more severe in patients with co-morbid conditions, those who are malnourished, immunosuppressed and immunocompromised. Inflammatory bowel disease (IBD) which includes ulcerative colitis and Crohn's disease is chronic remitting and relapsing disorders with intestinal and extraintestinal manifestation. IBD patients are often malnourished and on immunosuppressive medications and there is a hypothetical concern that IBD patients are at substantial risk of COVID-19 infection. The management of IBD patients is often complex and poses unique challenges for gastroenterologists during the pandemic. The purpose of this review article is to summarize the growing level of evidence and understanding of the management of IBD during the COVID-19 pandemic, in the light of international and national gastroenterology society guidelines. We performed a thorough literature search on IBD, SARS-CoV-2 and COVID-19 on PubMed, EMBASE, OVID Medline and Google Scholar and pertaining literature was critically examined and summarized. Per national and international society guidelines and recommendations, IBD is not a risk factor for SARS-CoV-2 infection. IBD patients should continue with their medications and they should

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follow universal precautions *i.e.* masks, hand and respiratory hygiene and avoidance of health care facilities and public toilets as general population. Among IBD patients older age, having active disease, and co-morbid conditions are risk factors for a severe SARS-CoV-2 infection. Furthermore, elective endoscopic and surgical procedures can be delayed or deferred until discussing the risks and benefits with patients.

Key Words: SARS-CoV-2; COVID-19; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Pandemic

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Core Tip: The current pandemic caused by coronavirus disease 2019 (COVID-19) has resulted in devastating consequences worldwide. This viral infection affecting the respiratory and gastrointestinal systems is the most severe in patients with co-morbid conditions, including the immunocompromised. Patients with inflammatory bowel disease are often malnourished and on immunosuppressive medications; management of this population poses unique challenges during this time. In this review, we summarize the current evidence and understanding of the management of inflammatory bowel disease during the COVID-19 pandemic. We further outline the current guidelines for treatment of those with inflammatory bowel disease and offer education for measures to prevent infections, including COVID-19.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), also known as the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, is a global health emergency with major public health concerns[1]. SARS-COV is a single-stranded RNA virus belonging to the genus Betacoronavirus and subgenus Sarbecoronavirus and recognized to cause severe acute respiratory syndrome for two decades. SARS-CoV is an emerging zoonotic disease that has the potential to infect humans, bats, and other mammals. There are hundreds of strains of the coronaviruses and over the last two decades different strains have caused three major documented epidemics globally, resulting in significant morbidity and mortality[2]. The current pandemic began at the end of 2019 as a cluster of new atypical pneumonia cases from an unknown cause, traced back to a wet animal market in the city of Wuhan, in the Hubei province in China. On December 31, 2019, the outbreak was reported to the WHO regional office in China and was linked to a novel strain of a coronavirus provisionally named as 2019-nCoV[3]. It was later designated as SARS-CoV-2 by the International Committee on Taxonomy of Viruses[4]. The SARS-CoV-2 virus rapidly spread in the entire world. As of October 9, 2020, there have been 36754395 confirmed cases of SARS-CoV-2 have been documented in over 187 countries and territories with a global death toll of 1064838. The first case of SARS-CoV-2 was reported in the United States of America (USA) on January 20, 2020, and since then the number of confirmed cases has been increased dramatically[5]. According to the Centers for Disease Control and Prevention (CDC) report from the USA, the overall cumulative hospitalization rate is 183.2 per 100000, with the highest rates in people 65 years and older (495.5 per 100000) and 50-64 years (273.2 per 100000)[6]. Other high risk groups for severe infection and hospital admission include male gender, smokers, residents at nursing home or long term care facilities, and people of all ages with pre-existing medical illness, *e.g.* hypertension, diabetes mellitus, obesity, chronic lung/liver/heart disease, chronic kidney disease undergoing dialysis, and immunocompromised or immunosuppressed patients[7].

Inflammatory bowel disease (IBD) which includes ulcerative colitis (UC) and

Crohn's disease (CD) is chronic remitting and relapsing disorders with intestinal and extraintestinal manifestation[8]. Globally, there are 6.8 million cases of IBD, and the age-standardized prevalence rate increased from 79.5/100000 population to 84.3/100000 population between 1990-2017[9]. There is evidence from translation research that angiotensin-converting enzyme 2 and TMPRS2 receptors are exhibited in abundance in the ileum and colon[10] which leads to the hypothesis that patients with CD and UC are more at risk of SARS-CoV-2 infection and its complications than the general public. Furthermore, patients with IBD are usually malnourished and often treated with immunomodulators and immunosuppressive medications to control inflammation and to prevent relapse and complications. These medications are known to be associated with opportunistic infections, particularly viral infections such as influenza and herpes zoster, reactivation of chronic hepatitis B with anti-tumor necrosis factors (anti-TNFs), and viral warts associated with the use of thiopurines[11]. In addition, frequent encounters with health care facilities for infusion of the biologics, follow up visits, blood draws and endoscopies put these patients at risk for exposure to SARS-CoV-2 infection. As a result, there is a concern among healthcare providers and patients that having IBD (quiescent or active) and being on medical therapies to control inflammation places them at a substantial risk to contract SAR-CoV-2 infection and progress to a more critical illness requiring intensive care unit (ICU) admission, ventilator use, and have increased mortality compared with the general population. However, there is strong evidence that having inflammatory bowel disease is not a risk factor for SARS-CoV-2 infection[12]. The management of IBD is complex, multifaceted and there are numerous questions and concerns related to IBD and COVID-19. The purpose of this review article is to summarize the growing level of evidence and understanding of the management of IBD during the COVID-19 pandemic, in the light of international and national gastroenterology society guidelines. To fulfil the objectives of the review, we did thorough literature search on PubMed, OVID and EMBASE through December 2019 to October 2020.

PRESENTATION, CHARACTERITICS AND RISK FACTORS OF SEVERE COVID-19 INFECTION IN IBD PATIENTS

The current literature suggests that digestive symptoms such as non-bloody diarrhea, nausea and vomiting are commonly exhibited by COVID-19 patients with a variable frequency of 11%-35%[13,14]. Nobel *et al*[15] published a case control study involving two-hundred and seventy-eight SARS-CoV-2 patients. According to the study results, patients with diarrhea, nausea and vomiting at the time of diagnosis of SARS-CoV-2 were 70% more likely to be tested positive for COVID-19 infection than other patients. Another retrospective cross-sectional study which included 207 consecutive patients with positive SARS-CoV-2 showed that the presence of digestive symptoms was associated with a higher severity of overall illness and the need of hospitalization. However, no significant differences were reported in ICU admissions, ventilator use and mortality in patients with predominantly gastrointestinal symptoms[16]. It is also now well established that there is fecal shedding of the coronavirus as detected by nucleic acid in more than 50% of patients' stool samples and persists even after nasopharyngeal samples becomes negative[17]. Recently, a case of a female patient with COVID-19 infection presenting as bloody diarrhea was published by Carvalho *et al*[18] Her evaluation was significant for leukocytosis, elevated C-reactive protein and evidence of colitis on computed tomography (CT) scan of the abdomen. With the presumed diagnosis of infectious colitis and to rule out inflammatory and ischemic colitis, a flexible sigmoidoscopy was performed which showed erythema without ulceration in the distal colon. Biopsies were negative for infectious colitis, ischemia, or inflammatory bowel disease. On day 4 of hospitalization, SARS-CoV-2 PCR from nasopharyngeal swab turned positive[18]. The clinical implication of these gastrointestinal symptoms is important especially in patients with IBD. Patients with IBD having new or worsening gastrointestinal symptoms with and without respiratory symptoms should be thoroughly evaluated using appropriate testing and non-invasive measure such as inflammatory markers, fecal calprotectin and imaging studies to differentiate acute flare with SARS-CoV-2 infection. In a systematic review published by D'Amico *et al*[19], the prevalence of COVID-19 in IBD cohort was found to be 0.4%. They included 23 studies and found more prevalence of COVID-19 infection in male patient with inflammatory bowel disease regardless of their age[19].

Patients with CD and UC are often immunosuppressed. However, the exact impact of the degree of immunosuppression on the severity of SARS-CoV-2 infection is not

known. It is also not certain if untreated or partially treated IBD increases the risk of getting SARS-CoV-2. Preliminary data of risk and characteristics of corona virus infections in IBD patients from the first epicenter i.e. Wuhan, China is scarce. A study published by An *et al*[20] included 318 patients with IBD registered between Jan 1, 2000, and Dec 8, 2019, in a prospective database were followed during the COVID-19 pandemic. Patients with IBD were contacted and educated about self-prevention measures such as hand hygiene, keeping track of pulmonary symptoms and fever and to seek medical assistance online *vs* in person. In addition, infliximab infusions and other immunosuppressive treatments were discontinued as per guidelines of the National Chinese Society of Gastroenterology[20]. In contrary, clinicians from Italy published their experience of managing seventy-nine patients with IBD who tested positive for COVID-19 from 24 referral IBD units from March 11th to 29th, 2020. Of these patients, 28% of those with IBD and COVID-19 positive were hospitalized and another 6% were diagnosed with COVID-19 infection when they were admitted to the hospital with IBD flares. After controlling for confounding factors, age more than 65 years, diagnosis of UC, IBD activity and Charlson Comorbidity Index > 1 were significantly associated with severe SARS-CoV-2 infection and hospitalization. In this study, the authors found no relation of IBD treatments with COVID-19 infection severity[21].

More recently, an international registry/database was developed called the Surveillance Epidemiology of Coronavirus Under Research Exclusion (SECURE)-IBD to report adult and pediatric SARS-CoV-2 cases and its outcome in terms outpatient management *vs* hospitalization, ICU admission, ventilator use and mortality in IBD patients. As of October 10, 2020, 2575 cases of IBD disease with concurrent COVID-19 patients were reported to the registry worldwide. Preliminary analysis of this database showed that young (under 40 years) IBD patients with COVID-19 infection were more likely to exhibit a milder form of disease and approximately 80% were managed in the outpatient setting. There is a direct correlation of increasing age with more severe pneumonia requiring hospitalization, ICU admission and ventilator use. Approximately 25% of the deaths were reported in the older IBD patient *i.e.* ≥ 80 years which is highest among all age groups. Furthermore, 54% of male IBD and COVID-19-positive patients needed hospital admission, and 5% developed severe respiratory disease as defined by ICU admission, ventilator use or even death. A total of 1447 patients with CD developed COVID-19 infection, and 78% of them were managed in the outpatient; of those with UC and COVID-19-positive, 70% were managed as outpatients. Those with UC were also more likely to develop severe disease, be admitted to the ICU, and require ventilator support. In terms of disease activity, IBD patients in remission who developed COVID-19 infection were more likely to develop a milder form of the viral illness, while those with moderate-to severe activity tended to be hospitalized and require ICU admission. Similarly, having multiple comorbid conditions was shown to be a risk factor for developing severe COVID-19 infection in those with underlying IBD[22].

MANAGEMENT OF IBD MEDICATIONS DURING THE COVID-19 PANDEMIC

The Crohn's and Colitis Foundation of America (CCFA), the European Crohn's and Colitis Organization (ECCO), and the International Organization for the study of Inflammatory Bowel Disease (IOIBD), recommend against discontinuation of immunosuppressive and biological drugs as a preventative strategy in IBD patients without symptoms and signs suggestive of COVID-19 infection[23-25]. In this section, we will discuss the safety of the medications (immunomodulators and immunosuppressive) in stable IBD patients without COVID-19 infection (Table 1).

Sulfasalazine and 5-aminosalicylates

Sulfasalazine and 5-aminosalicylates (5-ASA) are mild immunosuppressive medications and are often used as initial therapy for induction and maintenance of mild to moderate UC[26]. Overall, they are safe, effective and tolerated well. Preliminary data from the SECURE-IBD registry showed about 14% of IBD and COVID-positive patients on sulfasalazine and 5-ASA compounds developed severe infection (ICU/Ventilator/Death)[22]. However, the data should be interpreted with caution as the medication categories were not mutually exclusive. In summary, sulfasalazine and 5-ASA are considered safe even during the COVID-19 pandemic and should be continued.

Table 1 Management of inflammatory bowel disease medications during the Coronavirus disease 2019 pandemic

Medications	Clinical case scenarios		
	Stable IBD without SARS-CoV-2 infection	Quiescent IBD with positive SARS-CoV-2 infection	Active IBD with positive COVID-19 infection (irrespective of severity)
Sulfasalazine and 5-aminosalicylates	Continue therapy	Continue therapy	Continue therapy
Budesonide	Continue therapy	Unclear evidence	Unclear evidence
Oral/Parenteral steroids	Discontinue/Delay therapy	Discontinue/Delay therapy	Discontinue/Delay therapy
Immunomodulators (methotrexate and thiopurines)	Continue therapy	Discontinue/Delay therapy	Discontinue/Delay therapy
Anti-tumor necrosis factor inhibitors (infliximab, adalimumab, certolizumab and golimumab)	Continue therapy	Unclear evidence	Discontinue/Delay therapy
Anti-integrins (vedolizumab)	Continue therapy	Unclear evidence	Unclear evidence
Anti-interleukins (ustekinumab	Continue therapy	Unclear evidence	Discontinue/Delay therapy
Janus kinase inhibitors (tofacitinib)	Continue therapy	Discontinue/Delay therapy	Discontinue/Delay therapy

IBD: Inflammatory bowel disease; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2.

Corticosteroids

Corticosteroids are often used as a first-line treatment in IBD as an initial treatment to induce remission and management of acute flares. Prolonged use of steroids is associated with immunosuppression, opportunistic infections and increased mortality in IBD patients[27]. Due to these immunosuppressive and its anti-inflammatory properties, systemic steroids have been evaluated in multiple randomized clinical trials and found to be promising in the management of severe and critically ill COVID-19 cases[28,29]. There is a recent prospective meta-analysis by the World Health Organization REACT *i.e.*, Rapid Evidence Appraisal for COVID-19 Therapies Working Group. The meta-analysis included data from 7 clinical trials involving more than 1700 COVID-19 patients and concluded that corticosteroids has mortality benefits[30]. Though there is limited information on the use of steroids in COVID-positive IBD patients, the present evidence linked the concurrent steroid use to increased rates of hospitalization and severe COVID-19 infection in IBD patients[22,23].

Immunomodulators

Immunomodulators such as methotrexate and thiopurines (azathioprine and 6-mercaptopurine) are found to be effective as monotherapy and in combination therapy for IBD patients. Though immunomodulators are usually safe, their use has been associated with lymphopenia (which is associated with a poor prognosis in COVID-19 patients) and opportunistic viral infections such as herpes simplex and viral warts[31]. *In vitro* studies suggest that thiopurines have potential antiviral activity against SARS-CoV or MERS-CoV, but these results were not reproduced in humans[32]. Methotrexate and thiopurines are safe and should be continued in stable IBD patients without a COVID-19 infection. According to the SECURE-IBD registry data, methotrexate and thiopurines are associated with a severe SARS-CoV-2 infection in 10%-11% of IBD patients. Gastroenterologists can consider discontinuing immunomo-dulators in IBD patients with suspected and confirmed COVID-19 infections[22].

Biologics

Biologics such as anti-TNF agents (infliximab, adalimumab, certolizumab and golimumab), anti-integrin (vedolizumab) and anti-interleukin-12/23 biologics (ustekinumab) are safe and effective treatment modalities for moderate-to-severe CD and UC[33]. With anti-TNF therapy, there are risks of opportunistic viral infections, reactivation of hepatitis B infection, and latent tuberculosis[34]. According to current recommendations, biologics should be continued in stable IBD patient without COVID-19 infection to prevent acute flares and immunogenicity. However, the clinician can consider holding biological therapy for 2 weeks or more if an IBD patient is exposed to COVID-19 and discontinued if patients have an active COVID-19 infection until the patient clears the infection[35].

Tofacitinib, an oral JAK inhibitor, is approved for the management of UC.

According to expert opinion, tofacitinib should be continued in the lowest possible dose to maintain remission. Patients on tofacitinib in contact with someone positive for COVID-19 should consider temporarily withholding the medication for two weeks. In patients with confirmed COVID-19 infection, consideration for discontinuation of tofacitinib should be made until the patient clears the infection[31].

NON-PHARMACOLOGICAL MEASURES—PREVENTION OF COVID-19 INFECTION IN IBD PATIENTS

Emerging data reveals that patients with IBD are not at higher risk of a COVID-19 infection, and like the general population, should follow universal precautions to prevent infection. Current guidelines, expert opinion, and lessons learned from experience of epicenters such as Wuhan, China, Milan, Italy, New York City, USA and more recently Brazil, highlight the importance of patient education regarding self-prevention measures, minimizing contact with health care facilities through telemedicine, and ensuring continuation of IBD medicines to prevent COVID-19 infection and IBD flare[12,36-38].

Health optimization and malnutrition

Nutrition is an important determinant of health and malnutrition is established to be associated with multiple negative health outcomes in general. This is also true in the case of a COVID-19 infection[39]. Data from China and North America show that COVID-19 infections tend to be more severe (defined as the need for hospitalization, ICU admission, and ventilator use) and with higher mortality in the elderly, patients with underlying health conditions, and the malnourished[40]. Older age and co-morbid conditions are independently associated with sarcopenia (reduced muscle mass) and hypoalbuminemia. Patients with IBD are often malnourished due to their symptoms and medications such as steroids, sulfasalazine and methotrexate[41]. Optimization of nutritional status can prevent severe COVID-19 infections. IBD patients should continue mineral and vitamin supplementation and avoid smoking and foods that can trigger flares[42].

Minimize risk of COVID-19 infection

So far, the best preventive strategy to avoid COVID-19 is by minimizing the risk of exposure to the virus. This can be achieved by following universal precautions and avoiding contact with healthcare facilities.

Reduce the risk of transmission

All IBD patients must be educated about preventive strategies to mitigate the COVID-19 pandemic. According to the WHO guidelines, the general population including patients with IBD should clean their hands with an alcohol-based hand sanitizer or wash with soap and water regularly and thoroughly, maintain social distance of at least 3 feet, avoid going to crowded spaces, public toilets, touching the face/eyes/nose/mouth, cancel non-essential travel, follow good respiratory hygiene (*i.e.*, cover sneeze and cough), and to seek appropriate medical attention if symptoms develop suggestive of SARS-CoV-2 infection[43,44].

Immunization

IBD patients are at risk of vaccine-preventable viral and bacterial infections. They should be encouraged to receive pneumococcal (PCV13 and PPSV 23) and influenza vaccines to prevent co-infections[12].

Avoid exposure to health care facilities-Role of telemedicine services

To prevent spread of the COVID-19 infection, IBD patients and physicians are encouraged to use telemedicine to avoid unnecessary exposure to COVID-19. Telemedicine is defined by the Institute of Medicine as the use of electronic and telecommunications technology to provide and support healthcare when distance separates the participants[45]. Prior to COVID-19, the utilization of telemedicine in North America was limited due to several reasons which included low reimbursements from insurance companies, lack of training, unavailability of the equipment, software constraints, and other technical issues[46]. Telemedicine services are found to be useful for patient monitoring, advice, and education during this unprecedented time and has been adopted by health systems globally[47,48]. Similarly, in

the United States the use of telemedicine services is tremendously expanded due to improved reimbursements from insurance companies and Centers for Medicare and Medicaid Services.

Prevention of COVID-19 at infusion centers

To prevent the risk of flares and immunogenicity, IBD patients should continue with their infusions at scheduled times. However, patients and infusion center management should take measures to reduce the risk of coronavirus transmission. Examples of these measures include implementation of pre-admission screening protocols to assess for the symptoms of SARS-CoV-2 infection, preferably 1-2 days before their scheduled infusions. On the day of infusion, patients should use designated entry points to avoid contact with hospitalized patients[49]. No accompanying persons should be allowed in the infusion center. Patients with IBD should be re-screened for COVID-19 symptoms with temperature measurement on the day of infusion. All patients and staff should wear surgical masks with rearrangement of seats approximately 1.5 m apart[50].

ENDOSCOPY IN IBD PATIENTS DURING THE PANDEMIC

Due to the risk of community transmission from infected aerosols and potential fecal shedding of the SARS-CoV-2, endoscopies should be scheduled in carefully selected patients after weighing the risks and benefits. Currently, only urgent and emergent endoscopic procedures are recommended by the British Society of Gastroenterology (BSG), the IOIBD, the ECCO, and the CCFA to avoid potential exposure of SARS-CoV-2 to health care personnel and patients[23,35,51] (Figure 1). However, it is not clear when to consider endoscopy as urgent or emergent in patients with IBD. Iacucci *et al*[52] published a review articles in *Lancet Gastroenterology and Hepatology* to discuss four different scenarios that could justify the need for endoscopic evaluation (diagnostic and therapeutic) during the pandemic. In the first scenario, the authors discussed a case of a suspected new diagnosis of IBD with possible moderate-to-high degree of inflammation as evident by elevated inflammatory markers and fecal calprotectin. In this particular scenario, flexible sigmoidoscopy or colonoscopy with random biopsies were recommended before initiating biologics. Other indications for urgent endoscopy for IBD patients include acute flare of UC, especially if the last endoscopic procedure was more than 3 months ago, partial bowel obstruction in patients with IBD needing urgent endoscopic decompression or stent placement, and ascending cholangitis or worsening jaundice in patient with primary sclerosing cholangitis and IBD requiring endoscopic retrograde cholangiopancreatography with stent placement[52].

All IBD patients needing endoscopic procedures should be tested for COVID-19. Suspected and confirmed cases should be performed in a negative-pressure room and health care providers should follow standardized precautions including hand hygiene and use of disposable gowns, N95 mask with surgical mask, gloves, and head and shoe covers. In addition, the American and European gastrointestinal societies (ASGE and ESGE) published guidelines for infection control and scope reprocessing during the COVID-19 pandemic. After procedures, the endoscope should be placed in a fully enclosed and labeled container for transportation to the decontamination room. The reprocessing staff should also wear adequate personal protective equipment (PPE) to limit their exposure. Commonly used disinfectant (bactericidal, mycobactericidal, fungicidal and viricidal) can be used to clean scopes after procedures and no special handling instruction are recommended. Meticulous attention should be paid to clean the procedure room after each procedure and at the end of the day[53-55].

SURGICAL MANAGEMENT OF PATIENTS WITH IBD DURING THE COVID-19 PANDEMIC

Over the past decade, significant advances have been made in the medical management of IBD. However, timely surgery remains a vital part in the current multi-modal approach to manage both CD and UC and their complications. According to the estimates about half the patients with UC and up to two-third of people with CD will eventually require surgery[56]. Surgery in IBD patients can be performed electively, *e.g.* to eliminate risk of cancer and management of medical refractory IBD, or emergently, such as in cases of intestinal perforation, toxic megacolon, strictures, fistulas and abscesses[57].

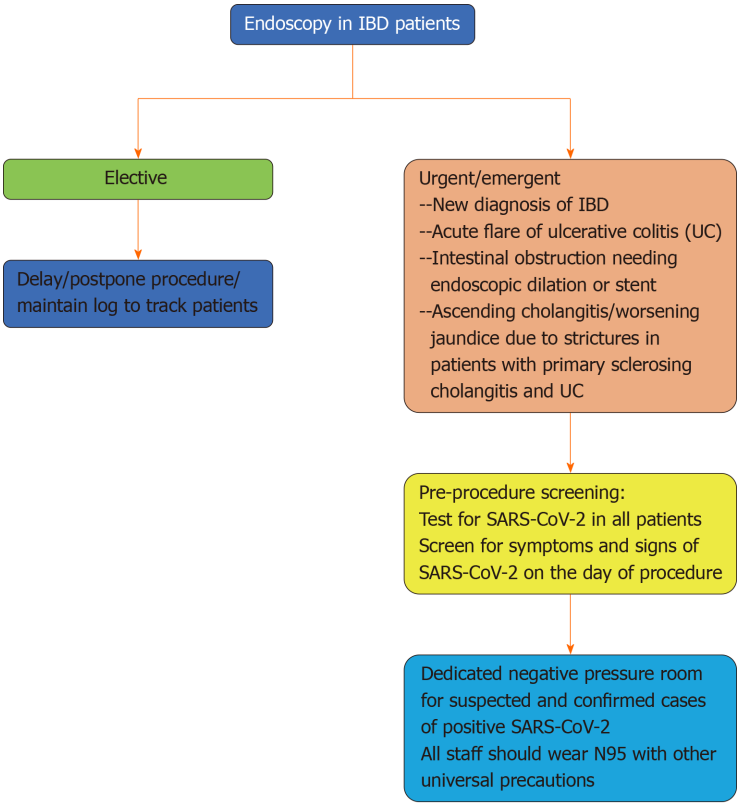


Figure 1 Simplified algorithm for endoscopic management of patients with inflammatory bowel disease during the coronavirus disease 2019 pandemic. IBD: Inflammatory bowel disease; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2.

Surgical management of IBD poses unique challenge during the current pandemic, especially in the timing of the surgery. Poor outcomes after elective surgery in COVID-19 patients were reported in a study published by Lei *et al*[58]. In this retrospective analysis, 34 patients who underwent unintentional elective surgeries during the incubation period of COVID-19 were included. All patients developed pneumonia from SARS-CoV-2 infection shortly after their procedure, approximately 44% of patients needed ICU admission, and 20.5% of patients died from complications such as adult respiratory distress syndromes, arrythmias and cardiac arrest. Patients with active IBD or in remission are susceptible to COVID-19 and the possibility of increased post-operative morbidity and mortality is an important factor to consider for scheduling non-emergent and elective procedures. In addition, there is always a risk of staff exposure from suspected or confirmed COVID-19 patients during the peri-operative period and nosocomial transmission to other patients[59]. Therefore, the CCFA, ECCO, IOIBD, and BSG recommend reducing contact in the healthcare settings by delaying elective non-essential endoscopies and surgical procedures to reduce possible exposure to SARS-CoV-2 infection and to conserve scarce resources in this time of uncertainty[23,35,51]. However, delays in IBD surgeries caused by COVID-19 pandemic can exert tremendous pressure on an already strained healthcare system, harm to IBD patients, and causes anxiety for patients and families.

In April 2020, the IOIBD and American Society of Colon and Rectal Surgeons developed expert-based recommendations for the surgical management of IBD patients during the COVID-19 pandemic. According to the recommendations, the peri-operative considerations includes testing all IBD patients requiring surgery for SARS-CoV-2 preoperatively due to the high risk of transmission from asymptomatic and pre-symptomatic COVID-19 infected patients and carriers. In addition, the experts recommend practicing universal precautions peri-operatively due to questionable reliability of the test, wide variations in the sensitivity and specificity resulting in high false positives and shedding of the virus through aerosol and fecal-oral route in asymptomatic and pre-symptomatic patients. To prevent nosocomial transmission, all staff should wear PPE such as N95 masks covered by surgical masks and plastic face shields. The surgeries should be performed in negative-pressure rooms on suspected and confirmed COVID-19 patients with IBD[60] (Figure 2).

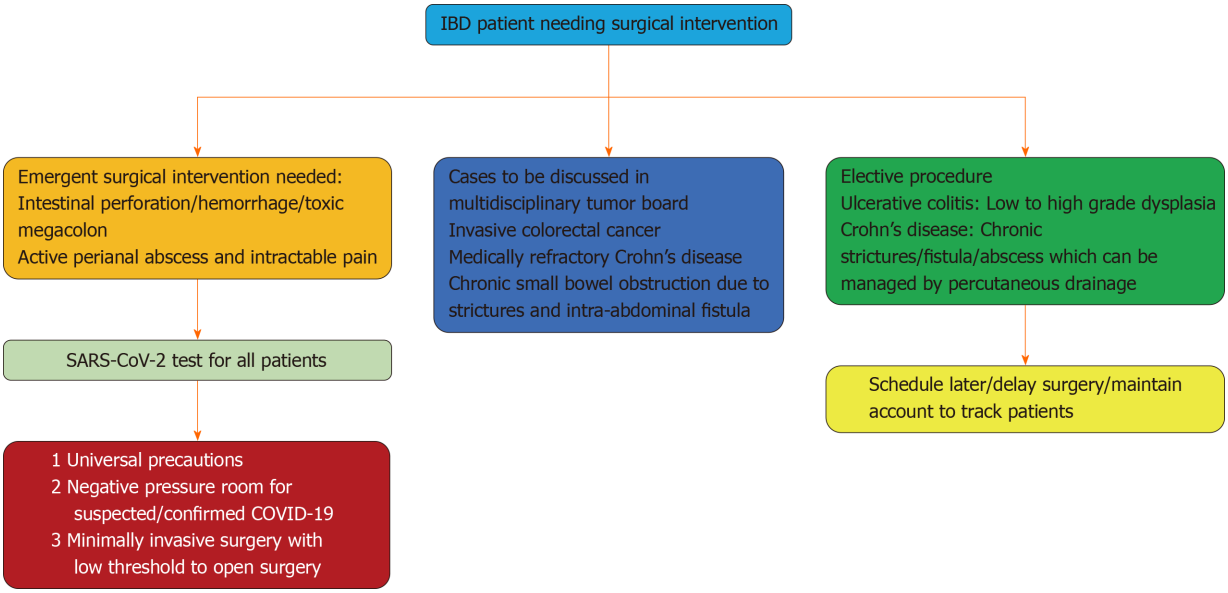


Figure 2 Simplified algorithm for surgical management of patients with inflammatory bowel disease during the coronavirus disease 2019 pandemic. IBD: Inflammatory bowel disease; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2.

RECOMMENDATIONS FOR SURGICAL MANAGEMENT OF CD

Patients with CD often need operative interventions to alleviate symptoms of severe inflammation in the intestine, treat complications such as intestinal hemorrhage, perforation, abscess formation, fistulas, strictures and to improve quality of life. Per experts' recommendations, indications for emergent surgical interventions in patient with CD during the COVID-19 pandemic includes medically unstable patient with active intestinal hemorrhage not amenable to or failure to controlled by angiography, acute small bowel obstruction, perforation, peritonitis or abscess not responding to percutaneous drainage and antibiotics. Small bowel obstruction and contained perforation in otherwise stable patients can be managed non-operatively and surgery is reserved for people who fail to respond conservative treatment. Invasive small bowel and colon cancer should be discussed in tumor board and scheduled for procedure promptly. Cases of medically refractory CD, chronic small bowel obstruction due to strictures, and intra-abdominal fistula should be discussed in multi-disciplinary team meetings and should prioritize these patients once surgical slots become available. Regarding perianal CD patient with active perianal abscess, intractable pain, and sepsis not responding to minimally invasive surgical methods and broad-spectrum antibiotics should also be scheduled for fecal diversion surgery urgently.

RECOMMENDATIONS FOR SURGICAL MANAGEMENT OF UC

Medical refractory disease and complications of severe UC such as intestinal hemorrhage, perforation and toxic megacolon are indications for emergent laparotomy and surgery should not be delayed. Invasive colorectal cancer with and without metastatic disease should be discussed in multi-disciplinary tumor board meetings and should be treated promptly. Surgeries for patients with UC with low-grade to high-grade dysplasia may be delayed in the short term and surgeons should maintain records of the deferred procedures to schedule them once slots are available[61].

IBD PATIENTS IN THE CLINICAL TRIALS

The purpose of the IBD clinical trial is to find the most effective and safest treatment. Currently there are several IBD clinical trials in process globally with hundreds of patients enrolled in them. The COVID-19 pandemic has made overall negative impacts on clinical trials due to disruptions, social distancing policies, travel limitations and

delays in the supply of investigational medications. According to the CCFA, IBD patients currently enrolled in clinical trials should contact clinical research staff for updates regarding any changes to trials through emails or phone calls and try to delay the follow up visits and arrange for home drug delivery.

GUIDELINE TO MANAGING PREGNANT IBD PATIENTS DURING THE PANDEMIC

In non-pandemic times, the management of IBD can be broken down into three phases: Pre-conception, pregnancy period, and post-partum. Prior to conception, IBD care should focus on disease and medication management with the goals to maintain the patient in remission and maintaining medications that are safe in the event a patient becomes pregnant[62]. Medications for IBD are thought to be safe in pregnancy with the exception of methotrexate and thalidomide which are category X[64]. If a patient is on methotrexate, the medication must be stopped at least 3 months in advance before conception, while biologics and thiopurines are lower risk and can be continued during pregnancy. During pregnancy, patients should follow closely with their gastroenterologist and have monitoring for symptoms every two weeks. If there is a concern of a flare, diagnostic work-up includes labs, imaging, and endoscopy studies or surgery if severely toxic. Medication management is similar to non-pregnant women with the exception of usage of methotrexate, tofacitinib, and thiopurine usage in thiopurine-naïve patients[62].

During these pandemic times, there have been reports of vertical transmission of COVID-19 from an infected mother to the newborn and neonates with COVID-19 have had a range of outcomes[65,66]. Currently, there are no studies that have focused on the outcomes of pregnant women with IBD who have become infected with COVID-19 regarding IBD activity, pregnancy course, and neonatal health. The CCFA recommends continuing current IBD management with no changes in the setting of the pandemic and to work closely with gastroenterologists. Rosen *et al*[66] reviewed the management of acute severe UC in pregnant women with SARS-CoV-2. They describe a case of young woman who tested positive for COVID-19 with an UC flare and respiratory symptoms that was managed with intravenous cyclosporin 2 mg/kg since she was unable to transition to oral prednisone and azithromycin and hydroxychloroquine. Unfortunately, she had a spontaneous abortion during her hospital course[67]. There is no known optimal management of IBD pregnant women in this pandemic, but given current recommendations, the usage of biologics or cyclosporin may be used for salvage therapy while restricting steroid usage.

MENTAL HEALTH MANAGEMENT IN IBD PATIENTS DURING THE COVID-19 PANDEMIC

The mental health and stress from the outbreak of COVID-19 can affect people of all ages. In those living with IBD, the severity of medical disease can greatly affect their mental health with the added stress of the COVID-19 pandemic. The CDC has recommended a range of therapies to cope with the stress of the pandemic including taking breaks from news stories, taking care of the body with breathing exercises, stretching, healthy eating habits, regular exercise, good sleep hygiene, and to avoid substances such as alcohol and drugs. They also recommend making time to unwind and to take time to connect with others[68]. In Italy, Sani *et al*[68] described that with protracted social isolation there will be concerns of an increase of disorders such as anxiety, mood, additive and thought disorders in patients' mental health during and after the COVID-19 pandemic. Furthermore, the authors recommended active ongoing participation of mental health professionals during this critical period[69].

In IBD patients, there are no specific guidelines on the optimal management of mental health. However, the CCFA recommend the same guidelines as the CDC with turning off media and news updates, setting a schedule, regular stress management, connecting with family and friends, enforcing a daily routine, and making use of meeting with mental health professionals[70]. Additionally, patients with IBD should find support groups either locally or through online resources.

MANAGEMENT OF IBD PATIENTS WITH COVID-19

Recommendations for the management of IBD patients without a concern for an active COVID-19 infection have been to maintain medications if IBD disease activity is stable[72]. Additionally, patients should maintain these medications to avoid relapse and its consequences[35]. However, it is unclear the appropriate management of SARS-CoV-2 positive IBD patients. Management of these patients can be divided into those with stable IBD disease or acute flares and further divided to patients with COVID-19 that are asymptomatic and symptomatic (Figure 3). Regardless of COVID-19 positivity, patients should adhere to strict social distancing per their local recommendations.

Quiescent IBD patient with active COVID-19

Since rapid testing has been increasing, patients with no or minimal symptoms may test positive for COVID-19. An expert commentary by Rubin *et al*[23] with the support of IOIBD stated that asymptomatic COVID-19-positive IBD patients should have their medications regimens changed to monitor for the development of symptoms. Specifically, monoclonal antibody therapies such as anti-TNF, ustekinumab, or vedolizumab held for 14 days with monitoring of COVID-19 symptoms and recommend reinitiating therapy after two weeks if these symptoms do not develop. Immunomodulators such as thiopurines, methotrexate, and tofacitinib should be held. However, stopping 5-aminosalicylates in patient who tested positive was felt to be inappropriate on the most recent survey by the IOIBD[23].

The IOIBD has different recommendations for IBD patients who develop symptomatic COVID-19. First off, survey participants of the IOIBD felt that it was inappropriate to hold 5-aminosalicylates and therefore were considered safe to continue. Monoclonal antibody therapy (such as anti-TNF and ustekinumab), immunomodulators (thiopurines, methotrexate, and tofacitinib), and corticosteroids (prednisone greater than 20 mg per day) should be discontinued. It is uncertain whether vedolizumab or non-systemic corticosteroids (such as budesonide) should be held in setting of COVID-19[23]. There is no consensus on the timing of recommencement once infection occurred, but one recommendation by Al-Ani *et al*[12] stated to reinitiate IBD therapy once infection is cleared defined by two negative nasopharyngeal swabs 24 hours apart at least 8 days after symptoms or waiting at least 14 days after symptom onset and clinical improvement with resolution of fever for at least three days.

Acute flare with active COVID-19

Exacerbations of IBD have been rare, less than 10 percent, during the pandemic; however, patients who develop flares may require endoscopic evaluation[72]. Given the concern of fecal-oral transmission with the detection of COVID-19 in stool specimens, there is a risk of transmission from patient to health-care provider[73,74]. Therefore, it is critical to ensure patients be tested or screened for COVID-19 prior to considering endoscopic evaluation or therapy.

In patients suspicious for flares, *i.e.* those with new abdominal pain, diarrhea, and fever, and COVID-19, treatment and evaluation should focus on evaluating gastroenterology symptoms which include ruling out enteric infections and confirming inflammation (with inflammatory markers such as fecal calprotectin or cross-sectional imaging like CT or MR enterography). To note, COVID-19 can illicit an inflammatory response on the gut therefore elevation of fecal calprotectin must be cautiously used to diagnose a flare and a threshold of < 100 mg/g should be used to rule out active inflammation[74]. If confirmed relapse, treatment focuses on the severity of flare and COVID-19. For mild COVID symptoms, flares should be treated as done as per usual. In more severe COVID symptoms, the risk-benefits must be weighed on starting IBD therapies, increasing therapy, or holding IBD therapy[35]. Steroids, specifically prednisone, should be decreased to the lowest possible dose (less than 20 mg per day) in non-severe cases as they are found to be associated with poor outcomes in SECURE IBD study and by expert opinion by Japan IBD COVID-19 task force[75,76]. However, patient with severe and progressive COVID-19 infection with requirement of mechanical ventilation current recommendations are to continue with IV steroids[30]. There is limited evidence of medications on COVID-19 and the risk of infection. The BSG reported that IBD medications including all classes of immunomodulators and biologics are safe with no added risk of SARS-CoV-2 infection[77]. However, with medications being trialed for the treatment of COVID-19, caution should be advised in IBD patients to avoid drug-drug interactions[12].



Figure 3 Simplified algorithm for management of patients with inflammatory bowel disease during the coronavirus disease 2019 pandemic. IBD: Inflammatory bowel disease; COVID-19: Coronavirus disease 2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2.

A well-known anti-malarial drug and treatment for rheumatoid arthritis and lupus that was used in the early stages of the pandemic was hydroxychloroquine/chloroquine. This is an immunomodulator against cytokines that has interactions with other immunomodulators, such as methotrexate, azathioprine, and tofacinib. Concurrent chloroquine usage with thiopurines or methotrexate can have an increased risk of myelosuppression; therefore, it is recommended to stop thiopurines and methotrexate in the event of chloroquine usage. Furthermore, chloroquine may increase tofacitinib levels; however, the clinically significant of this is unknown[12]. Overall, hydroxychloroquine/chloroquine has quickly fallen out of favor given the cardiac complications[78].

Remdesivir, a nucleoside prodrug, has antiviral properties a broad spectrum of

viruses[79]. There has been no medication interaction reported with concurrent usage of remdesivir with corticosteroids, thiopurines, methotrexate, tofacitinib, biologics or 5-ASA[12].

Kaletra (Lopinavir/Ritonvair) is a protease inhibitor used for the treatment of human immunodeficiency virus. A randomized controlled open label trial involving a total of one hundred and ninety-nine patients with SARS-CoV-2 underwent randomization and ninety-nine patients received lopinavir-ritonavir. Results of the trial showed no benefits of lopinavir-ritonavir as compared to standard care[80]. However, interactions have been documented with steroids and tofacitinib, causing increased levels of both latter drugs. Furthermore, tofacitinib should be stopped if necessary. No interactions were noted with thiopurines, methotrexate, 5-ASA, or biologics[12].

POST COVID-19 INFECTION CARE IN IBD PATIENT

While the COVID-19 pandemic continues to evolve, there is speculation on care in the post-pandemic period. As stated earlier, IBD therapy resumption is thought to be safe once the COVID infection is cleared, defined by negative testing over an 8 days period or improving symptoms after 14 days of symptom onset without repeat COVID testing[12] and/or after 2 nasopharyngeal PCR tests are negative. However, for post-infection endoscopy, there are numerous barriers including the concern of fecal-oral transmission, prevention of outbreaks, and finally the waitlist from cancelled procedures[52,73]. Recommendations from experts including Iacucci *et al*[52] suggest prioritizing patients according to symptoms, lab testing, medications, surgical history, and cancer risk.

CONCLUSION

Management of IBD is unique and challenging especially during this unprecedented time. There is always a concern of increased risk of SARS-CoV-2 among IBD patients due to nature of the disease and use of immunosuppressive medications. However, it is clear from current guidelines and expert opinion that the risk of SARS-CoV-2 infection in IBD patients is similar to that of the general population. Hence, those with IBD should continue immunomodulators and immunosuppressive agents except corticosteroids to prevent risk of flare and development of immunogenicity. Data from epicenters such as Milan and New York City, as well as the SECURE-IBD registry, show that older age, active IBD, co-morbid conditions and use of steroids are associated with risk of severe COVID-19 infection among IBD patients. Similarly, predictors of poor outcome of COVID-19 infection have been well documented in immunocompromised patients in the general population. Considering the current literature, gastroenterologists should focus on educating their IBD patients for measures to prevent infection such as universal precautions, use of telemedicine and deferring non-elective surgical and endoscopic procedures.

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META-ANALYSIS

Comparing the incidence of major cardiovascular events and severe microvascular complications in patients with type 2 diabetes mellitus: A systematic review and meta-analysis

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Abstract

BACKGROUND

Type 2 diabetes mellitus (T2DM) causes both macrovascular and microvascular complications. However, currently, selection of glycemic measures and their thresholds to diagnose T2DM, and efficacy outcomes in evaluation of anti-diabetic drugs is predominantly informed by the relation of T2DM to microvascular complications. We can be severely mistaken on T2DM by neglecting macrovascular complications which are generally more severe, if they also occur more commonly than microvascular complications.

AIM

To compare the incidence of major cardiovascular events (MACEs) and severe microvascular complications (SMICs) in T2DM patients.

METHODS

MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials were searched from inception to September 2017. Cohort studies or trials of T2DM patients aged 18 years or older that reported incidence of both MACEs and SMICs were included. MACEs were defined as nonfatal myocardial infarction and stroke, and cardiovascular death, while SMICs included serious retinopathy, nephropathy and diabetic disorder. The relative risk (RR) was estimated as the incidence of MACEs divided by that of SMICs in same patients and combined

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with meta-analysis in a random-effect model.

RESULTS
Twelve studies with a total of 16 cohorts and 387376 patients were included, and the combined RR was 2.02 (95%CI: 1.46–2.79). The higher incidence of MACEs remained in various subgroup and sensitivity analyses.

CONCLUSION
Patients with T2DM are much more likely to develop MACEs than SMICs. By taking more serious consequences and relatively higher incidence into consideration, macrovascular complications deserve more emphasis in developing the diagnostic criteria of T2DM and in evaluating the efficacy of anti-diabetic drugs.

Key Words: Diabetes mellitus; Diabetic complications; Cardiovascular disease; Diabetic retinopathy; Diagnostic criteria; Anti-diabetic drugs

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Core Tip: Microvascular complications currently predominate the definition and treatment evaluation of type 2 diabetes mellitus (T2DM). We can be severely mistaken by neglecting macrovascular complications which are generally more severe, if they also occur more commonly than microvascular complications. This systematic review and meta-analysis shows that major cardiovascular events are twice as common as severe microvascular complications in T2DM patients, suggesting that macrovascular complications should be emphasized over microvascular complications in developing the diagnostic criteria of T2DM and in evaluating the efficacy of anti-diabetic drugs.

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INTRODUCTION

Since 1980, the number of patients with diabetes mellitus has nearly quadrupled to 422 million in 2014 worldwide[1]. Type 2 diabetes mellitus (T2DM), accounting for over 90% of all diabetes cases, can cause both macrovascular (mainly cardiovascular disease) and microvascular (*e.g.*, retinopathy, neuropathy, diabetic foot) complications[2,3]. Hyperglycemia causes microvascular complications by activating the protein kinase C, resulting in abnormal microvasculature characterized by endothelial dysfunction, smooth muscle cell proliferation, increased vascular permeability and angiogenesis[4,5]. In addition, oxidative stress, advanced glycation end products and chronic low-grade inflammation also play important roles in the pathogenesis[6-8]. Microvascular complications are not limited to those occurring in the retina, kidneys, and nerves, but may also affect other organs[9]. For example, diabetes mellitus can cause coronary microvascular dysfunction, which reduces the coronary flow serve and leads to the failure of a normal functioning microvasculature[10].

Despite the potential damage to multiple organs caused by microvascular complications, they are generally less serious than macrovascular complications in terms of the number of deaths, health care cost and decreased quality of life they cause in T2DM patients[11-13]. However, for some historical reasons, macrovascular complications have been less considered in developing the definition of T2DM (*e.g.*, which glycemic measures and which diagnostic thresholds to use) which is largely dominated by concerns about microvascular complications, in particular retinopathy[14-17].

Moreover, the efficacy of anti-diabetic drugs is also evaluated according to their

effects on microvascular complications[18]. Macrovascular complications, if included, are often considered as safety indicators or adverse effects of anti-diabetic drugs[19,20]. However, it is indeed observed that some glucose-lowering drugs which can reduce the risk of microvascular complications increase the risk of macrovascular complications in T2DM patients[21,22]. Consequently, trials based only on microvascular complications could mistakenly endorse drugs that can reduce microvascular complications at the cost of increasing macrovascular complications.

If severe macrovascular complications occur as commonly as or more commonly than severe microvascular complications (SMICs) in patients with T2DM, the diagnostic criteria, evaluation of anti-diabetic drugs and practice guidelines may have to be reviewed by considering more about macrovascular complications. We thus conducted this systematic review and meta-analysis to compare the incidence of major cardiovascular events (MACEs) and SMICs in T2DM patients.

MATERIALS AND METHODS

This systematic review and meta-analysis summarized direct comparisons of the incidence of MACEs with that of SMICs in the same patients with T2DM in cohort studies or clinical trials. The PRISMA guidelines were followed in this review[23]. This review has not been registered.

Literature search

We searched MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials from inception to September 2017, with no language restriction. Terms for cardiovascular disease, microvascular disease, T2DM, and study design (cohort and trials) were used to identify potentially relevant studies. Detailed search strategies are included in Supplementary Tables 1 and 2. We also manually reviewed the reference lists of eligible articles and important reviews on the topic to look for additional studies.

Study selection

After initial scan of titles and abstracts of cohort studies and trials, full-texts of potentially included studies were further reviewed for their eligibility. A study was deemed eligible if its sample size was > 1000, included patients with T2DM aged ≥ 18 years, and reported absolute number of cases or incidence for both MACEs and SMICs. Although one thousand is an arbitrary cutoff, studies with fewer than 1000 participants would have too few microvascular events to allow for reliable estimation of the incidence of SMICs and add little to the included studies. These studies were thus excluded. We also excluded studies conducted in special subgroups of diabetic patients, such as those wholly with cancer or cardiovascular disease and those receiving dialysis at baseline.

In this systematic review, no matter for the MACEs and SMICs, we included the mainly serious types of macrovascular and microvascular complications respectively. MACEs were generally defined as nonfatal myocardial infarction and stroke, and cardiovascular death. SMICs, for which there was no standardized definition, were defined as serious retinopathy, nephropathy, and foot disorder. The reason for choosing these complications as SMICs was that they represented three serious types of microvascular complications, which have the equal severity with MACEs. Serious retinopathy included severe loss of vision, blindness, or laser therapy for retinopathy[24-26]. Serious nephropathy included renal failure, dialysis, end-stage renal disease or renal death[27]. Serious foot disorder included limb amputation or death from peripheral artery disease[3]. Noticeably, by taking retinopathy which requires laser therapy and amputation as SMICs, we slightly over-estimated the incidence of SMICs, which could lead to a more conservative estimate of the risk of MACEs relative to SMICs.

It takes a long time to have all these complications occur in T2DM patients and some studies may have no event for some complications, particularly for severe microvascular ones. Thus, we also included studies that lacked data on only one type of event just to ensure having enough eligible studies in this review.

Study selection was completed independently by three researchers (Zhu YY, Li P, Huang XY). The disagreements were settled by discussions with a more experienced reviewer (Yang ZY).

Data extraction

The following data were extracted from each eligible study: Bibliographic information (*e.g.*, first author, publication year, study location), baseline characteristics (*e.g.*, number of participants, mean age, sex, and diabetes duration), length of follow-up and outcomes including information on definition of MACEs and SMICs, number of people at risk, number or cumulative incidence of each outcome event).

For multiple reports of the same study, we extracted data from the report with relatively longer follow-up and more complete information. For clinical trials, data in the intervention and control groups were extracted separately and considered as two separate cohorts in the data analysis.

Data extraction was completed by three researchers (Zhu YY, Li P, Huang XY). This process was double-checked by another reviewer (Yang ZY). The disagreements were settled by discussion.

Quality assessment

Methodological quality of included studies was assessed by Zhu YY, using a 5-item scale adapted from the Institute of Health Economics and the Newcastle–Ottawa Quality Assessment Scale (Supplementary Table 3)[28,29]. The scale evaluated each study based on the population (1 items), outcome measurement (1 item), results (2 item), competing interests and source of support (1 item). For each assessed study, 1 point was assigned to each item if the criterion was met, and a score of 3.5 or more suggests high quality.

Data synthesis and analysis

In each study, the incidence was estimated as the number of MACEs and SMICs divided by the number of participants at risk respectively. Because some studies reported different numbers of people at risk for different types of SMICs or MACEs, the smallest number of people at risk for SMICs was used as denominator, and the largest was used for MACEs in order to avoid over-estimation of the macrovascular over microvascular complication ratio. In occasions where data on a particular type of MACEs or SMICs was missing, the average incidence estimated from all other studies was used to make it up in the analyses.

In each study, the relative risk (RR) was the ratio of the incidence of MACEs over that of SMICs. The random effect model was used in meta-analysis to estimate the overall RR and its 95%CI. Heterogeneity was evaluated using Cochran's Q test and I^2 statistics[30]. Heterogeneity was judged present if the P value ≤ 0.10 for Cochran's Q or an I^2 statistic ≥ 50%. Begg's funnel plot and Egger's test were used to test the potential publication bias[31]. Previous studies have found age, follow-up time, and diabetes duration are associated with macrovascular and microvascular events in patients with T2DM[32]. Besides, different types of study (cohort *vs* trial) were included in our review. In theory, the drugs tested in trials may affect the incidence of complications, thus affecting the risk ratio (although the result of subgroup analysis showed no significant difference). Therefore, subgroup analyses were conducted according to study design, age at baseline, follow-up period, duration of diabetes to investigate the potential sources of heterogeneity. We planned to do the multivariate meta-regression analysis incorporating all factors mentioned above, but did not actually do it because of the limited number of eligible studies (< 10 times the numbers of factors) as recommended by Cochrane Handbook.

Sensitivity analyses were conducted by excluding studies with missing data on a particular type of SMICs, by excluding those including laser photocoagulation therapy as serious retinopathy, and by combining the data in intervention and control groups from a trial into one cohort rather than taking them as two. A two-tailed significance level of 0.05 was used for all the statistical tests, except for Q tests of heterogeneity for which 0.10 was used. Analyses were performed using RevMan 5.3 and STATA 14. The statistical methods of this study were reviewed by Dr. Jean H. Kim from the Chinese University of Hong Kong.

RESULTS

We identified a total of 52577 references from the electronic databases. Figure 1 shows the flow chart of selection process. Finally, 12 studies (eight cohort studies[33-40] and four clinical trials[41-45]) were eligible for this review. The incidence of MACEs and that of SMICs in the ACCORD study were extracted from two different papers[42,43].

The characteristics of the included studies are summarized in Table 1. Among four

Table 1 Baseline characteristics of eligible studies

Characteristics	ACCORD[42,43]	ADVANCE[45]	Bethel *et al*[33], 2007	Cheng *et al*[35], 2015	Gordon *et al*[36], 2017	Hansen *et al*[34], 2013	Johnson *et al*[37], 2013	Kanaya *et al*[38], 2011	Leal *et al*[39], 2013	Marre *et al*[44], 2004	Martin *et al*[40], 2007	VADT[41]
Study design	Trial	Trial	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Cohort	Trial	Cohort	Trial
Treatment	Intensive *vs* Standard	Intensive *vs* Standard	/	/	/	/	/	/	/	Ramipril *vs* Placebo		Intensive *vs* Standard
Country/Territory	USA	Multiple	USA	Taiwan	UK	Denmark	USA	USA	UK	Multiple	German	USA
No. of Participants	10251	11140	33772	226310	10484	1381	15951	64211	4031	4912	3142	1791
Female (%)	39	42	58	51	46	47	59	47	43	30	51	3
Age (yr)	62	66	74	59	73	65	51	60	62	65	63	60
BMI (kg/m^2)	32	28	-	25.8	31	-	-	-	29	-	30	31
Diabetes duration (yr)	10	7	0	5.6	6.0	0	-	9.8	11	9.8	0	11.5
Length of follow-up (yr)	3.5	5	10	6.7	2.4	19	1.6	7.2	8.41	3.9	6.5	5.6
Current smoking (%)	14	14	-	-	14	35	26	12	20	15	-	17
Drinking alcohol (%)	-	-	-	-	-	-	-	-	-	46	-	-
FPG (mmol/L)	9.7	8.5	-	-	-	13.7	-	-	-	9.8	9.2	11.4
HbA1c (%)	8.3	7.5	-	8.5	8.25	10.2	-	-	8.2	7.8	7.6	9.4
SBP (mmHg)	136	145	-	134	136	148	-	-	139	145	149	132
DBP (mmHg)	75	81	-	80	75	85	-	-	-	82	87	76
Prevalence of MaC (%)[1]	35	32	0.88	0	12.5	-	15	-	6.4	10.2	22.4	40
Prevalence of MiC (%)[1]	39	10	0.78	0	13.5	33	17	14	3.6	73.8	-	8

[1]The highest prevalence was chosen if more than one macrovascular or microvascular complications were reported. /: not applicable; -: No relevant data; BMI: Body mass index; FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MaC: Macrovascular complications; MiC: microvascular complications.

clinical trials, three (ACCORD[43], ADVANCE[45], and VADT[41]) were conducted to assess the effect of the intensive glucose lowering treatment (*vs* standard treatment) and Marre *et al*[44] study for the effect of ramipril (*vs* placebo) on macrovascular and microvascular complications. Totally, 387376 patients with T2DM (50% males) were

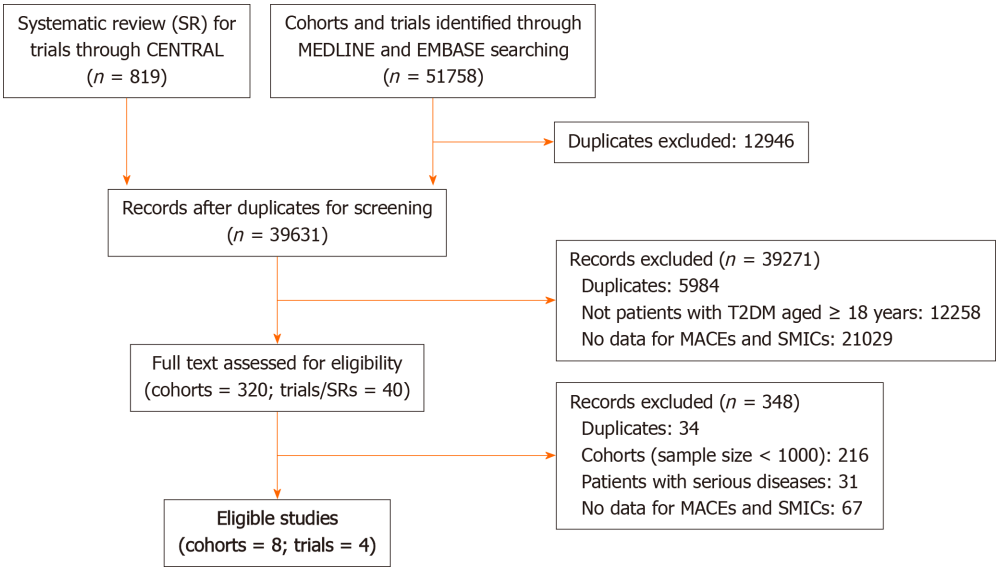


Figure 1 The flow chart of search and selection of eligible studies. T2DM: Type 2 diabetes mellitus; SMICs: Severe microvascular complications; MACEs: Major cardiovascular events.

included in this review. The patients had a mean age of 61 (range: 51-74) years old, mean duration of diabetes of 6 (range: 0-11.5) years, and mean HbA1c level of 8.4% (range: 7.5%-10.2%). The patients were followed up for a mean duration of 6.6 (range: 1.6-19) years. The details about definitions and numbers of MACEs and SMICs reported in each eligible study are shown in Supplementary Tables 4 and 5 separately. In the 12 eligible studies, two studies (ACCORD[43] and ADVANCE[45]) did not report data on serious diabetic foot and one (Kanaya *et al*[38]) on serious retinopathy. No missing data were found on MACEs.

Among the 12 eligible studies, participants from five studies (Bethel *et al*[33], Cheng *et al*[35], Hansen *et al*[34], Kanaya *et al*[38], and Martin *et al*[40]) were considered representative of the general patients with T2DM. Except Cheng *et al*[35]'s study, all studies did not exclude those with known macrovascular or microvascular complications at baseline. The studies' scores ranging from two to five. As a result, six studies[33-35,38,41,43] were considered methodologically as high quality (≥ 3.5). Detailed results are shown in Supplementary Table 6.

Incidence data from the intervention and control arms of clinical trials were considered as two separate cohorts in the data analysis. Therefore, 16 cohorts (eight cohort studies plus eight cohorts from four clinical trials) were finally included in the meta-analyses. Figure 2 summarizes the incidence of MACEs relative to that of SMICs in patients with T2DM in each cohort. The pooled RR for all 16 cohorts is 2.02 (95%CI: 1.46–2.79). Substantial heterogeneity was found (I^2 = 99%). The funnel plot (Supplementary Figure 1) was visually and statistically symmetrical (Egger's test t = -1.46, P = 0.167), suggesting no evidence of publication bias in the included studies. Results of subgroup analyses according to age, study design, length of follow-up and duration of diabetes are shown in Table 2. The incidence of MACEs was consistently higher than that of SMICs in all subgroups. However, study design is statistically significantly associated with the RR. The summarized RR is higher in cohort studies than in clinical trials. Study design explained 43.7% of the overall heterogeneity.

The RRs became even larger in all sensitivity analyses. After excluding five cohorts from three studies (Kanaya *et al*[38], ACCORD[43], and ADVANCE[45]) that had missing data on a particular type of SMICs, the combined RR was even larger and remained statistically significant (RR = 2.66, 95%CI: 1.85-3.81). As anticipated, the RR was also increased considerably (RR = 2.26, 95%CI: 1.67-3.06) after excluding six cohorts from four studies (Cheng *et al*[35], Hansen *et al*[34], VADT[41], and ADVANCE[45]) that included laser photocoagulation as severe retinopathy. Besides, combining the intervention and control cohorts in each trial[41-45] into one cohort also made the combined RR larger (RR = 2.22, 95%CI: 1.53-3.24).

Table 2 The risk of major cardiovascular events to that of severe microvascular complications: Subgroup analysis

Subgroup	Relative risk	95%CI	I^2 (%)	*P* difference
Age (yr)			0	0.45
< 65	1.83	1.17-2.84		
≥ 65	2.34	1.46-3.75		
Study design			80.3	0.02
Cohort	2.67	1.84-3.87		
Trial	1.48	1.04-2.11		
Length of follow-up (yr)			3.1	0.31
≤ 5	1.74	1.19-2.53		
> 5	2.30	1.56-3.39		
Diabetes duration (yr)			0	0.75
≤ 9	2.19	1.46-2.87		
> 9	1.93	1.27-2.91		

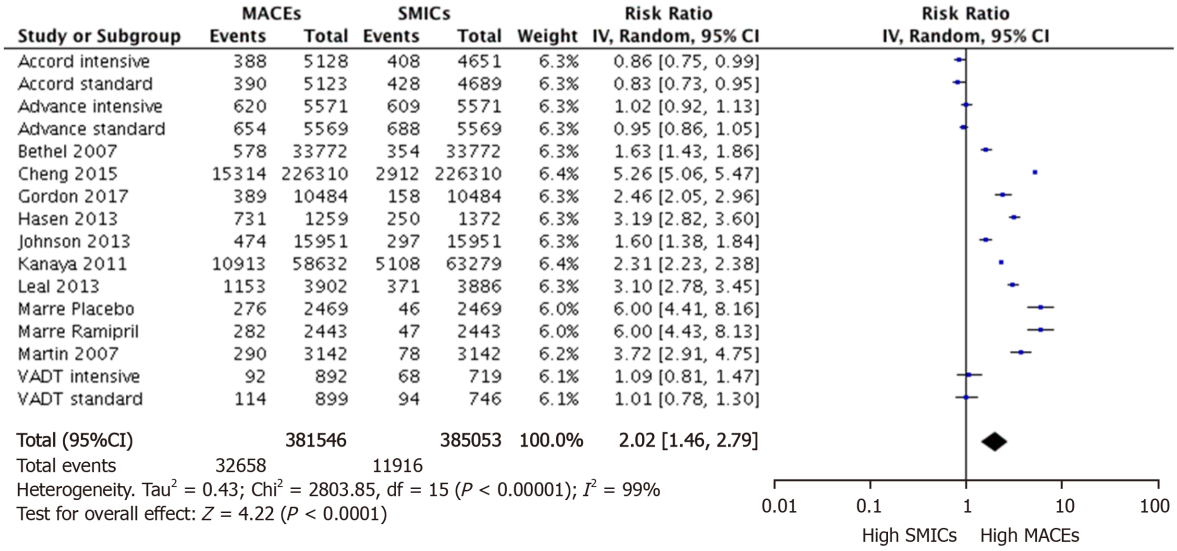


Figure 2 Meta-analysis of the incidence of major cardiovascular events over severe microvascular complications in type 2 diabetes patients. SMICs: Severe microvascular complications; MACEs: Major cardiovascular events.

DISCUSSION

We identified 16 eligible cohorts and the combined result showed that in patients with T2DM, MACEs occurred twice as commonly as SMICs. Qualitatively, the conclusion remained consistent regardless of age, study design, length of follow-up, and duration of diabetes.

Quantitatively, the relative risk differed considerably according to study design. The summarized RR was much lower in the clinical trials than in cohort studies. This may be partly attributed to the use of a much broader definition of SMICs by including laser photocoagulation as serious retinopathy in the two of these four trials (VADT[41] and ADVANCE[45]). T2DM patients with mild-to-moderate retinopathy are recommended laser photocoagulation therapy to relieve the symptoms and delay or prevent vision loss, suggesting majority of patients taking laser therapy in these trials did not have severe retinopathy[46]. In addition, the number of T2DM patients receiving laser therapy in the same trials is 2-3 times more than that in those who developed severe vision loss or blindness[33,39,47]. Therefore, including laser photocoagulation as serious retinopathy in these two trials may partly explain why trials are more likely to report a lower RR.

Meanwhile, clinical trials, like ACCORD and ADVANCE, usually set the follow-up frequency as every 1 or 2 months, while patients in cohort studies were visited every 1 or 2 years, or even longer. Thus, another possible reason for the difference between cohort studies and clinical trials is the intensive follow-up and rigorous examination in clinical trials which may detect more SMICs outcomes in clinical trials than in cohort studies. Noticeably, even though its summarized RR is relatively smaller, the conclusion remains consistent in clinical trials that MACEs occur more often than SMICs in T2DM patients.

Previous meta-analysis has shown that the risk of MACEs was more than doubled in diabetic patients than in non-diabetic people[48]. Meanwhile, our meta-analysis showed that the incidence of MACEs was two times that of SMICs in diabetic patients. Based on these information, it can be inferred that in a given population the absolute number of MACEs attributable to diabetes is greater than that of SMICs, even if the attributable risk for SMICs is 100%. The difference would be bigger if deaths caused by MACEs were compared with those by SMICs.

Therefore, the findings of this study have important implications regarding T2DM by taking the severity and frequency of complications into consideration. First, macrovascular complications should be given a greater weight than microvascular complications in developing or modifying the diagnostic criteria for T2DM. For example, is the relation between blood glycemic measures and the risk of macrovascular complications similar to that for microvascular complications? Do they suggest the same cutoff values for diagnosing diabetes? Further investigations are thus entailed to address these questions.

Second, our findings also suggest that macrovascular complications should be also considered in the evaluation of the efficacy of anti-diabetic drugs in patients with T2DM. Currently, microvascular complications are frequently used in quantifying the effect of glucose-lowering drugs, while macrovascular complications that are more common as shown in this study and more severe than microvascular complications are sometimes not considered. There is even evidence that some anti-diabetic drugs can reduce the risk of microvascular complications but at the same time show no effect or even increase the risk of macrovascular complications[18,21,22]. Our study suggests that it is time to review the current practice in the evaluation of glucose-lowering drugs in which macrovascular complications should be used as the primary outcome for quantifying the benefit of the drugs.

There are some limitations in this systematic review. First, participants in trials and some cohort studies may not fully represent the general population of those with T2DM. But we have not found any good reasons that more representative populations would have a smaller difference between the two types of vascular complications. Second, a fraction of T2DM patients in this review already have microvascular or macrovascular complications at baseline and could not be excluded in analysis, which may bias the comparison but is unlikely to qualitatively reverse the risk ratio between these two types of complications[49,50].

CONCLUSION

Patients with T2DM are much more likely to develop MACEs than SMICs. This suggests by taking more serious consequences and higher incidence into consideration, macrovascular complications deserve more emphasis in developing the diagnostic criteria of type 2 diabetes mellitus and in evaluating the efficacy of anti-diabetic drugs.

ARTICLE HIGHLIGHTS

Research background

Type 2 diabetes mellitus (T2DM) causes both macrovascular and microvascular complications. However, currently, selection of glycemic measures and their thresholds to diagnose T2DM, and efficacy outcomes in evaluation of anti-diabetic drugs is predominantly informed by the relation of T2DM to microvascular complications.

Research motivation

We can be severely mistaken by neglecting macrovascular complications in

developing the diagnostic criteria of T2DM and in evaluating the efficacy of anti-diabetic drugs if they also occur more commonly than microvascular complications.

Research objectives

This study aimed to compare the incidence of major cardiovascular events (MACEs) and severe microvascular complications (SMICs) in the same T2DM patients.

Research methods

The systematic review and meta-analysis with a random-effect model were conducted to combine the relative risk (RR) estimated as the incidence of MACEs divided by that of SMICs in the same T2DM patients of eligible cohort studies or trials.

Research results

Twelve studies with a total of 16 cohorts and 387376 patients were included, and the combined RR was 2.02 (95%CI: 1.46–2.79). The higher incidence of MACEs remained in various subgroup and sensitivity analyses.

Research conclusions

Patients with T2DM are much more likely to develop MACEs than SMICs.

Research perspectives

With their severity and incidence taken into consideration, macrovascular complications deserve more emphasis in developing the diagnostic criteria of T2DM and in evaluating the efficacy of anti-diabetic drugs.

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META-ANALYSIS

Effects of antithrombotic agents on post-operative bleeding after endoscopic resection of gastrointestinal neoplasms and polyps: A systematic review and meta-analysis

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Abstract

BACKGROUND

There are some studies investigating the relationship between antithrombotic medication and postoperative bleeding after endoscopic resection (ER) with controversial results.

AIM

To perform a meta-analysis evaluating the effects of antithrombotic therapy on postoperative bleeding after ER.

METHODS

A systematic search was conducted on PubMed, Web of Science, Cochrane Library. The Newcastle-Ottawa scale was used to evaluate the quality of studies. Stata 12.0 was used for statistical analysis. The odds ratio (OR) and 95%CI were calculated and heterogeneity was quantified using Cochran's *Q* test and I^2.

RESULTS

Total 66 studies were included in the meta-analysis. Pooled data suggested that antithrombotic therapy was significantly associated with postoperative bleeding (OR = 2.302, 95%CI: 2.057-2.577, *P* = 0.000) after ER. The risk of postoperative bleeding after endoscopic submucosal dissection, endoscopic mucosal resection and polypectomy in the antithrombotic group was higher than the non-antithrombotic group (OR = 2.439, 95%CI: 1.916-3.105; OR = 2.688, 95%CI: 1.098-6.582; OR = 2.112, 95%CI: 1.434-3.112).

CONCLUSION

The risk of postoperative bleeding after ER correlated with the types and management of antithrombotic agents by our meta-analysis.

Key Words: Endoscopic resection; Antithrombotic; Anticoagulants; Postoperative

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Core Tip: In recent years, more and more people suffering from cardiovascular disease and/or cerebrovascular disease receive antithrombotic therapy which change patients' coagulation status and may lead to high risk of postoperative bleeding after endoscopic resection (ER). The relationship between the postoperative bleeding after ER and antithrombotic agents is still uncertain. With this reason, a systematic review and meta-analysis was carried out to identify whether the use of antithrombotic drugs increases the risk of the postoperative bleeding after ER.

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INTRODUCTION

Endoscopic resection (ER) is deemed as an effective method for gastrointestinal neoplasia and polyp. ER is an acceptable technique to enable *en bloc* resection of gastric adenomas, early oesophageal, gastric and colorectal cancer and incidence and its related mortality of colorectal cancer[1-3]. This includes polypectomy, endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). For example, patients with oesophageal neoplasia receiving ER can maintain the integrity of oesophageal structure and function, whereas the quality of life can be affected by oesophagectomy[4].

Although the therapeutic effect of ER has been greatly affirmed, Postoperative bleeding as a major complication is still a problem to be solved. Postoperative bleeding after ER is defined as bleeding within 30 d from a mucosal defect shown by massive melena, a decrease in blood hemoglobin level of more than 2 g/dL, or requirement of endoscopic hemostasis or transfusion[1,5,6]. A study has shown that the incidence rate of postoperative bleeding after esophageal or colorectal ESD ranged from 0.0% to 4.6%[7]. And the incidence rate of postoperative bleeding after ESD due to gastric neoplasm ranged from 1.8% to 15.6%[7]. A study that included 3788 cases of polypectomy by Choung found that postoperative bleeding occurred in 42 cases (1.1%)[8]. Another study with 30881 cases of polypectomy by Rutter also reported that the postoperative bleeding developed in 291 cases (0.94%)[9]. Preventive strategies such as acid secretion inhibitors and prophylactic clipping have been developed to reduce the postoperative bleeding risk after ER, but postoperative bleeding cannot be completely avoided. Some factors such as the size of polyp and a patient's coagulation status have been reported to be associated with the risk of postoperative bleeding after ER.

In recent years, more and more people suffering from cardiovascular disease and/or cerebrovascular disease receive antithrombotic therapy which change patients' coagulation status and may lead to high risk of postoperative bleeding after ER. The relationship between the postoperative bleeding after ER and antithrombotic agents is still uncertain. With this reason, a systematic review and meta-analysis was carried out to identify whether the use of antithrombotic drugs increases the risk of the postoperative bleeding after ER.

MATERIALS AND METHODS

We carried out a systematic review and meta-analysis of the hemorrhagic data of different antithrombotic users after ER from published studies. The review and analysis was performed in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines[10].

Search method

We used PubMed, Web of Science and Cochrane Library to search for articles published in English from inception to February 2019. The search queries were: (1) (((antithrombotic OR anticoagulant OR antiplatelet OR heparin OR warfarin OR aspirin)) AND (endoscopic submucosal dissection OR ESD)) AND (bleeding OR hemorrhage); (2) (((antithrombotic OR anticoagulant OR antiplatelet OR heparin OR warfarin OR aspirin)) AND (EMR OR endoscopic mucosal resection)) AND (bleeding OR hemorrhage); (3) (((antithrombotic OR anticoagulant OR antiplatelet OR heparin OR warfarin OR aspirin)) AND (endoscopic polypectomy)) AND (bleeding OR hemorrhage); and (4) (((antithrombotic OR anticoagulant OR antiplatelet OR heparin OR warfarin OR aspirin)) AND (APC OR argon plasma coagulation)) AND (bleeding OR hemorrhage).

Study selection

The studies that met the following inclusion criteria were included: (1) Polypectomy, EMR, ESD, polypectomy incorporated argon plasma coagulation and the hot and cold snare; (2) Randomized controlled trials, retrospective studies or cohort studies were performed to investigate the risk of postoperative bleeding after ER in patients with gastrointestinal neoplasm receiving antithrombotic medication; (3) The incidence rate of postoperative bleeding can be extracted in the antithrombotic medication group and the non-antithrombotic medication group; and (4) Anticoagulants and antiplatelet drugs were incorporated in antithrombotic agents.

The studies were excluded if: (1) The postoperative bleeding rate or antithrombotic therapy information could not be extracted; (2) Antithrombotic drugs and NSAIDS were recorded together; (3) Endoscopic treatment such as biopsy, sphincterotomy or ampullectomy was carried out; (4) Reviews, case reports, guidelines, or animal studies were screened out; (5) The articles were not written in English; and (6) The full text could not be obtained.

Methodological quality assessment

The Newcastle-Ottawa scale was used to evaluate the quality of the included studies. And the Newcastle-Ottawa scale includes three aspects: Selection, comparability, exposure (retrospective studies) or outcome (cohort studies)[11].

Data extraction

Two authors worked together to extract the basic information about the first author, publication year, country, research method (retrospective/cohort), ER method (ESD/EMR/polypectomy), number, age and gender. Moreover, the odds ratio (OR) and 95%CI of the postoperative bleeding rate were calculated in the antithrombotic group (continued/discontinued) and the non-antithrombotic group.

Statistical analysis

Statistical analysis was performed by Stata 12.0. The Cochran's Q test and I^2 (P < 0.10 was considered significant) were used to identify heterogeneity. The value I^2 of 0-25% indicated insignificant heterogeneity; 26%-50%, low heterogeneity; 51%-75%, moderate heterogeneity; and greater than 75%, high heterogeneity[12]. If there was no significant heterogeneity, the OR and 95%CI were calculated in a fixed-effect model. Otherwise, a random-effect model was used. The funnel plot was used to assess publication bias.

RESULTS

Assessment of the studies

The initial literature yielded 1258 articles (454 articles from PubMed, 679 articles from Web of Science, 125 articles from Cochrane Library). After the exclusion of 929 articles due to duplicates and lack of relevance, 329 articles were retrieved for full text evaluation. 263 articles were excluded after reviewing the full text (Figure 1). Ultimately, 66 studies were included in the meta-analysis (Fifty-nine retrospective studies, seven prospective observational studies). The characteristics of included studies were described in the Table 1. The included studies were carried out from different countries (Fifty from Japan, six from Korean, five from USA, two from Italy, one from UK, one from Australia, one from Holland). The mean age was older than 60 years old in most studies.

Table 1 Characteristics of included studies and participants

Ref.	Country	Research method	Location	Age (yr)	Gender male, %
So *et al*[49], 2019	South Korea	Retrospective study	Gastric lesion	68.8/68.5	954, 79.7%
Kishida *et al*[45], 2019	Japan	Retrospective study	Colorectal lesion	64/68	55, 41.66%
Inoue *et al*[65], 2019	Japan	Prospective observational study	Gastrointestinal lesion	67.4 ± 8.3	201, 58.6%
Harada *et al*[56], 2019	Japan	Retrospective study	Gastric lesion	72.3 ± 8.82	414, 69.3%
Arimoto *et al*[54], 2018	Japan	Retrospective study	Colorectal lesion	68.5	492, 58.3%
Azumi *et al*[39], 2018	Japan	Retrospective study	Gastric lesion	73 (41-94)	284, 64.8%
Fujita *et al*[67], 2018	Japan	Retrospective study	Colorectal lesion	72.2 ± 7.4/72.9 ± 8.3	63, 73.8%
Horikawa *et al*[58], 2018	Japan	Retrospective study	Gastric lesion	78 (56-89)	77, 77%
Izumikawa *et al*[40], 2018	Japan	Retrospective study	Gastric lesion	-	255, 75.25%
Kono *et al*[41], 2018	Japan	Retrospective study	Gastric lesion	72 (66-78)	652, 74.77%
Oh *et al*[60], 2018	South Korea	Retrospective study	Gastric lesion	70 (49-85)	173, 80.47%
Park *et al*[63], 2018	South Korea	Prospective observationalstudy	Colorectal lesion	55.8 ± 11.9/52.4 ± 12.3	2661, 68.46%
Sanomura *et al*[59], 2018	Japan	Retrospective study	Gastric lesion	69.8 ± 9.2	719, 70%
Seo *et al*[55], 2018	South Korea	Retrospective study	Colorectal lesion	63 (55-69.5)	723, 60.8%
Sakai *et al*[64], 2018	Japan	Retrospective study	Colorectal lesion n	72.6 ± 7.2/69.1 ± 10.9	669, 66.63%
Yamashita *et al*[36], 2018	Japan	Retrospective study	Colorectal lesion	66.6 ± 10.6	373, 57.4%
Yanagisawa *et al*[35], 2018	Japan	Retrospective study	Gastrointestinal lesion	-	314, 72.02%
Matsumoto *et al*[46], 2018	Japan	Retrospective study	Colorectal lesion	70/65	551, 65.44%
Harada *et al*[61], 2017	Japan	Prospective observational study	Gastric lesion	76.8 ± 6.0/72.7 ± 7.9	40, 88.88%
Yano *et al*[33], 2017	Japan	Retrospective study	Gastric lesion	72 (33-94)	1319, 74.65%
Ueki *et al*[14], 2017	Japan	Retrospective cohort study	Gastric lesion	71.2 ± 8.4	264, 72.5%
Yoshio *et al*[78], 2017	Japan	Retrospective study	Gastric lesion	75/76	90, 90.91%
Gotoda *et al*[15], 2017	Japan	Retrospective study	Gastric lesion	75, 68.8-81.0	410, 77.5%
Furuhata *et al*[17], 2017	Japan	Retrospective study	Gastric lesion	69	1377, 77.3%
Shibuya *et al*[1], 2017	Japan	Retrospective study	Colonic lesion	-	Unclear
Bronsgeest *et al*[42], 2017	Holland	Retrospective study	Colorectal lesion	67.4 ± 8.3	201, 58.6%
Ishigami *et al*[34], 2017	Japan	Retrospective study	Lower gastrointestinal lesion	64.9 ± 11.1	526, 68%
Pigò *et al*[3], 2017	Italy	Retrospective study	Colorectal lesion	65.4	385, 63.2%
Kono *et al*[76], 2017	Japan	Prospective observationalstudy	Upper gastrointestinal lesion	74 ± 8.3	44, 89.8%
Lin *et al*[75], 2017	United States	Retrospective study	Colorectal lesion	-	Unclear
Sato *et al*[38], 2017	Japan	Retrospective study	Gastric lesion	71.1	1786, 75.1%
Igarashi *et al*[27], 2017	Japan	Retrospective study	Gastric lesion	72.4	758, 77.7%
Amato *et al*[31], 2016	Italy	Prospective observational study	Gastrointestinal lesion	59 ± 12.1	54.3%
Kubo *et al*[32], 2016	Japan	Retrospective study	Gastrointestinal lesion	63.9	467,59.3%
Shindo *et al*[25], 2016	Japan	Retrospective study	Gastric lesion	71 ± 8, 32-87	190, 72.5%
Yoshida *et al*[52], 2016	Japan	Retrospective study	Colorectal lesion	68.2 ± 10.3	Unclear
Ninomiya *et al*[53], 2015	Japan	Retrospective study	Colorectal lesion	67 ± 11.1	410, 70.4%
Al-Mammari *et al*[4], 2015	United Kingdom	Prospective observational study	Oesophageal lesion	71, 65-78	85, 72.6%
Odagiri *et al*[16], 2015	Japan	Retrospective cohort study	Colorectal lesion	-	4495, 59.4%
Namasivayam *et al*[5], 2014	United States	Retrospective study	Gastrointestinal lesion	69	Unclear
Terasaki *et al*[21], 2014	Japan	Retrospective study	Colorectal lesion	66.9 ± 11.2	233, 64.2%

Tounou *et al*[50], 2014	Japan	Retrospective study	Gastric lesion	71.8, 36-92	257, 73.4%
Suzuki *et al*[18], 2014	Japan	Retrospective study	Colorectal lesion	65.5, 29-86	183, 57.7%
Matsumura *et al*[23], 2014	Japan	Retrospective study	Gastric lesion	72.1 ± 8.6	302, 71.1%
Beppu *et al*[74], 2014	Japan	Retrospective study	Colorectal lesion	59.5 ± 11.6	176, 84.6%
Inoue *et al*[77], 2014	Japan	Retrospective study	Colorectal lesion	69.2	95, 81.2%
Sanomura *et al*[66], 2014	Japan	Retrospective study	Gastric lesion	73.7 ± 8.9	64, 82.1%
Yoshio *et al*[47], 2013	Japan	Retrospective study	Gastric lesion	70	951, 76.1%
Takeuchi *et al*[29], 2013	Japan	Retrospective study	Gastric lesion	5.2	477, 57.2%
Koh *et al*[37], 2013	Japan	Retrospective study	Gastric lesion	70.3 ± 8.6	817, 74%
Mukai *et al*[6], 2012	Japan	Retrospective study	Gastric lesion	72.4 ± 8.8	116, 72%
Lim *et al*[51], 2012	South Korea	Retrospective study	Gastric lesion	62.6	1143, 71.8%
Miyahara *et al*[48], 2012	Japan	Retrospective study	Gastric lesion	71.7 ± 8.9, 36-92	763, 70.5%
Cho *et al*[57], 2012	South Korea	Retrospective study	Colorectal lesion	62.2	385, 74.9%
Toyokawa T *et al*[24], 2011	Japan	Retrospective study	Gastric lesion	26-95	811, 72.2%
Higashiyama *et al*[19], 2011	Japan	Retrospective study	Gastric lesion	69, 29-91	702, 76%
Metz *et al*[2], 2011	Australia	Prospective observational study	Colonic lesion	68, 26-93	Unclear
Tokioka *et al*[30], 2011	Japan	Retrospective study	Gastric lesion	69.4	378, 73.4%
Okada K *et al*[22], 2011	Japan	Retrospective study	Gastric lesion	68.4, 33-94	425, 73%
Mannen *et al*[20], 2010	Japan	Retrospective study	Gastric lesion	71.6 ± 8.6, 36-91	323, 74.1%
Goto *et al*[13], 2010	Japan	Retrospective study	Gastric lesion	68.3	347, 76.4%
Witt *et al*[44], 2009	United States	Retrospective cohort study	Colorectal lesion	69.6	691, 56.4%
Ono *et al*[28], 2019	Japan	Retrospective study	Gastric lesion	67	Unclear
Takizawa *et al*[26], 2008	Japan	Retrospective study	Gastric lesion	66 ± 10, 29-93	779, 80.5%
Sawhney *et al*[62], 2007	United States	Retrospective study	Colorectal lesion	65.1	169, 97.7%
Yousfi *et al*[43], 2004	United States	Retrospective study	Gastrointestinal lesion	70.5, 45-91	100, 61.7%

Effect analysis

A total of 48691 cases after ER were enrolled, of which 8918 cases were receiving antithrombotic medication and 39773 cases were not taking any antithrombotic drugs[1,2,4,6,13-33]. The average postoperative bleeding rate in the antithrombotic group was 8.44%, while it was 5.28% in the non-antithrombotic group. With the random-effects model, the risk of postoperative bleeding in the antithrombotic group was higher than the non-antithrombotic group (OR = 2.421, 95%CI: 1.831-3.200, P = 0.000, I^2 = 82.5%). In addition, a more homogeneous analysis (I^2 = 36.0%) was carried out after six articles[3,5,29,34-36] were screened out in the sensitivity analysis and the results remained unchanged (OR = 2.302, 95%CI: 2.057-2.577, P = 0.000) (Figure 2). Besides this, the results were not changed when data from retrospective and prospective studies were separately analyzed.

A total of 27014 cases after ESD were enrolled in this meta-analysis (3624 cases were receiving antithrombotic medication and 23390 cases were not taking antithrombotic drugs[1,6,13-30,33,37-41]). The average postoperative bleeding rate after ESD in the antithrombotic group was 13.91%, while it was 7.77% in the non-antithrombotic group. With the random-effects model, the risk of postoperative bleeding after ESD in the antithrombotic group was higher than the non-antithrombotic group (OR = 2.439, 95%CI: 1.916-3.105, P = 0.000, I^2 = 63.5%). Moreover, a more homogeneous analysis (I^2 = 0.0%) was carried out after six articles[6,20,24,26,29,36] were screened out in the sensitivity analysis and the results remained unchanged (OR = 2.507, 95%CI: 2.185-2.875, P = 0.000, Figure 3). The risk of postoperative bleeding after gastric ESD in the antithrombotic group was higher than the non-antithrombotic group (OR = 2.295, 95%CI: 1.757-2.998, P = 0.000, I^2 = 64.1%)[6,13-15,17,19,20,22-29,33]. Meanwhile, the risk of postoperative bleeding after colorectal ESD in the antithrombotic group was higher than the non-antithrombotic group (OR = 3.305, 95%CI: 1.561-6.998, P = 0.002, I^2 =

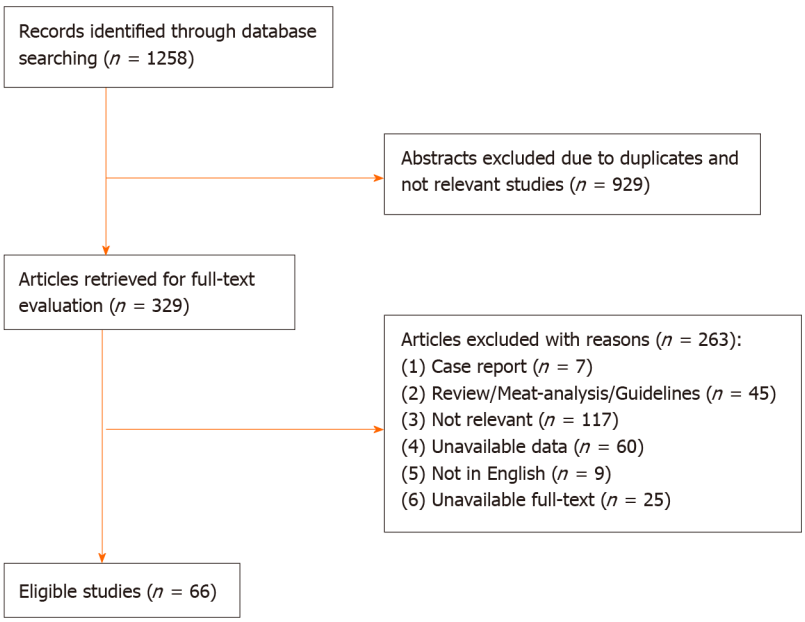


Figure 1 A flow diagram of articles retrieved and inclusion progress through the stage of meta-analysis.

65.0%)[1,15,18,21,36].

A total of 5514 cases after EMR were enrolled in this meta-analysis (1475 cases were receiving antithrombotic medication and 4039 cases were not taking any antithrombotic drugs[1,2,4,5,42]). The average postoperative bleeding rate after EMR in the antithrombotic group was 2.85%, while it was 1.29% in the non-antithrombotic group. With the random-effects model, the risk of postoperative bleeding after EMR in the antithrombotic group was higher than the non-antithrombotic group (OR = 2.688, 95%CI: 1.098-6.582, P = 0.030. I^2 = 72.7%). Furthermore, a more homogeneous analysis (I^2 = 5.3%) was carried out after one article[5] was screened out in the sensitivity analysis and the results remained unchanged (OR = 3.765, 95%CI: 2.380-5.954, P = 0.000, Figure 4). The risk of postoperative bleeding after colorectal EMR in the antithrombotic group was higher than the non-antithrombotic group (OR = 3.711, 95%CI: 2.332-5.904, P = 0.005, I^2 = 32.9%). But the analysis on the risk of postoperative bleeding after gastric EMR could not be carried out due to insufficient data.

A total of 10709 cases of polypectomy were enrolled in this meta-analysis (2554 cases were receiving antithrombotic medication and 8155 cases were not taking any antithrombotic drugs[1,3,35,43-46]). The average postoperative bleeding rate in the antithrombotic group was 4.89%, while it was 1.69% in the non-antithrombotic group. With the random-effects model, there was no significant difference (OR = 2.338, 95%CI: 0.610-8.954, P = 0.215, I^2 = 93.6%) in the postoperative bleeding rate between the two groups. Another more homogeneous analysis (I^2 = 44.4%) was carried out after two articles[3,35] were screened out in the sensitivity analysis and the results were found to have changed (OR = 2.112, 95%CI: 1.434-3.112, P = 0.006, Figure 5). The risk of postoperative bleeding after colorectal polypectomy in the antithrombotic group was higher than the non-antithrombotic group (OR = 2.921, 95%CI: 1.821-4.687, P = 0.000, I^2 = 31.9%). Table 2 shows the number of cases with or without antithrombotic agents and hemorrhagic outcome.

Quality assessment and publication bias

The Newcastle-Ottawa scale was used to assess the quality of the included studies in this meta-analysis. Thirteen articles had 6 stars, twenty-three articles had 7 stars, twenty-eight articles had 8 stars, and the others had 9 stars (Table 3). At the same time, the funnel plot did not show any features associated with publication bias (Figure 6).

Subgroup analyses

Among the ESD group, we performed several subgroup analyses to independently evaluate the effects of different types of antithrombotic agents in postoperative bleeding: (1) In gastric ESD retrospective comparison studies of single

Table 2 Number of cases with or without antithrombotic agents and hemorrhagic outcome

Ref.	Resection method	Total	Drug	Post-bleeding	No bleeding
So *et al*[48], 2019	ER	1197	Antithrombotic agent (+/-)	40/50	359/748
			Continued antithrombotic agent (+/-)	11/7	69/138
			Discontinued antithrombotic agent (+/-)	29/43	330/657
			HR (+)	5	9
Kishida *et al*[45],2019	Polypectomy	6382	Antithrombotic agent (+/-)	15/40	986/5341
			Single APT (+)	4	683
			Single anticoagulants (+)	2	85
			Multiple APT (+)	3	163
			Multiple antithrombotic agents (+)	2	39
			Single antithrombotic agent (+)	13	947
			HR (+)	4	16
Inoue *et al*[65], 2019	EMR	102	VKA (+)	12	73
			Discontinued VKA (+)	0	4
			Continued VKA (+)	0	2
			HR (+)	15	98
			DOAC (+)	3	14
			Discontinued DOAC (+)	0	3
Inoue *et al*[65], 2019	ESD	54	VKA (+)	14	31
			Discontinued VKA (+)	1	2
			Continued VKA (+)	0	1
			HR (+)	13	31
			DOAC (+)	2	7
			Discontinued DOAC (+)	2	4
Harada *et al*[56], 2019	ESD	597	Antithrombotic agent (-)	21	422
			Single-LDA (+)	10	85
			DAPT (+)	10	49
			Continued LDA (+)	15	80
			Discontinued APT (+)	5	54
Arimoto *et al*[54], 2018	ESD	919	Antithrombotic agent (-)	26	757
			APT (+)	5	131
			Discontinued APT (+)	5	105
			Continued APT (+)	0	26
Azumi *et al*[39], 2018	ESD	438	Antithrombotic agent (+/-)	6/15	72/345
Fujita *et al*[67], 2018	EMR	84	Discontinued anticoagulants (+)	1	42
			HR (+)	4	37
Horikawa *et al*[58], 2018	ESD	100	Antithrombotic agent (-)	1	49
			Continued LDA	1	49
Izumikawa *et al*[40], 2018	ESD	273	Antithrombotic agent (+/-)	15/11	66/207
Kono	ESD	872	Antithrombotic agent (+/-)	23/38	159/652

et al[41], 2018			Single antithrombotic agent	12	130
			Multiple antithrombotic agents (+)	11	29
			Discontinued antithrombotic agent (+)	8	120
			Discontinued		
			Single APT (+)	3	88
			Multiple APT (+)	3	16
			Single anticoagulants (+)	1	13
			Continued		
			Single APT (+)	1	16
			Multiple APT (+)	4	2
			Single anticoagulants (+)	7	13
			HR (+)	10	21
Oh *et al*[60], 2018	ESD	215	Single APT (+)	14	147
			Multiple APT (+)	15	39
			LDA (+)	12	82
			Thienopyridine (+)	2	54
			Continued APT (+)	23	130
			Discontinued APT (+)	6	56
Park *et al*[63], 2018	Polypectomy	3887	APT (+)	12	339
			Anticoagulants (+)	0	15
Sanomura *et al*[58], 2018	ESD	1243	Antithrombotic agent (-)	40	1127
			Anticoagulants (+)	11	65
			Warfarin (+)	5	32
			DOAC (+)	4	14
Seo *et al*[55], 2018	ESD	1189	Antithrombotic agent (-)	26	945
			APT (+)	7	175
			Aspirin (+)	2	139
			Warfarin (+)	0	10
			DOAC (+)	1	2
			Single antithrombotic agent (+)	10	326
			Multiple antithrombotic agents (+)	0	23
			Discontinued antithrombotic agent (+)	7	206
			Continued antithrombotic agent (+)	0	5
Sakai *et al*[64], 2018	Polypectomy	1004	Discontinued anticoagulants (+)	12	0
			HR (+)	8	70
			Warfarin (+)	7	55
			DOAC (+)	1	15
Yamashita *et al*[36], 2018	ESD	650	Antithrombotic agent (+/-)	7/18	21/652
			Warfarin (+)	5	14
			DOAC	2	7
Yanagisawa *et al*[35], 2018	Polypectomy	436	Antithrombotic agent (+/-)	30/2	188/216
			Discontinued anticoagulants (+)	0	23

			Continued anticoagulants (+)	10	83
			HR (+)	20	82
			Continued warfarin (+)	2	41
			Continued DOAC (+)	8	42
			Warfarin (+)	20	125
			DOAC (+)	10	63
Matsumoto *et al*[46], 2018	Polypectomy	1003	Antithrombotic agent (+/-)	2/2	184/815
Harada *et al*[61], 2017	ESD	45	Continued warfarin (+)	2	20
			HR	5	18
Yano *et al*[33], 2017	ESD	144	Antithrombotic agent (+/-)	47/103	287/1330
Ueki *et al*[14], 2017	ESD	364	Antithrombotic agent (+/-)	7/17	67/273
			Discontinued antithrombotic agent (-)	7	67
			Discontinued single APT (+)	4	57
			Discontinued single anticoagulants (+/-)	2	4
			Aspirin (+)	4	43
			Thienopyrindine (+)	0	7
Yoshio *et al*[78], 2017	ESD	97	Warfarin (+)	18	55
			DOAC	5	19
Gotoda *et al*[15], 2017	ESD	529	Antithrombotic agent (+/-)	12/14	96/407
			APT (+)	8	80
			Single antithrombotic agent (+)	6	80
			Multiple antithrombotic agents (+)	7	17
			Single APT (+)	3	69
			Multiple APT (+)	5	11
			Warfarin (+)	3	11
			Aspirin (+)	2	33
			Thienopyridine (+)	0	10
Furuhata *et al*[17], 2017	ESD	1781	Antithrombotic agent (+/-)	33/68	220/1460
			Single antithrombotic agent (+)	11	139
			Multiple antithrombotic agents (+)	6	30
			Continued single APT (+)	1	14
			HR (+)	15	37
Shibuya *et al*[1], 2017	ESD	259	Antithrombotic agent (+/-)	4/6	32/217
Shibuya *et al*[1], 2017	EMR	3018	Antithrombotic agent (+/-)	16/15	510/2477
Shibuya *et al*[1], 2017	Polypectomy	892	Antithrombotic agent (+/-)	3/5	163/721
Bronsgeest *et al*[42], 2017	EMR		Antithrombotic agent (+/-)	13/15	107/277
			APT (+)	4	53
			Anticoagulants (+)	4	43
Ishigami *et al*[34], 2017	ER	773	Antithrombotic agent (+/-)	10/14	35/714
			HR (+)	10	35
Pigò *et al*[3], 2017	Polypectomy	609	Antithrombotic agent (+/-)	38/32	72/467
			Single APT	14	57

			Multiple APT	3	8
			HR (+)	21	7
			Aspirin (+)	10	32
			Thienopyridine	4	25
Kono *et al*[76], 2017	ESD/EMR	49	Single antithrombotic agent (+)	4	24
			Multiple antithrombotic agents (+)	7	14
			Discontinued antithrombotic agent (+)	5	20
			Continued antithrombotic agent (+)	6	18
			HR (+)	4	12
Lin *et al*[75], 2017	Polypectomy	4923	Aspirin (+)	36	3897
			Thienopyridine (+)	5	590
Sato *et al*[38], 2017	ESD	2378	Antithrombotic agent (+/-)	46/76	401/1855
			APT (+)	35	270
			Anticoagulants (+)	2	33
			HR (+)	6	33
			Aspirin (+)	12	199
			Thienopyridine (+)	0	19
			Warfarin (+)	1	16
			DOAC (+)	1	17
Igarashi *et al*[27], 2017	ESD	976	Antithrombotic agent (+/-)	35/30	332/692
			Discontinued antithrombotic agent (+)	26	250
			Continued antithrombotic agent (+)	5	49
			HR	4	33
			Multiple antithrombotic agents (+)	9	70
			Single antithrombotic agent (+)	26	262
			Continued aspirin (+)	4	29
			Discontinue aspirin (+)	19	152
			Continued thienopyridine (+)	1	17
			Discontinued thienopyridine (+)	9	63
			Continued anticoagulants (+)	1	11
			Discontinued anticoagulants (+)	3	27
Amato *et al*[31], 2016	ER	2692	Antithrombotic agent (+/-)	16/16	595/2069
			APT (+)	11	461
			Anticoagulants (+)	5	134
Kubo *et al*[32], 2016	ER	788	Antithrombotic agent (+/-)	16/13	194/565
			APT (+)	8	146
			Anticoagulants (+)	11	72
			HR (+)	10	63
Shindo *et al*[25], 2016	ESD	262	Antithrombotic agent (+/-)	10/13	38/201
			Discontinued antithrombotic agent (+)	0	25
			Continued APT (+)	2	8

			HR (+)	8	5
Yoshida *et al*[52], 2016	ESD	678	Antithrombotic agent (-)	10	585
			APT (+)	3	60
			Anticoagulants (+)	3	17
Ninomiya *et al*[53], 2015	ESD	609	Antithrombotic agent (-)	28	537
			Discontinued APT (+)	2	11
			Continued APT (+)	5	26
Al-Mammari *et al*[4], 2015	EMR	117	Antithrombotic agent (+/-)	1/1	14/101
Odagiri *et al*[16], 2015	ESD	7567	Antithrombotic agent (+/-)	49/282	440/6796
Namasivayam *et al*[5],2014	EMR	1712	Antithrombotic agent (+/-)	4/10	772/912
			APT (+)	3	521
			Anticoagulants (+)	0	89
			Single antithrombotic agent (+)	1	617
			Multiple antithrombotic agents (+)	3	111
			Thienopyridine (+/-)	0/10	17/912
Terasaki *et al*[21], 2014	ESD	363	Antithrombotic agent (+/-)	4/20	36/303
Tounou *et al*[50], 2014	ESD	350	Antithrombotic agent (-)	16	245
			Discontinued single APT (+)	7	37
			Continued single APT (+)	2	12
			Dual APT (+)	11	20
			Aspirin (+)	9	44
			Thienopyridine (+)	0	5
Suzuki *et al*[18], 2014	ESD	317	Antithrombotic agent (+/-)	1/13	27/276
			HR	0	6
Matsumura *et al*[23], 2014	ESD	425	Antithrombotic agent (+/-)	10/10	77/328
			Discontinued antithrombotic agent (+)	2	39
			Continued antithrombotic agent (+), HR (-)	3	22
			HR (+)	5	16
Beppu *et al*[74], 2014	ER	208	APT (+)	9	18
			Anticoagulants (+)	12	9
			Aspirin (+)	6	11
			Thienopyridine (+)	3	7
Inoue *et al*[77], 2014	Polypectomy	117	Discontinued antithrombotic agent (+)	1	71
			HR (+)	9	36
Sanomura *et al*[66], 2014	ESD	78	Continued LDA (+)	1	27
			Discontinued LDA (+)	3	63
Yoshio *et al*[47], 2013	ESD	1250	Antithrombotic agent (-)	45	972
			Discontinued antithrombotic agent (-)	12	197
			HR (+)	9	15
Takeuchi *et al*[29], 2013	ESD	833	Antithrombotic agent (+/-)	21/15	69/728
Koh *et al*[37], 2013	ESD	1166	Antithrombotic agent (+/-)	17/45	158/946

Mukai *et al*[6], 2012	ESD	161	Antithrombotic agent (+/-)	4/17	29/111
Lim *et al*[51], 2012	ESD	1591	Antithrombotic agent (-)	68	1249
			Discontinued APT (+)	6	96
			Continued APT (+)	20	152
Miyahara *et al*[48], 2012	ESD	1082	Antithrombotic agent (-)	68	883
			Discontinued antithrombotic agent (+)	7	124
Cho *et al*[57], 2012	ESD	514	Antithrombotic agent (-)	15	424
			Discontinued APT (+)	2	54
			Continued APT (+)	4	15
Toyokawa *et al*[24], 2011	ESD	1123	Antithrombotic agent (+/-)	8/48	175/892
Higashiyama *et al*[19], 2011	ESD	924	Antithrombotic agent (+/-)	123/773	3/25
Metz *et al*[2], 2011	EMR	269	Antithrombotic agent (+/-)	8/11	30/220
			APT (+)	6	18
			Anticoagulants (+)	1	10
			HR (+)	1	2
			Aspirin (+)	5	12
			Thienopyridine (+)	1	6
Tokioka *et al*[30], 2011	ESD	515	Antithrombotic agent (+/-)	3/23	37/452
Okada *et al*[22], 2011	ESD	582	Antithrombotic agent (+/-)	4/24	70/484
Mannen *et al*[20], 2010	ESD	436	Antithrombotic agent (+/-)	1/38	32/365
Goto *et al*[13],2010	ESD	454	Antithrombotic agent (+/-)	5/21	52/376
Witt *et al*[44], 2009	Polypectomy	1225	Antithrombotic agent (+/-)	11/2	414/798
Ono *et al*[28], 2019	ESD	444	Antithrombotic agent (+/-)	6/20	50/368
Takizawa *et al*[26], 2008	ESD	968	Antithrombotic agent (+/-)	3/60	74/831
Sawhney *et al*[62], 2007	Polypectomy	173	APT (+)	17	51
			Anticoagulants (+)	14	12
Yousfi *et al*[43], 2004	Polypectomy	162	Antithrombotic agent (+/-)	32/49	27/54
			APT (+)	32	27

ER: Endoscopic resection; ESD: Endoscopic submucosal dissection; EMR: Endoscopic mucosal resection; APT: Antiplatelet; LDA: Low dose of aspirin; HR: Heparin replacement; DOAC: Direct oral anticoagulant; Thienopyridine: Thienopyridine derivatives.

antithrombotic user (No. bleeding/total = 43/524) *vs* non-antithrombotic agent user (No. bleeding/total = 112/2671)[15,17,27]: The risk of postoperative bleeding in single antithrombotic agent group was significantly higher than the non-antithrombotic agent group [OR = 2.061, 95%CI: 1.405-3.024, P = 0.000 (I^2 = 0.0%)]; (2) In gastric ESD retrospective comparison studies of multiple antithrombotic user (No. bleeding/total = 33/179) *vs* non-antithrombotic agent user (No. bleeding/total = 150/3361)[15,17,27,41]: The risk of postoperative bleeding in multiple antithrombotic agents group was significantly higher than the non-antithrombotic agent group [OR = 4.985, 95%CI: 3.251-7.561, P = 0.000 (I^2 = 40.6%)]; (3) In gastric ESD retrospective comparison studies of multiple antithrombotic (No. bleeding/total = 33/179) user *vs* single antithrombotic user (No. bleeding/total = 55/666)[15,17,27,41]: The risk of postoperative bleeding in multiple antithrombotic agents group was higher than the single antithrombotic agent group [OR = 2.492, 95%CI: 1.563-3.974, P = 0.000 (I^2 = 43.9%)]; (4) In gastric ESD retrospective comparison studies of discontinued antithrombotic user *vs* (No. bleeding/total = 81/1074) non-antithrombotic agent user (No. bleeding/total = 216/3894)[14,25,27,47-49]: The risk of postoperative bleeding in discontinued antithrombotic agent group was slightly higher than the non-antithrombotic agent group [OR = 1.405, 95%CI: 1.069-1.848, P = 0.015 (I^2 = 34.4%)]; (5)

Table 3 The quality assessment of included studies

Ref.	Selection				Comparability	Outcome/exposure			Stars
	1	2	3	4		1	2	3	
So *et al*[49], 2019	*	*	*	*	**	*	*	*	9
Kishida *et al*[45], 2019	*	*			*	*	*	*	6
Inoue *et al*[65], 2019	*	*	*		**	*	*	*	8
Harada *et al*[56], 2019	*	*		*	**	*	*	*	8
Arimoto *et al*[54], 2018	*	*	*	*	*	*	*	*	8
Azumi *et al*[39], 2018	*	*		*	**	*	*	*	8
Fujita *et al*[67], 2018	*	*		*	**	*	*	*	8
Horikawa *et al*[58], 2018	*	*		*	**	*	*	*	8
Izumikawa *et al*[40], 2018	*	*			*	*	*	*	6
Kono *et al*[41], 2018	*	*		*	*	*	*	*	7
Oh *et al*[60], 2018	*	*		*		*	*	*	6
Park *et al*[63], 2018	*	*	*		**	*	*	*	8
Sanomura *et al*[59], 2018	*	*		*	*	*	*	*	7
Seo *et al*[55], 2018	*	*	*	*	*	*	*	*	8
Sakai *et al*[64], 2018	*	*		*	*	*	*	*	7
Yamashita *et al*[36], 2018	*	*		*	*	*	*	*	7
Yanagisawa *et al*[35], 2018	*	*		*	**	*	*	*	8
Matsumoto *et al*[46], 2018	*	*		*		*	*	*	6
Harada *et al*[61], 2017	*	*		*	*	*	*	*	7
Yano *et al*[33], 2017	*	*		*	*	*	*	*	7
Ueki *et al*[14], 2017	*	*		*	*	*	*	*	7
Yoshio *et al*[78], 2017	*	*	*	*	*	*	*	*	8
Gotoda *et al*[15], 2017	*	*		*	*	*	*		6
Furuhata *et al*[17], 2017	*	*		*	**	*	*	*	8
Shibuya *et al*[1], 2017	*	*		*	**	*	*	*	8
Bronsgeest *et al*[42], 2017	*	*	*	*	*	*	*	*	8
Ishigami *et al*[34], 2017	*	*		*	*	*	*	*	7
Pigò *et al*[3], 2017	*	*		*	*	*	*	*	7
Kono *et al*[76], 2017	*	*	*	*	*	*	*	*	8
Lin *et al*[75], 2017	*	*		*	*	*	*	*	7
Sato *et al*[38], 2017	*	*		*	**	*	*	*	8
Igarashi *et al*[27], 2017	*	*		*	**	*	*		7
Amato *et al*[31], 2016	*	*	*		*	*	*	*	7
Kubo *et al*[32], 2016	*	*	*	*	*	*	*		7
Shindo *et al*[25], 2016	*	*		*	*	*	*		6
Yoshida *et al*[52], 2016	*	*		*	*	*	*		6
Ninomiya *et al*[53], 2015	*	*		*	*	*	*		6
Al-Mammari *et al*[4], 2015	*	*	*		*	*	*	*	7
Odagiri *et al*[16], 2015	*	*	*	*	*	*	*		7
Namasivayam *et al*[5], 2014	*	*		*	*	*	*		6

Terasaki *et al*[21], 2014	*	*		*	**	*	*		7
Tounou *et al*[50], 2014	*	*		*	*	*	*	*	7
Suzuki *et al*[18], 2014	*	*		*	*	*	*	*	7
Matsumura *et al*[23], 2014	*	*			**	*	*		6
Beppu *et al*[74], 2014	*	*		*	**	*	*	*	8
Inoue *et al*[77], 2014	*	*		*	**	*	*	*	8
Sanomura *et al*[66], 2014	*	*		*	**	*	*	*	8
Yoshio *et al*[47], 2013	*	*	*	*	*	*	*		7
Takeuchi *et al*[29], 2013	*	*		*	**	*	*	*	8
Koh *et al*[37], 2013	*	*		*	**	*	*	*	8
Mukai *et al*[6], 2012	*	*		*	*	*	*		6
Lim *et al*[51], 2012	*	*		*	**	*	*	*	8
Miyahara *et al*[48], 2012	*	*	*	*	**	*	*		8
Cho *et al*[57], 2012	*	*		*	**	*	*	*	8
Toyokawa T *et al*[24], 2011	*	*	*	*	*	*	*		7
Higashiyama *et al*[19], 2011	*	*		*	*	*	*	*	7
Metz *et al*[2], 2011	*	*	*		**	*	*	*	8
Tokioka *et al*[30], 2011	*	*		*	**	*	*	*	8
Okada K *et al*[22], 2011	*	*		*	*	*	*		6
Mannen *et al*[20], 2010	*	*		*	*	*	*		6
Goto *et al*[13], 2010	*	*		*	**	*	*	*	8
Witt *et al*[44], 2009	*	*		*	*	*	*	*	7
Ono *et al*[28], 2019	*	*		*	**	*	*		7
Takizawa *et al*[26], 2008	*	*		*	**	*	*	*	8
Sawhney *et al*[62], 2007	*	*		*	**	*	*	*	8
Yousfi *et al*[43], 2004	*	*	*	*	**	*	*	*	9

In gastric ESD retrospective comparison studies of continuous antithrombotic user (No. bleeding/total = 18/144) *vs* non-antithrombotic user (No. bleeding/total = 50/1081)[25,27,49]: The risk of postoperative bleeding in continuous antithrombotic agent group was higher than the non-antithrombotic agent group [OR = 2.886, 95%CI: 1.513-5.504, P = 0.001 (I^2 = 0.0%)]; (6) In gastric ESD retrospective comparison studies of continuous antithrombotic user (No. bleeding/total = 18/144) *vs* dis-continued antithrombotic user (No. bleeding/total = 55/660)[25,27,49]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 1.615, 95%CI: 0.919-2.837, P = 0.096 (I^2 = 32.9%)]; (7) In gastric ESD retrospective comparison studies of antiplatelet (APT) (No. bleeding/total = 100/891) user *vs* non-antithrombotic user (No. bleeding/total = 212/4620)[15,38,41,50,51]: The risk of postoperative bleeding in the APT agent group was higher than the non-antithrombotic agent group [OR = 2.545, 95%CI: 1.979-3.273, P = 0.000 (I^2 = 38.8%)]. In colorectal ESD retrospective comparison studies of APT user (No. bleeding/total = 22/425) *vs* non-antithrombotic user (No. bleeding/total = 90/2914)[52-55]: The risk of postoperative bleeding in the APT agent group was higher than the non-antithrombotic agent group [OR = 1.821, 95%CI: 1.127-2.944, P = 0.014 (I^2 = 25.8%)]; (8) In gastric ESD retrospective comparison studies of discontinued APT user (No. bleeding/total = 17/271) *vs* non-antithrombotic user (No. bleeding/total = 127/2450)[41,51,56]: There was no significant difference in the risk of postoperative bleeding risk between the two groups [OR = 1.218, 95%CI: 0.721-2.060, P = 0.461 (I^2 = 0.0%)]. In colorectal ESD retrospective comparison studies of discontinued APT user (No. bleeding/total = 9/179) *vs* non-antithrombotic user (No. bleeding/total = 69/1787)[53,54,57]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 1.494, 95%CI: 0.725-3.081, P = 0.277 (I^2 = 0.0%)]; (9) In gastric ESD retrospective comparison

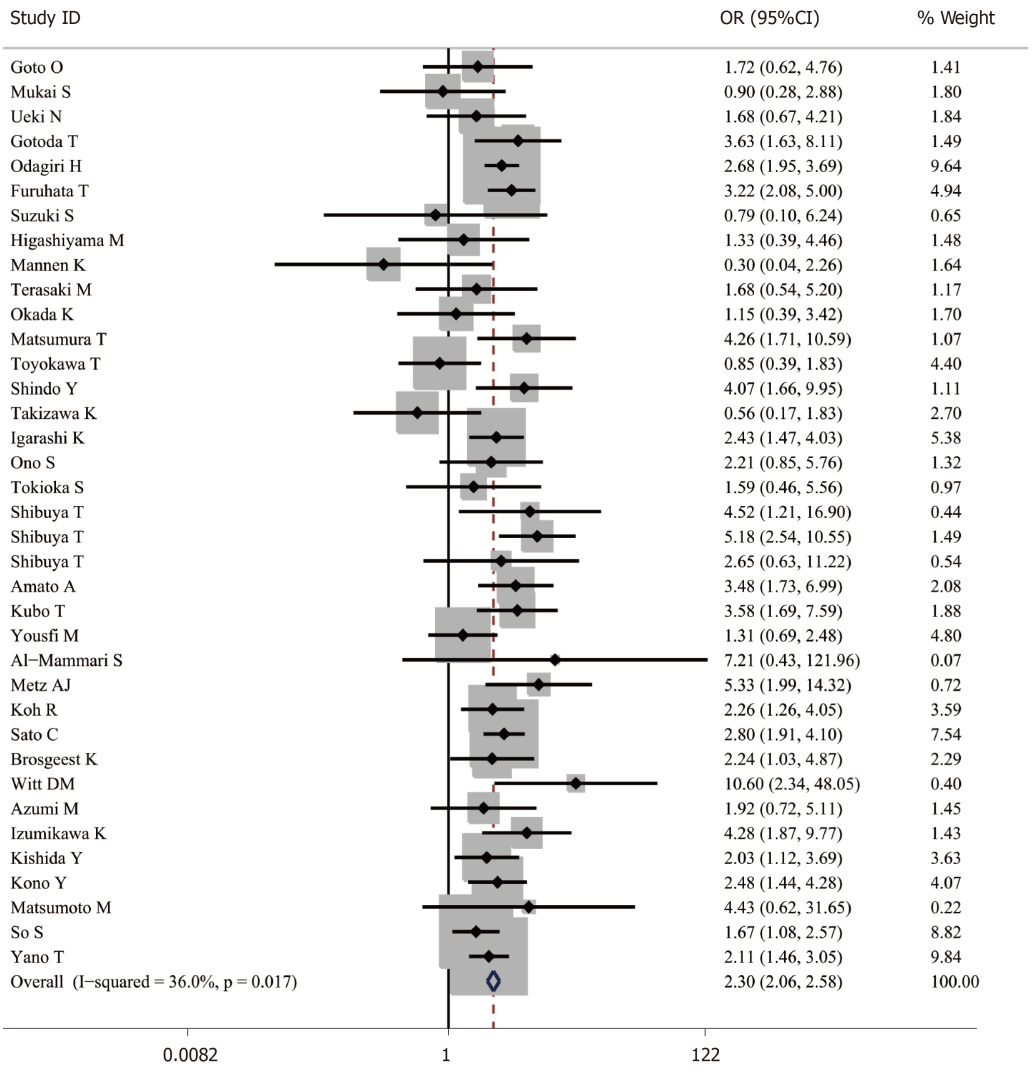


Figure 2 Forest plot of antithrombotic group *vs* non-antithrombotic group in endoscopic resection.

studies of continuous APT user (No. bleeding/total = 43/350) *vs* non-antithrombotic user (No. bleeding/total = 141/2710)[25,41,51,56,58]: The risk of postoperative bleeding in continuous APT agent group was higher than the non-antithrombotic agent group [OR = 2.955, 95%CI: 2.026-4.310, P = 0.000 (I^2 = 0.0%)]. In colorectal ESD retrospective comparison studies of continuous APT user (No. bleeding/total = 9/75) *vs* non-antithrombotic user (No. bleeding/total = 69/1787)[53,54,57]: The risk of postoperative bleeding risk in continuous APT agent group was higher than the non-antithrombotic agent group [OR = 3.409, 95%CI: 1.652-7.036, P = 0.001 (I^2 = 43.9%)]; (10) In gastric ESD retrospective comparison studies of continuous APT user (No. bleeding/total = 44/299) *vs* discontinued APT user (No. bleeding/total = 20/297)[41,56,59,60]: The risk of postoperative bleeding in continuous APT agent group was higher than the discontinued APT agent group [OR = 2.004, 95%CI: 1.095-3.668, P = 0.024 (I^2 = 0.0%)]. In colorectal ESD retrospective comparison studies of continuous APT user (No. bleeding/total = 9/75) *vs* discontinued APT user (No. bleeding/total = 9/179)[53,54,57]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 1.740, 95%CI: 0.616-4.910, P = 0.296 (I^2 = 50.6%)]; (11) In gastric ESD retrospective comparison studies of multiple APT user (No. bleeding/total = 33/131) *vs* non-antithrombotic user (No. bleeding/total = 89/1815)[15,41,50,56]: The risk of postoperative bleeding in multiple APT agent group was higher than the non-antithrombotic agent group [OR = 6.437, 95%CI: 4.048-10.237, P = 0.000 (I^2 = 7.3%)]; (12) In gastric ESD retrospective comparison studies of multiple APT user (No. bleeding/total = 48/185) *vs* single APT user (No. bleeding/total = 40/494)[15,41,50,56,60]: The risk of postoperative bleeding in multiple APT agent group was higher than the single APT agent group [OR = 3.606, 95%CI: 2.270-5.726, P = 0.000 (I^2 = 39.4%)]; (13) In gastric ESD retrospective comparison studies of continuous single APT

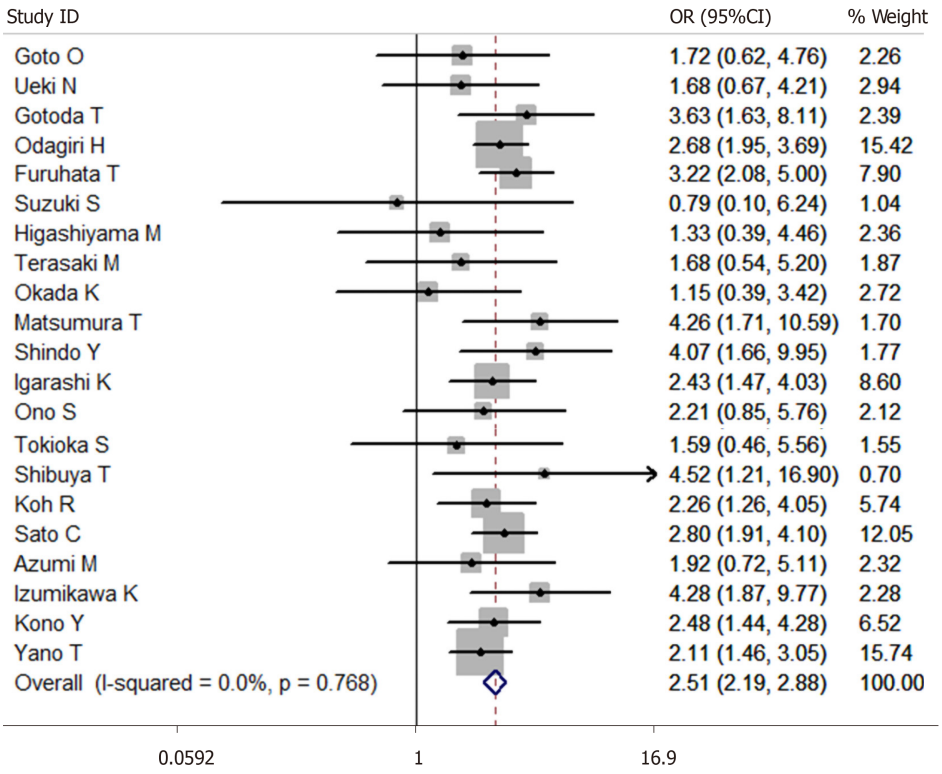


Figure 3 Forest plot of antithrombotic group *vs* non-antithrombotic group in endoscopic submucosal dissection.

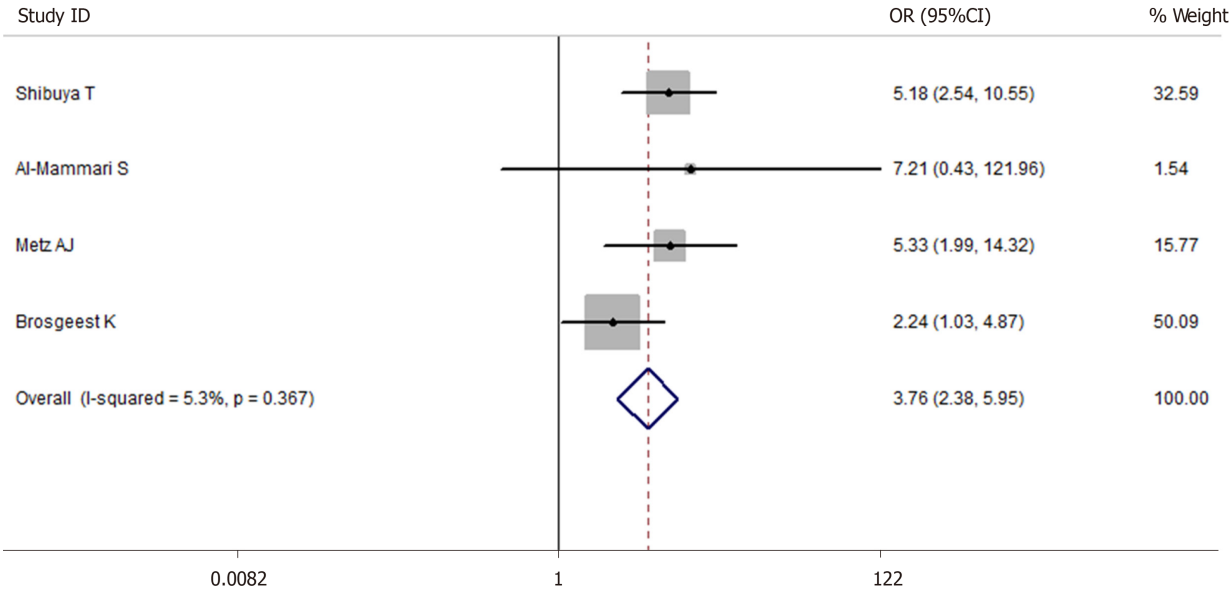


Figure 4 Forest plot of antithrombotic group *vs* non-antithrombotic group in endoscopic mucosal resection.

user (No. bleeding/total = 5/96) *vs* non-antithrombotic user (No. bleeding/total = 71/1262)[17,41,50,58]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 1.427, 95%CI: 0.524-3.886, P = 0.486 (I^2 = 0.0%)]; (14) In gastric ESD retrospective comparison studies of aspirin user (No. bleeding/total = 38/491) *vs* non-antithrombotic user (No. bleeding/total = 145/3396): The risk of postoperative bleeding in aspirin agent group was higher than the non-antithrombotic agent group [OR = 1.889, 95%CI: 1.293-2.759, P = 0.000 (I^2 = 47.0%)]; (15) In gastric ESD retrospective comparison studies of continuous aspirin user (No. bleeding/total = 36/320) *vs* discontinued aspirin user (No. bleeding/total = 34/391): There was no significant difference in the postoperative bleeding risk between the two groups [OR = 1.430, 95%CI: 0.786-2.603, P = 0.241 (I^2 = 0.0%)]; (16) In gastric ESD

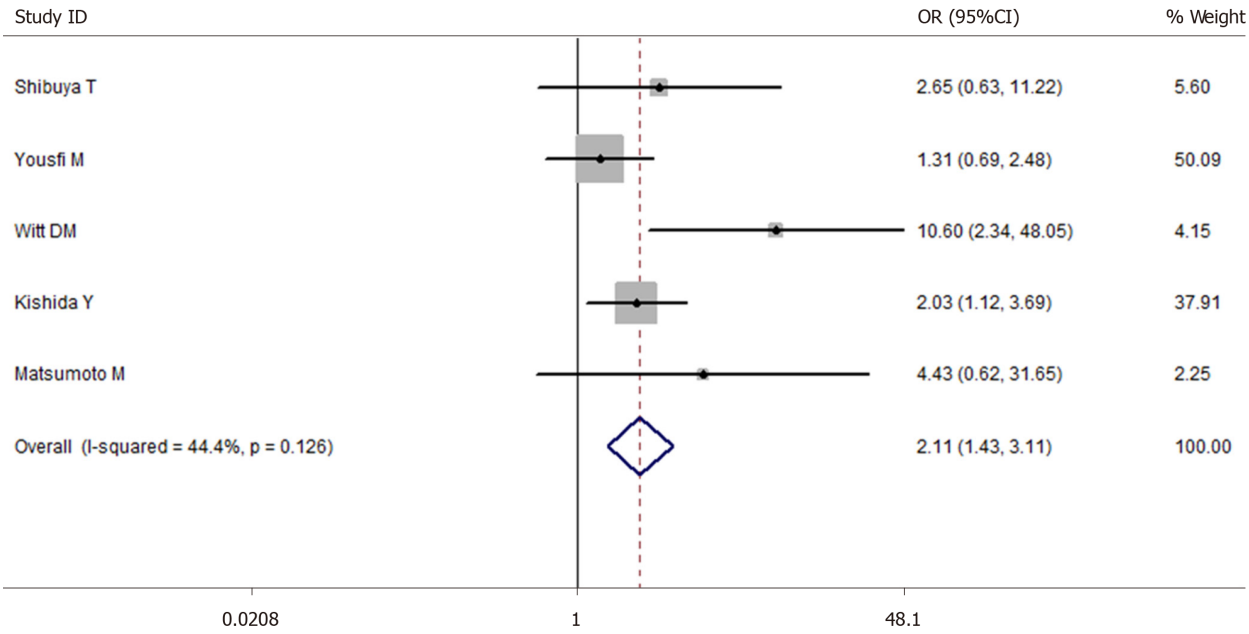


Figure 5 Forest plot of antithrombotic group *vs* non-antithrombotic group in polypectomy.

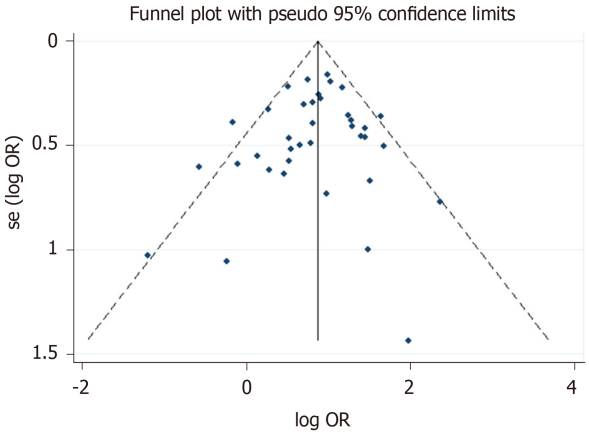


Figure 6 Funnel plot of antithrombotic group *vs* non-antithrombotic group in endoscopic resection.

retrospective comparison studies of discontinued aspirin user (No. bleeding/total = 31/325) *vs* non-antithrombotic user (No. bleeding/total = 147/3047)[27,51,53,56]: The risk of postoperative bleeding in discontinued aspirin agent group was higher than the non-antithrombotic agent group [OR = 2.093, 95%CI: 1.349-3.246, P = 0.001 (I^2 = 33.1%)]; (17) In gastric ESD retrospective compatison studies of thienopyridine derivatives user (No. bleeding/total = 0/41) *vs* non-antithrombotic user (No. bleeding/total = 123/2903)[14,15,38,50]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 0.983, 95%CI: 0.234-4.132, P = 0.981 (I^2 = 0.0%)]; (18) In gastric ESD retrospective comparison studies of aspirin user (No. bleeding/total = 39/440) *vs* thienopyridine derivatives user (No. bleeding/total = 78/2009)[14,15,38,50,60]: The risk of postoperative bleeding in the aspirin agent group was higher than the thienopyridine derivatives agent group [OR = 1.806, 95%CI: 1.062-3.037, P = 0.029 (I^2 = 47.0%)]; (19) In gastric ESD comparison studies (two retrospective studies and one prospective study) of anticoagulant user (No. bleeding/total = 21/145) *vs* non-antithrombotic user (No. bleeding/total = 154/3788)[38,41,59]: The risk of postoperative bleeding [OR = 4.029, 95%CI: 2.442-6.646, P = 0.000 (I^2 = 18.1%)] in the anticoagulant agent group was significantly higher than the non-antithrombotic agent group; (20) In gastric ESD comparison studies (three retrospective studies and one prospective study) of warfarin user (No. bleeding/total = 24/127) *vs* direct oral anticoagulants (DOAC) user (No. bleeding/total = 10/60)[38,47,59]: There was no significant difference

in the risk of postoperative bleeding between the two groups [OR = 0.940, 95%CI: 0.407-2.171, P = 0.885 (I^2 = 0.0%)]; (21) In gastrointestinal ESD retrospective comparison studies of anticoagulant user (No. bleeding/total = 13/89) *vs* APT user (No. bleeding/total = 49/501)[38,41,52]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 1.677, 95%CI: 0.852-3.302, P = 0.135 (I^2 = 64.1%)]; (22) In gastric ESD retrospective comparison studies of heparin replacement (HR) (No. bleeding/total = 25/128) user *vs* non-antithrombotic user (No. bleeding/total = 154/3681)[23,27,38,41]: The risk of postoperative bleeding [OR = 5.547, 95%CI: 3.457-8.900, P = 0.000 (I^2 = 16.9%)] in HR agent group was significantly higher than the non-antithrombotic agent group; (23) In gastric ESD retrospective comparison studies of HR user (No. bleeding/total = 32/125) *vs* continuous antithrombotic user (No. bleeding/total = 10/101)[17,25,27,61]: The risk of postoperative bleeding in the HR agent group was significantly higher than the continuous antithrombotic agent group [OR = 2.859, 95%CI: 1.257-6.503, P = 0.012 (I^2 = 0.0%)]; and (24) In gastric ESD retrospective comparison studies of HR user (No. bleeding/total = 29/120) *vs* continuous single APT user (No. bleeding/total = 7/83)[17,27,41]: The risk of postoperative bleeding in HR agent group was significantly higher than the continuous single APT agent group (OR = 2.988, 95%CI: 1.173-7.761, P = 0.000 (I^2 = 3.1%)].

Among the EMR group, we performed several subgroup analyses to evaluate the effects of different types of antithrombotic agents on postoperative bleeding: (1) APT (No. bleeding/total = 13/605) user *vs* non-antithrombotic user (No. bleeding/total = 36/1445)[2,5,42]: OR = 1.744, 95%CI: 0.398-7.643, P = 0.461 (I^2 = 78.8%). There were two retrospective studies and one prospective study in the subgroup analysis. There were two studies about colorectal EMR and one study about gastric EMR in the subgroup analysis; (2) Anticoagulant user (No. bleeding/total = 44/567) *vs* non-antithrombotic user (No. bleeding/total = 218/8131)[2,5,42]: There was no significant difference in the risk of postoperative bleeding risk between the two groups [OR = 1.409, 95%CI: 0.552-3.597, P = 0.474 (I^2 = 0.0%)]. There were two retrospective studies and one prospective study in the subgroup analysis. There were two studies about colorectal EMR and one study about gastric EMR in the subgroup analysis; and (3) Anticoagulant user (No. bleeding/total = 5/147) *vs* APT user (No. bleeding/total = 13/605)[2,5,42]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 0.768, 95%CI: 0.261-2.261, P = 0.631 (I^2 = 0.0%)]. There were two retrospective studies and one prospective study in the subgroup analysis. There were two studies about colorectal EMR and one study about gastric EMR in the subgroup analysis.

Among the polypectomy group, we also performed several subgroup analyses to evaluate the effects of different types of antithrombotic agents on postoperative bleeding: (1) APT (No. bleeding/total = 56/994) user *vs* non-antithrombotic user (No. bleeding/total = 121/5983)[3,43,45]: OR = 1.766, 95%CI: 1.192-2.616, P = 0.005 (I^2 = 73.9%) (retrospective studies). There were two studies about colorectal polypectomy and one study about gastric polypectomy in the subgroup analysis; (2) Anticoagulant user (No. bleeding/total = 16/128) *vs* APT user (No. bleeding/total = 33/1106)[45,62,63]: The risk of postoperative bleeding after colorectal polypectomy in the anticoagulant agent group was significantly higher than the APT agent group [OR = 3.132, 95%CI: 1.442-6.803, P = 0.004 (I^2 = 9.0%)] (retrospective studies); and (3) Warfarin user (No. bleeding/total = 32/226) *vs* DOAC (No. bleeding/total = 13/98)[35,36,64]: There was no significant difference in the risk of postoperative bleeding between the two groups [OR = 1.126, 95%CI: 0.557-2.275, P = 0.741 (I^2 = 0.0%)] (retrospective studies). There were two studies about colorectal polypectomy and one study about gastric polypectomy in the subgroup analysis.

A subgroup analysis was planned to assess the risk of postoperative bleeding according to the difference in the size of the lesion, dosage and cessation period of antithrombotic agent, but we failed to perform the analysis because of insufficient data.

Thromboembolic event

Thromboembolic event is defined as arterial thromboembolism. This includes stroke, transient ischemic attack and infarction perioperative period. These thromboembolic events in included studies were available in nineteen articles (one event in the heparin therapy group[17], five events in the antithrombotic group[5,27], three events in the HR group[35,47,64], one event in the discontinued anticoagulant therapy group[30], one event in the discontinued antithrombotic therapy group[32], two events in the withdrawal period of antiplatelet therapy group[2,51], one event in the anticoagulant therapy group[44], one event in the withdrawal period of anti-vitamin K antagonisis therapy

group[65], four events in the low dose aspirin interrupted group[66]). No thromboembolic events occurred in seven studies[36,41,49,58,59,67].

DISCUSSION

Despite several practice guidelines about the cessation or continuation of antithrombotic drugs before ER made by the British Society of Gastroenterology[68], the European Society of Gastrointestinal Endoscopy[68], the American Society for Gastrointestinal Endoscopy[69] and the Japan Gastroenterological Endoscopy Society[70], the effect of antithrombotic drugs on the risk of postoperative bleeding was still controversial in some studies[4,6,13,14,16,19-22,24,26,27,31,37,48,57]. Our study found that antithrombotic agents confer a higher risk for postoperative bleeding after ESD and EMR. But the risk of postoperative bleeding after polypectomy was not significantly elevated in the patients with antithrombotic drugs from our study, which was in consistent with the results of a study by Matsumoto *et al*[46]. Nevertheless, there was significant heterogeneity in the analysis of antithrombotic group *vs* non-antithrombotic group. To explain the heterogeneity (I^2 = 82.5%) of our meta-analysis, we got the following findings: (1) Different methods were used to prevent postoperative bleeding; (2) Different definitions on postoperative bleeding[2,19]; (3) Different types and doses of antithrombotic agents; and (4) Different follow-up time, ranging 24 h to 3 mo. In order to reduce the heterogeneity, we have done the subgroup analyses to assess the effect of different types of antithrombotic agents in the risk of postoperative bleeding.

Some studies found that APT did not correlate with the risk of postoperative bleeding[32,52]. At the same time, the risk of delayed postoperative bleeding after ESD was not increased in a single APT agent (continued or discontinued)[17]. In contrast, it has been demonstrated that APT (especially dual APT) increases the risk of postoperative bleeding[50]. A retrospective study by Singh *et al*[71] showed that clopidogrel alone was not an independent risk factor for postoperative bleeding, but a randomized trial by Chan *et al*[72] showed that continued clopidogrel use results in a higher risk of postoperative bleeding compared to the discontinued clopidigrel use group. Our study found that continued single APT agent use did not increase the risk of postoperative bleeding, but multiple APT agents increased the risk of postoperative bleeding after ER.

Some studies found that low dose aspirin and continued use of aspirin didn't induce a higher risk of postoperative bleeding after polypectomy and gastric ESD[23,43,50]. However, Ninomiya *et al*[53] found that continued use of aspirin increased the risk of postoperative bleeding after colorectal ESD. A study by Metz *et al*[2] demonstrated that the use of aspirin within 7 d of the operation was an independent risk factor for postoperative bleeding after colonic EMR. In a meta-analysis by Shalman *et al*[73], the risk of immediate bleeding in patients with aspirin was not increased, but the risk of delayed bleeding in patients with aspirin or thienopyridine derivatives was increased. Our study found that the use of aspirin significantly increased the risk of postoperative bleeding, but thienopyridine derivatives did not increase the risk of postoperative bleeding after ER. Nevertheless, the guidelines recommend continuing aspirin and withdrawing thienopyridine derivatives in the endoscopic resection[68-70]. Therefore, more prospective or randomized controlled trials are needed to determine the effects of aspirin and thienopyridine on the risk of postoperative bleeding after ER.

Several guidelines about gastroenterological endoscopy recommend that anticoagulant agent should be discontinued with HR[68-70]. APT plus HR (meaning that anticoagulants were substituted by heparin before polypectomy) were not correlated with postoperative bleeding, but anticoagulant or anticoagulant plus HR were risk factors for postoperative bleeding[32]. Besides, HR alone was related to postoperative bleeding in univariate analysis but was not in multivariate analysis[32]. And our study has reached the same conclusion. Cessation of antithrombotic therapy could result in thromboembolic events such as cerebral infarction and hemorrhagic shock. But the risk of the thromboembolic events in the included studies is relatively low.

There were several drawbacks in this meta-analysis. First of all, the results of our meta-analysis were derived from retrospective studies. Retrospective studies may underestimate the risk of postoperative bleeding. Further prospective studies are needed to confirm our results. Secondly, the surveillance periods of included studies were not exactly the same. Finally, different types and doses of antithrombotic agents were used in the included studies, which may lead to bias.

CONCLUSION

In conclusion, the risk of postoperative bleeding after ER (polypectomy, EMR and ESD) correlated with the types and management of the antithrombotic agents according to our meta-analysis. Interrupting or switching antithrombotic therapy might result in the increased risk of serious thromboembolic events. Therefore, it is important to comprehensively assess the risk of postoperative bleeding and thromboembolic events in the patients with antithrombotic drugs after ER.

ARTICLE HIGHLIGHTS

Research background

Endoscopic resection (ER) is deemed as an effective method for gastrointestinal neoplasia, polyp, gastric adenomas, early oesophageal, gastric and colorectal cancer. More and more people suffering from cardiovascular disease and/or cerebrovascular disease receive antithrombotic therapy which change patients' coagulation status and may lead to high risk of postoperative bleeding after ER. The relationship between the postoperative bleeding after ER and antithrombotic agents is still uncertain.

Research motivation

This study explored the relationship between the postoperative bleeding after ER and antithrombotic agents.

Research objectives

The aim of this study is to identify whether the use of antithrombotic drugs increases the risk of the postoperative bleeding after ER by a systematic review and meta-analysis.

Research methods

A systematic search was conducted on PubMed, Web of Science, Cochrane library. The Newcastle-Ottawa scale was used to evaluate the quality of studies. Stata 12.0 was used for statistical analysis. The odds ratio and 95%CI were calculated and heterogeneity was quantified using Cochran's Q test and I^2.

Research results

Total 66 studies were included in the meta-analysis. Pooled data suggested that antithrombotic therapy was significantly associated with postoperative bleeding after ER. The risk of postoperative bleeding after endoscopic submucosal dissection, endoscopic mucosal resection and polypectomy in the antithrombotic group was higher than the non-antithrombotic group.

Research conclusions

The risk of postoperative bleeding after ER correlated with the types and management of antithrombotic agents by our meta-analysis.

Research perspectives

Our results can guide the use of antithrombotic drugs before ER and evaluate the risk of postoperative bleeding.

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MINIREVIEWS

COVID-19-associated stroke risk: Could nutrition and dietary patterns have a contributing role?

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Abstract

The novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 has created a life-threatening world pandemic. Unfortunately, this disease can be worse in older patients or individuals with comorbidities, having dangerous consequences, including stroke. COVID-19–associated stroke widely increases the risk of death from COVID-19. In addition to the personal hygiene protocols and preventive policies, it has been proven that immune-compromised, oxidative, and pro-coagulant conditions make a person more susceptible to severe COVID-19 complications, such as stroke; one of the most effective and modifiable risk factors are poor nutritional status. Previous literature has shown that healthy dietary patterns, such as the Mediterranean diet, some food groups, and specific micronutrients, reduce the risk of ischemic and hemorrhagic stroke. In this work, for the first time, we hypothesized that a healthy diet could also be a protective/preventive factor against COVID-19–associated stroke risk. In order to prove this hypothesis, it is required to study nutritional intake and dietary patterns in patients suffering from COVID-19–associated stroke. If this hypothesis is proven, the chronic supportive role of a healthy diet in critical situations will be highlighted once again.

Key Words: COVID-19; COVID-19–associated stroke; Nutrition; Dietary patterns; Food

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Core Tip: The novel coronavirus disease 2019 (COVID-19) can be worse in older patients or individuals with comorbidities, having dangerous consequences, including stroke. COVID-19–associated stroke widely increases the risk of death from COVID-19. Previous literature has shown that healthy dietary patterns, such as the Mediterranean diet, some food groups, and specific micronutrients, reduce the risk of ischemic and hemorrhagic stroke. Recently, healthy nutrition and certain nutrients or nutraceuticals reported to be effective in common relief functions in stroke and COVID-19 pathways. If growing studies confirm our hypothesis, it means that healthy nutrition could be a protective/preventive factor against COVID-19–associated stroke risk.

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INTRODUCTION

Due to the significance of the current pandemic, many studies have paid attention to its consequent clinical outcomes. Clinical manifestations can astonishingly vary from asymptomatic to the most life-threatening conditions resulted from cytokine storm[1,2]. Currently, the novel coronavirus disease 2019 (COVID-19)–associated stroke is not common and, simultaneously, is one of the most deteriorate consequences of this infection, particularly in those with severe infection[3,4], which is responsible for the mortality of about 40% of affected patients if it occurs[5,6]. Stroke, in turn, is the second cause of death and the leading cause of disability[7]. Although it is not yet exactly clear COVID-19 to be a culprit in the co-occurrence of stroke in infected patients[8], studies have shown that the incidence of stroke in COVID-19 patients is about 0.9% to 2.7%[6,8]. COVID-19–associated stroke is often an acute ischemic stroke, while hemorrhagic stroke also is rarely reported, especially in elderly[5,9]. Although some studies have reported stroke in young COVID-19 patients[10], a systematic review and metasummary of the literature have shown a mean age of 63.4 ± 13.1 years and the simultaneous presence of cardiovascular comorbidities[6]. Hypertension, diabetes, and inappropriate cholesterol levels are the main comorbidities associated with the severity of COVID-19[11]. On the other hand, the available data, indeed, suggest that severe COVID-19 can lead to stroke[6,9]. Preventive strategies and available health-promoting protocols based on antiviral therapies, immune modulators, and anticoagulants to reduce the severity of this disease appear to be significant and interesting points to be studied[12]. However, lifestyle has always been the most modifiable factor influencing diseases, of which nutrition is a vital part[13-15]. The role of healthy nutrition in the prevention and treatment of hypertension, diabetes, high serum levels of cholesterol, all of which predispose a person to stroke, and most non-communicable diseases has also been proven[16-18]. Recently, researchers have discussed immune-modulatory properties and other various effects of healthy nutrition on COVID-19 and its complications[13-15,19-21], among which no attention has been paid to COVID-19–associated stroke.

HYPOTHESIS

A vast body of evidence-based data from the past to the present has shown that healthy eating can prevent hemorrhagic and ischemic stroke[22-24]. Protection against hyper-inflammatory and hypercoagulable states and a pleasant change in metabolites derived from intestinal microbiota leading to cardiovascular health are the mainstream mechanisms involved[25]. Although there is no study concerning nutrition in COVID-

19–associated stroke yet, there are growing studies addressing the impact of healthy diets, certain food groups, and some specific nutrients on COVID-19[13-15,19-21]. In these studies, healthy nutrition and some nutrients or nutraceuticals are considered mitigators of the disease severity and cytokine storm. On the other hand, some data have shown stroke as the worst event following COVID-19, occurring under cytokine storm and hypercoagulable conditions[26]. Altogether, the above-mentioned evidence creates the speculation that people who adherence to a healthy diet and lifestyle may be less likely to have a stroke following COVID-19 affliction.

EVALUATION OF THE HYPOTHESIS

To evaluate our hypothesis, we conducted a comprehensive review by searching both pubmed.gov and scholar.google.com to first understand the association between stroke and COVID-19 and second review the studies in which nutrition has been linked to stroke and COVID-19. The former results are shown in Table 1, and the latter in Table 2. We also explain them in detail below.

COVID-19–ASSOCIATED STROKE RISK

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection enters the target cell *via* the angiotensin-converting enzyme 2 (ACE2) receptor[12]. In addition to the lung, this receptor is present in the endothelial cells, heart, kidney, and intestine[27]. This is an underlying cause of beyond respiratory manifestations of COVID-19. Once the virus infects the endothelial cells lining blood vessels beds, inflammatory cells and apoptotic bodies are accumulated; this results in vascular endothelial dysfunction, shifting vascular autoregulation to vasoconstriction, and organ ischemia, tissue edema, and pro-coagulant condition consequently[27,28]. On the other hand, immune-mediated recruitment of immune cells elevates the inflammatory responses and hyper-inflammatory states, leading to cytokine storm in severe cases. Cytokine storm plays a key role in the pathogenesis of severe COVID-19 manifestations, including acute ischemic stroke caused by hypercoagulable state[26]. Similar to other viral syndromes, SARS-CoV-2 is associated with an increased risk of stroke[29,30]. Elevated interleukin-6 (IL-6) levels following cytokine storm cause hyperviscosity[8]. Thus, besides the mechanisms, including infection-induced hypercoagulability, viral cardiomyopathy, and a diffuse hyperinflammatory state[27,28,31], proposed so far for the association between COVID-19 and acute ischemic stroke, a mechanical factor as dehydration can also increase stroke risk[8,32]. By comparing measured biomarkers in severe COVID-19 patients with moderate cases, a study has shown higher levels of alanine aminotransferase, lactate dehydrogenase, C-reactive protein (CRP), ferritin, D-dimer, IL-2R, IL-6, IL-10, and TNF-α in severely infected patients. Furthermore, the total number of T lymphocytes, $CD4^+$ T cells, and $CD8^+$ T cells significantly are lower in severe cases than those moderate. Therefore, they have concluded the cytokine storm to be related to disease severity[33]. A recently published systematic review further reveals the outstanding presence of raised D-dimer, fibrinogen, and anti-phospholipid antibodies in COVID-19 patients with coincide acute ischemic stroke[6]. Thus, the speculate on COVID-19–associated stroke is consistent with previous studies, which have well demonstrated that higher levels of D-dimer significantly associated with older age and pre-existing comorbidities are related to worse complications and death from COVID-19[9,34]. According to a report by Ntaios *et al*[35], COVID-19-associated ischemic stroke is more severe with poorer outcomes and higher mortality than ischemic strokes unrelated to COVID-19. Although most studies have discussed ischemic stroke, there is little data to suggest that SARS-CoV-2-induced diffuse endothelial inflammation also could be a mechanism resulting in hemorrhagic stroke[31]. On the other hand, as stated, the infection caused by SARS-CoV-2 is mediated by the host cells ACE2 receptors; this, in turn, downregulates the expression of ACE2. Hence, the availability of angiotensin II increases, leading to severe blood pressure fluctuates, especially in patients with a history of hypertension that makes them prone to hemorrhagic stroke[9]. Finally, it is hypothesized that therapeutic targeting of protease-activated receptor (PAR), as a protein engaged in the neuroinflammatory toxicity, may be useful in controlling various complications caused by COVID-19[32].

Table 1 Summary of available evidence-based associations between stroke and coronavirus disease 2019

Ref.	Study design	Sample/ Aim	The main culprits suggested in the occurrence of stroke result from COVID-19 affliction
Bhaskar *et al*[26]	The REPROGRAM consortium position paper	An overview of cytokine storm and its implications in COVID-19	(1) Cytokine storm; (2) Thromboembolic events; and (3) Large vessel occlusion
Rothstein *et al*[31]	Retrospective, observational study	844 COVID-19 patients, 28 of them had a stroke	(1) Endothelialitis; (2) Diffuse endothelial inflammation; (3) Infection-induced hypercoagulability; (4) Viral cardiomyopathy; and (5) Diffuse hyperinflammatory state
Valderrama *et al*[8]	Case study	A 52-year-old man with co-occurrence of stroke and COVID-19 infection	(1) Inflammation; (2) Injury to the myocardium; (3) Thrombogenesis; (4) Increased D-dimer levels; (5) Increased interleukin-6 levels; (6) Hyperviscosity; (7) Vascular endothelial damage; (8) Intracerebral hemorrhage; (9) Microthrombosis; and (10) Fibrinogen consumption coagulopathy
Tan *et al*[6]	Systematic review and meta-summary of the literature	A total of 39 studies comprising 135 patients, pooled incidence of co-occurrence of stroke and COVID-19 was 1.2 %	(1) Elevated D-dimer; (2) Elevated fibrinogen; and (3) The presence of antiphospholipid antibodies
Wang *et al*[9]	A review article	Summarizing the potential contribution of COVID-19 to hemorrhagic stroke in the elderly and proposi-ng possible mechanisms	(1) Downregulation of ACE2 expression; (2) Increased angiotensin II availability; (3) Severe blood pressure fluctuates; (4) Predisposition to hemorrhagic stroke; (5) Elevated plasma D-dimer levels; (6) Classical inflammatory biomarkers; (7) Viral CNS infections; (8) Cytokine, chemokine, and protease; and (9) Increasing BBB permeability
Ntaios *et al*[35]	Special report	Pooled all patients who were hospitalized with confirmed COVID-19 and AIS in 28 sites from 16 countries	(1) Endotheliopathy; (2) Potentiate the prothrombotic milieu; (3) Immune-mediated platelet activation; (4) Dehydration; and (5) Infection-induced cardiac arrhythmias

COVID-19: Coronavirus disease 2019; ACE2: Angiotensin-converting enzyme 2; CNS: Central nervous system; BBB: Blood-brain barrier; AIS: Acute ischemic stroke.

DIETARY PATTERNS AND STROKE RISK

According to several national studies, nearly 90% of the stroke burden can be assigned to the modifiable risk factors, including poor diet[36,37]. Therefore, a healthy diet is necessary and important as an approved preventive strategy against stroke[22]. Many articles are now available, all of which indicate that healthy eating and adherence to the certain dietary patterns, such as the healthy Nordic diet[23], Dietary Approaches to Stop Hypertension (DASH) diet[22], and especially the Mediterranean diet[24], significantly reduce the risk of both ischemic and hemorrhagic strokes. Hansen *et al*[23], in their cohort study with a median follow-up period of 13.5 years identifying 2283 cases of stroke, have found that adherence to a healthy Nordic diet (containing fish, apples, pears, cabbages, root vegetables, rye bread, and oatmeal) has a positive effect on stroke risk so that the higher Healthy Nordic Food Index score is associated with a lower risk of total stroke. Clinical trials have also shown that adherence to a healthy Nordic diet leads to weight loss, decreased blood pressure, and improved serum lipid status[38-40]. This diet is rich in potassium and fiber since it contains high amounts of fruits and vegetables associated with decreased blood pressure. Further, high fiber in whole grains is associated with lower total and low-density lipoprotein cholesterol. On the other hand, abundant flavonoids in apples, kale, and broccoli are also related to a reduced risk of stroke[23]. The next most examined dietary approach is the DASH diet, which includes a high intake of fruits, vegetables, whole grains, low-fat dairy, legumes, nuts, low sodium intake, sweetened beverages, and red/processed meat[22]. A meta-analysis of prospective studies by Feng *et al*[22] has shown that every 4 points increase in the score of adherence to the DASH diet reduces the risk of stroke in a dose-response manner by 4%. This is true for both ischemic and hemorrhagic strokes, although less data is available on the latter. Randomized clinical trials have also shown that adherence to this diet, in addition to lowering blood pressure, can reduce the stroke risk by improving lipid profile, controlling body weight, besides reducing the risk of type 2 diabetes and metabolic syndrome, all of which are important in the pathogenesis of stroke[41-45]. Another dietary pattern emphasized to be followed to prevent stroke is the Mediterranean diet. The PREDIMED study[46] with 7447 participants has found that adherence to the traditional Mediterranean diet characterized by high consumption of olive oil, fruits, nuts, vegetables, and cereals, moderate intake of fish and poultry, low intake of dairy products, red meat, and

Table 2 Summary of available studies related to dietary patterns, some foods, and micronutrients in context of stroke and/or coronavirus disease 2019

Ref.	Study design	Sample/ Aim	Possible pathways in which nutritional factors may be involved in pathogenesis of stroke and/ or COVID-19–associated stroke
Hansen *et al*[23]	A Danish cohort study	Incident cases of stroke among 55338 men and women	(1) Higher Healthy Nordic Food Index score, was associated with a lower risk of total stroke; (2) Reduction in blood pressure; (3) Improved blood lipids; (4) Induced weight loss; (5) Lower total and low-density lipoprotein cholesterol; and (6) Increased antioxidant and flavonoid levels
Feng *et al*[22]	A meta-analysis of prospective studies	Included 12 prospective cohort studies comprising a total of 548632 participants	(1) Higher adherence to the DASH diet was related to a reduced risk of developing stroke; (2) Blood pressure-lowering effects; (3) Improved lipid profiles and body weight; (4) Decreased the risk of metabolic syndrome; (5) Improved serum inflammatory biomarkers; (6) Reduced oxidative stress; and (7) Anti-inflammatory and antioxidative effects
Estruch *et al*[24]	Randomized clinical trial	A total of 7447 persons randomly assigned to 3 diets: a Med-diet suppl with extra-virgin olive oil, a Med-diet suppl with mixed nuts, or a control diet	(1) A Med-diet suppl with extra-virgin olive oil or nuts reduced the incidence of major cardiovascular events; (2) High biologic plausibility; (3) Anti-inflammatory and antioxidative effects; (4) Improved endothelial dysfunction; (5) Resistance to vasoreactivity; (6) Insulin sensitivity; (7) Improved blood lipids
Chiu *et al*[47]	2 Prospective cohort study	Cohort 1, n = 5050; Cohort 2, n = 8302	(1) Taiwanese vegetarian diet is associated with a lower risk of ischemic and hemorrhagic strokes; (2) Shift gut microbial community to reduce the production of trimethylamine N-oxide; and (3) So, decreased platelet hyperreactivity and thrombosis
D'Elia *et al*[49]	A meta-analysis	Pooled analysis of 14 cohorts (overall 333250 participants and 10659 events)	(1) An inverse and significant association between K intake and risk of stroke; and (2) Decreased blood pressure
Zhao *et al*[50]	A systematic review and meta-analysis	18 prospective cohort studies on Mg intake and the incidence of stroke	(1) Increasing Mg intake may be a crucial component of stroke prevention that acts in a dose-dependent manner; and (2) Improved cardiovascular health
Iacoviello *et al*[51]	A systematic review	Prospective studies that focused on primary prevention of stroke by nutrition	Nutrients, food groups and dietary patterns are effective in preventing stroke, such as: (1) Vitamin D, dairy calcium, folate (not supplementation), vitamin C, chocolate, coffee, tea, and the regular and moderate alcohol consumption; (2) Calcium and dairy intake are inversely associated with low-grade systemic inflammation; (3) Low-fat dairy and milk consumption decreased the incidence of hypertension; (4) Vitamin D involved in the modulation of the renin-angiotensin system, endothelial function, vascular smooth muscle proliferation, insulin sensitivity, and systemic inflammation; (5) Vitamin C, beta-carotene, and flavonoids are antioxidant compounds can be reduce blood pressure and improve microvascular function; and (6) Chocolate intake resulted in increased HDL, decreased LDL oxidation, improved endothelial function and reduced blood pressure
Bousquet *et al*[52]	Review article	The role of diet in COVID-19 death rates	(1) Countries where the major daily intake originates from uncooked or fermented kale, cabbages, and fermented dairy products have lower COVID-19 death rates than the others; (2) Mentioned foods known as good sources for anti-ACE activity and rich in antioxidants; and (3) Improving high blood pressure and subsequent hemorrhagic stroke
Kalantar-Zadeh *et al*[20]	Perspective study	The impact of dietary patterns and the commensal microbiome on susceptibility to and severity of COVID-19	(1) A healthy, diverse, and balanced diet based on plants, high fiber, and fermented foods could be a preventative strategy, mitigating harmful effects of SARS-CoV-2; and (2) Probiotics can modulate the immune system, thereby reducing the susceptibility to affliction or morbidity and mortality from COVID-19
Tan *et al*[55]	Prospective observational study	AIS patients (n = 140), healthy controls (n = 92)	(1) Gut-brain axis has been brought to attention; (2) Probiotics and beneficial microbes exert pleasant alter on the gut microbiome towards producing more SCFAs; (3) There was dysbiosis (low fecal SCFAs level) in AIS patients; and (4) The SCFAs levels were negatively correlated with stroke severity and prognosis
Zabetakis *et al*[14]	Review article	This review speculates the importance of nutrition as a mitigation strategy to support immune function amid the COVID-19 pandemic	(1) Person's nutritional status and nutrients and foods may exert anti-inflammatory and immunomodulatory effects; (2) Nutrients such as vitamin C, vitamin D, and zinc may hold some promise for the treatment of COVID-19; (3) Nutrients with anti-inflammatory, antithrombotic, and antioxidant properties may prevent or attenuate the inflammatory and vascular manifestations associated with COVID-19; and (4) It is vitally important to maintain a healthy diet and lifestyle during the pandemic
Kanki *et al*[56]	Animal study	Adult male C57BL6N mice included in two group NCD or HFD	(1) Mice with a HFD have higher levels of endogenous thrombin; (2) Thrombin was considered as a stimulator of PAR-1-based signaling; (3) PAR signaling involved in the neuroinflammatory complications; and (4) HFD mice experience larger infarct and worse outcomes after stroke induction
Wallace *et al*[21]	Review article	A summary of clinical and prospective cohort studies assessing the relationship of Mg with IL-6, a prominent drug target for	(1) Nutrition plays an important and safe role in helping mitigate patient morbidity and mortality from COVID-19; (2) Mg participates in the function of many enzymes involved in the severe immune and inflammatory responses that are manifestations of COVID-19; (3) Mg modulates IL-6, nuclear factor-kB, and CRP; (4) Mg enhances vitamin D functionality; (5) Mg is essential in preventing the serious consequences of COVID-19; (6) K restores ACE2

		treating COVID-19	functionality; and (7) Hypokalemia is seen in most patients with severe and critical COVID-19
Rhodes *et al*[2]	Perspective study	To review the evidence relevant to vitamin D and COVID-19	(1) Vitamin D is potential to reduce COVID-19 risk, severity, and mortality; (2) Inflammation and cytokine storm suppression; (3) Inverse modulatory role in the renin-angiotensin system; (4) Downregulating renin and increasing ACE2; and (5) Inverse association between serum level of vitamin D and anti-phospholipid antibodies
Infusino *et al*[57]	A scoping review	Nutraceuticals and supplements studied thus far on COVID-19	(1) Vitamin E, D, C, carotenoids, minerals (Zn, Mn, Cu, and Se), polyphenols, and curcumin have; and (2) Anti-inflammatory, anticoagulant, antioxidant, binding to SARS-CoV-2 target receptor, and antiviral properties

DASH: Dietary approaches to stop hypertension; Med-diet: Mediterranean dietary pattern; suppl: supplemented; K: Potassium; Mg: Magnesium; HDL: High density lipoproteins; LDL: Low density lipoproteins; COVID-19: Coronavirus disease 2019; ACE: Angiotensin-converting enzyme; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; AIS: Acute ischemic stroke; SCFAs: Short-chain fatty acids; NCD: Normal diet; HFD: High fat diet; PAR: Protease-activated receptor; IL-6: Interleukin-6; CRP: C-reactive protein; Zn: Zinc; Mn: Manganese; Cu: Copper; Se: Selenium.

processed/sweetened products, besides moderate consumption of wine, fortified with a mixture of nuts and virgin olive oil for a median of 4.8 years, could reduce the stroke risk by 40% compared to the control. The results of a recent meta-analysis have also shown that any 4 points increase in score of adherence to the Mediterranean dietary pattern in both the Mediterranean and non-Mediterranean populations are significantly associated with a 14% and 17% reduction in the risk of ischemic and hemorrhagic stroke, respectively[25]. Although the Mediterranean dietary pattern, similar to the DASH, is not a low sodium diet, following this diet high in potassium can probably lead to a reduction in dietary sodium due to the less consumption of processed foods[25]. A newly published cohort study by Chiu *et al*[47] has demonstrated that a vegetarian diet reduces the risk of ischemic and hemorrhagic stroke, and higher serum levels of vitamin B12 weaken the relationship. Since a vegetarian diet is a meat and egg free diet, one of the proposed mechanisms is that the adherence to this diet could reduce substrates of intestinal microbiota to produce trimethylamine N-oxide, thus, reduce the platelet hyperactivation and thrombosis functions, and the risk of stroke, consequently[47]. In this study, vitamin B12 deficiency is in favor of a decrease in the incidence of stroke, while other studies are the opposite[48].

SOME STUDIED MICRONUTRIENTS OR FOODS AND STROKE RISK

A meta-analysis study has shown that potassium intake significantly inverses association with stroke risk. It is, therefore, recommended to consume potassium-rich foods to support cardiovascular and prevent stroke[49]. The same relationship in a dose-response manner is true for dietary/supplemental magnesium and total stroke[50]. Moreover, stroke studies have rigorously recommended the protective effect of vitamin D on stroke incidence[51]. Further, other dietary components recommended in the context of stroke incidence have been listed in their work, among which, other than what said thus far, dairy calcium (not supplementation), folate (not supplementation), vitamin C, chocolate, coffee, tea, and the regular and moderate alcohol consumption (not alcohol abuse) are observed[51]. Due to controversy and lack of consensus, there is no recommendation for using B12, folate, and B6 to reduce the risk of stroke, and the results are uncertain[51]. Hence, more studies are needed in this regard.

HOW COULD DIETARY PATTERNS BE ATTRIBUTED TO COVID-19–ASSOCIATED STROKE RISK?

A study has found that countries where the major daily intake originates from uncooked or fermented kale, cabbages, and fermented dairy products known as good sources for anti-ACE activity and rich in antioxidants have lower COVID-19 death rates than the others[52]. ACE converts angiotensin I to angiotensin II, while ACE2 does the opposite in the renin-angiotensin system[52], in addition to being the entry point for SARS-CoV-2 into host cells as mentioned earlier[12,52]. Therefore, in the case of infection with SARS-CoV-2, ACE inhibitors may be effective in improving complications such as high blood pressure and subsequent hemorrhagic stroke[52]. The blood levels of ACE respond very quickly to food intake. Hence, dietary patterns can affect this enzyme levels; thus, it is suggested that a diet rich in saturated fatty acids increases ACE levels[52]. While many functional foods are listed as the ACE inhibitor and

antioxidant[53,54]. A recent study has discussed that a healthy, diverse, and balanced diet based on plants, high fiber, and fermented foods rich in the beneficial probiotics, such as *Bifidobacteria* and *Lactobacilli* species, could be a preventative strategy, mitigating harmful effects of SARS-CoV-2[20]. Probiotics can modulate the immune system by controlling the gut microbiota, thereby reducing the susceptibility to affliction or morbidity and mortality from COVID-19[20]. Whereas following a high-fat diet and frequent snacks between meals can lead to dysbiosis. Therefore, its frequency should be kept minimum and often include fruits and vegetables[20]. In this respect, interestingly, Tan *et al*[55] have described that the gut microbiome and the short-chain fatty acids (SCFAs) produced by it could regulate brain functions in the path of the gut-brain axis, thus playing a major role in the prevention of stroke. In the study of 140 acute ischemic stroke patients compared with 92 healthy controls, they have observed dysbiosis and low levels of fecal SCFAs in patients with acute ischemic stroke than in controls, inversely related to stroke severity and prognosis. Therefore, SCFAs are introduced as possible prognostic markers and potential targets for stroke remedy[55]. Furthermore, interestingly a few recent studies have discussed the Mediterranean diet and COVID-19[14]. This dietary pattern is anti-inflammatory; it is rich in bioactive components with anti-inflammatory and antioxidant properties and a variety of vitamins and trace elements but limited in processed foods[14]. Mechanisms involved in reducing stroke following adherence to the Mediterranean diet, which may also be considered for COVID-19–associated stroke, include protection against inflammation, oxidative stress, platelet aggression, endothelial dysfunction, and a pleasant change in metabolites derived from intestinal microbiota that leads to cardiovascular health[25]. On the other hand, as one study suggested, therapeutic targeting of PAR could be promising in the management of COVID-19 neuroinflammatory complications[32]. In this regard, according to an animal study, mice with a high-fat diet (HFD) have higher levels of endogenous thrombin compared to those with a normal diet, being considered as a model for stimulation of PAR-1-based signaling. Whereas β-arrestin-2, unlike thrombin, has a positive effect on this signaling under ischemic stroke condition. HFD mice experience larger infarct and worse outcomes after stroke induction[56]. Therefore, it seems that the role of diet to be also prominent in many signaling pathways related to COVID-19 neuroinflammatory complications. Thus, more human studies in this scope are essential. Apart from nutritional recommendations, due to elevated IL-6 Levels followed by hyperviscosity state[8], it seems necessary to maintain hydration and adequate fluid intake in COVID-19 patients. Altogether, this is important long before and immediately after the COVID-19 pandemic, since long-term and continuous following these healthy dietary patterns, as said, can prevent or reduce comorbidities; this, in turn, may be decrease predisposition to severe COVID-19 and, consequently, its worse complications, such as stroke.

HOW COULD SOME MICRONUTRIENTS OR FOODS BE ATTRIBUTED TO COVID-19–ASSOCIATED STROKE RISK?

Some studies have shown the importance role of potassium and magnesium in COVID-19. Since hypokalemia has been observed in most patients with severe and critical COVID-19[21], potassium-rich foods might also play a supportive role in these patients. Magnesium is also important since it participates in the function of many enzymes involved in the severe immune and inflammatory responses that are manifestations of COVID-19. It, due to its modulator role in IL-6, nuclear factor-kB, and CRP, besides its ability to activate and enhance the function of vitamin D, is essential in preventing the serious consequences of COVID-19 and reducing its morbidity and mortality[21]. In addition to vitamin D, widely discussed due to potential to reduce COVID-19 risk, severity, and mortality *via* mechanisms, including inflammation and cytokine storm suppression, besides the inverse modulatory role in the renin-angiotensin system by downregulating renin and increasing ACE2[2,21], other nutrients, such as vitamins, minerals, and functional foods have been discussed in the context of COVID-19. All of these components have also been studied separately concerning stroke. However, no study has ever considered them all together to discuss their association with stroke as a life-threatening complication of COVID-19. For example, some studies have shown that the prevalence of vitamin D deficiency is about 70% among patients with the anti-phospholipid syndrome with elevated levels of anti-phospholipid antibodies. Furthermore, antibodies titers in healthy controls have been lower in the summer season than in other months of the year[2]. This is a fascinating common point in parallel with studies that have shown increased levels of

anti-phospholipid antibodies in COVID-19–associated stroke cases[6]. However, the causal relationship between vitamin D levels or supplementation and serum levels of anti-phospholipid antibodies and thrombotic events has not yet been established[2]. Some of the main nutraceuticals and supplements studied thus far on COVID-19 include vitamin E, vitamin D, vitamin C, carotenoids, minerals (Zn, Mn, Cu, and Se), polyphenols, and curcumin. Possible mechanisms of their positive effect on COVID-19 pathways have been proposed as anti-inflammatory, anticoagulant, antioxidant, binding to SARS-CoV-2 target receptor, and antiviral properties[57].

CONSEQUENCES OF THE HYPOTHESIS

The present hypothesis stated for the first time in literature proposes a link between a healthy diet and COVID-19–associated stroke risk. Adherence to healthy dietary patterns and more consumption of some foods and nutrients in the recommended daily allowance has always been flagged as a prevention approach in chronic and acute conditions. Here, it is speculated that such a role of healthy nutrition can be highlighted once again in preventing COVID-19–associated stroke too. Since no study to date has proven this, nutritional intake evaluations or food frequency questionnaire-based studies are strongly recommended in order to elicit dietary patterns in COVID-19–associated stroke patients. Also, certain nutrients reported to be effective in common relief functions in stroke and COVID-19 pathways can be prescribed in trials for COVID-19 patients; then, the incidence of stroke in them can be investigated prospectively. If these confirm our hypothesis, it means that healthy nutrition could be a protective/preventive factor against COVID-19–associated stroke risk.

CONCLUSION

Due to the significance of the current pandemic, many studies have paid attention to its consequent clinical outcomes. COVID-19 can be more severe in older patients or individuals with comorbidities, having dangerous consequences, including stroke. Adherence to healthy dietary patterns and more consumption of some foods and nutrients in the recommended daily allowance has always been emphasized as a prevention approach in chronic and acute conditions. However, it should be noted that the present study did not intend to give dietary advice to definite prevention of COVID-19–associated stroke. It only aimed to review the existing literature to evaluate the hypothesis stating that nutrition could be related to COVID-19 and its associated stroke. As far as we know, nutrition cannot be effective in preventing this crisis in the short time, but it can be found that the history of dietary patterns and nutritional intake in patients with stroke or other serious COVID-19–associated complications what is the difference with those in COVID-19 patients without serious complications or even in healthy population by further nutritional intake evaluations or food frequency questionnaire-based studies. If the future studies confirm the present study's hypothesis (*i.e.*, people who adherence to the healthy dietary patterns and habits are less likely to suffer from severe COVID-19–associated complications such as stroke) the chronic supportive role of a healthy diet in critical situations will be highlighted once again.

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SYSTEMATIC REVIEWS

How far has panic buying been studied?

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Abstract

BACKGROUND

Although panic buying (PB) is a ubiquitous behavior, it became prominent during the coronavirus disease 2019 pandemic. However, studies are inadequate to explore the different aspects of it, even though it covers several perspectives of life and academic domains.

AIM

To assess the research that have been conducted on PB.

METHODS

A search was conducted to identify the articles in PubMed, PubMed Central, Scopus, and Google Scholar using the search term "panic buying" on November 15, 2020. A total of 104 articles was extracted from the initial search. After removing duplicates and initial and full-text screening, 42 articles were included in the study. We only considered peer-reviewed published articles that can be downloaded in a full portable document format. Articles published in other languages and preprints were excluded.

RESULTS

Among the 42 articles, 27 were original contributions, 6 were correspondences, 3 were commentaries, 3 were review articles, and there was one each for editorial, opinion, and discussion type of articles. Several domains have been researched

s/by-nc/4.0/

Manuscript source: Invited manuscript

Specialty type: Behavioral Sciences

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such as psychology, responsible factors, supply chain, management, disaster preparedness, e-commerce, consumer behavior, marketing, prevention strategies, media, social network, economics, personality, and engineering. Authors from several disciplines, such as psychiatry, management, economics, business, sales and marketing, consumer behavior, public health, communication, information management, sociology, engineering, business administration, psychology, nursing, health economics, food policy, epidemiology, and community health, have been studied it. Definition, causative model, econometric model, controlling strategy, and measuring instrument have been reported. A total of 18 papers had cross-country collaboration, and ten were funded projects. Most of the authors were affiliated with the institutions of Australia, Bangladesh, China, India, Singapore, and the United States.

CONCLUSION
PB is a relatively newer concept to get the attention of the research community. Further robust studies with replication of the findings are warranted to explore, predict, and control during crises.

Key Words: Panic buying; Systematic review; COVID-19; Pandemic; Disaster; Supply chain

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Core Tip: Panic buying is an under-researched emerging research problem. Although it covers several aspects of human life, there is a dearth of studies. This review was aimed to assess the extent of research that has been done on panic buying. A total of 42 papers were included after a systematic search. Several domains have been researched such as psychology, responsible factors, supply chain, management, disaster preparedness, e-commerce, consumer behavior, marketing, prevention strategies, media, social network, economics, personality, and engineering. Definition, causative model, econometric model, controlling strategy, and measuring instrument have been reported.

Citation: Arafat SMY, Hussain F, Kar SK, Menon V, Yuen KF. How far has panic buying been studied? *World J Meta-Anal* 2020; 8(6): 446-460
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INTRODUCTION

Panic buying (PB) is an interesting behavioral phenomenon, usually noticed among the public in the face of disasters. The term "panic buying" consists of two words "panic" and "buying," which refer to the affective and behavioral components of this phenomenon. It has some common roots with stockpiling[1]. The coronavirus disease 2019 (COVID-19) pandemic has witnessed an increase in PB behavior globally, irrespective of the socioeconomic status of the country[2,3]. It has been conceptualized as "the phenomenon of a sudden increase in buying of one or more essential goods in excess of regular need provoked by adversity, usually a disaster or an outbreak resulting in an imbalance between supply and demand"[4]. Several intermingled factors interact with each other to influence it, pointed out as primary (provoking stimuli), secondary (media and psychosocial aspects), and tertiary (utility, demand, and price of the goods) factors[2]. Multiple psychological explanations have been proposed such as anticipation of shortage and price hike, supply disruption, fear, uncertainty, maladaptive coping, and maintaining control over the environment to attribute it[2,5-7]. There are complex interactions between media, environment, rumor, and increased demand and price as these factors can have a bidirectional role with PB[2,3,6,8]. It has been traced in response to an adverse stimulus, such as COVID-19, pandemic, war, government's declaration, any policy change, disaster, *etc.*[2,9].

Theoretically, PB shares multiple aspects of human life and several domains of

academia. It is related to behavior, public health, disaster preparedness, mass media, economics, sociology, business, marketing, supply chain, industrial buying and production, e-commerce, and so on[8]. However, there is a dearth of studies exploring the different aspects of it. Although studies are recently coming out, the issue is still under-researched. With this background, we aimed to conduct a review to assess the extent of research on PB that has been conducted.

MATERIALS AND METHODS

Article search

A search was done to identify the articles in PubMed, PubMed Central (PMC), Scopus, and Google Scholar (GS) with the search term "panic buying" on November 15, 2020. A total of 104 (PubMed = 19, PMC = 22, Scopus = 30, GS = 33) articles were extracted from the initial search. Subsequently, 44 duplicate articles were removed, and the remainder of the 60 articles were screened. After screening the abstract, another 14 articles were removed as PB was not identified as a study variable. Then, full texts were screened resulting in the removal of four articles because the articles only mentioned PB without discussing its aspects. Finally, 42 articles were included in the study (Figure 1).

Inclusion criteria

We included peer-reviewed published articles that were extracted from the search and downloadable as full portable document format in the study.

Exclusion criteria

Articles published in other languages (for example, Bangla, German, Chinese, and French) and preprints were excluded from the study.

Outcome variables

Type of research, publishing year, applied method, key findings, the geographical distribution of the authors, specialty of authors, collaboration (intracountry or intercountry), a major subject of the journal where the articles published, a principal domain discussed in the article, funding status, and keywords were outcome variables. We considered the first authors' and corresponding authors' affiliated institutions' location to describe the authors' geographical locations.

Statistical analysis

In the current study, we assessed the aspects of PB research, detail statistical analysis was not performed. We did a word cloud of the keywords of the articles to reveal highlighting search terms mentioned in the articles.

Ethical statement

The study was conducted complying with the Declaration of Helsinki (1964). As we analyzed the publicly available media reports, no formal ethical approval was sought.

RESULTS

Distribution of the studies

In this review, we searched articles from PubMed, PMC, Scopus, and GS, and 42 articles were reviewed. The articles were published between 2002 and 2020 whilst 36 papers were published in 2020 (Table 1). Among the selected articles, 27 were original articles, 6 were correspondences, 3 were commentaries, 3 were review articles, and 1 each were editorial, opinion, and discussion type of articles (Table 1).

Applied methods

Nine studies surveyed the target population online or offline, five studies applied cross-sectional data analysis, four papers studied social media, three studies analyzed media reports, and two studies mined data from Google search volume using Google Trends application programming interface (Table 1).

Table 1 Summary of the articles

No.	Ref.	Year	Type	Title	Method applied	Key message
1	Ahmed *et al*[10]	2020	Original	The COVID-19 pandemic and the antecedents for the impulse buying behavior of US citizens	Survey (online and offline)	This study sorted out nine variables from the literature that may influence impulsive buying and tested them by conducting surveys in major United States cities. The variables include fear of complete lockdown, peer buying, the limited supply of essential goods, empty shelves, United States stimulus checks, panic buying, fear appeal, social media fake news, and COVID-19.
2	Alchin[11]	2020	Commentary	Gone with the wind		This paper proposes a definition of "panic buying," with references to literature, philosophy, and contemporary neurobiology. The self-fulfilling prophecy, the contagion model of emotional propagation, the Polyvagal Theory, and Nietzsche's study of the classical tragedy were discussed in relation to panic buying.
3	Alfa *et al*[12]	2020	Original	Effect of panic buying on individual savings: The COVID-19 lockdown experience	Cross-sectional	The paper assessed the microeconomic effect of PB on the savings of an individual. This study's findings revealed that price fluctuation, price differential, and spending hurt the individual saving rate.
4	Arafat *et al*[2]	2020	Original	Responsible factors of panic buying: An observation from online media reports	Analysis of media reports	The authors analyzed 784 media reports to find out the reported responsible factors of panic buying. A sense of scarcity, increased demand, importance of the product, and anticipation of the price hike were the major contributing force towards PB, as mentioned in the reports. The authors postulated a causative model of PB.
5	Arafat *et al*[3]	2020	Original	Media portrayal of panic buying: A content analysis of online news portals	Analysis of media reports	This study analyzed content published in media to determine how media is depicting PB during COVID-19.The findings suggested that the media have been portraying more negative aspects of PB. The authors recommended developing media guidelines to censor news that influences impulse buying behavior.
6	Arafat *et al*[4]	2020	Original	Panic buying: An insight from the content analysis of media reports during COVID-19 pandemic	Media report analysis	The authors proposed a definition of PB. This study analyzed information extracted from English media reports to evaluate the nature, extent, and impact of PB.
7	Arafat *et al*[5]	2020	Correspondence	Psychological underpinning of panic buying during pandemic (COVID-19)		The authors studied psychological reasons of PB, which include fear of scarcity, insecurity, losing control over the environment, social learning, and exacerbation of anxiety.
8	Arafat *et al*[8]	2020	Correspondence	Possible controlling measures of panic buying during COVID-19		The authors mentioned possible measures to control PB during a pandemic. The recommendations included positive role-playing by media. Promotion of feeling of kinship and encouraging generosity can reduce it from the public end. Setting a quota policy and subsidiary sales for necessity items could be a potential strategy.
9	Arafat *et al*[9]	2020	Correspondence	Panic buying: Is it really a problem?		The paper mentioned some challenges to study PB in detail to explore its several aspects
10	Benker[13]	2020	Original	Stockpiling as resilience: Defending and contextualising extra food procurement during lockdown	Online interview	This study analyzed 19 invited interviews taken online in the United Kingdom. The study found that though food shortages were common for a couple of weeks, food hoarding didn't make impulsive buying. The United Kingdom households considered food procurement as a single resilience strategy among the taken six strategies.
11	Chen *et al*[14]	2020	Discussion	A discussion of irrational stockpiling behaviour during crisis		The authors discussed the current and long-term impact of PB on the economy, society, and local communities. They think that stopping impulse buying is impossible, but it should be controlled by improving the supply chain and maintaining communication with the stakeholders.
12	Dammeyer[15]	2020	Original	An explorative study of the individual differences associated with consumer stockpiling during the early stages of the 2020 Coronavirus outbreak in Europe	Online survey	This study answered whether individual differences influenced PB during crises. The authors found a high tendency of stockpiling on extroversion and neuroticism and a relatively low tendency on conscientiousness and openness.

13	Dickins *et al*[16]	2020	Original	Food shopping under risk and uncertainty	Authors analyzed super market sales data	In this study, the authors showed the importance of food security and suggested optimality models of foraging under risk and uncertainty as foraging correlates to PB.
14	Dulam *et al*[17]	2020	Original	Development of an agent-based model for the analysis of the effect of consumer panic buying on supply chain disruption due to a disaster	Simulation model	This study used an agent-based simulation model to analyze how a supply chain responds to consumer PB caused by a natural disaster. The authors found this model useful in applying a quota policy per person to protect the supply chain from disruption.
15	Du *et al*[18]	2020	Original	COVID-19 increases online searches for emotional and health-related terms	Data mining from Google Trends	This study measured fear-related emotions, protective behaviors, seeking health-related knowledge, and PB due to COVID-19 prevalence in the United States, the United Kingdom, Canada, and Australia using internet search volumes in Google Trends. The results found that the increased prevalence of COVID-19 was associated with panic buying consistently in all four countries.
16	Hall *et al*[19]	2020	Original	Beyond panic buying: consumption displacement and COVID-19	Cross-sectional	The authors analyzed consumer spending data acquired from financial third parties and found instances of PB for grocery, home, hardware, and electrical categories that happened in the Canterbury region of New Zealand before the lockdown that lasted less than a week. The study showed a high consumption displacement in the hospitality and retailing sectors that dominate this area's economy.
17	Hao *et al*[20]	2020	Original	Impact of online grocery shopping on stockpile behavior in COVID-19	Online survey	It investigated how online shops affect the food stockpiling manner among urban consumers in China using bivariate probit models. The authors recommended improved and resilient supply chains that can withstand intense PB phenomena during emergencies.
18	Islam *et al*[21]	2020	Original	Panic buying in the COVID-19 pandemic: A multi-country examination	Online survey	The authors surveyed 1081 people from United States, China, India, and Pakistan to test their conceptual model and hypotheses. The research revealed that stimuli such as Limited Quantity Scarcity and Limited Time Scarcity affect emotional stress, which eventually influences impulse buying. The findings also correlated excessive social media use to PB and discussed some managerial implications.
19	Jeżewska-Zychowicz *et al*[22]	2020	Original	Consumers' fears regarding food availability and purchasing behaviors during the COVID-19 pandemic: The importance of trust and perceived stress	Cross-sectional	It investigated how the public perception of food availability changed based on the trust in the received information from media and friends. The participants showed less trust in media for COVID updates but high trust in media and friends for food availability updates and increased buying more food than usual. The consumers were highly afraid of empty shelves in the market, which also motivated them to stockpile food.
20	Kar *et al*[23]	2020	Correspondence	Online group cognitive behavioral therapy for panic buying: Understanding the usefulness in COVID-19 context		The authors postulated to explain the usefulness of online group CBT in COVID19 context for controlling the PB.
21	Keane *et al*[24]	2020	Original	Consumer panic in the COVID-19 pandemic	Data mining using Google Health Trends API	The authors developed an econometric model of consumer panic using Google search data for 54 countries from January 1st to April 30th, 2020. Findings included limited movement notice announced by local or foreign governments generated a week-long short-run panic. The study found little impact of stimulus offerings and no consumer panic due to travel restrictions.
22	Kostev *et al*[25]	2020	Original	Panic buying, or good adherence? Increased pharmacy purchases of drugs from wholesalers in the last week before Covid-19 lockdown	Retrospective cross-sectional analysis of the IMS RPM® (Regional Pharmaceutical Market) Weekly database	The paper assessed the PB of medication during the COVID-19 pandemic in Germany. The study suggested that Germany's lockdown was associated with a sharp increase in purchasing behavior in pharmacies for different markets, including psychotropic, neurological, and cardiovascular drugs.
23	Laato *et al*[26]	2020	Original	Unusual purchasing behavior during the early stages of the COVID-19 pandemic: The stimulus-organism-response approach	Online survey	The authors conducted a web survey with 211 Finnish participants for a week to test a hypothetical research model. The study found a positive link between voluntary self-isolation and unusual purchases. The online information overload caused cyberchondria, which eventually motivated self-isolation followed by PB.
24	Lins *et al*[27]	2020	Original	Development and initial psychometric properties of a panic buying scale during COVID-19 pandemic	Online survey	This study developed the first PB scale that was psychometrically acceptable in the Brazilian context.

25	Loxton *et al*[28]	2020	Review	Consumer behavior during crises: Preliminary research on how coronavirus has manifested consumer panic buying, herd mentality, changing discretionary spending and the role of the media in influencing behaviour	Literature review and cross-sectional data analysis	This study reviewed consumer behavior data, including impulse buying, herd instinct, and prioritization of purchasing decisions of past crises and shock events. The authors then analyzed consumer spending data acquired from data services that confirmed the sorted variables in the COVID-19 context.
26	Martin-Neuninger *et al*[29]	2020	Opinion	What does food retail research tell us about the implications of coronavirus (COVID-19) for grocery purchasing habits?		The paper discussed the consequences of lockdowns on consumer grocery purchasing habits, focusing on New Zealand. In avoidance of PB, the authors suggested few recommendations to the food companies so that people can enjoy visiting supermarkets without compromising safety. They also asked to improve online delivery services to gain trust and customer confidence.
27	Micalizzi *et al*[1]	2020	Original	Stockpiling in the time of COVID-19	Survey	This study aimed to discuss stockpiling behavior during COVID-19 and investigated individual predictors of stockpiling. Those affiliated with conservative politics, worry much about COVID-19, and self-isolated were prone to stockpiling behavior.
28	Naeem[30]	2020	Original	Do social media platforms develop consumer panic buying during the fear of Covid-19 pandemic	Telephonic interview	The study revealed how social media aggravated PB by arousing fear appeal. Along with some exacerbating factors like uncertainties, anxiety, persuasive buying, empty shelves, and exert opinion, a huge load of information at users' fingertips made them more anxious about what was to come, leading to panic buying.
29	Prentice *et al*[31]	2020	Original	Timed intervention in COVID-19 and panic buying	Semantic analysis, secondary data and big data analysis	This paper depicted PB as a side effect of the Australian government's timed-intervention policy. The authors supported their findings with real-life evidence.
30	Rosita[32]	2020	Review	Panic buying in the COVID-19 pandemic era in Indonesia	Literature review	This paper proposed a definition of PB and extrapolated some underlying reasons for it. It also mentioned the negative impact of impulsive buying and recommended some stakeholders' measures to control it.
31	Shorey *et al*[33]	2020	Original	Perceptions of the public on the COVID-19 outbreak in Singapore: A qualitative content analysis	Qualitative content analysis	This study analyzed 2075 comments made to the 29 published news by local media outlets on their Facebook pages to find common concerns shared by Singapore's public. The five main themes derived from the qualitative thematic analysis were fear and concern, PB and hoarding, reality and expectations about the situation, staying positive amid the 'storm,' and worries about the future. The authors recommended clear communication, timely updates, and support measures from the government to maintain social peace and cohesion.
32	Sim *et al*[6]	2020	Correspondence	The anatomy of panic buying related to the current COVID-19 pandemic		The paper mentioned two episodes of PB in Singapore due to a new alert level set by the local authority and the declaration of COVID-19 as a pandemic by the World Health Organization. The authors found some underlying reasons for PB and suggested some recommendations to facilitate it.
33	Singh *et al*[34]	2020	Commentary	A critical analysis to comprehend panic buying behaviour of Mumbaikar'sin COVID-19 era		The authors studied different driving factors of PB and suggested how the retailers should adapt inventory when the supply chain is under disruption. They recommended stopping the panic buying so that others can get the share of the products.
34	Turambi *et al*[35]	2020	Original	Panic buying perception in Waliansatu sub-district, Tomohon City	Online survey	This study analyzed the perception of city dwellers towards PB due to COVID-19. It described different PB episodes that appeared in a city in Indonesia.
35	Yuen *et al*[7]	2020	Review	The psychological causes of panic buying following a health crisis	Systematic review	It was the first systematic review on PB. The authors identified four major themes responsible for PB.
36	Zheng *et al*[36]	2020	Original	Supply disruption management under consumer panic buying and social learning effects	Analytical study	The study analyzed how social learning among customers can influence buying decisions when adequate supply is at risk. When the panic intensity is at a moderate level, social learning can help to adjust the consumer demand, but it will work negatively when the panic intensity is very low or very high. The authors also introduced an optimal inventory ordering strategy for retailers.
37	Tsao *et al*[37]	2019	Original	Product substitution in different weights and brands considering customer segmentation and	Mathematical model	It proposed a mathematical model for managing wholesaler's inventory to maximize the profit during PB. The authors suggested substituting the same products of different weights and brands between high- and

				panic buying behavior		low-indexed stores during a supply disruption. The model can determine optimal order quantity, the number of substitutable units, leftover units, and the unsated demand to improve the store services.
38	Wei *et al*[38]	2011	Original	Research on emergency information management based on the social network analysis — A case analysis of panic buying of salt	Data mining	This research studied how to manage information in an emergency analyzing social network to control PB.
39	Fung *et al*[39]	2010	Original	Disaster preparedness of families with young children in Hong Kong	Survey	This study surveyed households' heads to explore their perception and preparedness for future disastrous events most likely to occur in Hong Kong. These families experienced PB for necessities during disasters especially for children's items and drugs.
40	Kulemeka[40]	2010	Commentary	United States consumers and disaster: Observing "panic buying" during the winter storm and hurricane seasons		This article was an update of ongoing research. This article narrated predisaster shopping and claimed that such shoppers do not go for panic buying rather help each other.
41	Bonneux *et al*[41]	2006	Correspondence	An iatrogenic pandemic of panic		The authors mentioned humans' overreactions to the perceived threat of a hypothetical pandemic accompanied by clever marketing for the panic buying of antiviral drugs.
42	Thomas[42]	2002	Editorial	Panic buying ahead?		The author highlighted the preparedness for future PB influenced by herd instinct in the semiconductor industry.

CBT: Cognitive behavior therapy; COVID-19: Coronavirus disease 2019; PB: Panic buying.

Reported aspects of PB

Definition of PB, causative model, econometric model, media reporting of PB, responsible factors for PB, psychological reasons for PB, controlling strategy, measuring instrument, challenges to explore the problem, and geographical distribution have been identified.

Academic domains

Several domains have been researched such as psychology, supply chain, management, disaster preparedness, e-commerce, consumer behavior, marketing, prevention strategies, media, social network, economics, personality, and engineering (Table 2).

Distribution of authors

Authors from several disciplines, such as psychiatry, management, economics, business, sales and marketing, consumer behavior, public health, communication, information management, sociology, engineering, business administration, psychology, nursing, health economics, food policy, epidemiology, and community health, have been studied it. Most of the authors were affiliated with institutions from Australia, Bangladesh, China, India, Singapore, and the United States (Table 2). Eighteen papers had cross-country collaboration, and ten were funded projects. The average number of authors per paper was 3.3; Arafat SMY has published the maximum number of papers (6) as the first author on the topic.

Table 2 Bibliometric summary of the articles

Ref.	Author No.	Month of publication	Country of the 1st author	Country of the corresponding author	Specialty of the 1st author	Specialty of the corresponding author	Collaboration	Journal	Subject of the journal	Domain of the discussed topic	Funding
Ahmed *et al*[10]	4	Aug 20	Pakistan	Pakistan	Sales and Marketing	Sales and Marketing	Cross-country	*J of Competitiveness*	Competitiveness	Impulse buying behavior	No info
Alchin[11]	1	Jul 20	Australia	Australia	Public health	Public health		*Australasian Psychiatry*	Psychiatry	Panic buying during disaster	No funding
Alfa *et al*[12]	2	Sep 20	Nigeria	Nigeria	Economics	Economics	Intracountry	*Lapai J of Economics*	Economics	Economics	No info
Arafat *et al*[2]	7	Nov 20	Bangladesh	Bangladesh	Psychiatry	Psychiatry	Cross-country	*Frontiers in Public Health*	Public health	Media	No funding
Arafat *et al*[3]	9	Sep 20	Bangladesh	Bangladesh	Psychiatry	Psychiatry	Cross-country	*Global Psychiatry*	Psychiatry	Media	No funding
Arafat *et al*[4]	9	Jul 20	Bangladesh	Bangladesh	Psychiatry	Psychiatry	Cross-country	*Neurology, Psychiatry and Brain Research*	Psychiatry	Media	No funding
Arafat *et al*[5]	6	May 20	Bangladesh	Bangladesh	Psychiatry	Psychiatry	Cross-country	*Psychiatry Research*	Psychiatry	Psychological causes of panic buying	No funding
Arafat *et al*[8]	3	May 20	Bangladesh	Bangladesh	Psychiatry	Psychiatry	Cross-country	*Int J of Mental Health and Addiction*	Psychiatry	Controlling of panic buying	No funding
Arafat *et al*[9]	3	Sep 20	Bangladesh	India	Psychiatry	Psychiatry	Cross-country	*Int J of Social Psychiatry*	Psychiatry	Challenges of scientific studying	No funding
Benker[13]	1	Oct 20	United Kingdom	United Kingdom	Sociology	Sociology		*Appetite*	Behavioral science	Emergency preparedness	No funding
Chen *et al*[14]	7	Jun 20	Australia	Australia	Engineering	Engineering	Intracountry	*J of Safety Science and Resilience*	Disaster	Economics	No funding
Dammeyer[15]	1	Jul 20	Denmark	Denmark	Psychology	Psychology		*Personality and Individual Differences*	Psychology	Individual personality and PB	No funding
Dickins *et al*[16]	2	Oct 20	United Kingdom	United Kingdom	Psychology	Psychology	Intracountry	*Learning and Motivation*	Psychology	Uncertainty and PB	No funding
Dulam *et al*[17]	3	Apr 20	Japan	Japan	Engineering	Engineering	Intracountry	*J of Advanced Simulation in Science and Engineering*	Engineering	Technology and PB	No info
Du *et al*[18]	4	Oct 20	China	China	Psychology	Psychology	Intracountry	*Applied Psychology: Health and Well-Being*	Psychology	Behavioral perspectives	National Natural Science Foundation of China
Hall *et al*[19]	4	Jul 20	New Zealand	New Zealand	Management	Management	Cross-country	*J of Service Management*	Management	Consumer behavior during a disaster	No info
Hao *et al*[20]	3	Aug 20	China	China	Economics	Economics	Cross-country	*China Agricultural*	Economics	E-commerce's role	Beijing Municipal

								Economic Review		on food hoarding	Education Commission Social Science
Islam *et al*[21]	7	Oct 20	China	China	Economics and Management	Economics and Management	Cross-country	*J of Retailing and Consumer Services*	Marketing	Reasons of panic buying	National Social Science Fund of China
Jeżewska-Zychowicz *et al*[22]	3	Sep 20	Poland	Poland	Business	Business	Intracountry	*Nutrients*	Human nutrition	Behavioral perspectives	Warsaw university of life sciences
Kar *et al*[23]	3	Oct 20	India	India	Psychiatry	Psychiatry	Cross-country	*Indian J of Psychiatry*	Psychiatry	Behavioral perspectives for Controlling of panic buying	No funding
Keane *et al*[24]	2	Aug 20	Australia	Australia	Health Economics and Marketing	Consumer Behaviour, Econometrics	Intracountry	*J of Econometrics*	Econometrics	Econometrics model of PB	Australian Research Council grants
Kostev *et al*[25]	2	Jul 20	Germany	Germany	Epidemiology, public health	Epidemiology, public health	Intracountry	*J of Psychiatric Research*	Psychiatry	Drug purchasing surge during COVID 19	No funding
Laato *et al*[26]	4	Jul 20	Finland	Norway	Technologies	Economics and management	Cross-country	*J of Retailing and Consumer Services*	Marketing	Media and panic buying	No info
Lins *et al*[27]	2	Sep 20	Portugal	Portugal	Social psychology	Social psychology	Cross-country	*Heliyon*	Medical sciences	Measurement instrument	No funding
Loxton *et al*[28]	6	Jul 20	Australia	China	Business	Economics	Cross-country	*J of Risk and Financial Management*	Finance and Risk	Consumer behavior during disasters	No funding
Martin-Neuninger *et al*[29]	2	Jun 20	New Zealand	New Zealand	Food Policy and Security	Food Policy and Security	Cross-country	*Frontiers in Psychology*	Psychology	Consumer behaviour	No funding
Micalizzi *et al*[1]	3	Oct 20	United States	United States	Behavioral and Social Sciences	Behavioral and Social Sciences	Intracountry	*British J of Health Psychology*	Psychology	Hoarding during emergency	National Institutes on Drug Abuse
Naeem[30]	1	Sep 20	United Kingdom	United Kingdom	Business administration	Business administration		*J of Retailing and Consumer Services*	Marketing	Social media and panic buying	No funding
Prentice *et al*[31]	3	Aug 20	Australia	Australia	Marketing and Consumer Behavior	Marketing and Consumer Behavior	Intracountry	*J of Retailing and Consumer Services*	Marketing	Government timed-intervention policy and PB	Inspector General Emergency Management Queensland, Australia
Rosita[32]	1	Oct 20	Indonesia	Indonesia	Consumer behavior	Consumer behavior		*Int J of Multiscience*	All disciplines	Consumer behavior	No info
Shorey *et al*[33]	4	Jul 20	Singapore	Singapore	Nursing	Nursing	Intracountry	*Journal of Public Health*	Public health	Responses during emergencies	No funding

Sim *et al*[6]	4	Apr 20	Singapore	Singapore	Psychiatry	Psychiatry	Cross-country	*Psychiatry Research*	Psychiatry	Panic buying distribution	No info
Singh *et al*[34]	2	Mar 20	India	India	Arts humanities & communication	Arts humanities & communication	Intracountry	*Studies in Indian Place Names*	History	Supply chain	No info
Turambi *et al*[35]	2	No info	Indonesia	Indonesia	Economics	Economics	Intracountry	*International Journal of Applied Business and International Management*	Business	Behavioral perspectives	No info
Yuen *et al*[7]	4	May 20	Singapore	China	Economics and Supply Chain Management	Economics and Supply Chain Management	Cross-country	*Int J Environ Res Public Health*	Environmental and Public Health	Psychological causes of panic buying	Nanyang Technological University, Singapore
Zheng *et al*[36]	3	Mar 20	China	Hong Kong	Management	Management	Cross-country	*Omega*	Management	Supply chain	Hongkong & China
Tsao *et al*[37]	3	Feb 19	Taiwan	Taiwan	Industrial management	Industrial management	Intracountry	*Industrial Marketing Management*	Industrial marketing	Marketing and supply chain	Partially supported by Ministry of Sci and Tech Taiwan
Wei *et al*[38]	3	Sep 11	China	China	Information management	Information management	Intracountry	2011 International Conference on Management Science & Engineering (18th)	Management Science and Engineering	Social network data mining and PB	No info
Fung *et al*[39]	2	Sep 10	Hong Kong	Hong Kong	Family and Community Health	Family and Community Health	Intracountry	*Scandinavian J of Public Health*	Public health	Disaster preparedness	No funding
Kulemeka[40]	1	No info	United States	United States	Business and Economics	Business and Economics		*Advances in Consumer Research*	Consumer research	Disaster preparedness	No info
Bonneux *et al*[41]	2	Mar 06	Belgium	Belgium	Public health	Public health	Intracountry	*BMJ*	Medical sciences	Drug stockpiling	No info
Thomas[42]	1	Aug 02	No info		Semiconductor			*III-Vs Review*	Semiconductor industry	Possible pb in semiconductor industry	No info

Distribution of journals

The maximum number of papers were published in behavioral health (13: Psychiatry 9; psychology 4), followed by business (including marketing and management) (12), public health (4), and economics (3) (Table 2). *Journal of Retailing and Consumer Services* hosted the maximum number of papers on PB (4) followed by *Psychiatry Research* (2).

Distribution of keywords

A wide spectrum of keywords was found in articles with a prominence of COVID-19, PB, Coronavirus, and pandemic. Figure 2 shows the word cloud analysis of different keywords extracted from research publications on "panic buying." Words with a larger font size refer to the most frequently used keywords and vice versa. Different colors are used to differentiate words from each other. Colors do not have any

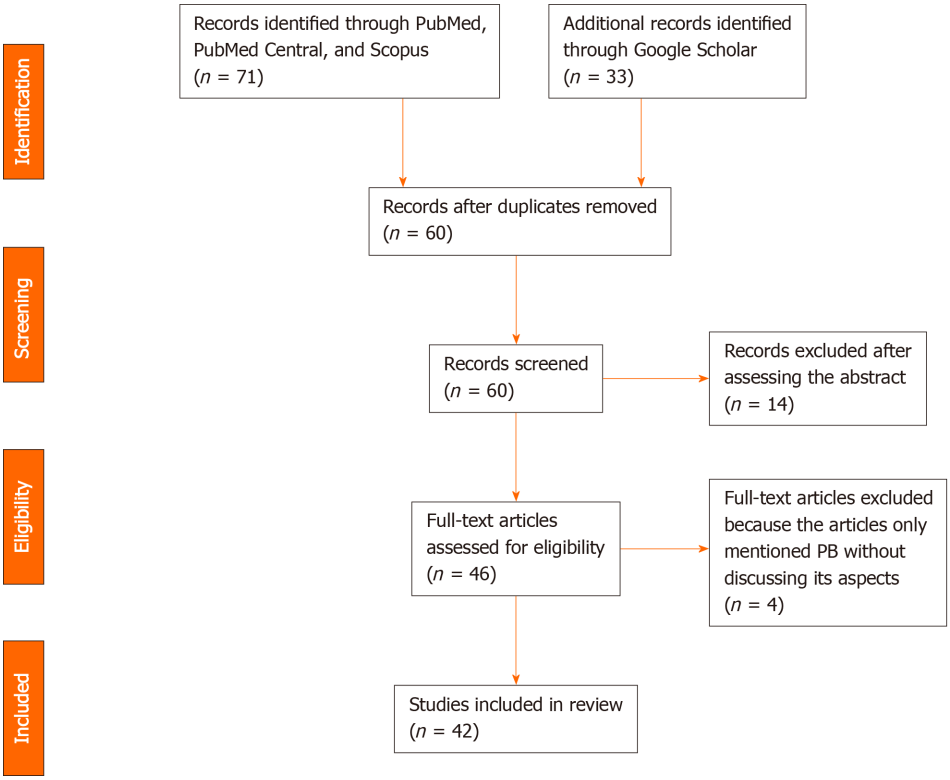


Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow diagram.

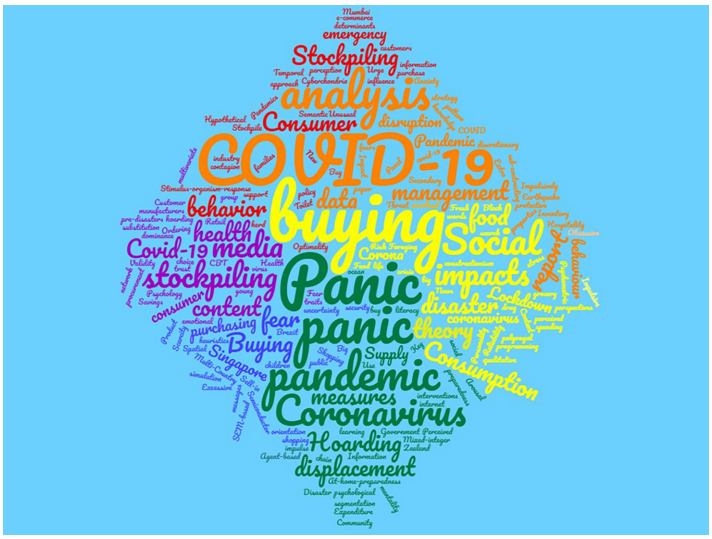


Figure 2 Word cloud analysis of key words. The color of the words does not have any significance.

statistical significance.

DISCUSSION

We aimed to identify the aspects of PB already explored and research trends. We searched PubMed, PMC, Scopus, and GS with the search term "panic buying" on November 15, 2020. A total of 42 articles were collected and scrutinized (Figure 1). The type of research, publishing year, applied method, key findings, geographical distribution of the authors, specialty of authors, collaboration, subject of the journal, and research funding were assessed (Table 1 and Table 2).

Main findings

The main findings of the review revealed that some aspects of PB have been addressed such as the definition of PB[4], causative model[2], econometric model[21], media reporting of PB[3], responsible factors for PB[2], psychological reasons for PB[5-7], controlling strategy[8], measuring instrument[27], challenges to explore PB[9], and geographical distribution[2]. However, the methods were superficial that instigated further studies to replicate the observations.

Distribution of the studies

The study revealed that more than three-quarters (85.71%) of the research output on PB has occurred in the wake of the COVID-19 pandemic. This is an indication of the growing public health relevance of this phenomenon. More than sixty percent (64.28%) of the papers were original contributions indicating that the more empirical studies are coming out gradually (Table 1). Most of the studies applied survey and cross-sectional study design, which may be explained by the pattern of PB as it appears irregularly, episodically, and erratically in response to the adverse stimuli[2,9]. However, longitudinal studies are better in order to explore the behavior. Online media reports, social media, and Google Trends were also used, which may be explained by the COVID-19 pandemic, and the lockdown was applied.

Responsible factors

Several studies have looked at the etiological underpinnings of PB, mainly in terms of psychological and social factors that may contribute to the phenomenon[5-7]. The results are intuitive and point to the significance of perception of commodity and time scarcity, sense of uncertainty as well as the herd instinct, which has its basis in the social learning theory, as potential contributors to the genesis of PB[2,5-7]. Further, perceptions of price differential and price fluctuations have been found to correlate with PB behavior; this has implications from a management perspective and highlights the importance of maintaining supply chains.

Controlling measures of PB

Little empirical evidence is available on the management of PB. One group of authors proposed an online cognitive behavior therapy model for PB, but it was not tested[23]. The media should play a central role in curbing PB by spreading awareness about the phenomenon and adopting responsible reporting practices. A collaborative approach between the media, government, and health sector would foster a collective sense of responsibility and bring about sustainable changes in reporting practices, a key strategy to control PB[3].

Drawing upon these insights, it appears that PB can be controlled by adequate, timely, and consistent information on the evolving situation with an additional focus on clarifying misinformation or rumors. To reduce visual cues, big retail stores can encourage online shopping at least for those who are young/internet savvy. This will reduce long queues outside shopping stores, which is a visual signal for other buyers to join, and will reduce the possibility of such images being circulated in the mass media and social media, another important cue for PB[10]. Opening fair price shops where commodity prices are tightly regulated may be helpful in curbing panic purchases.

Academic domains

Researchers from several specialties took part in PB research, multiple academic domains have been researched, and articles have been published in several specialty journals such as psychiatry, psychology, business, sales and marketing, public health, supply chain, economics, management, consumer behavior, disaster preparedness, e-commerce, consumer behavior, marketing, prevention strategies, media, social network, economics, communication, information management, sociology, personality, nursing, health economics, food policy, epidemiology, community health, and engineering (Table 2). Consumer behavior patterns have been studied during different situations such as pandemic[28] and seasonally recurring disasters[40]. Insights from these studies can be used to spread awareness about PB, facilitate the identification of hoarders, and take steps to mitigate supply chain disruptions[37]. Reducing conflicting information from different media sources and giving advance information about impending seasonal disasters would assist people in staggering their purchases and reduce eleventh-hour PB. Concordance of information from media sources is likely to promote trust in the media, a key element that has been linked to an increased likelihood of distress purchases[22].

Distribution of authors

Most of the authors were affiliated with institutions from Australia, Bangladesh, China, India, Singapore, and the United States (Table 2). The fact that more than half of the studies reviewed originated from Asia may reflect the proneness of countries in the region for PB, probably due to a combination of structural issues, public mistrust, and lack of adequate governmental action. More than forty percent (42.85%) of the papers had intercountry collaborations signifying the common interest of the research. Among the 10 funded projects, China had the highest funding (3 full; 1 partial); Australia (2), Hong Kong (1 partial), United States (1), Poland (1), Taiwan (1), and Singapore (1).

Strengths of the study

This is the first systematic approach to provide an overview and identify the research gaps on the emerging research topic. Only peer-reviewed published articles were reviewed.

Limitations

The search was done cross-sectionally by a single individual (first author). Only articles published in the English language were included. Preprints were not included.

CONCLUSION

PB is a relatively newer concept to get the attention of the research community. The study revealed several important aspects of PB research including research trends, major studied areas, geography, collaboration, and funding of studies. This review would help policymakers, researchers, funders, and other stakeholders to shape their decision while studying PB. Further robust studies with replication of the findings are warranted to fill the research gaps identified in this review.

ARTICLE HIGHLIGHTS

Research background

Panic buying is an under-addressed research entity.

Research motivation

Sporadic evidence is coming out in recent days.

Research objectives

We aimed to see the perspectives of panic buying that have been studied through November 15, 2020.

Research methods

We did a systematic search in PubMed, PubMed Central, Scopus, and Google Scholar and reviewed 42 articles.

Research results

The study identified the distribution of study, aspects of panic buying, academic domain related to panic buying, distribution of authors, the specialty of the authors and journals, funding, and collaborations of the identified articles.

Research conclusions

Although the study identified some important perspectives, further studies are warranted in a systematic manner.

Research perspectives

The review provides a good insight into the different stakeholders to plan further studies and prevent panic buying.

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META-ANALYSIS

Split-dose *vs* same-day bowel preparation for afternoon colonoscopies: A meta-analysis of randomized controlled trials

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Abstract

BACKGROUND

Quality of bowel preparation in afternoon colonoscopies has been a struggle. Currently, a choice of same-day preparation (SaD) or split-dose preparation (SpD) exists; however, randomized controlled trials' results have varied.

AIM

To examine the outcomes of SaD and SpD for afternoon colonoscopies.

METHODS

An extensive literature search was conducted using multiple databases. Only randomized controlled trials (RCTs) in adults that compared SaD to SpD with Ottawa bowel preparation score (OBPS) were included. Odds ratio (OR) or mean difference was used to analyze outcomes.

RESULTS

Eleven RCTs were included (n = 1846). No difference was observed for satisfactory bowel preparation based on OBPS among participants receiving SaD *vs* SpD (OR 0.77; 95%CI: -0.57-1.03; P = 0.07; I^2 = 5%). Subgroup analysis showed no difference in terms of satisfactory bowel preparation based on OBPS between the two groups when receiving same preparation formula (polyethylene glycol) (OR 0.83; 95%CI: 0.51-1.35; P = 0.46; I^2 = 39%) as well as receiving same formula

PRISMA guidelines.

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and volume (4 L polyethylene glycol) (OR 1.14; 95%CI: 0.65-2.01; $P = 0.64$; $I^2 = 0\%$).

CONCLUSION

In patients undergoing afternoon colonoscopies, SaD is comparable with SpD in terms of satisfactory bowel preparation. Further studies are needed to validate these results and determine the optimal formula and dosages.

Key Words: Afternoon; Colonoscopy; Preparation; Split-dose; Same-day; Meta-analysis

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Core Tip: Afternoon colonoscopies have considerably more inadequate bowel preparations than morning colonoscopies. Different bowel preparation regimens have been tried to help improve preparation quality in afternoon colonoscopies, including split-dose and same-day bowel preparations. Studies have shown conflicting results on which preparation regimen is optimal. Therefore, we conducted a meta-analysis on this subject and found that split-dose bowel preparation shows no difference in satisfactory bowel preparations *vs* same-day bowel preparation for afternoon colonoscopies. Therefore, either preparation may be utilized.

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INTRODUCTION

Colorectal cancer (CRC) is a common and devastating disease resulting in significant cancer deaths around the world[1,2]. Colonoscopy remains the screening test of choice for CRC and the only method which encompasses both diagnostic and therapeutic potential[3]. Afternoon colonoscopies have higher rates of suboptimal bowel preparation[4]. Suboptimal bowel preparations are associated with prolonged procedure time, low adenoma detection rate, and increased patient discomfort, complications, and healthcare costs[5-7].

Several studies have suggested that the quality of bowel cleansing for afternoon colonoscopies depends on timing and quantity of the bowel preparation[8,9]. Some studies have shown that split-dosing bowel preparations (SpD) is superior to same-day preparation (SaD, the morning of the procedure) with regard to both cleansing efficacy and tolerability, while other studies report that SaD has a better cleansing and tolerability compared with SpD. Currently, the U.S. Multi-Society Task Force on Colorectal Cancer (USMSTF) recommends SaD regimen as an alternative for SpD for colonoscopies in the afternoon[10] This recommendation was made based on the results of one prospective study comparing the SaD regimen with the SpD regimen and two randomized controlled trials (RCTs) using controls with day-prior regimens[6,11,12].

Recently published RCTs on this topic report mixed results. Moreover, data pooling from the RCTs is challenging given varied bowel preparation regimens and bowel preparation scales. Therefore, a meta-analysis of the RCTs to compare the SaD with the SpD regimens for afternoon colonoscopies was performed.

MATERIALS AND METHODS

Literature search and study selection

Literature search was conducted with a three-fold system. First, multiple databases, including EMBASE, Cochrane databases, MEDLINE/PubMed, Google Scholar, CINAHL, and Scopus were searched in November 2019 for afternoon and colonoscopy. Second, major conference proceeding abstracts (Digestive Disease Week,

American College of Gastroenterology, United European Gastroenterology meetings) were searched through November 2019. Third, references from identified studies were searched for any potentially omissions. If data required clarification, we communicated with the authors.

Data extraction

All RCTs on adults comparing the SaD with SpD regimen for afternoon colonoscopies, using the Ottawa bowel preparation score (OBPS) were included. Exclusion criteria were patients < 18 years old or non-RCTs. To reduce confounding, subgroup analyses were performed for the same formulation and volume of bowel preparation using polyethylene glycol with electrolytes (PEG). Two authors (Parsa NP and Grisham EA) independently reviewed all the studies for inclusion and extracted data using standard forms. Any disagreements on inclusion or data extraction were settled by the senior author (Bechtold ML).

Quality assessment of studies

The Cochrane's Collaboration Risk of Bias Tool was used to assess the quality of studies[13-15]. For each study, a grade, as described as low, moderate, or high, was based on the assessment of limitations, effect magnitude, precision, publication and other forms of bias, and consistency of results[13-15].

Statistical analysis

A meta-analysis was conducted comparing SaD and SpD for afternoon colonoscopies by calculating pooled estimates of quality of bowel preparation. Outcomes were analyzed using mean difference (MD) or odds ratio (OR) by the DerSimonian and Laird method (the random-effects model). The I^2 measure of inconsistency was used to assess heterogeneity ($P < 0.10$ or $I^2 > 50\%$ was deemed significant). If heterogeneity was discovered, researchers used performed a sensitivity analysis to remove the least amount of studies necessary to reach non-significant heterogeneity by comparing results to the original pooled data. RevMan 5.3 (Review Manager, Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012) was used for statistical analysis. Funnel plots assessed for publication bias.

RESULTS

Article search and quality assessment

Evaluation of titles and abstracts resulted in 663 articles being identified. After review, 21 articles remaining in which 11 satisfied the inclusion criteria (n = 1846) with mean age range of 51.6-61.8 years[16-26] (Figure 1). Studies were global, including many countries (United States, Spain, Italy, China, Korea, India) (Table 1). Most of the studies were deemed high-quality studies based on quality assessment (Table 2).

Overall results

Eight RCTs reported the mean OBPS (n = 1328)[17-21,23-25]. Eight studies reported the number of satisfactory bowel preparations (n = 1483)[16,19,21-26]. Of these, 1202 had satisfactory bowel preparations with 578 in the SaD group and 624 in the SpD group. There was no difference between SaD and SpD for the mean OBPS (MD 0.33; 95%CI: -0.09-0.75; P = 0.13; I^2 = 74%) (Figure 2A) or the number satisfactory bowel preparations (79.1% *vs* 83%; OR 0.77; 95%CI: 0.57-1.03; P = 0.07; I^2 = 5%) (Figure 2B) despite a trend favoring SpD. Given significant heterogeneity in the mean OBPS analysis, a sensitivity analysis was performed which showed similar results without significant heterogeneity when one study[23] was eliminated (OR 0.18; 95%CI: -0.11-0.46; P = 0.22; I^2 = 36%).

Same formulation bowel preparation (PEG)

Five studies reported the mean OBPS (n = 877)[17,20,21,23,25]. There was no difference between SaD and SpD for mean OBPS (MD 0.45; 95%CI: -0.13-1.02; P = 0.13; I^2 = 78%) (Figure 3A). Five studies reported the number of satisfactory bowel preparations (n = 1045)[21-23,25,26]. Of these, 862 had satisfactory bowel preparations (82.5%) with 415 in the SaD group and 447 in the SpD group. There was no difference between SaD and SpD for number satisfactory bowel preparations (81% *vs* 84%; OR 0.83; 95%CI: 0.51-1.35; P = 0.46; I^2 = 39%) (Figure 3B). Given significant heterogeneity in the mean OBPS analysis, a sensitivity analysis was performed which showed similar results without

Table 1 Description of studies included in the meta-analysis

Ref.	Country	Number of patients (*n*)	Bowel preparation times (dose 1)	Patients per group (*n*)	Bowel preparation scale	Satisfactory bowel preparations (*n*)	OBPS (mean score ± SD)
Parra-Blanco *et al*[16], 2006	Spain	88	Same day PEG 3 L	43	Ottawa	34	ND
			Split-dose NaP 45 mL/45 mL	45	Ottawa	36	ND
Kang *et al*[24], 2014	South Korea	196	Same day NaP 1/1	97	Ottawa	59	4.05 ± 1.56
			Split-dose PEG 2 L/2 L	99	Ottawa	71	3.8 ± 1.55
Shah *et al*[17], 2014	India	159	Same day PEG 2 L	80	Ottawa	ND	6.02 ± 1.34
			Split-dose PEG 1 L/1 L	79	Ottawa	ND	5.52 ± 1.23
Cesaro *et al*[18], 2013	Italy	101	Same day Halflytely 2 L/10-20 mg Bisacodyl	50	Ottawa	ND	2.78 ± 1.95
			Split-dose PEG 3 L/1 L	51	Ottawa	ND	3.41 ± 1.90
de Leone *et al*[19], 2013	Italy	154	Same day Halflytely 2 L/10-20 mg Bisacodyl	78	Ottawa	70	3.09 ± 2.4
			Split-dose PEG 2 L/2 L	76	Ottawa	70	2.39 ± 2.55
Kim *et al*[25], 2014	South Korea	100	Same day PEG 4 L	50	Ottawa	41	4.98 ± 1.78
			Split-dose PEG 2 L/2 L	50	Ottawa	42	4.98 ± 1.57
Kotwal *et al*[20], 2014	United States	103	Same day PEG 3 L	51	Ottawa	ND	7.15 ± 3.58
			Split-dose PEG 2 L/2 L	52	Ottawa	ND	7.38 ± 3.65
Seo *et al*[21], 2013	South Korea	197	Same day PEG 2 L	97	Ottawa	72	3.76 ± 2.07
			Split-dose PEG 2 L/2 L	100	Ottawa	75	3.67 ± 1.57
Zhang *et al*[23], 2014	China	318	Same day PEG 2 L	159	Ottawa	126	4.4 ± 2.7
			Split-dose PEG 1 L/2 L	159	Ottawa	143	2.9 ± 2.4
Alkhairi *et al*[26], 2017	United States	300	Same day PEG 4 L	142	Ottawa	142	ND
			Split-dose PEG 2 L/2 L	158	Ottawa	156	ND
Castro *et al*[22], 2019	United States	130	Same day PEG 4 L	65	Ottawa	34	ND
			Split-dose PEG 2 L/2 L	65	Ottawa	31	ND

OBPS: Ottawa bowel preparation score; PEG: Polyethylene glycol; ND: Not detected.

significant heterogeneity when one study[23] was eliminated (OR 0.26; 95%CI: -0.02-0.54; P = 0.07; I^2 = 0%).

Same formulation and volume bowel preparation (4 L PEG)

Three studies reported the mean OBPS (n = 362)[17,20,25]. There was no difference between SaD and SpD for mean OBPS (MD 0.30; 95%CI: -0.08-0.68; P = 0.12; I^2 = 12%) (Figure 4A). Three studies reported the number of satisfactory bowel preparations (n = 530)[22,25,26]. Of these, 446 had satisfactory bowel preparations with 217 in the SaD group and 229 in the SpD group. There was no difference between SaD and SpD for number satisfactory bowel preparations (84.4% *vs* 83.9%; OR 1.14; 95%CI: 0.65-2.01; P = 0.64; I^2 = 0%) (Figure 4B).

Publication bias

For any outcome, no significant publication bias was identified (Figure 5).

DISCUSSION

The USMSTF currently recommends the SaD bowel preparation as an alternative to SpD for afternoon colonoscopies[10]. This recommendation, which is based on "high-

Table 2 Quality assessment of studies included in meta-analysis based upon Cochrane's Collaboration Risk of Bias tool

Ref.	Study design	Random sequence generation	Allocation concealment	Blinding	Blinding outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Quality assessment
Parra-Blanco *et al*[16], 2006	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Kang *et al*[24], 2014	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Shah *et al*[17], 2014	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Cesaro *et al*[18], 2013	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
de Leone *et al*[19], 2013	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Kim *et al*[25], 2014	RCT	Not described	Adequate	Single-blinded	Adequate	None	None	None	Moderate
Kotwal *et al*[20], 2014	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Seo *et al*[21], 2013	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Zhang *et al*[23], 2014	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Castro *et al*[22], 2019	RCT	Adequate	Adequate	Single-blinded	Adequate	None	None	None	High
Alkhairi *et al*[26], 2017	RCT	Not described	Not described	Single-blinded	Adequate	None	None	None	Moderate

RCT: Randomized controlled trial.

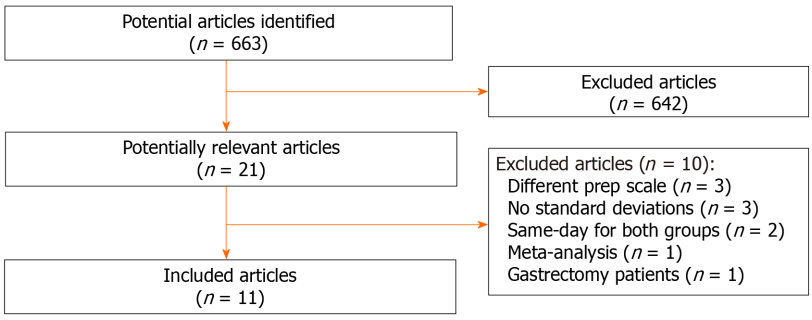


Figure 1 Details of search algorithm.

quality evidence", is based on one prospective study and two RCTs that their control groups received a day-prior bowel preparation regimen[6,11,12]. Since this recommendation, several high-quality trials have evaluated and compared the efficacy of SaD *vs* SpD for afternoon colonoscopies, supporting the value of this study.

The practice of SaD bowel preparation was supported by two meta-analyses published in 2017. Both studies concluded the noninferiority of the SaD compared with the SpD regimen with regards to bowel preparation for afternoon colonoscopies, consistent with the findings of our study[27,28]. Avalos *et al*[27] conducted a meta-analysis on 11 RTCs comparing the efficacy of bowel preparation quality between the SaD and SpD regimens and reported a similar results for the bowel preparation quality, patient willingness to repeat the procedure and adenoma detection rate, although SaD patients reported less bloating and improved quality of sleep. Cheng *et al*[28] pooled the results of 14 RTCs and reported comparable results between the SaD and SpD regimens for bowel preparation with substantial heterogeneity (I^2 = 60%), so subgroup

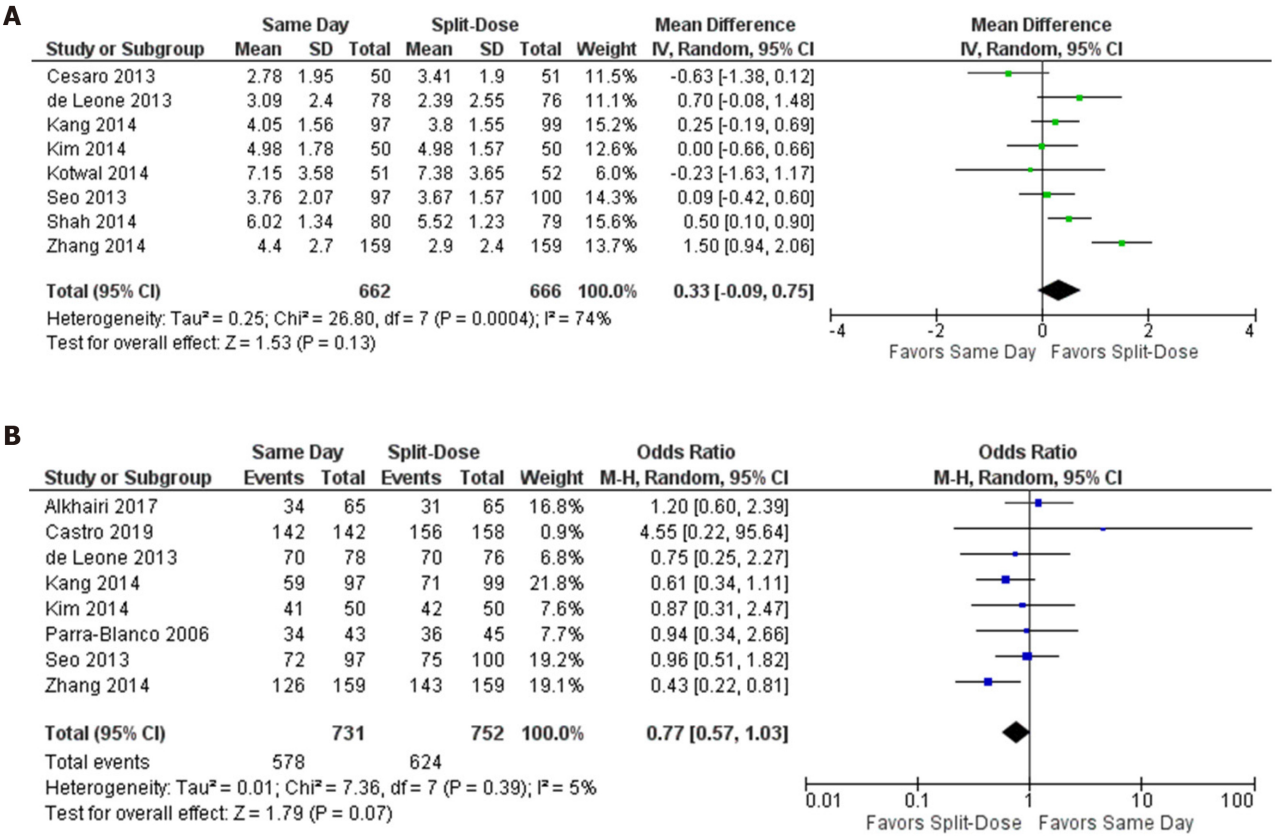


Figure 2 Forest plot showing overall bowel preparation results between same-day preparation *vs* split-dose preparation for afternoon colonoscopies. A: Mean Ottawa bowel preparation score; B: Number of satisfactory bowel preparations.

analysis was performed to evaluate the influence of bisacodyl on bowel preparation. Comparing SaDs with bisacodyl to SpDs without it the previous evening showed the results favored SaDs ($I^2 = 0\%$). If both arms eliminated adjuvants, the analysis revealed that patients in the SpD arm had better bowel preparation with no heterogeneity (OR 0.66; 95%CI: 0.49-0.88). Heterogeneity was a significant limitation of these meta-analyses as many studies used varied bowel preparation scales in the study arms. Furthermore, neither compared the SaD and SpD regimens among patients who received same formula and volume bowel preparation.

This meta-analysis is the first comparing SaD *vs* SpD bowel preparation for afternoon colonoscopies that used identical validated scales to evaluate the bowel preparation quality. No significant differences were identified between the SaD and SpD regimens for quality of bowel preparation by total OBPS. Moreover, we performed a comprehensive subgroup analysis in order to minimize potential confounding factors. Further subgroup analyses showed no differences in terms of satisfactory bowel preparation based on OBPS between the two groups when receiving the same preparation formula (PEG) as well as receiving same preparation formula and volume (4 L PEG). Only RCTs in adult patients were evaluated and used in this meta-analysis. Moreover, by using the OBPS, which evaluates the bowel preparation quality before the application of any cleansing maneuvers, the amount of time and adequacy of cleaning was not an issue, thereby limiting confounding variables. Results of our study can help guide clinicians and patients to select the optimal method for bowel preparation. The current guidelines indicate that providers are responsible for maintaining optimal bowel preparations at greater than 85%, which is often affected by inadequate bowel preparation ingestion by patients[10]. Given a lack of clinical differences, both SaD and SpD regimens should be offered to patients and their preference should be considered in order to maximize their adherence. This may potentially minimize procedure cancellations and increase the success rates of afternoon colonoscopies.

The strengths of this meta-analysis are abundant. Inclusion of worldwide RCTs in varying populations, including China, Korea, Spain, Italy, India, and United States, allows for generalization to many populations. Second, the quality of RCTs included were moderate-to-high. Given the lack of ability to blind the patient to the bowel

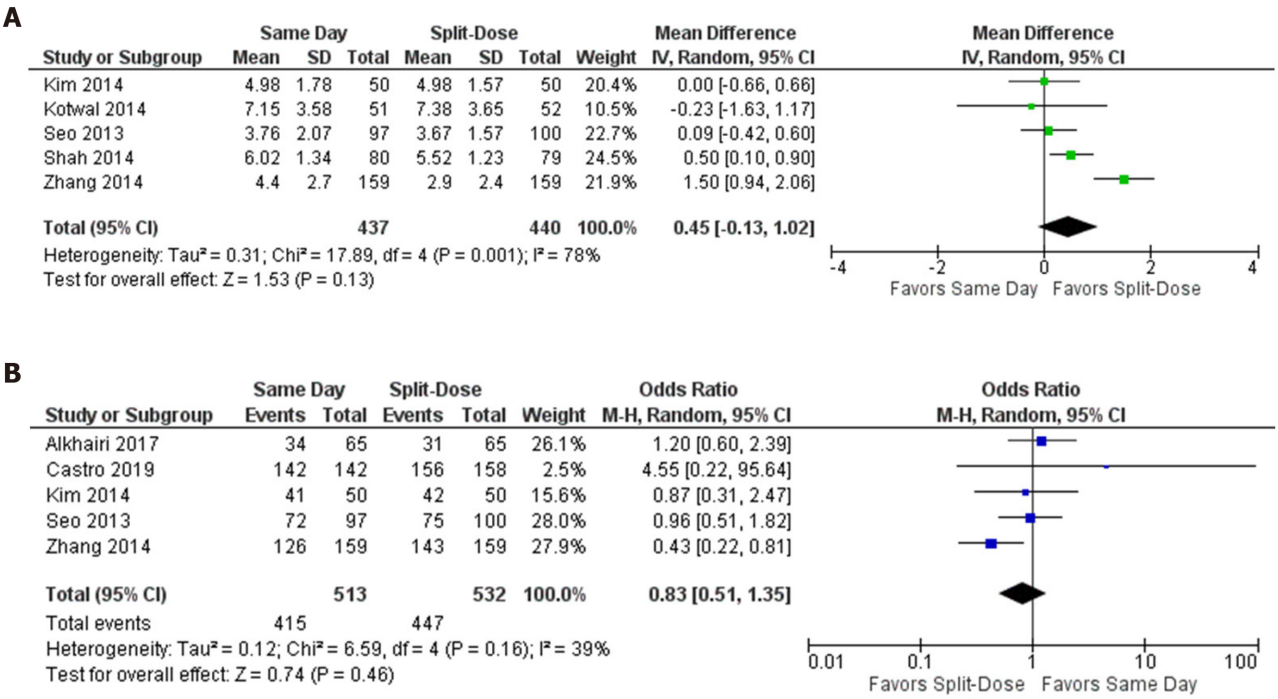


Figure 3 Forest plot showing same bowel preparation results between same-day preparation *vs* split-dose preparation for afternoon colonoscopies. A: Mean Ottawa bowel preparation score; B: Number of satisfactory bowel preparations.

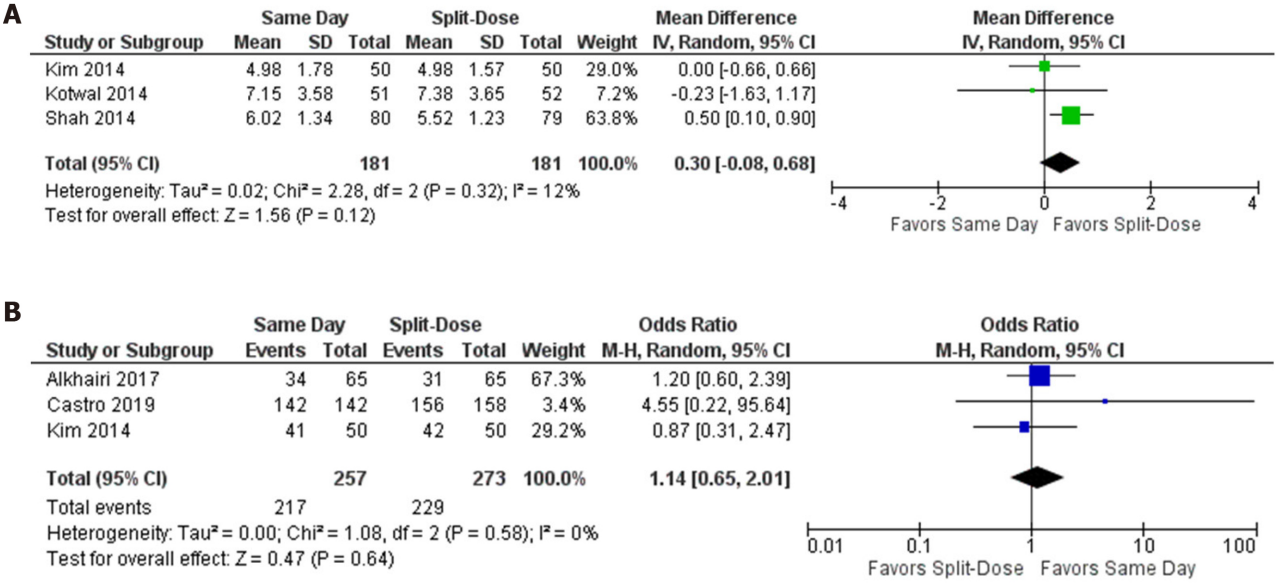


Figure 4 Forest plot showing same bowel preparation (type and volume) results between same-day preparation *vs* split-dose preparation for afternoon colonoscopies. A: Mean Ottawa bowel preparation score; B: Number of satisfactory bowel preparations.

preparation, the included studies were the highest exceptional quality possible. Third, to minimize confounding factors, extensive subgroup analyses were performed and only studies with the same bowel preparation and the same bowel preparation with same volume were evaluated. This effort limits significant confounding factors. Finally, the OBPS was used which limits confounding variables of cleaning effort and cleaning time since evaluated prior to cleaning. Limitations of this meta-analysis were observed. First, significant heterogeneity was observed in two outcomes. Besides a diversity of bowel preparation across studies, there were slightly varied preparations within study arms. Furthermore, some using SaD or SpD preparations with or without

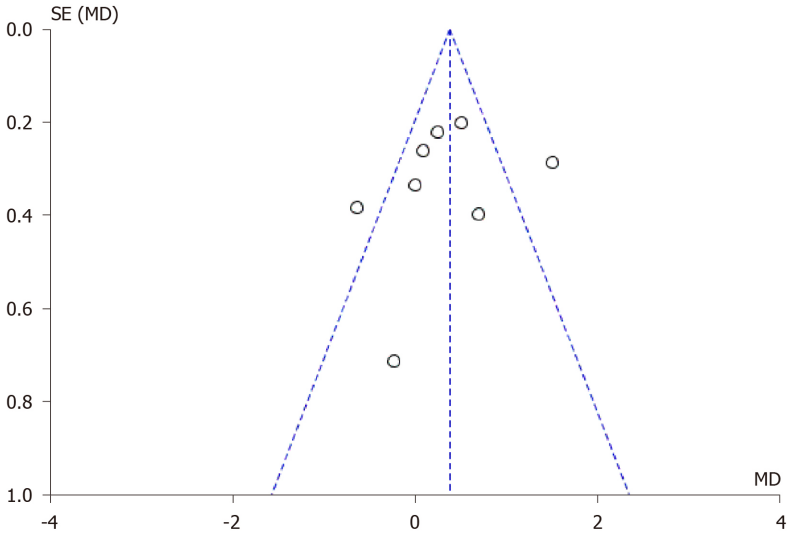


Figure 5 Funnel plot showing no publication bias.

bisacodyl. For those two outcomes with significant heterogeneity, sensitivity analyses were performed with similar results without significant heterogeneity when Zhang *et al*[23] was removed. Second, the type of diet as well as the length of diet restriction during preparation varied among studies and therefore, the influence of diet on bowel preparation could not be further analyzed.

CONCLUSION

In conclusion, our meta-analysis showed that no difference exists between SaD and SpD bowel preparation for the number of satisfactory bowel preparations in the afternoon colonoscopies. Both options should be offered to patients in order to maximize adherence and increase afternoon colonoscopy success rates.

ARTICLE HIGHLIGHTS

Research background

Bowel preparation for afternoon colonoscopies is important for screening for colorectal cancer.

Research motivation

Bowel preparation for afternoon colonoscopies is controversial. Examining the best approach would be beneficial for patients and those performing colonoscopies.

Research objectives

This meta-analysis examines the use of same-day preparation (SaD) or split-dose preparation (SpD) for afternoon colonoscopies.

Research methods

An extensive literature search was conducted using multiple databases. Only randomized controlled trials in adults that compared SaD to SpD with Ottawa bowel preparation score (OBPS) were included. Odds ratio or mean difference was used to analyze outcomes.

Research results

No differences were observed for satisfactory bowel preparation based on OBPS among participants receiving SaD *vs* SpD overall (P = 0.07), when the two groups received the same preparation formula (polyethylene glycol, PEG) (P = 0.46), and when the two groups received the same formula and volume (4 L PEG) (P = 0.64).

Research conclusions

In patients undergoing afternoon colonoscopies, SpD is comparable with SaD in terms of satisfactory bowel preparations.

Research perspectives

Patients and proceduralists may be confident in using either SaD or SpD for afternoon colonoscopies.

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META-ANALYSIS

Comparison of hand-assisted laparoscopic radical gastrectomy and laparoscopic-assisted radical gastrectomy: A systematic review and meta-analysis

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Author contributions: Gan W, Chen ZY, Liu LY, and Chen GB designed the research study; Gan W, Chen ZY, and Liu LY performed the research; Zhou J and Song YN contributed analytic tools; Gan W, Chen GB, and Cao YK analyzed the data and wrote the manuscript; all authors have read and approved the final manuscript.

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Abstract

BACKGROUND

Gastrectomy is the optimal treatment for gastric cancer. Laparoscopic-assisted gastrectomy (LAG) has been extensively employed, while hand-assisted laparoscopic gastrectomy (HALG), which is similar to LAG, remains controversial. Although HALG is popular in China, some surgeons do not accept it as a minimal-access technique.

AIM

To assess the safety and practicability of HALG by comparing the short-term outcomes of HALG and LAG.

METHODS

The electronic databases of EMBASE, PubMed, China National Knowledge Infrastructure, and Cochrane Library were thoroughly searched, and randomized controlled trials (RCTs) comparing HALG and LAG were included. The study results, including surgery time, blood loss, retrieved lymphatic nodes, incision length, time to first flatus, hospitalization duration, and all postsurgical complications, were compared between the two groups.

RESULTS

Five RCTs, which included 302 cases with HALG and 298 cases with LAG, were considered eligible for inclusion. Meta-analysis showed that HALG significantly reduced surgery time ($P < 0.01$), hospital duration ($P < 0.01$), and overall postsurgical complications ($P < 0.01$). Additionally, HALG significantly increased the number of retrieved lymphatic nodes ($P = 0.01$) and incision length ($P < 0.01$)

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compared with LAG. The blood loss and time to first flatus were similar between the two groups ($P > 0.05$).

CONCLUSION

Compared with LAG, HALG is a simpler and safer technique. Additionally, HALG should be used as a minimal-access technique, especially in technologically undeveloped areas.

Key Words: Gastric cancer; Hand-assisted laparoscopy; Gastrectomy; Laparoscopic-assisted gastrectomy; Meta-analysis; Systematic review

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Core Tip: No consensus is available in the literature about which technique is more beneficial to the patients between hand-assisted laparoscopic gastrectomy (HALG) and laparoscopic-assisted gastrectomy (LAG). This is the first systematic review and meta-analysis comparing HALG and LAG. We compared these two techniques in terms of estimated surgery time, blood loss, retrieved lymphatic nodes, incision length, time to first flatus, hospitalization duration, and all postsurgical complications from selected randomized controlled trials. Compared with LAG, HALG is a simpler and safer technique.

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INTRODUCTION

Gastric cancer is associated with high mortality and morbidity rates in China[1-3]. Gastrectomy is the optimal treatment for gastric cancer, but the surgical approach has numerous choices such as traditional open gastrectomy, laparoscopic-assisted gastrectomy (LAG), totally laparoscopic gastrectomy, robot-assisted gastrectomy, and hand-assisted laparoscopic gastrectomy (HALG). Hunter predicted an exciting prospect for hand-assisted laparoscopic surgery (HALS) in gastrectomy[4]. HALS combines the advantages of laparoscopic surgery and laparotomy; thus, it is popular in China[5,6].

Compared with laparoscopic-assisted or totally laparoscopic surgery, HALS retains the tactile sensation of the surgeon's hand, which can make the operation faster and safer. Besides, it also has advantages of laparoscopy, such as being minimally invasive and having a zooming surgical field. For young surgeons, it also has the advantage of having a short-learning curve[7]. To date, HALG has formed the unique surgical approach called three-step HALG[8-10], which makes gastrectomy more convenient and simpler.

Recently, the number of studies on HALG is increasing. Although certain studies have compared HALG and LAG[11,12], controversy about its useful meaning still exists. Therefore, the present study conducted a systematic review and meta-analysis, with an aim to evaluate the safety and practicability of HALG, and compare the short-term outcomes of HALG and LAG.

MATERIALS AND METHODS

Search strategy

EMBASE, PubMed, Cochrane Library, and China National Knowledge Infrastructure were searched for primary studies published up to August 2019. The search terms 'hand-assisted laparoscopic' and 'gastrectomy' and 'gastric cancer' were used in

English and Chinese. Additionally, the references cited in retrieved articles were reviewed in order to select studies that better suit our criteria. Studies with only abstracts or unpublished reports were not included.

Inclusion and exclusion criteria

Two authors (Gan W and Liu LY) independently reviewed the search results. Any studies that met the following criteria were considered: (1) All patients were diagnosed with gastric cancer; (2) The study compared HALG and LAG; (3) It was a randomized controlled trial (RCT); and (4) The endpoints included postsurgical complications. If there were two or more articles by the same authors or research institutions, the one with larger sample size was selected.

To limit heterogeneity across the studies, the following exclusion criteria were used: (1) The study included totally laparoscopic or robotic radical gastrectomy; (2) It did not provide sufficient data to calculate the risk ratio (RR) ant its 95% confidence interval (CI) of different procedures for overall postsurgical complications; and (3) The article was an abstract presented at meetings, a case series, a cohort study, a review, or a letter.

Data extraction

Data were extracted independently by two authors, and discrepancies were resolved by consensus. The following details were extracted: Name of the first author, institution, country, study period, publication year, sample size, mean age, gender, tumor stage, surgery time, blood loss, retrieved lymphatic nodes, incision length, time to first flatus, hospitalization duration, and all postsurgical complications.

Statistical analysis

All statistical analyses were performed using RevMan 5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). For the meta-analysis, the results were presented as RR for dichotomous variables and weighted mean difference (WMD) for continuous variables. If the I^2 value was ≤ 50%, a fixed effects model was employed, and if the value was > 50%, a random effects model was selected. Two-sided $P < 0.05$ was considered to indicate a statistically significant difference. χ^2 test was used to evaluate statistical heterogeneity, and I^2 statistic was calculated to evaluate the extent of variability attributable to statistical heterogeneity between trials. To assess the publication bias, a funnel plot was applied.

RESULTS

Selected studies

During the initial search, 126 publications were obtained from electronic databases. A total of 17 articles were reviewed in detail. Two studies derived from the same research institution[13,14]; thus, the biggest sample size study was selected[13]. Finally, five RCT studies were selected for the meta-analysis[13,15-18]. The details of the search strategy are shown in Figure 1.

Study characteristics

The basic characteristics of the included studies are shown in Table 1. Five RCTs were included. A total of 302 patients were included in the HALG group, and 298 patients were included in the LAG group. Five studies were reported from different regions of China.

Study quality

The Jadad scoring system was used to assess the quality of the selected RCT studies. Due to being open-label RCTs, those studies only scored 2 or 3 points (Table 2). It was known that operation was impossible to blind patients and surgeons. Thus, studies with a score ≥ 2 were classified as methodologically sound studies.

Intraoperative outcome

The surgery time, blood loss, incision length, and retrieved lymphatic nodes were evaluated. The HALG group had a shorter surgery time compared with the LAG group (WMD, -23.81 min; 95%CI, -38.80 to -8.81; $P = 0.002$; Figure 2A). There was no significant difference in blood loss between the two groups (WMD, -8.61 mL; 95%CI, -19.66 to 2.44; $P = 0.13$; Figure 2B). Only four studies reported the incision length, and

Table 1 Characteristics of the included studies

Ref.	Year	Nation	Geographical region	Study period	Sample size		Age, mean (yr)		Gender (M/F)		Tumor stage		Types of operation					
					HALG	LAG	HALG	LAG	HALG	LAG	HALG	LAG	HALG			LAG		
											I/II/III/IV	I/II/III/IV	TG	DG	PG	TG	DG	PG
Gong *et al*[13]	2014	China	Southwest	2008-2013	120	113	58.94	59.29	75/45	82/31	15/19/46/40	9/15/52/37	46	61	13	31	59	23
Wang[16]	2015	China	North	2010-2013	61	65	NA	NA	NA	NA	NA	NA	0	61	0	0	60	0
Yang *et al*[18]	2016	China	Southwest	2013-2015	42	42	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Xue *et al*[17]	2018	China	Central	2015-2016	28	28	52.68	52.74	21/7	20/8	3/7/18/0	2/5/21/0	0	28	0	0	28	0
Gao *et al*[15]	2019	China	East	2013-2014	51	50	57.6	58.2	32/19	36/14	10/12/29/0	13/15/22/0	12	39	0	17	33	0

TG: Total gastrectomy; DG: Distal gastrectomy; PG: Proximal gastrectomy; NA: Not reported; HALG: Hand-assisted laparoscopic gastrectomy; LAG: Laparoscopic-assisted gastrectomy.

the value was longer in the HALG group than in the LAG group (WMD, 0.89 cm; 95%CI, 0.45 to 1.33; $P < 0.01$; Figure 2C). The HALG group had a greater number of retrieved lymphatic nodes compared with the LAG group (WMD, 2.02; 95%CI, 0.40 to 3.64; $P = 0.01$; Figure 2D).

Postsurgical outcomes

The time to first flatus and the duration of postsurgical hospitalization were evaluated in the postoperative recovery. There was no significant difference in the time to first flatus between the two groups (WMD, 0.02 d; 95%CI, -0.22 to 0.25; $P = 0.90$; Figure 2E). The HALG group had shorter hospital duration, compared with the LAG group (WMD, -0.60 d; 95%CI, -0.95 to -0.26; $P < 0.01$; Figure 2F).

The overall postsurgical complications were evaluated in all the included studies. The pooled result showed that the HALG group had a lower risk of overall postsurgical complications than the LAG group (RR, 0.57; 95%CI, 0.37 to 0.88; $P < 0.01$; Figure 2G).

Publication bias

The funnel plot of overall postsurgical complications was used to examine the potential publication bias. Based on the approximate symmetry, there was no evidence of publication bias in this meta-analysis (Figure 3).

Table 2 Jadad scores of the included randomized controlled trials

Ref.	Randomization	Blind	Withdrawals and dropouts	Total
Gong *et al*[13], 2014	1	0	1	2
Wang[16], 2015	1	0	1	2
Yang *et al*[18], 2016	1	0	1	2
Xue *et al*[17], 2018	2	0	1	3
Gao *et al*[15], 2019	2	0	1	3

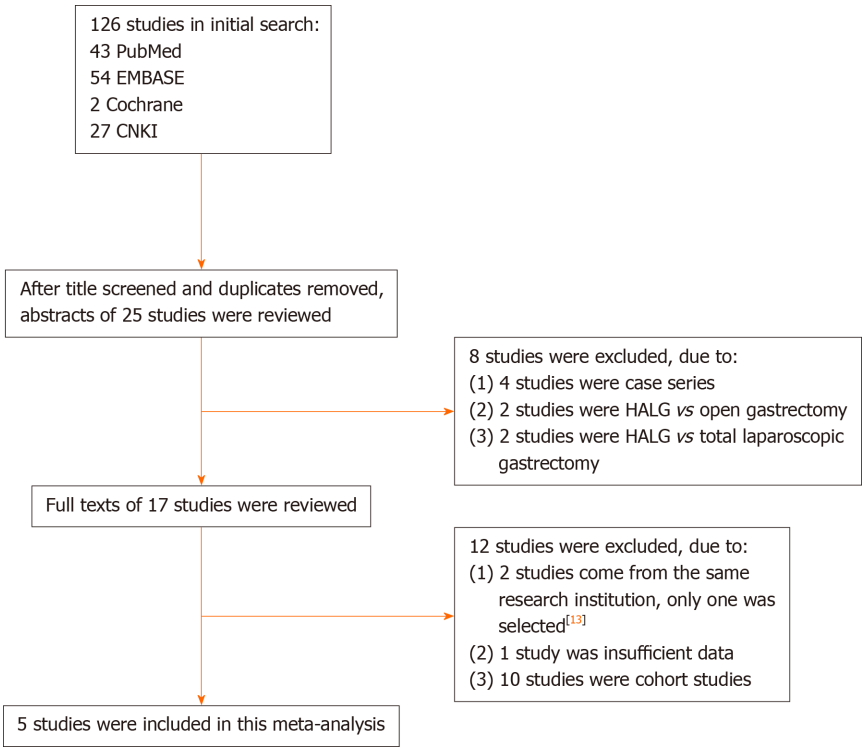


Figure 1 Flowchart of the search strategy. HALG: Hand-assisted laparoscopic gastrectomy.

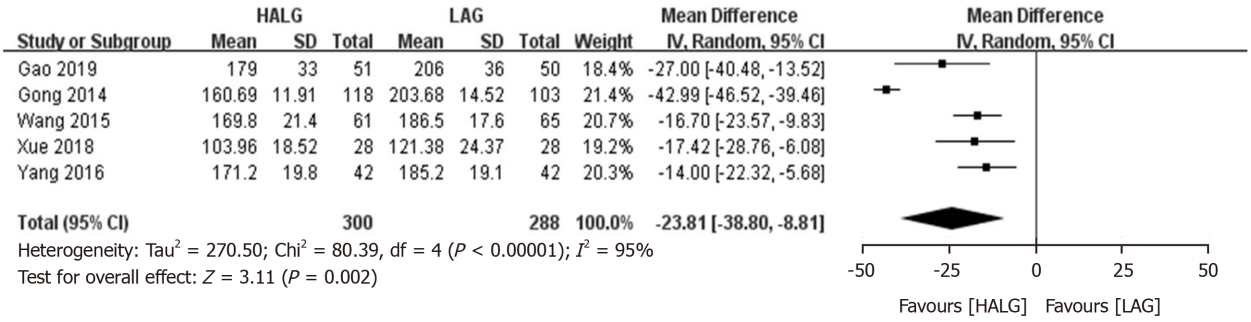
DISCUSSION

The therapeutic effect of LAG has been confirmed for gastric cancer in previous studies[19-21]. Likewise, several studies confirmed the therapeutic effect of HALS on gastrointestinal tumors[22-24]. There are numerous similarities between HALG and LAG, such as the use of laparoscopy, a small incision, and digestive reconstruction. However, they also differ in various aspects such as the surgical procedure and the function of incision. 'Three-step HALG' has become the standardized procedure in our hospital[8,25]. The application of HALG has been gradually increasing, especially in China. Nevertheless, due to the lack of consistency across different studies, controversy exists on the therapeutic effects and advantages of HALG. In this meta-analysis, in order to improve the reliability, we only included RCTs on HALG and LAG.

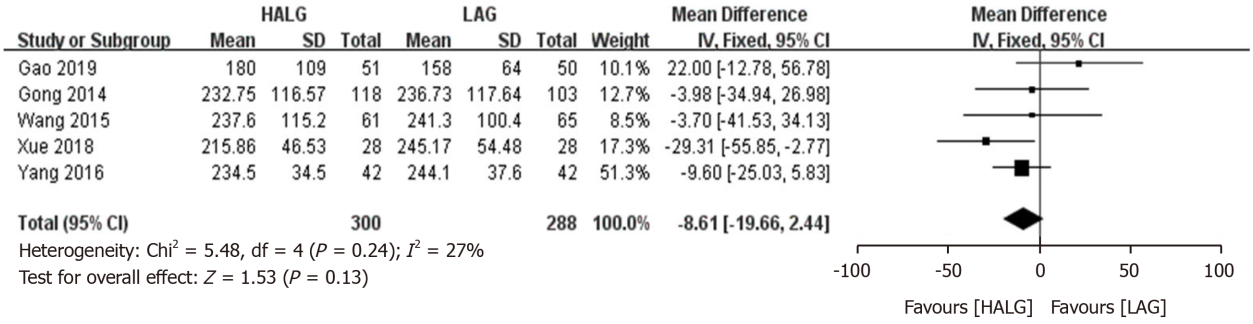
RCTs are the gold standard in study design; however, randomized controlled surgical trials, especially blinding, remain controversial, since the surgeon cannot be blinded to the procedure, and there are practical and ethical barriers to blind patients[26]. Due to the absence of blinding, five studies automatically scored poorly on the Jadad score in this meta-analysis. Although the poor-quality RCTs may be biased due to their inherent design limitations, there is no satisfactory program to resolve this issue. In addition, two of the studies did not report the exact value of the groups' baseline[16,18], although they clearly reported that the groups were similar at the baseline in the article.

Previously, HALG has been considered the transitional bridge from traditional open

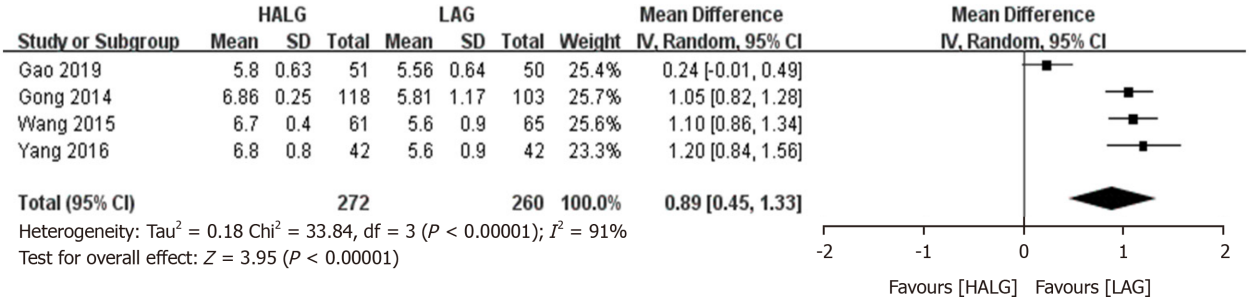
A


HALG
LAG
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Gao 2019 179 33 51 206 36 50 18.4% -27.00 [-40.48, -13.52]
Gong 2014 160.69 11.91 118 203.68 14.52 103 21.4% -42.99 [-46.52, -39.46]
Wang 2015 169.8 21.4 61 186.5 17.6 65 20.7% -16.70 [-23.57, -9.83]
Xue 2018 103.96 18.52 28 121.38 24.37 28 19.2% -17.42 [-28.76, -6.08]
Yang 2016 171.2 19.8 42 185.2 19.1 42 20.3% -14.00 [-22.32, -5.68]
Total (95% CI) 300 288 100.0% -23.81 [-38.80, -8.81]
Heterogeneity: Tau² = 270.50; Chi² = 80.39, df = 4 (P < 0.00001); I² = 95%
Test for overall effect: Z = 3.11 (P = 0.002)
-50 -25 0 25 50
Favours [HALG] Favours [LAG]

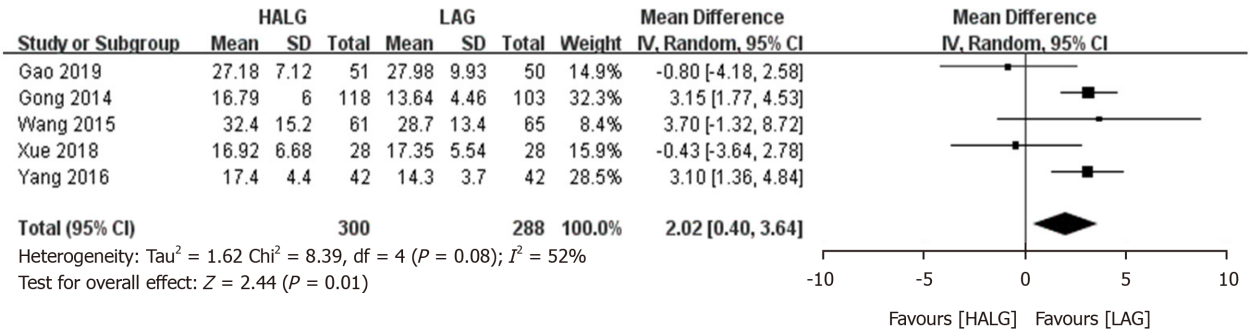
B


HALG
LAG
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Fixed, 95% CI
IV, Fixed, 95% CI
Gao 2019 180 109 51 158 64 50 10.1% 22.00 [-12.78, 56.78]
Gong 2014 232.75 116.57 118 236.73 117.64 103 12.7% -3.98 [-34.94, 26.98]
Wang 2015 237.6 115.2 61 241.3 100.4 65 8.5% -3.70 [-41.53, 34.13]
Xue 2018 215.86 46.53 28 245.17 54.48 28 17.3% -29.31 [-55.85, -2.77]
Yang 2016 234.5 34.5 42 244.1 37.6 42 51.3% -9.60 [-25.03, 5.83]
Total (95% CI) 300 288 100.0% -8.61 [-19.66, 2.44]
Heterogeneity: Chi² = 5.48, df = 4 (P = 0.24); I² = 27%
Test for overall effect: Z = 1.53 (P = 0.13)
-100 -50 0 50 100
Favours [HALG] Favours [LAG]

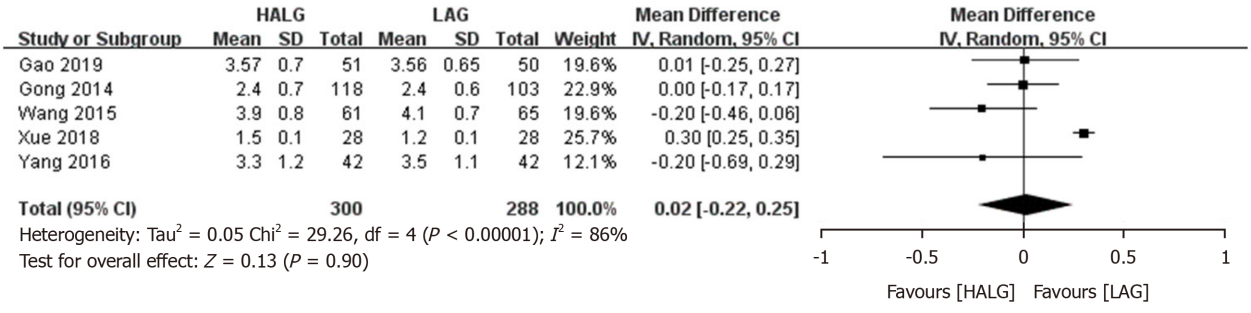
C


HALG
LAG
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Gao 2019 5.8 0.63 51 5.56 0.64 50 25.4% 0.24 [-0.01, 0.49]
Gong 2014 6.86 0.25 118 5.81 1.17 103 25.7% 1.05 [0.82, 1.28]
Wang 2015 6.7 0.4 61 5.6 0.9 65 25.6% 1.10 [0.86, 1.34]
Yang 2016 6.8 0.8 42 5.6 0.9 42 23.3% 1.20 [0.84, 1.56]
Total (95% CI) 272 260 100.0% 0.89 [0.45, 1.33]
Heterogeneity: Tau² = 0.18 Chi² = 33.84, df = 3 (P < 0.00001); I² = 91%
Test for overall effect: Z = 3.95 (P < 0.00001)
-2 -1 0 1 2
Favours [HALG] Favours [LAG]

D


HALG
LAG
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Gao 2019 27.18 7.12 51 27.98 9.93 50 14.9% -0.80 [-4.18, 2.58]
Gong 2014 16.79 6 118 13.64 4.46 103 32.3% 3.15 [1.77, 4.53]
Wang 2015 32.4 15.2 61 28.7 13.4 65 8.4% 3.70 [-1.32, 8.72]
Xue 2018 16.92 6.68 28 17.35 5.54 28 15.9% -0.43 [-3.64, 2.78]
Yang 2016 17.4 4.4 42 14.3 3.7 42 28.5% 3.10 [1.36, 4.84]
Total (95% CI) 300 288 100.0% 2.02 [0.40, 3.64]
Heterogeneity: Tau² = 1.62 Chi² = 8.39, df = 4 (P = 0.08); I² = 52%
Test for overall effect: Z = 2.44 (P = 0.01)
-10 -5 0 5 10
Favours [HALG] Favours [LAG]

E


HALG
LAG
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
Gao 2019 3.57 0.7 51 3.56 0.65 50 19.6% 0.01 [-0.25, 0.27]
Gong 2014 2.4 0.7 118 2.4 0.6 103 22.9% 0.00 [-0.17, 0.17]
Wang 2015 3.9 0.8 61 4.1 0.7 65 19.6% -0.20 [-0.46, 0.06]
Xue 2018 1.5 0.1 28 1.2 0.1 28 25.7% 0.30 [0.25, 0.35]
Yang 2016 3.3 1.2 42 3.5 1.1 42 12.1% -0.20 [-0.69, 0.29]
Total (95% CI) 300 288 100.0% 0.02 [-0.22, 0.25]
Heterogeneity: Tau² = 0.05 Chi² = 29.26, df = 4 (P < 0.00001); I² = 86%
Test for overall effect: Z = 0.13 (P = 0.90)
-1 -0.5 0 0.5 1
Favours [HALG] Favours [LAG]

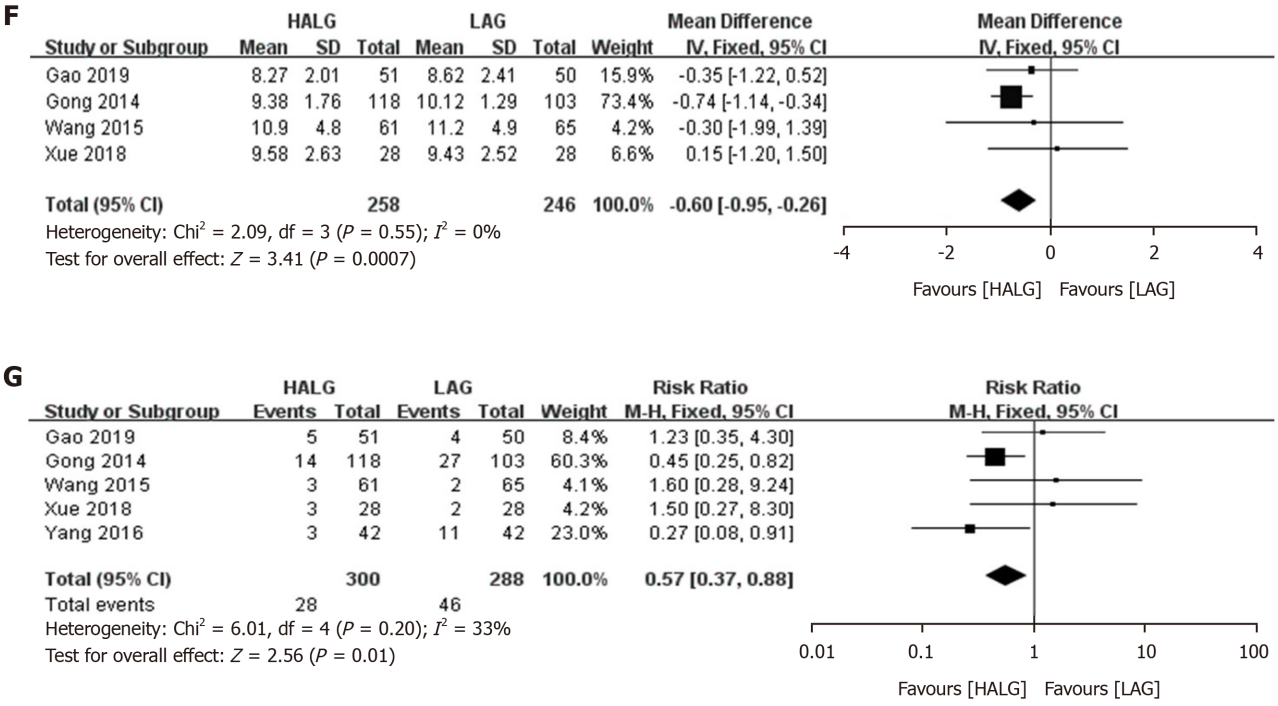


Figure 2 Forest plots based on intraoperative and postoperative clinical data. A: Surgery time; B: Blood loss; C: Incision length; D: Retrieved lymphatic nodes; E: Time to first flatus; F: Postsurgical hospitalization; G: Overall postsurgical complications. HALG: Hand-assisted laparoscopic gastrectomy; LAG: Laparoscopic-assisted gastrectomy.

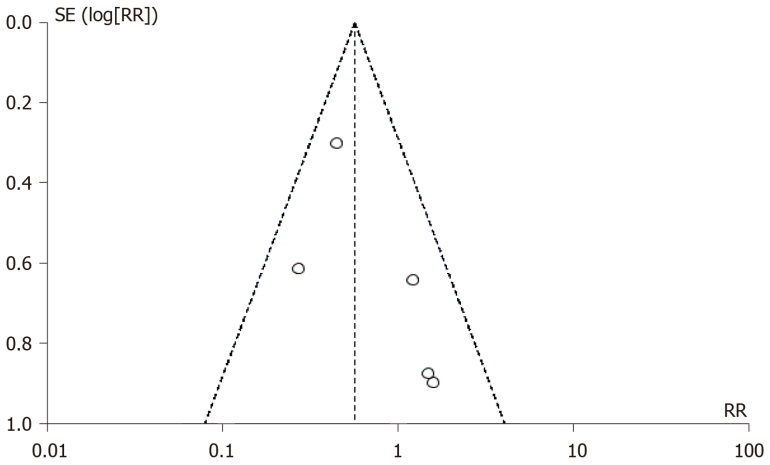


Figure 3 Funnel plot of the overall postsurgical complications. RR: Risk ratio.

surgery to laparoscopic surgery[27-29]. Currently, HALG is universally used for gastrointestinal tumors in China. However, most surgeons may prejudice HALG's minimal invasiveness because of one hand into the abdominal cavity. In fact, several studies have shown that the important prognostic indicators of HALG, with the exception of incision length, are more advantageous than those of LAG[9,27,30-32]. Additionally, HALG should not be denied as being minimally invasive just because of slightly longer incision (WMD, 0.89 cm; 95%CI, 0.45 to 1.33). There are several possible reasons for this. First, HALG is simpler for isolating the omentum, and groups 5, 6, and 12 lymph nodes under direct vision through the upper abdomen incision, so that it can greatly reduce the laparoscopic operation time. Second, the surgeon's left hand is more agile than that of the LAG's assistant. Third, the initial learning-curve of HALG is shorter; thus, surgeons can better acquire the skill to decrease the operation time. Fourth, the incision length is appropriate for digestive reconstruction.

Severe postsurgical complications could increase the hospitalization duration and affect the prognosis. In this meta-analysis, the rates of all postsurgical complications of HALG were lower than those of LAG, which could be attributed to the following

reasons: First, HALG have a clearer operative field to isolate groups 5, 6, and 12 lymph nodes, and to complete the digestive reconstruction by the comfortable incision. Second, due to the direct hand assistance, the important structures, particularly the splenic lymph node, could more intuitively be revealed *via* the laparoscope. Third, HALG has a lower requirement for pneumoperitoneum pressure, so that it favorably maintains the stability of the internal environment. However, those studies did not evaluate the long-term outcomes. Thus, it is important to evaluate the long-term survival of HALG in the future.

To date, surgeons have multiple options to complete gastrectomy, especially the novel totally laparoscopic gastrectomy and robotic gastrectomy. However, the reconstruction process of totally laparoscopic gastrectomy or robotic gastrectomy is difficult[33-35]. Compared with HALG, it also has a longer-learning curve to complete operation[7]. The robotic gastrectomy is similar to minimal need for experienced assistance with HALG. However, the robotic approach is not widely used because of its high price[36,37]. Compared with the cheap equipment of HALG, many hospitals cannot pay for initial purchasing costs and maintenance costs of robotic procedures, especially in undeveloped areas. Additionally, the high hospitalization costs of robotic gastrectomy also affect the choice of patients. Collectively, we still recommend this ordinary HALG to the undeveloped areas in this analysis.

Nevertheless, this study has certain limitations. First, all the included studies were conducted in China, which limited the universal application of the results. Second, all the studies are RCTs, but there are no uniform criteria and no uniform training of surgeons. Due to the poor-quality RCTs, there is an indeterminate risk of bias. Third, although the present study included all the relevant publications from our search, the sample size is still not sufficient. Fourth, three types of gastrectomy were included in this meta-analysis, and the difference between these types is ignored, which may lead to high heterogeneity.

CONCLUSION

In conclusion, our meta-analysis suggests that HALG is a simpler and safer technique than LAG. HALG should be used as a minimal-access technique, particularly in technologically undeveloped areas. However, further high-quality RCTs with larger sample size should be conducted in order to evaluate this issue.

ARTICLE HIGHLIGHTS

Research background

Hand-assisted laparoscopic gastrectomy (HALG) is a popular operation in China, but some surgeons do not accept it as a minimal-access technique.

Research motivation

If the safety and practicability of HALG can be confirmed by comparing with laparoscopic-assisted gastrectomy (LAG), HALG should be used as a minimal-access technique.

Research objectives

This research aimed to assess the safety and practicability of HALG by comparing the short-term outcomes of HALG and LAG.

Research methods

The electronic databases of EMBASE, PubMed, China National Knowledge Infrastructure, and Cochrane Library were thoroughly searched, and only randomized controlled trials (RCTs) comparing HALG and LAG were included.

Research results

This meta-analysis included five RCTs with 600 cases. Compared with LAG, HALG reduced surgery time, hospital duration, and overall postsurgical complications, and increased the number of retrieved lymphatic nodes and incision length.

Research conclusions

HALG is simpler and safer technique than LAG. HALG should be used as a minimal-access technique, especially in technologically undeveloped areas.

Research perspectives

It is important to evaluate the long-term survival of hand-assisted laparoscopic gastrectomy in the future.

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