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World J Meta-Anal 2020 April 28; 8(2): 41-172



OPINION REVIEW

- 41 European Specialty Examination in Gastroenterology and Hepatology examination – improving education in gastroenterology and hepatology
Cremers I, Pisani A, Majerović M, Lillienau J, Michopoulos S, Wiencke K, Ellis T, Barrison I
- 48 Effectiveness and safety of sedation in gastrointestinal endoscopy: An opinion review
Ichijima R, Esaki M, Suzuki S, Kusano C, Ikehara H, Gotoda T

REVIEW

- 54 Probiotics in inflammatory bowel disease: Does it work?
Silva NOE, de Brito BB, da Silva FAF, Santos MLC, de Melo FF
- 67 Pathological characterization of occult hepatitis B virus infection in hepatitis C virus-associated or non-alcoholic steatohepatitis-related hepatocellular carcinoma
Elalfy H, Besheer T, Elhamady D, El Mesery A, Shaltout SW, Abd El-Maksoud M, Amin AI, Bekhit AN, Abd El Aziz M, El-Bendary M
- 78 Hypoxia and oxidative stress: The role of the anaerobic gut, the hepatic arterial buffer response and other defence mechanisms of the liver
Hewawasam SP
- 89 *Helicobacter pylori* and gastric cardia cancer: What do we know about their relationship?
Yin JJ, Duan FJ, Madhurapantula SV, Zhang YH, He G, Wang KY, Ji XK, Wang KJ
- 98 Treatment strategies and preventive methods for drug-resistant *Helicobacter pylori* infection
Li RJ, Dai YY, Qin C, Li XH, Qin YC, Pan Y, Huang YY, Huang ZS, Huang YQ

MINIREVIEWS

- 109 Utility of gastrointestinal ultrasound in functional gastrointestinal disorders: A narrative review
Ong AML

SYSTEMATIC REVIEWS

- 119 Systematic review with meta-analysis of the epidemiological evidence relating smoking to type 2 diabetes
Lee PN, Coombs KJ

META-ANALYSIS

- 153 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
Gu Y, Shi X, Yang Y, Ye XF, Wu Q, Yang ZP, He SX

SCIENTOMETRICS

- 163** Chinese research into ulcerative colitis from 1978 to 2017: A bibliometric analysis
Zhu M, Mu JX, Jiang MS, Mukherjee A, Zeng Z, Chen YD, Yang XL, Zhang H

ABOUT COVER

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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 where 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.

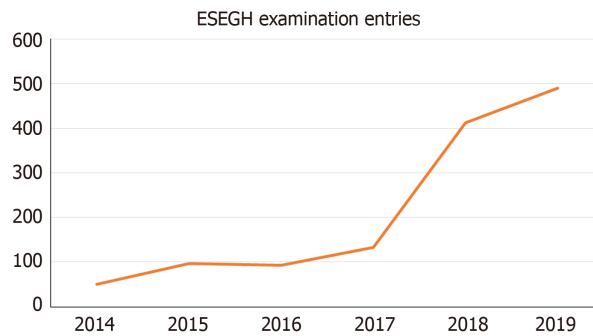


Figure 1 Annual entries to the European Specialty Examination in Gastroenterology and Hepatology. ESEGH: European Specialty Examination in Gastroenterology and Hepatology.

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

¹This 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.

²Other 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 where the ESEGH is mandated and a third (Portugal) where 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 Kingdom 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.

¹Candidates 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.

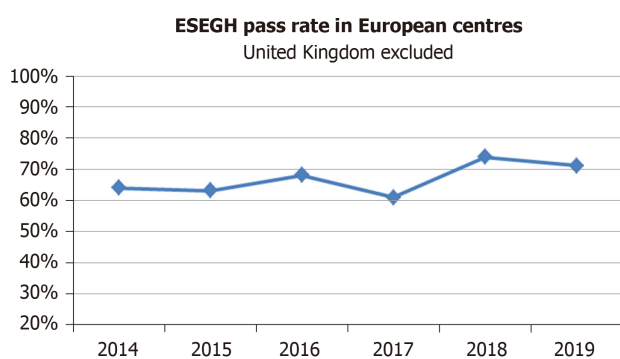


Figure 2 European Specialty Examination in Gastroenterology and Hepatology pass rate in European centres (United Kingdom excluded). ESEGH: European Specialty Examination in Gastroenterology and Hepatology.

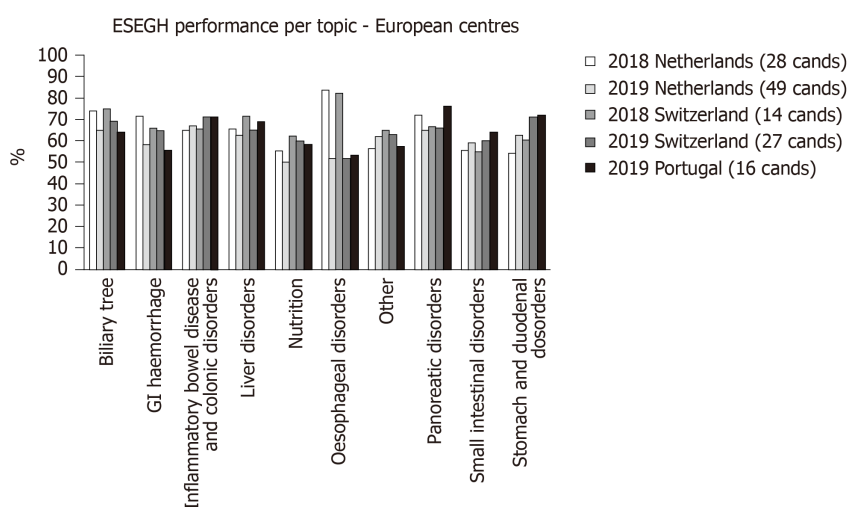


Figure 3 European Specialty Examination in Gastroenterology and Hepatology performance per topic in European centres (2018 and 2019). ESEGH: European Specialty Examination in Gastroenterology and Hepatology.

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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 increase 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 United 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 induced 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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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 metabolism 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-Brocá *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

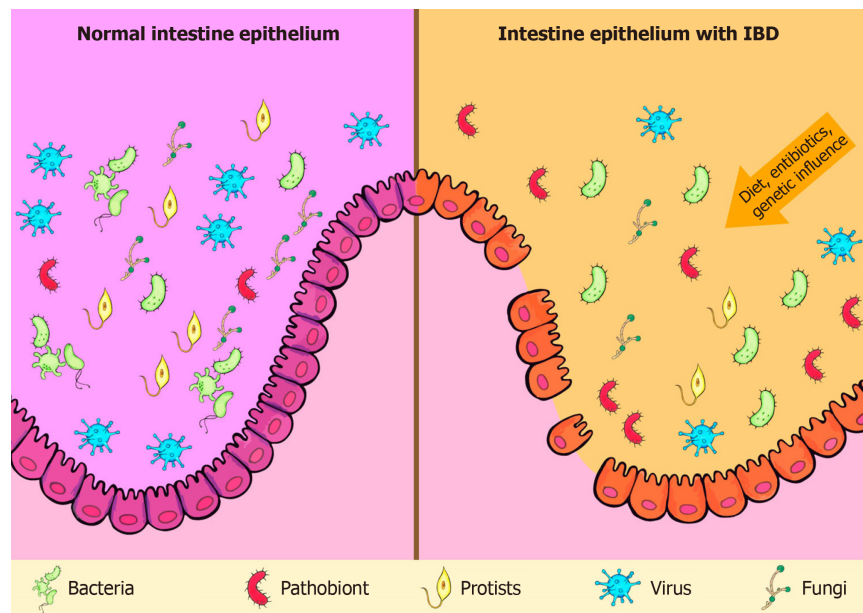


Figure 1 Comparison between normal intestinal microbiota and intestinal microbiota with inflammatory bowel disease. IBD: Inflammatory bowel disease.

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 phenomena 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- β , 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.
<i>Escherichia coli</i> 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]
<i>Saccharomyces boulardii</i>	Clinical remission of UC	[68-70]
<i>Bifidobacterium longum</i>	Objective improvements in UC parameters	[71]
<i>Lactobacillus acidophilus</i> La-5 + <i>Bifidobacterium</i> 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 interurrences ($P < 0.01$). 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- κ B and TNF- α expression by means of TLR4-NF- κ B signal pathway might play an important role in such promising VSL#3 effects on UC^[124]. Recently, a meta-analysis concluded that VSL#3 is effective in preventing pouchitis episodes and may have beneficial effects in inducing 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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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 accidentally 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^[5].

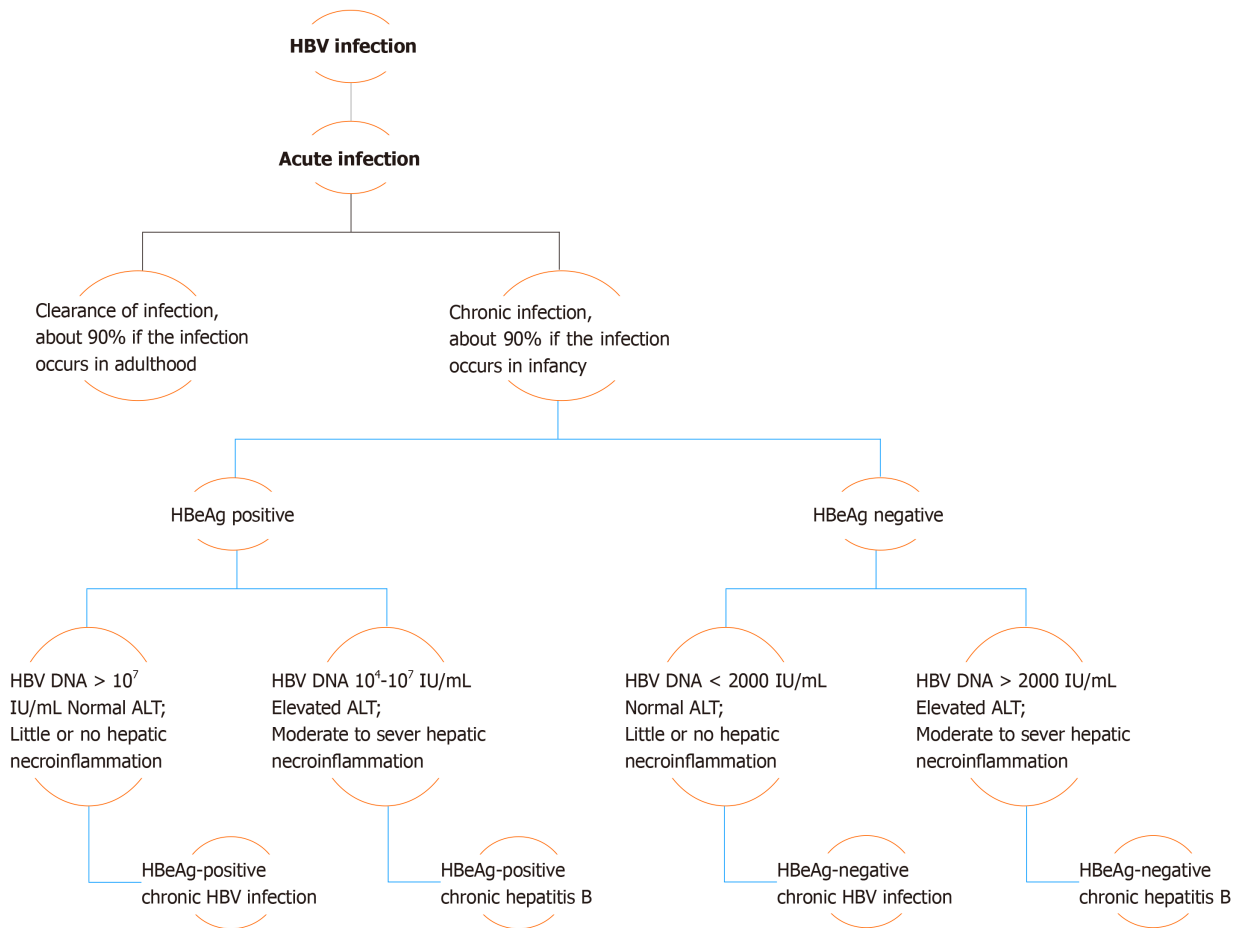


Figure 1 Natural history and assessment of patients with chronic hepatitis B virus infection. HBV: Hepatitis B virus.

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

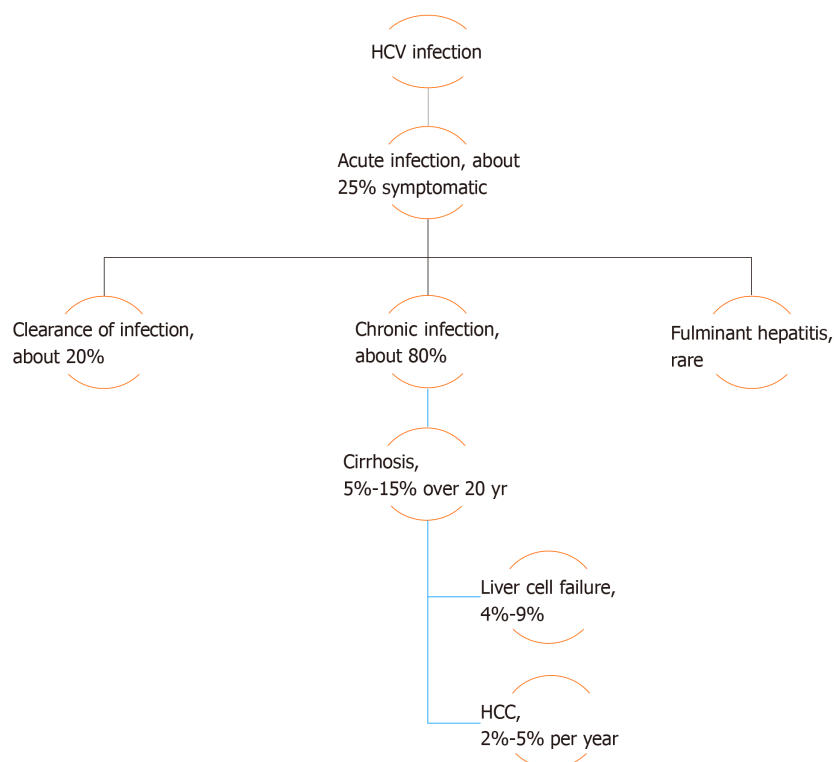


Figure 2 Natural history of hepatitis C virus infection. HCV: Hepatitis C virus.

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 HBcAg 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 increasingly 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-independent 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 indistinguishable 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 tissue^[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 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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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 putrefaction 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 10⁴ 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

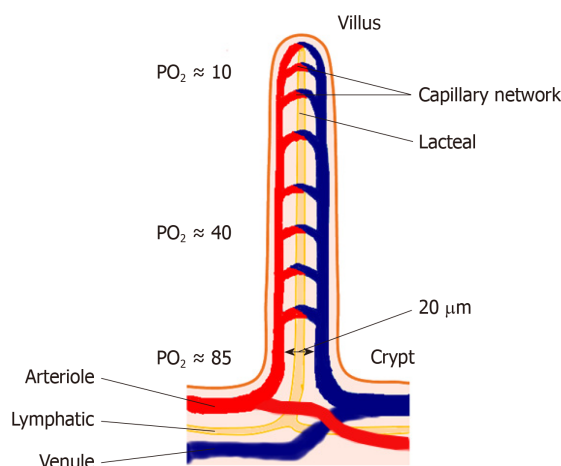


Figure 1 Schematic diagram of countercurrent blood flow within the small intestinal villus allowing diffusion of O_2 from the arteriole to the venule, creating a luminal hypoxia from the crypt to the villous tip. PO_2 is shown in mmHg.

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 Lauth *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 Lauth'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 Lauth's theorem. According to Lauth's hypothesis, adenosine for the HABR is derived from the demethylation of S-adenosylhomocysteine, a reaction that is oxygen independent. According to Lauth, 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", Lauth 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

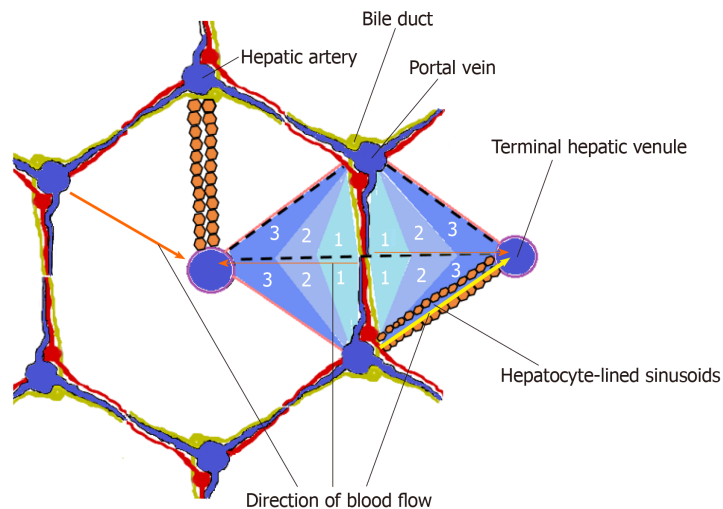


Figure 2 Schematic representation of a hepatic acinus within a conventional hepatic lobule. The demarcation of the hepatic acinus is shown by the dashed lines. Arrows indicate the direction of blood flow within hepatic sinusoids in a periportal-to-perivenous direction, giving rise to zones 1, 2 and 3 (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 CO₂ 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

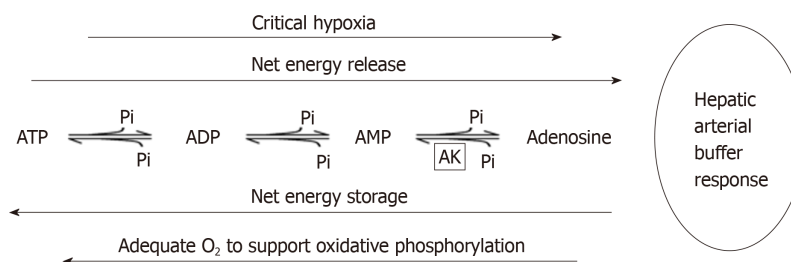


Figure 3 Production of adenosine from adenine nucleotides. ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate; Pi: Inorganic phosphate; AK: Adenosine kinase.

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.

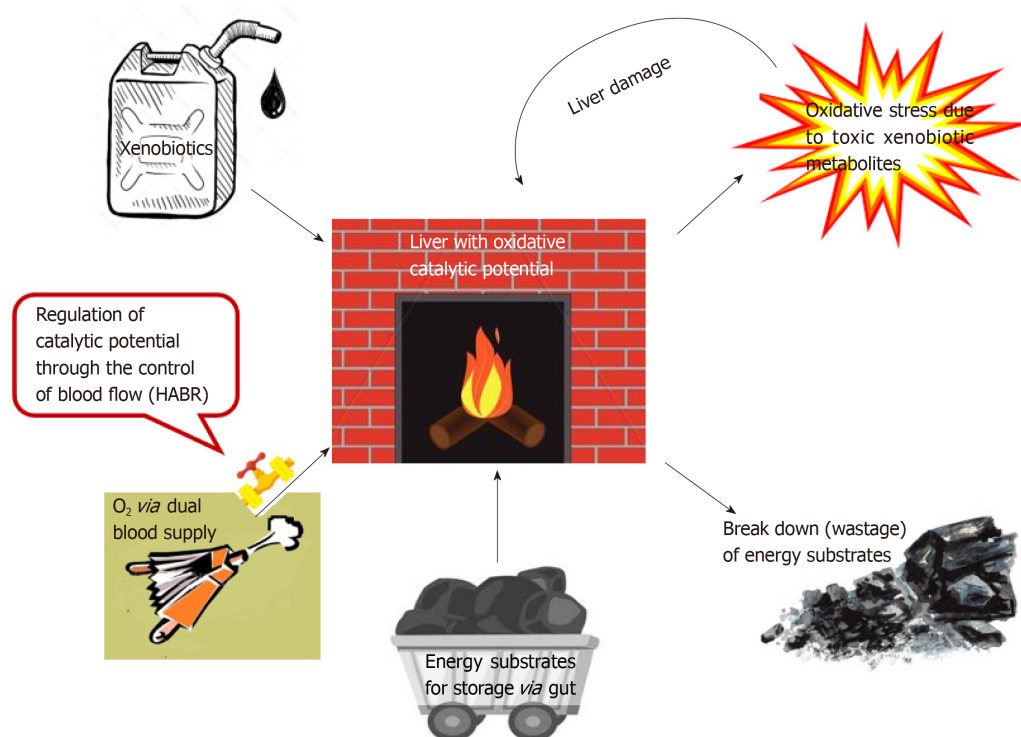


Figure 4 Liver as the “chemical smithy” of the body.

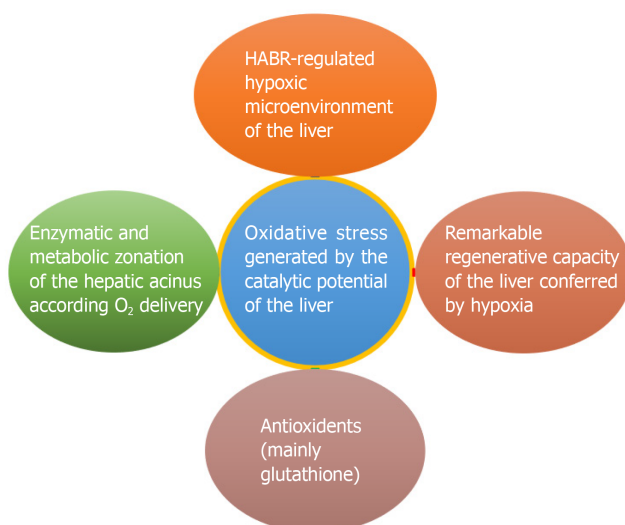


Figure 5 Protective adaptations of the liver against oxidative stress. HABR: Hepatic arterial buffer response.

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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; $I^2 =$

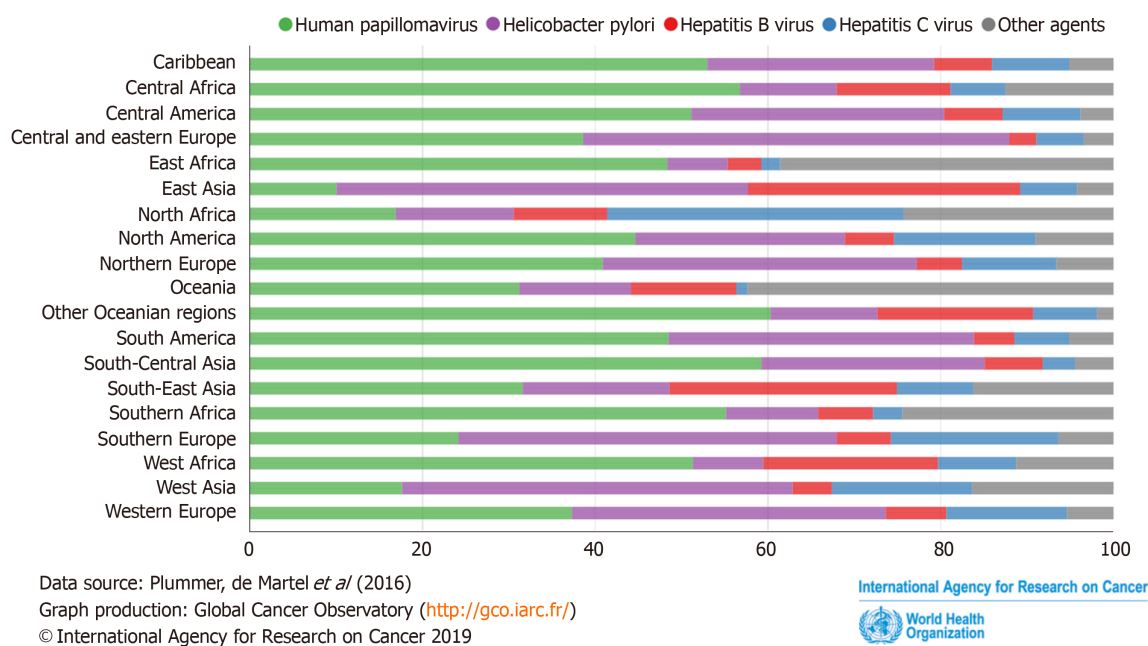


Figure 1 Proportions of cancer cases among both sexes attributable to infections in 2012 (by region).

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 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		<i>H. pylori</i> 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	

¹Regions 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.

²Total *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

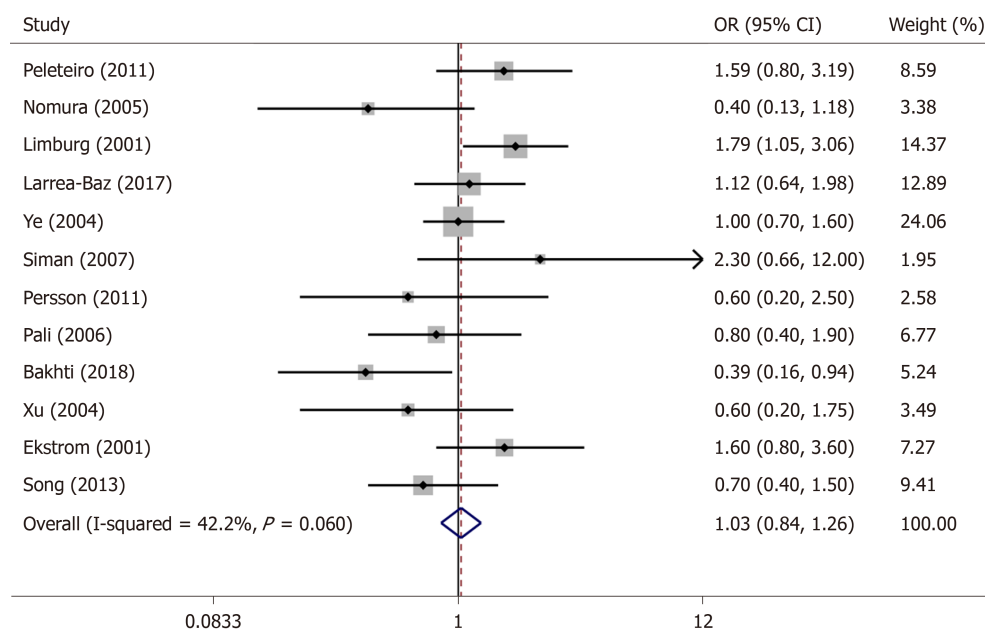


Figure 2 Forest plot of studies evaluating the odds ratios of *Helicobacter pylori* cagA positive expression for gastric cardia cancer.

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/m²) 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 SNPs 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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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

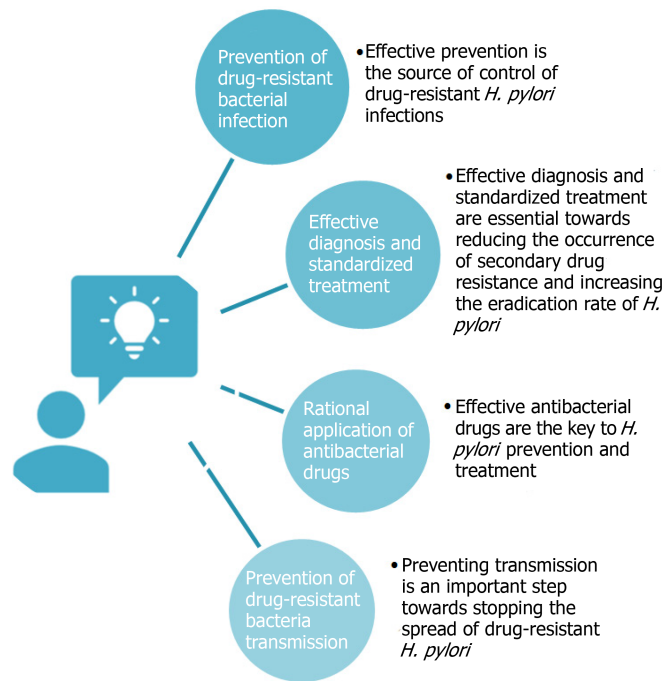


Figure 1 Strategies for prevention and treatment drug-resistant *Helicobacter pylori* infection.

method, and bacterial culture^[19]. Non-invasive detection methods include ¹³C 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

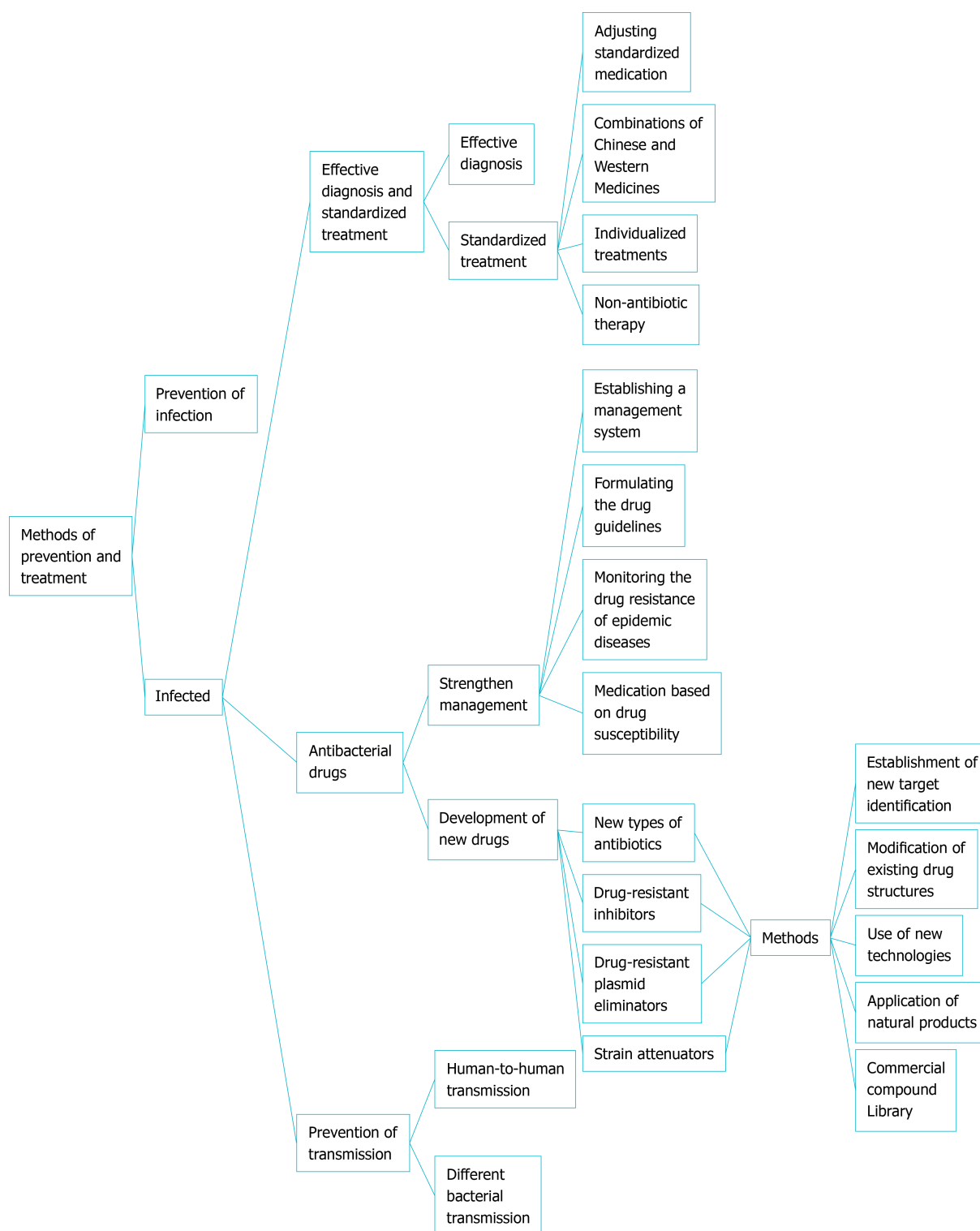


Figure 2 Methods for prevention and treatment of drug-resistant *Helicobacter pylori* infection.

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.*








	NO.1 Amoxicillin + CLA (1 g, 2 times/d + 0.5 g, 2 times/d)
	NO.2 Amoxicillin + Furazolidone (1 g, 2 times/d + 0.1 g, 2 times/d)
	NO.3 Amoxicillin + metronidazole (1 g, 2 times/d+0.4 g, 3 to 4 times/d)
	NO.4 Amoxicillin + tetracycline (1 g, 2 times/d + 0.5 g, 3 to 4 times/d)
	NO.5 Tetracycline + metronidazole (0.5 g, 3 to 4 times /d + 0.4 g, 3 to 4 times /d)
	NO.6 Tetracycline + Furazolidone (0.5 g, 3 to 4 times/d + 0.1 g, 2 times/d)
	NO.7 Amoxicillin + Levofloxacin (1 g, 2 times/d + 0.5 g, 1 time /d or 0.2 g, 2 times /d)

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 Blair'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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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^[59] 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

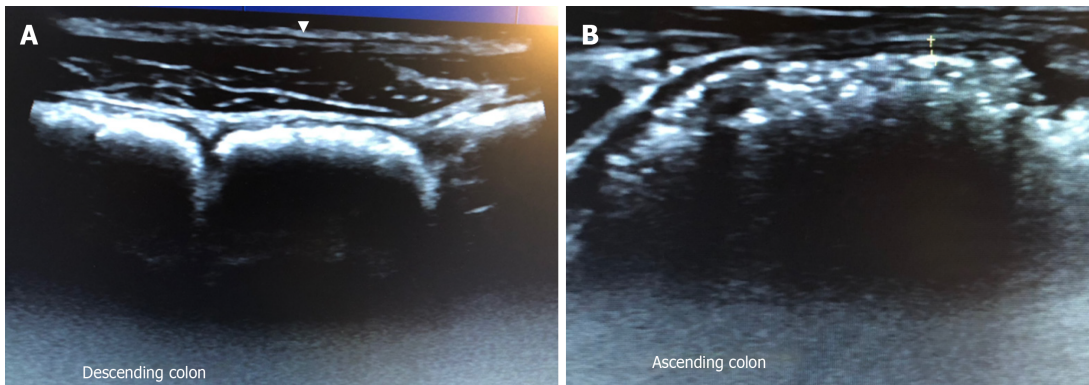


Figure 1 Gastrointestinal ultrasound images. A: Gastrointestinal ultrasound images of descending colon showing hausta-shaped reflections with acoustic shadow behind, suggestive of severe faecal loading B: Gastrointestinal ultrasound images of strong reflection with acoustic shadow behind, also suggestive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

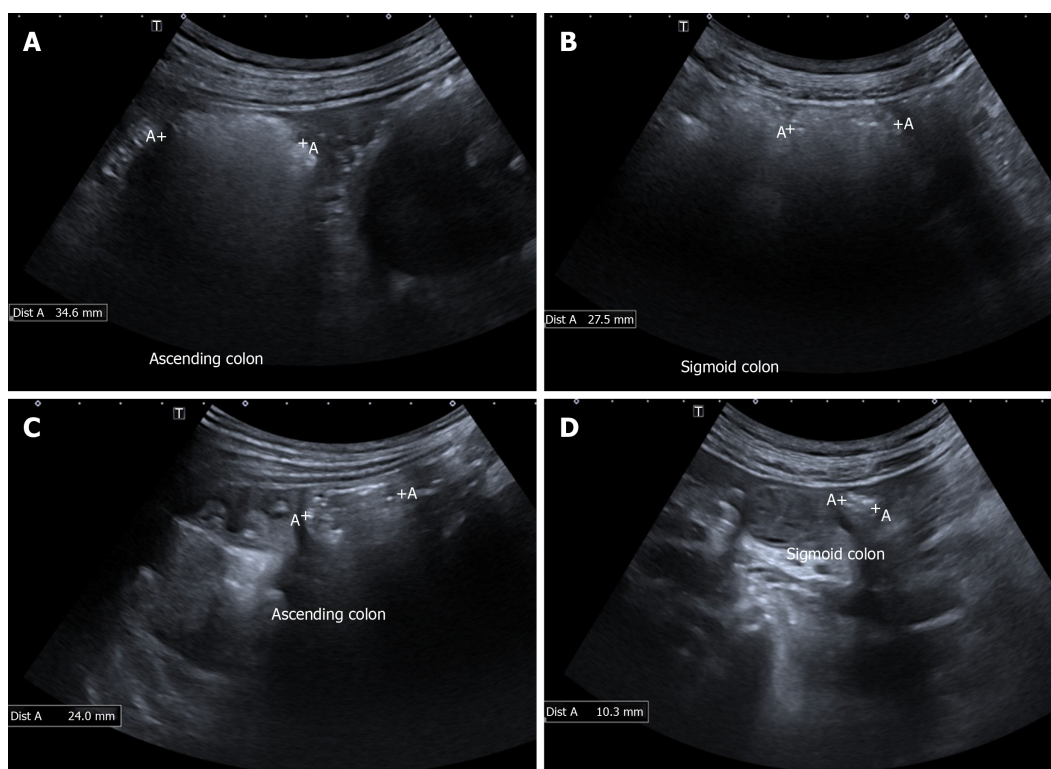


Figure 2 shows the ascending colon and sigmoid colon images of a constipated patients. A: Image of ascending colon pre-treatment; B: Image of sigmoid colon pre-treatment; Note the strong reflections with acoustic shadow behind, suggestive of severe faecal loading; Colonic diameters are 34.6 mm and 27.5 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 <i>vs</i> 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 <i>et al</i> ; 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 <i>vs</i> lower GI tract, endoscopy <i>vs</i> 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 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 chi-squared 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

¹Numbers of papers originally selected exclude those already identified in a previous step.

²Reasons for rejection are summarized in [Supplementary file 1](#).

³Or for separate periods of follow-up.

⁴From 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 characteristics¹ 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, Ansan 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+
BED-FORD	[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	Switzerland, 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 intervention	M+F	1986	23.0	3	568	436	Any
DEHGHA	[49]	Europe	Netherlands, Ommoord	General population	M+F	1990-1993	10.8	2	6935	645	55+
DE-PLAN	[11]	Europe	Spain, Navarra, Reus and Barcelona	Participants in clinical trial on Mediterranean 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	Netherlands, 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	Subset of participants 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
HEINZ	[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+
HIPOPOH	[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 professionals	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
KAN-GBUK	[81]	Asia	South Korea, Seoul	Individuals undergoing health screening	M+F	2002	5.6	3	174314	5544	18+
KAWA-HA	[82]	Asia	Japan, Kitakyushu City	City workers	M+F	2008	3.7	2	52781	4369	20-89
KAWA-KA	[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
LIEITO	[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 representative	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	^[119]	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, Gothenburg	General population (intervention 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	Participants 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	Undergoing health examinations	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	Participants in clinical trial on Mediterranean 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	Netherlands, Groningen	General population	M+F	1997-1998	11.4	3	7953	447	Any
REGARDS	^[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, São 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	Undergoing health examinations, Korean	M	2006	6.0	3	1774	180	20+
SAPALDIA	^[138]	Europe	Switzerland	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 businessmen	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	Participants 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	Participants in study of menopause transition, Black or White	F	1996	16.0	3	424	157	42-52
TCS	^[151]	Asia	Thailand	Students at Sukothai Thammarachathirak Open University	M+F	2005	8.0	1	39507	698	15-88
TERATA	^[152]	Asia	Japan, Chiba	Steelworkers	M	2002	8.0	2	8423	464	Any
TLSA	^[153]	Asia	Taiwan (China), Non-aboriginal areas	Participants in ongoing survey on aging, Taiwanese	M+F	1999	4.0	1	2995	277	53+
TOPICS6	^[154]	Asia	Japan, Toranomon	Government 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
VETERAN	^[157]	North America	United States	Veterans	M+F	2002-2003	4.0	2	239057	33453	18-99
VIP	^[158]	Europe	Sweden, Västerbotten County	General population	M+F	1990-2012	9.9	3	32120	2211	35-55
WHI	^[159]	North America	United States	Postmenopausal women in a clinical trial or an observational 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	Netherlands, Zutphen	General population	M	1960	25.0	2	841	58	40-59

¹Where relevant, characteristics are shown for the main reference, given first in the column Main/Other Ref.

²If location not stated, then national.

³All races are included unless stated otherwise.

⁴NA 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.

⁵1 = self-report only; 2 = medical records only; 3 = both.

⁶Studies HPFUS, NHS and NHSII.

⁷France, Italy, Spain, United Kingdom, Netherlands, Germany, Sweden and Denmark.

⁸Los Angeles, Oakland, San Antonio and San Juis Valley.

⁹Maryland, Illinois, North Carolina, California, New York and Minnesota.

¹⁰North Carolina, California, Maryland and Pennsylvania.

¹¹California, Florida, Louisiana, New Jersey, North Carolina and Pennsylvania.

¹²Subset 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 **Supplementary 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		
		<i>n</i> ²	RR (95%CI)	<i>P</i>	<i>n</i>	RR (95%CI)	<i>P</i>
Overall		99	1.33 (1.28-1.38)	<i>P</i> < 0.001, <i>P</i> < 0.05	156	1.28 (1.24-1.32)	<i>P</i> < 0.001, <i>P</i> < 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)	<i>P</i> < 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)	<i>P</i> < 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)	<i>P</i> < 0.05	29	1.22 (1.15-1.30)	<i>P</i> < 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)	<i>P</i> < 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

¹For 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.

²Number of estimates combined.

³NS 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 [Supplementary 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

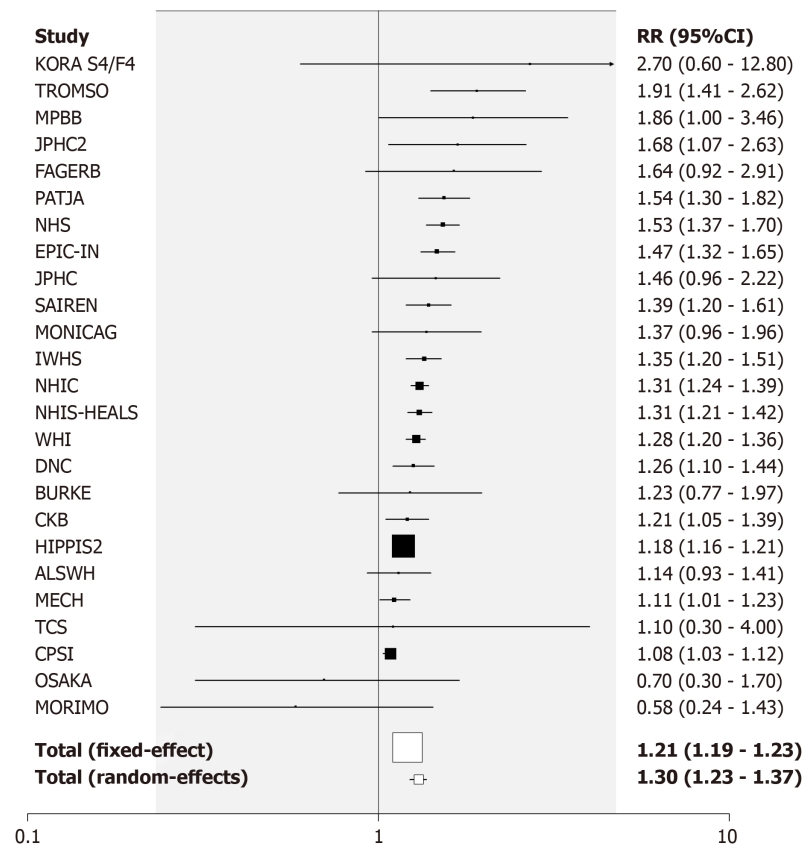


Figure 1 Forest plot for current vs never smoking, results for females. For each selected relative risk (RR), the figure shows the study ref. (see Table 2) and the RR and 95% confidence interval, both numerically and plotted as a line on a log scale from 0.1 to 10. The RRs are plotted from highest to 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

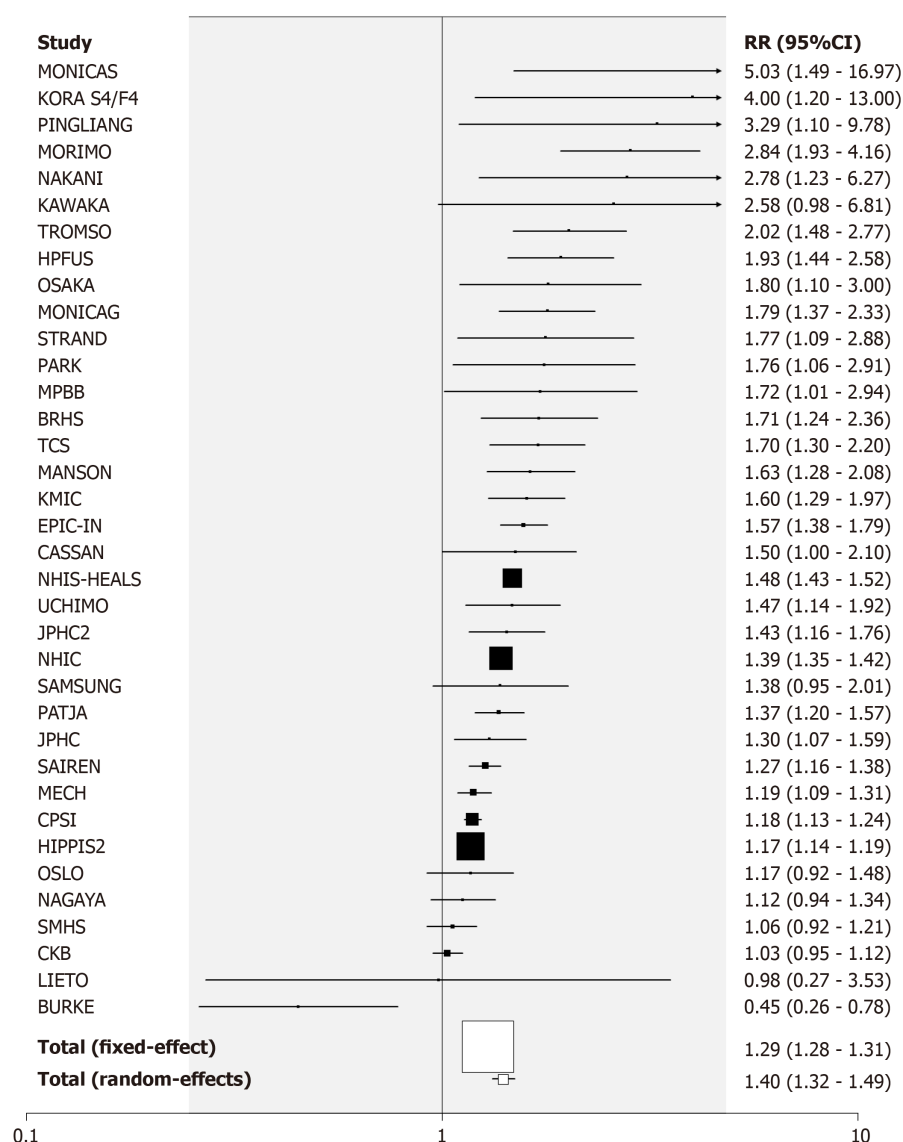


Figure 2 Forest plot for current vs never smoking, results for males. For each selected relative risk (RR) the figure shows the study ref. (see Table 2) and the RR and 95% confidence interval, both numerically and plotted as a line on a log scale from 0.1 to 10. The RRs are plotted from highest to lowest, 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	
	<i>n</i> ²	RR (95%CI)	<i>n</i>	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)

¹The 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.

²Number of estimates combined. CI: Confidence interval; RR: Relative risk.

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

Grouping ¹	<i>n</i> ²	RR (95%CI)	<i>P</i>
Overall	100	1.13 (1.11-1.16)	<i>P</i> < 0.001, <i>P</i> < 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)	<i>P</i> < 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)	<i>P</i> < 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

¹For 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.

²Number of estimates combined.

³NS 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)

¹The 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.

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

¹For 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.

²Number of estimates combined.

³NS 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.

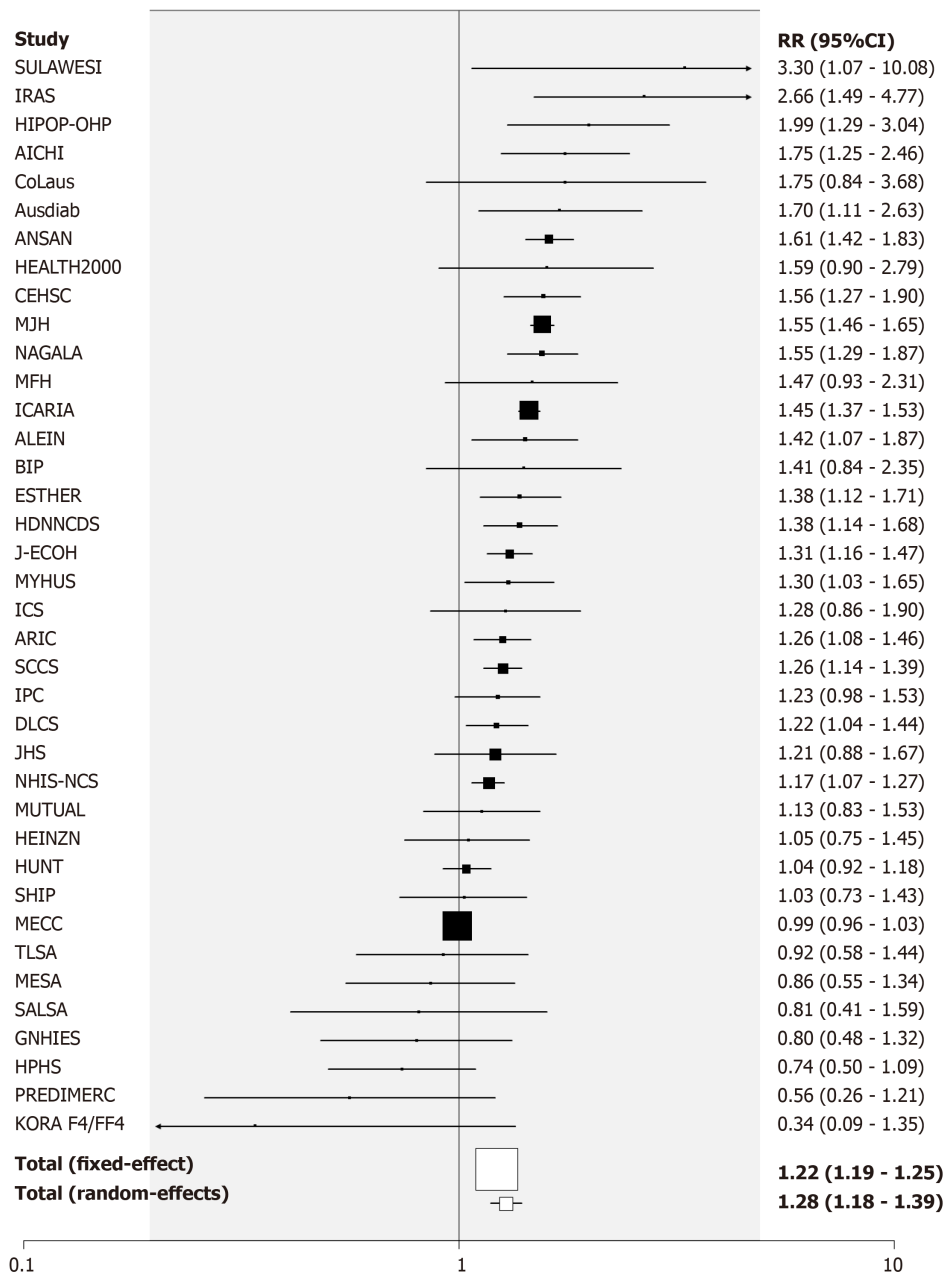


Figure 3 Forest plot for current vs never smoking, results for sexes combined. For each selected relative risk (RR), the figure shows the study ref. (see Table 2) and the RR and 95% confidence interval, both numerically and plotted as a line on a log scale from 0.1 to 10. The RRs are plotted from highest 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.

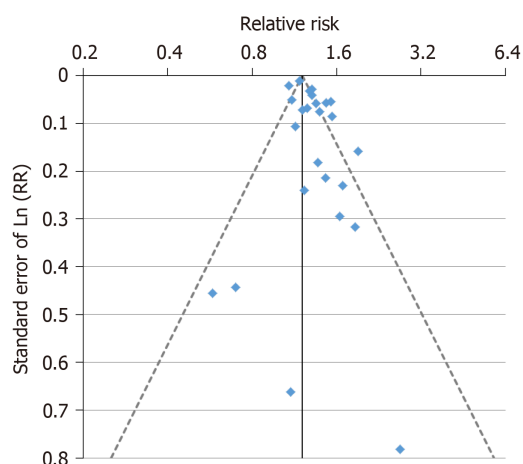


Figure 4 Funnel plot for current vs never smoking, results for females. Each of the selected relative risks (RRs) is shown as a diamond, plotted against its value on the x-axis (on a log scale) and the standard error of \log_e RR on the y-axis. The vertical line indicates the overall fixed-effect estimate, 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.

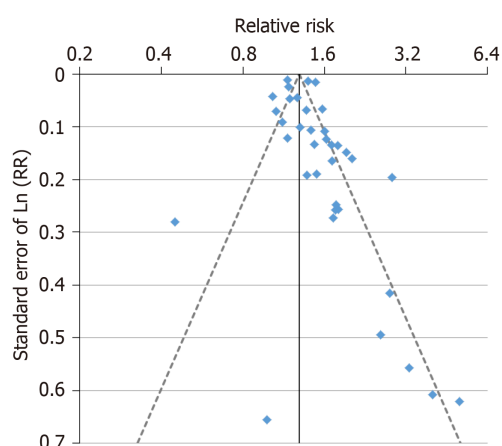


Figure 5 Funnel plot for current vs never smoking, results for males. Each of the selected relative risks (RRs) is shown as a diamond, plotted against its value on the x-axis (on a log scale) and the standard error of \log_e RR on the y-axis. The vertical line indicates the overall fixed-effect estimate, 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.

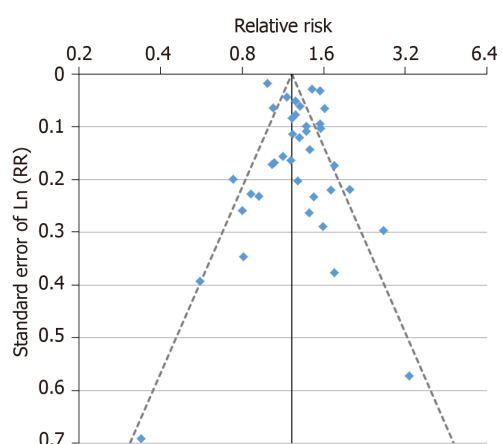


Figure 6 Funnel plot for current vs never smoking, results for sexes combined. Each of the selected relative risks (RRs) is shown as a diamond, plotted against its value on the x-axis (on a log scale) and the standard error of \log_e RR on the y-axis. The vertical line indicates the overall fixed-effect 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, 1.28 (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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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 enteroscopy (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 barrier.

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 resolved 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 <i>et al</i> ^[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 <i>et al</i> ^[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 <i>et al</i> ^[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 <i>et al</i> ^[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 <i>et al</i> ^[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 <i>et al</i> ^[11] , 2011	Germany	RCT	DBE	10	6/4	69 ± 12	10/0
			SE	10	6/4	69 ± 12	10/0
Frieling <i>et al</i> ^[12] , 2010	Germany	Prospective	DBE	17	7/10	61 ± 17	14/3
			SE	18	10/8	58 ± 25	11/7
Efthymiou <i>et al</i> ^[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 <i>et al</i> ^[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 <i>et al</i> ^[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 <i>et al</i> ^[4] , 2011	China	RCT	DBE	50	35/15	42.3	25/25
			SBE				

¹Median (IQR).²Median (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*

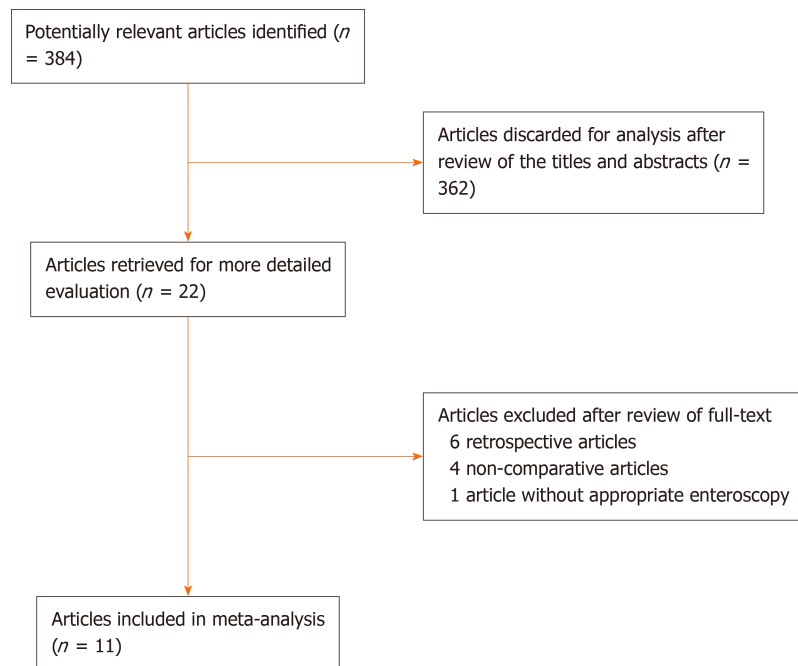


Figure 1 Flow chart.

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, DML, 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 \pm SD (cm)	Time to maximum insertion, mean \pm SD (min)	Examination time, mean \pm SD (min)	
								Oral	Anal
Moran <i>et al</i> ^[7] , 2018	SBE	17	7/17 (41.2)	4/17 (24)	NA	285.3 \pm 80.8	NA	36.1 \pm 11.4	NA
	SE	13	9/13 (69.2)	6/13 (46)	NA	330.0 \pm 88.2	NA	34.8 \pm 4.7	NA
Oka <i>et al</i> ^[8] , 2015	DBE	10	10/10 (100)	NA	NA	228 \pm 22.0	32.9 \pm 5.2	38.9 \pm 5.0	NA
	SE	10	9/10 (90)	NA	NA	214 \pm 16.9	19.5 \pm 2.6	28.2 \pm 4.3	NA
Despott <i>et al</i> ^[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 <i>et al</i> ^[2] , 2013	DBE	13	6/13 (46)	12/13 (92)	12/13 (92.3)	346.2 \pm 63.3	46.2 \pm 12.5	59.6 \pm 14.5	76.1 \pm 21.8
	SE	13	9/13 (69)	12/13 (92)	1/13 (7.7)	268.5 \pm 76.3	26.2 \pm 7.7	43.4 \pm 10.2	51.9 \pm 11.0
Rahmi <i>et al</i> ^[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 <i>et al</i> ^[11] , 2011	DBE	10	NA	14 ¹	NA	310 (220-430) ³	46.5 \pm 19.3	65.0 \pm 12.8	NA
	SE	10	NA	9 ¹	NA	250 (200-340) ³	24.3 \pm 11.8	43.3 \pm 9.3	NA
Frieling <i>et al</i> ^[12] , 2010	DBE	17	8/17 (47.1)	NA	NA	265 \pm 88.1	NA	41.8 \pm 9.5	
	SE	18	6/18 (33.4)	NA	NA	216 \pm 54.1	NA	46.5 \pm 12	
Efthymiou <i>et al</i> ^[3] , 2012	DBE	66	35/66 (53)	17/66 (26)	0/5	234.1 \pm 99.3	NA	60 (45-70) ²	
	SBE	53	30/53 (57)	16/53 (30)	0/1	203.8 \pm 87.6	NA	60 (45-67) ²	
Domagk <i>et al</i> ^[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 <i>et al</i> ^[5] , 2011	DBE	20	10/20 (50)	7/20 (35)	8/14 (57)	NA	NA	70.4 \pm 26.5	90.4 \pm 13.7
	SBE	18	11/18 (61)	5/18 (28)	0/14	NA	NA	92.8 \pm 20.6	93.1 \pm 22.6
Ren <i>et al</i> ^[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

¹Angiodysplasias treated with APC.²Median (IQR).³Median (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

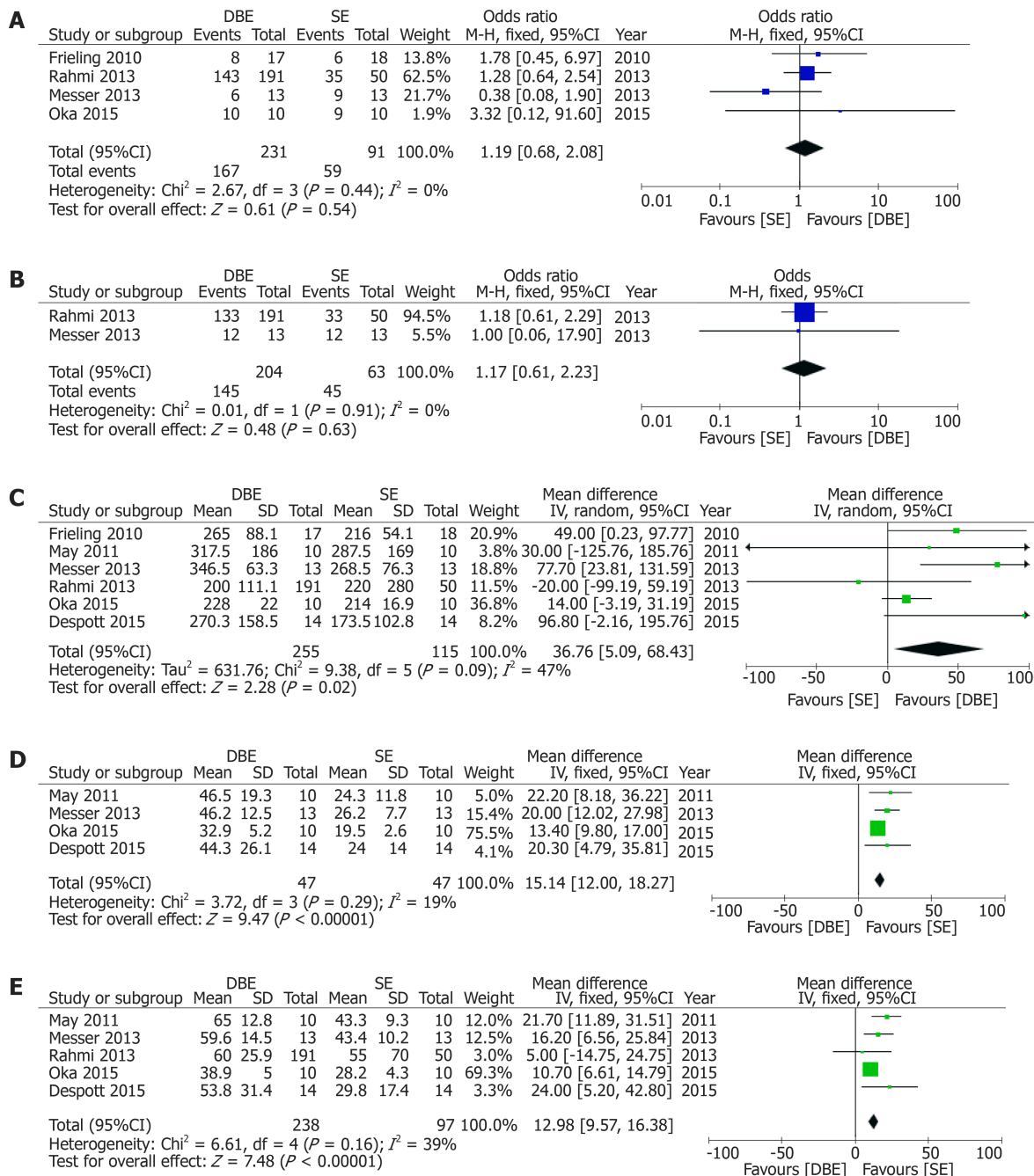


Figure 2 Outcome measures between double-balloon enteroscopy and spiral enteroscopy. A: Diagnostic yield; B: Therapeutic yield; C: Depth of maximal insertion; D: Time to maximum insertion; and E: Examination time.

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

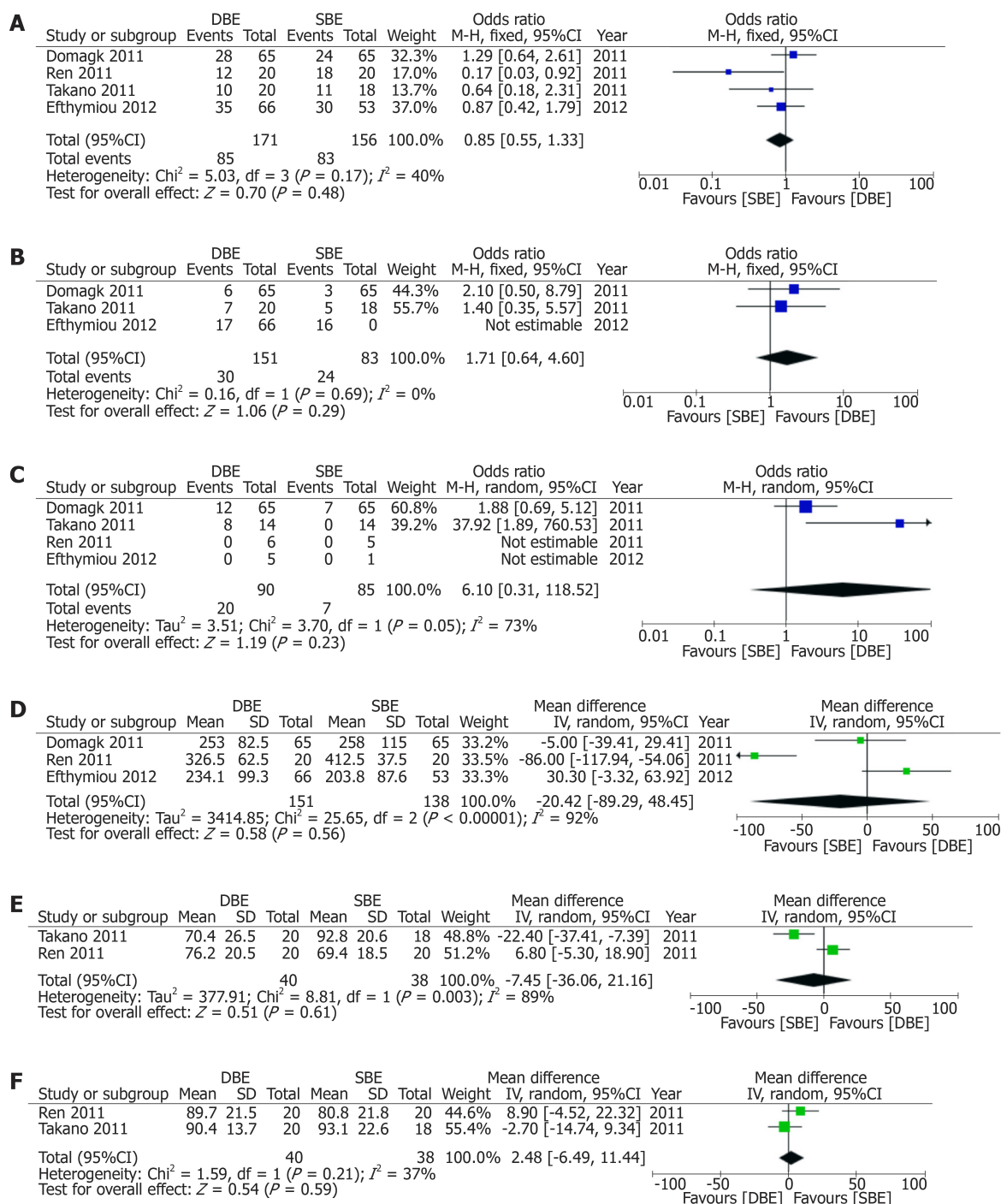


Figure 3 Outcome measures between double-balloon enteroscopy and single-balloon enteroscopy. A: Diagnostic yield; B: Therapeutic yield; C: Total enteroscopy; D: Depth of maximal insertion; E: Examination time (per-oral); and F: Examination time (per-anal).

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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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

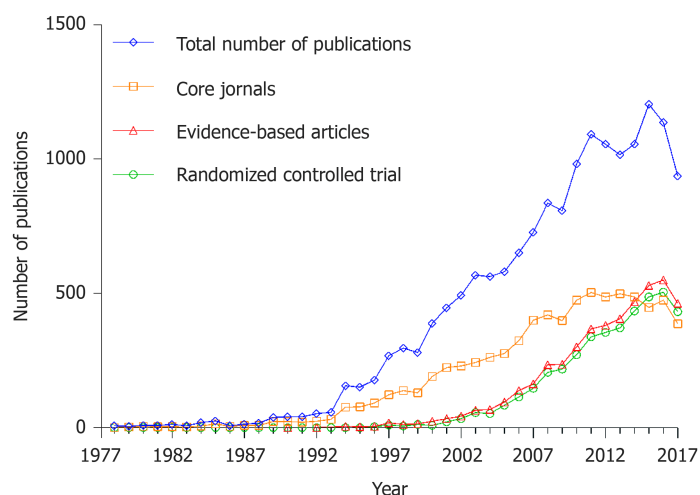


Figure 1 Number of papers focused on ulcerative colitis in China from 1978 to 2017. A total of 16257 papers were published for the period of 1978-2017. Amongst them, 7561 papers were published in core journals, 4641 met the requirements of evidence-based articles, and 4177 were publications on randomized 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

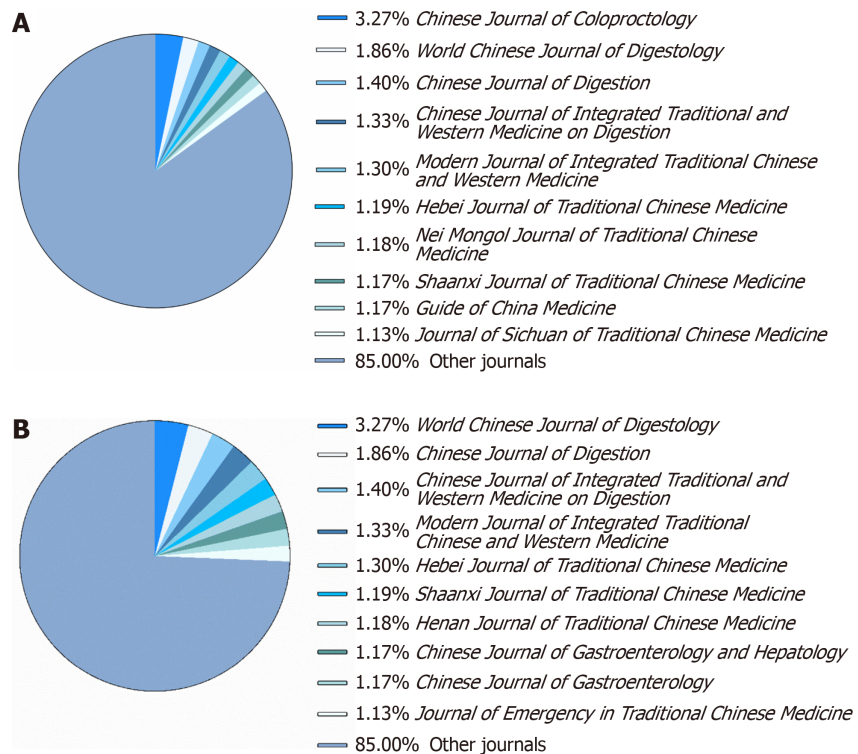


Figure 2 Journal distribution of publications focused on ulcerative colitis. A: Proportion of papers focused on ulcerative colitis (UC) published by each journal; B: Proportion of papers addressing UC published by each core journal. A total of 16257 papers focused on UC were published in 1119 journals. 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.

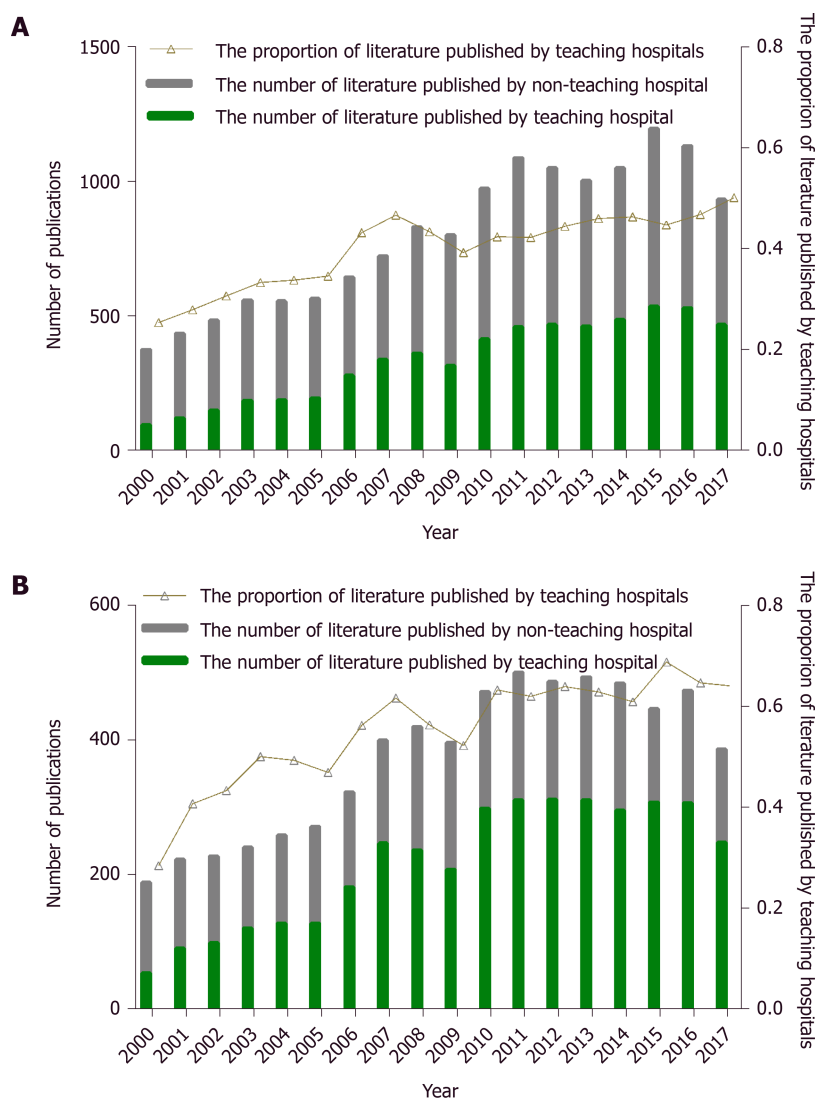


Figure 3 Organization distribution of publications focused on ulcerative colitis. A: Proportion of papers published by academic institutions from 2000 to 2017; B: Papers published by academic institutions in core journals from 2000 to 2017. In recent years, the proportion of papers published by academic 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 investigators 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*

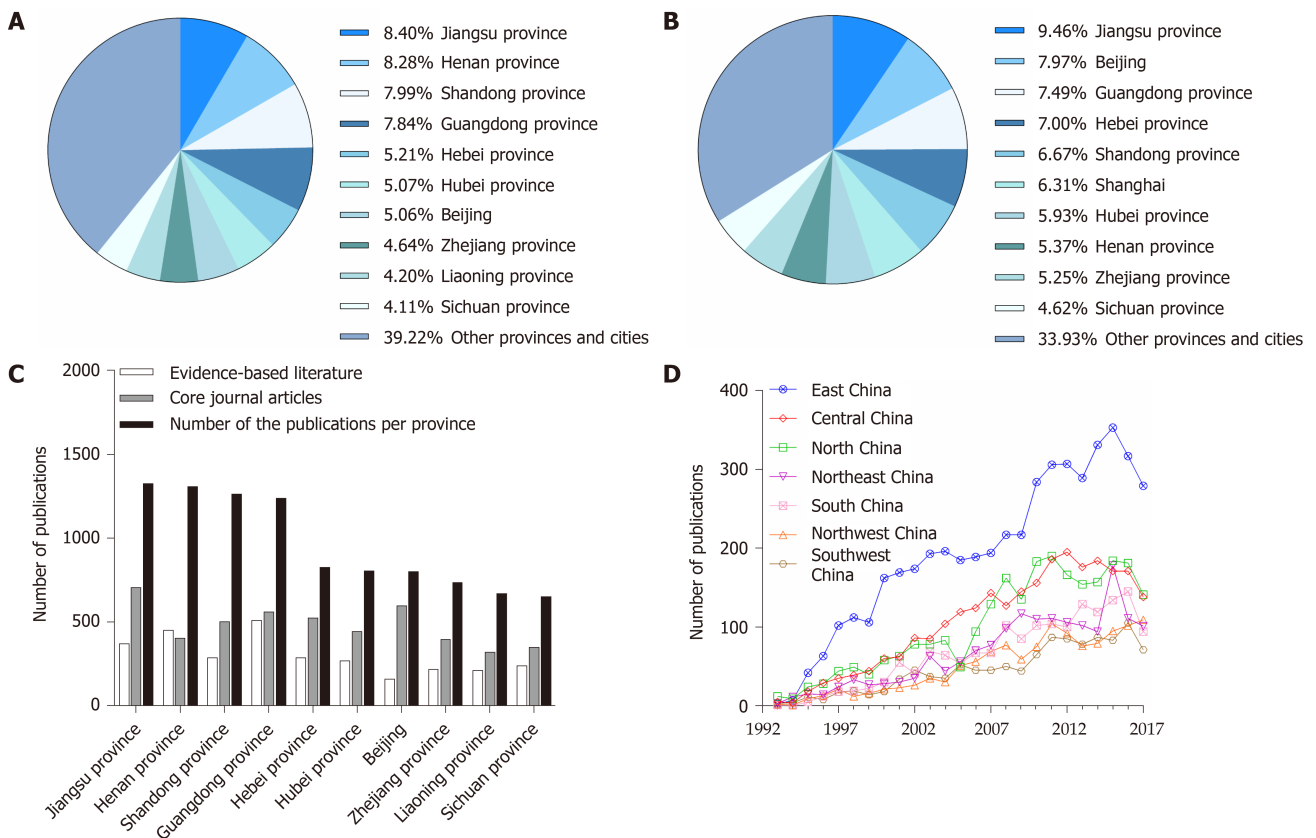


Figure 4 Reginal distribution of publications focused on ulcerative colitis. A: Proportion of publications in each province; B: Proportion of papers published by core journals in each province; C: Province distribution for the literature on ulcerative colitis (UC) in China; D: Regional distribution of 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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