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# Reviews in gastroenterology

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# Reviews in gastroenterology

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# Editorial: Reviews in gastroenterology

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Editorial on the Research Topic
Reviews in gastroenterology

## Introduction

Gastroenterology is a rapidly evolving discipline which covers diseases of the digestive tract (esophagus, stomach, and intestine) and digestive glands (liver, biliary tree, and pancreas). This Research Topic presents eight reviews which focus on the following specific conditions and diseases: gastroesophageal reflux disease, implications following cholecystectomy, locally advanced right colon cancer, appendiceal cancer, enteral nutrition, lumen-apposing metal stent, non-alcoholic fatty liver disease, and acute-on-chronic liver failure. Each of these complications, conditions and diseases account for an overwhelming disease burden in the fields of gastroenterology and hepatology.

## Summary of the reviews

The standard treatments for gastroesophageal reflux disease (GERD) are lifestyle adjustment and proton pump inhibitors. The agonist of the gamma-aminobutyric acid receptor, baclofen reduces reflux by inhibiting lower esophageal sphincter relaxation, and is used as an alternative management for GERD (1). However, the efficacy and side effects of baclofen in GERD treatment still need investigations. Arabpour et al. systemically reviewed 26 clinical trials and highlighted that baclofen benefited four populations with GERD as below: adults, children, patients with gastroesophageal reflux-induced chronic cough, and hiatal hernia patients. Baclofen ameliorates reflux symptoms, as well as improves pH-monitoring and manometry results. It should be noted that most of the published clinical studies on baclofen treatment in GERD had small sample sizes, and approximately one third of them were not randomized clinical trials. Although baclofen seems to be a promising medication and diversifies the treatment of GERD, the use of baclofen in the treatment of GERD needs further investigation.

Cholecystectomy can disturb bile flow and bile acid circulation, thus dysregulating gut microbiota (2). Growing evidence has linked these changes after cholecystectomy to the development of colorectal cancer (CRC) (3, 4). Jiang et al. discussed how cholecystectomy results in altered bile acid homeostasis and gut microbiota. In addition, they highlight the

mechanisms involved in these changes, which pose a higher risk of CRC compared to non-cholecystectomized patients. The authors also summarized the potential tumorigenic effect of secondary bile acids on CRC. The potential of bile acid treatment for CRC was also discussed. As there are a considerable number of patients receiving cholecystectomy worldwide, it alerts that these patients have a higher risk for CRC, and it is meaningful to enhance CRC screening in these populations.

Locally advanced right colon cancer (LARCC) invades neighboring organs such as the pancreas and the duodenum, which challenges surgical interventions (5). En bloc resection is considered a suitable surgical method for this situation but strong evidence from clinical trials is lacking (6). To identify the effect of en bloc resection in LARCC cases with neighboring organ invasion, the data of 117 patients showed that en bloc resection plus right hemicolectomy was superior to pancreaticoduodenectomy with respect to survival of LARCC (Ri et al.). Although more data are needed to validate this conclusion, en bloc resection plus right hemicolectomy might be a better surgical procedure for LARCC.

Appendiceal cancer is a rare gastrointestinal tumor with limited knowledge on its risk stratification, which hinders treatment strategies for this disease. To this end, Liu et al. collected and analyzed the clinical data from a cohort with a large number of patients with primary appendiceal cancer from the Surveillance, Epidemiology, and End Results database. Age, pathological stage, surgery, number of lymph nodes removed, T stage, N stage, M stage, and CEA are the independent prognosis factors for appendiceal cancer. A monogram survival prediction model was established and validated to predict 1, 3, and 5-year overall survival for patients with appendiceal cancer and stratify the risk to give the individualized treatment.

Enteral nutrition (EN) consists of the delivery of nutrients via tubes and is suggested as the first-line treatment to induce remission in pediatric inflammatory bowel disease (IBD) (7). However, the application of specific EN, such as exclusive EN, has not reached a consensus. Luo et al. reviewed 12 questionnaire survey studies regarding exclusive EN application in pediatric IBD, and summarized current opinions on EN methods, including treatment course, formula, and food reintroduction, which might help to standardize the use of EN in children IBD.

The lumen-apposing metal stent (LAMS) is a large-channel metal stent used in endoscopic ultrasonic-guided intervention and has been applied recently for pancreatic fluid collection drainage (8). However, this method has suffered from several drawbacks, such as complicated and difficult procedures. Yi et al. introduced a recently developed endoscopic approach combining LAMS and electro-cautery cyctotome, namely electro-cautery LAMS (EC-LAMS). EC-LAMS shows several advantages compared to conventional LAMS and double pigtail plastic stents, including easy deployment, lower cost, shorter processing time, broader indications, and lower risk of complications such as bleeding. In general, EC-LAMS seems to be a novel and promising method for stent placing.

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease with increasing disease burden (9, 10). Grip strength (GS) is an index of muscle strength and is associated with metabolic function (11). As NAFLD patients are often accompanied by metabolic disorders, GS might be considered as an indicator of NAFLD. Han et al. systemically reviewed 10 cross-section studies to reveal the relationship between GS and NAFLD. They demonstrated that people with low GS have higher risk of NAFLD, while NAFLD patients are associated to lower GS than healthy population. However, these cross-section studies were only conducted in China and Korea, and it is still unclear whether these conclusions can be applied in other populations.

Acute-on-chronic liver failure (ACLF) is caused by acute decompensation based on chronic liver diseases (12). Laboratory studies are trying to clarify the pathogenesis of ACLF and discover potential therapeutic targets. However, one of the major obstacles is the lack of suitable ACLF animal models. To this end, Zhai et al. compared the characteristics of current ACLF mouse models. Moreover, they established an ACLF mouse model by sequential injection of carbon tetrachloride ($CCl_4$) for 8 weeks, followed by a double dosage of $CCl_4$ for 72 hours, and *Klebsiella pneumoniae* administration. This model recapitulates the usual clinical course of human ACLF and represents a potential animal model for future ACLF studies.

## Conclusion

The reviews of this special issue summarize the recent advances in the field of gastroenterology and hepatology. These reviews encompass a wide range of diseases spanning the gastrointestinal tract and the liver. We are optimistic that the knowledge gaps identified by these reviews are the first step toward delivering better care for patients.

## Author contributions

All authors drafted the manuscript. EK and HT revised the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# External therapy of traditional Chinese medicine for treating irritable bowel syndrome with diarrhea: A systematic review and meta-analysis

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**Background:** Irritable bowel syndrome with diarrhea (IBS-D) is a chronic functional gastrointestinal disorder that has a significant impact on quality of life, work productivity, and healthcare resources. External therapy of traditional Chinese medicine (TCM) has positive effects on IBS-D and is simple, convenient, and low-cost. This study aimed to systematically evaluate the efficacy and safety of external therapy of TCM for IBS-D.

**Methods:** This study was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), Chinese Scientific Journals (VIP), Wan Fang, and Chinese Biomedical (CBM) databases were electronically searched to collect randomized controlled trials comparing external therapy of TCM with Western medicine for IBS-D from inception to 31 December 2021. Two authors independently screened, extracted, and assessed the selected studies. The Jadad scale and Cochrane Collaboration Risk of Bias tool were used to evaluate study quality. The certainty of evidence was assessed using the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE). The meta-analysis was performed using the Review Manager software (version 5.3).

**Results:** Twenty-one studies involving 1,862 subjects were included. Acupuncture and moxibustion were the most commonly used external therapies. The meta-analysis showed that based on total effective rate with moderate certainty of evidence ($n$ = 21 studies, $n$ = 1,862 participants, RR = 1.25, 95% CI [1.2, 1.31], $I^2$ = 0%, $P < 0.00001$), clinical cure rate with low certainty of evidence ($n$ = 17 studies, $n$ = 1,502 participants, RR = 1.66, 95% CI [1.4, 1.96], $I^2$ = 1%, $P < 0.00001$), recurrence rate with very low certainty of evidence ($n$ = 5 studies, $n$ = 260 participants, RR = 0.44, 95% CI [0.34, 0.58], $I^2$ = 0%, $P < 0.00001$), total symptom score (MD = −4.9,

95% CI [−7.34, −2.47]), and IBS severity scoring system score (IBS-SSS) with moderate certainty of evidence (MD = −52.72, 95% CI [−63.9, −41.53]), the experimental group had significant advantages compared with the control group. The sensitivity analysis further confirmed the robustness of the primary outcomes. The improvement in quality of life associated with IBS (IBS-QOL) was superior in the experimental group compared to the control group, and the difference was statistically significant; however, the clinical heterogeneity was strong. The inverted funnel plot of the included studies indicated a potential publication bias.

**Conclusion:** External therapy of TCM for IBS-D alleviated abdominal symptoms, improved clinical effectiveness, and reduced recurrence with great safety. However, because of the limitations of publication bias in trials, more rigorous studies with a clinical design are necessary for further verification of the outcomes.

**Systematic Review Registration:** [https://www.crd.york.ac.uk/PROSPERO/], identifier [CRD42020222993].

KEYWORDS

**systematic review, meta-analysis, irritable bowel syndrome with diarrhea, external therapy of TCM, randomized controlled trial, complementary therapy**

## Introduction

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterized by recurrent abdominal pain and bloating, altered bowel habits, and stool irregularities without structural or biochemical abnormalities (1). IBS is further categorized into four subtypes depending on stool consistency rather than stool frequency: IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), IBS with a mixed stool pattern (IBS-M), and IBS unsubtyped (IBS-U). However, 40% of all cases are IBS-D (2, 3). Globally, the pooled prevalence of IBS is 10–20% (4). In China, the population of outpatients with IBS comprises more than half of the population attending clinics for digestive system problems, and 75% of these cases are mainly the IBS-D subtype (5). The pathophysiology of IBS is complex and poorly understood; it includes genetic predisposition, the gut-brain axis, visceral hypersensitivity, changes in the gut microbiome, alterations in gastrointestinal motility and intestinal permeability, low-grade mucosal inflammation, and immune system activation (6–10). Antidiarrheal medications, antispasmodic therapy, microecological preparations, and central neuromodulators are commonly used as medical therapies for IBS-D (11). However, their clinical efficacy for intestinal discomfort is unsatisfactory (12). Notably, IBS-D might become increasingly common because of trends in the Westernized diet and lifestyle behavior changes, thus representing a considerable burden to both healthcare service and the society because of costs of diagnosis and treatment (13, 14). IBS-D has a strong impact on the quality of life (QOL), work productivity, healthcare resources, and the society, and it has a strong economic impact because of its refractoriness and recurrence (15, 16).

The external therapy of traditional Chinese medicine (TCM) has a long history and culture. It provides a solid theoretical basis for treating various diseases and has the benefits of simplicity, convenience, and low cost. Additionally, it has been widely used for IBS-D. Several studies have shown that external therapy of TCM, such as acupuncture, moxibustion, and acupoint application, can be used to relieve symptoms and reduce the recurrence rate and adverse reactions associated with IBS-D (17–19). A variety of external therapies involving TCM has gained increasing attention because of their use as an IBS-D treatment. Furthermore, external therapy involving TCM has been included in the consensus on the diagnosis and treatment of IBS (2017 edition) for clinical guidance.

However, the clinical efficacy and safety of various external therapies involving TCM have not yet been statistically and systematically assessed. To more objectively investigate the curative effects of external therapy involving TCM for IBS-D, we collected randomized controlled trials (RCTs) of the IBS-D treatment. Then, we compared the efficacy of the external treatment of TCM with that of conventional Western medicine and conducted a meta-analysis. Additionally, we expected that our results would provide evidence-based suggestions for clinical practice and guide clinical applications more effectively.

# Materials and methods

## Study protocol

The protocol was registered with the International Prospective Register of Systematic Reviews (registration no. CRD42020222993).[1] This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines of 2020 (20).

## Search strategy and data sources

A comprehensive search strategy for relevant clinical trials was independently performed by two reviewers (YW and YW) using the following eight databases: Web of Science, Embase, PubMed, Cochrane Library, Chinese Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI), Wan Fang, and Chinese Scientific Journals (VIP). Dates ranged from the inception of each of the different databases to 31 December 2021. There were no language restrictions. Because the methods of external therapy of TCM are abundant, we conducted a literature search of the eight aforementioned databases to include the maximum number of clinical trials. Search strategies and specific details are shown in **Supplementary materials**.

## Study selection

### Inclusion criteria

The following inclusion criteria for participants, intervention, comparator, study design, and study quality were used:

1) Participants: Patients diagnosed with IBS-D using specific criteria or internationally recognized criteria.
2) Intervention: The experimental group was treated with external therapy of TCM alone without any oral Chinese medicine or Western medicine.
3) Comparator: The control group was treated with conventional Western medicine treatment and without external therapy.
4) Study design: RCTs were designed with a sample size of $\geq$60. Moreover, treatment duration was $\geq$ 28 days.
5) Study quality: The methodological quality of each included study was assessed and had a Jadad score $\geq$3 (indicating a high-quality study) (21).

### Exclusion criteria

The following exclusion criteria were used:

1) Duplicate publications (only the earliest publication by the same author was included).
2) Cases were not identified as the IBS-D subtype.
3) Publications were reviews, meta-analyses, animal experiments, conference abstracts, books, theses, or study protocols.
4) The control group was a self-control group or included healthy subjects without intervention.
5) Outcome measures of interest for the meta-analysis were not included.

### Primary outcomes

The primary outcomes were total effective rate and clinical cure rate.

Criteria for total efficacy evaluation: IBS-D is a functional gastrointestinal disorder. The treatment goals for IBS-D are alleviation of abdominal pain and abdominal distension, reduction of the frequency of defecation, and improvement in stool form; the achievement of these goals was based on the participants' self-assessment of diarrhea symptoms. Clinical effect was assessed based on changes in the patients' self-reported major symptom scores. Total effectiveness rate was assessed using the comprehensive symptom score index (CSSI). The formula for calculating the CSSI was as follows: CSSI (%) = (score before−score after)/score before × 100%. When the CSSI (%) was $\geq$30%, a clinical effect was considered. If the CSSI (%) reached 90% or higher, then clinical recovery was considered. Treatments were recorded as effective, markedly effective, and clinically curative.

### Secondary outcomes

Secondary outcomes were recurrence rate, total symptom score, IBS severity scoring system (IBS-SSS) questionnaire score, and IBS-QOL scale score.

## Research screening process

First, duplicate records were eliminated according to the parameters of the title and author information using the NoteExpress software (Beijing Aegean Sea Music Technology Co., Ltd., Beijing, China). Subsequently, the remaining abstracts and full texts were independently reviewed by two authors using the inclusion and exclusion criteria (YW and YW). Disagreements were resolved by negotiation.

## Data extraction and management

The two authors (W and YW) separately extracted the following data from each included study using predesigned tables: general information such as title, first author, and

1 https://www.crd.york.ac.uk/PROSPERO/

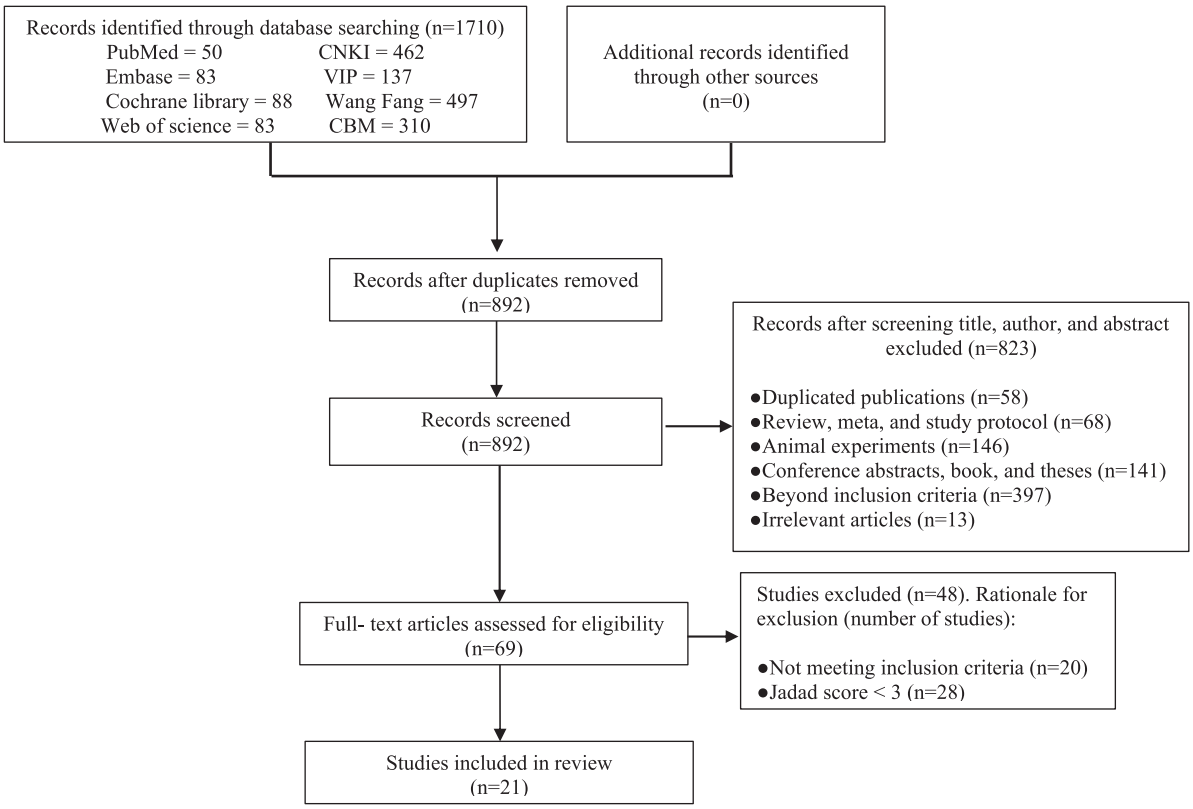


FIGURE 1
Flow chart of study selection process.

year of publication, study features such as method of randomization, allocation sequence generation, assignment concealment, blinding, and withdrawal, and data details such as sample size, age, disease duration, diagnostic criteria, outcome measures, intervention, duration, and adverse events. The included clinical trials were scored using the Jadad scale (22). Discrepancies between the two authors were resolved by a discussion resulting in a consensus, or an assessment by a third reviewer (XM).

## Quality assessment

The methodological quality of the trials was assessed independently by two reviewers using the Jadad scale and the Cochrane Collaboration Risk of Bias tool. The quality of the studies used during this review was evaluated using the Jadad scale, which included randomization, blinding, and withdrawals and dropouts (22). Moreover, the risk of bias of the RCTs was appraised using the Cochrane Collaboration tool, which is composed of the following seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of the outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Three levels of bias, high risk, low risk, and unclear risk, were used to assess each domain, and graphs depicting this information were visualized. A funnel plot was used to assess for publication bias when the pooled analyses included more than 10 studies.

## Grading certainty of evidence

The quality of evidence assessment of the primary and secondary outcomes was determined using Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) (23).

## Statistical analysis

Data synthesis and statistical analysis using a random effects model were performed using Review Manager (version 5.3). For dichotomous outcomes, the relative risk (RR) and 95% confidence intervals (CIs) were presented as effects measured using the Mantel-Haenszel method. For continuous variable outcomes, weighted mean differences (MDs) and 95% CIs were

TABLE 1 Basic characteristics of the included studies.

| Study | Sample size(n) (E/C) | Age (E/C) | Disease course (E/C) | Diagnostic criteria | Diagnostic criteria of traditional Chinese medicine | Syndrome types of traditional Chinese medicine | Evaluation Criteria for outcomes | Interventions | | Treatment durtion | Follow-up duration | Adverse event | | Jadad | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | E | C | | | E | C | | |
| Liu et al. (24) | 31/32 | 38.15 ± 7.85/ 38.45 ± 8.22 | 26.22 ± 7.08m/ 25.88 ± 7.02m | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Acupuncture; 40 sessions over 8 weeks | Trimebutine maleate capsules (0.2g, 3 × /day, 5days/week) | 8 weeks | NA | NA | NA | 3 | ①②④⑤ |
| Mao (25) | 40/40 | 46.38 ± 11.47/ 47.49 ± 12.39 | 96.49 ± 45.54m/ 90.13 ± 47.93m | Rome III | Not mentioned | Not mentioned | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Acupuncture; 18 sessions over 6 weeks | Pinaverium bromide (50mg, 3 × /day) | 6 weeks | 18 weeks | 1 case with anxiety and depression, 1 case with mild blood sickness | 4 cases with mild nausea and vomiting | 3 | ①②⑤⑥ |
| Li et al. (26) | 35/35 | 39.1 ± 11.8/ 37.9 ± 11.5 | 4.33 ± 3.93y/ 5.23 ± 7.35y | Rome II | Consensus on TCM Diagnosis and Treatment of Irritable Bowel Syndrome | Gan- stagnancy Pi-deficiency syndrome | Improvement of clinical symptoms | Acupuncture; 12∼16 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | 3 months | 0 | 0 | 3 | ①②③④⑥ |
| Lu (27) | 38/38 | 54.59 ± 12.50/ 54.54 ± 11.96 | 5.86 ± 3.44y/ 5.86 ± 3.68y | Rome III | Consensus on TCM Diagnosis and Treatment of Irritable Bowel Syndrome and Guidelines for the Diagnosis and Treatment of Digestive Diseases in Traditional Chinese Medicine (2006 Edition) | Gan- stagnancy Pi-deficiency syndrome | Improvement of clinical symptoms | Acupuncture; 12 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | NA | NA | NA | 3 | ①②④ |
| Sun et al. (28) | 30/30 | 38.81 ± 11.80/ 38.59 ± 11.45 | 4.23 ± 3.96y/ 5.63 ± 7.35y | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Acupuncture; 20 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | NA | 0 | 0 | 3 | ①②④⑥ |
| Zhang et al. (29) | 31/30 | 39.5 ± 2.1/ 39.9 ± 2.1 | 71.0 ± 8.4m/ 69.4 ± 7.6m | Rome III | Not mentioned | Not mentioned | Improvement of IBS-SSS score | Acupuncture; 12 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | NA | NA | NA | 3 | ①②⑤ |

*(Continued)*



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TABLE 1 (Continued)

| Study | Sample size(n) (E/C) | Age (E/C) | Disease course (E/C) | Diagnostic criteria | Diagnostic criteria of traditional Chinese medicine | Syndrome types of traditional Chinese medicine | Evaluation Criteria for outcomes | Interventions | | Treatment durtion | Follow-up duration | Adverse event | | Jadad | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | E | C | | | E | C | | |
| Cao et al. (30) | 35/35 | 44.36 ± 8.61/ 44.05 ± 8.72 | 8.52 ± 5.03y/ 8.84 ± 5.25y | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicineand and the Standard of Cure and Improvement of Clinical Diseases | Acupuncture; 80 sessions over 16 weeks | Pinaverium bromide (50mg, 3 × /day) | 16 weeks | NA | NA | NA | 3 | ①②⑤ |
| Li et al. (31) | 51/26 | 46 ± 13/ 48 ± 13 | 143.6 ± 125.9m/ 133.3 ± 116.7m | Rome III | Not mentioned | Not mentioned | Improvement of IBS-SSS score | Acupuncture; 18 sessions over 6 weeks | Pinaverium bromide (50mg, 3 × /day) | 6 weeks | NA | 0 | 1 patient had severe diarrhea after taking pinaverium bromide, and withdrew. | 3 | ①② |
| Li et al. (32) | 30/30 | 46 ± 16/ 44 ± 16 | 13.6 ± 9.8y/ 13.3 ± 10.1y | Roma III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Acupuncture; 28 sessions over 8 weeks | Pinaverium bromide (50mg, 3 × /day) | 8 weeks | NA | NA | NA | 3 | ① |
| Guo et al. (33) | 154/77 | 46 ± 12/ 44 ± 13 | 6∼480m/ 6∼348m | Rome III | Not mentioned | Not mentioned | Improvement of IBS-SSS score | Acupuncture; 18 sessions over 6 weeks | Pinaverium bromide (50mg, 3 × /day) | 6 weeks | NA | 7 cases with subcutaneous hemorrhage | 2 cases with dry mouth, 2 cases with dizziness, and 1 case with nausea | 3 | ①②⑤⑥ |
| Shi et al. (34) | 60/60 | 40.2 ± 10.8/ 38.5 ± 9.1 | 8.6 ± 3.8y/ 7.3 ± 2.1y | Rome III | Not mentioned | Not mentioned | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Electro acupuncture | Trimebutine maleate capsules (0.2g, 3 × /day) | 8 weeks | NA | NA | NA | 3 | ① |
| Wan et al. (35) | 58/57 | 37.23 ± 10.21/ 40.07 ± 11.67 | 4.04 ± 1.13y/ 4.12 ± 1.78y | Rome III | Diagnosis and Treatment of Irritable Bowel Syndrome with Integrated Chinese and Western Medicine | Gan- stagnancy Pi-deficiency syndrome, Deficiency of both Spleen and stomach syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Eye-acupuncture; 28 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | NA | NA | NA | 3 | ①②③④ |

*(Continued)*



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TABLE 1 (Continued)

| Study | Sample size(n) (E/C) | Age (E/C) | Disease course (E/C) | Diagnostic criteria | Diagnostic criteria of traditional Chinese medicine | Syndrome types of traditional Chinese medicine | Evaluation Criteria for outcomes | Interventions | | Treatment durtion | Follow-up duration | Adverse event | | Jadad | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | E | C | | | E | C | | |
| Zhang et al. (36) | 50/50 | 21∼71/ 23∼68 | 3m∼15y/ 8m∼20y | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Head-acupuncture; 30 sessions over 30 days | Bifidobacterium capsule (2 capsules, 2 × /day) | 30 days | NA | NA | NA | 3 | ①② |
| Liu et al. (37) | 38/37 | 37.05 ± 7.88/ 38.01 ± 8.01 | 25.39 ± 6.25m/ 24.97 ± 7.79m | Rome IV | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Herb-separated moxibustion; 40 sessions over 8 weeks | Pinaverium bromide (50mg, 3 × /day) | 8 weeks | NA | NA | NA | 3 | ①②④⑤ |
| Hao and Shi (38) | 42/42 | 38 ± 8/ 37 ± 7 | 23 ± 7m/ 24 ± 8m | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Herb-separated moxibustion; 40 sessions over 8 weeks | Pinaverium bromide (50mg, 3 × /day) | 8 weeks | NA | NA | NA | 3 | ①②④⑤ |
| Geng et al. (39) | 30/30 | 35.98 ± 7.39/ 35.29 ± 7.12 | 3.45 ± 1.03y/ 3.12 ± 0.98y | Rome III | Not mentioned | Spleen and kidney yang deficiency | Improvement of clinical symptoms | Long-snake moxibustion; 8 sessions over 8 weeks | Loperamide hydrochloride capsule(2mg, 3 × /day) + Bacillus licheniformis live capsule(0.5g, 3 × /day) | 8 weeks | NA | NA | NA | 3 | ①②④⑤ |
| Ge and Zeng (40) | 60/60 | 38.9 ± 11.2/ 39.1 ± 10.3 | 1∼13y/ 1∼12y | Rome II | Not mentioned | Not mentioned | Improvement of clinical symptoms | Warming acupuncture-moxibustion; 24 sessions over 4 weeks | Loperamide hydrochloride capsules (2mg, 3 × /day) | 4 weeks | 6 months | NA | NA | 3 | ①②③ |
| Li et al. (41) | 30/30 | 44.27 ± 11.95/ 44.17 ± 13.78 | 6.77 ± 2.93y/ 7.27 ± 3.04y | Rome III | Consensus on TCM Diagnosis and Treatment of Irritable Bowel Syndrome | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Umbilical moxibustion; 20 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | 3 months | NA | NA | 3 | ①②③ |
| Gu (42) | 30/30 | 38.24 ± 11.32/ 37.53 ± 10.21 | 5.63 ± 1.22y/ 6.55 ± 1.54y | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Gan- stagnancy Pi-deficiency syndrome | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Acupuncture and acupoint application; 20 sessions over 4 weeks | Pinaverium bromide (50mg, 3 × /day) | 4 weeks | NA | NA | NA | 3 | ①④ |

*(Continued)*



10.3389/fmed.2022.940328

TABLE 1 (Continued)

| Study | Sample size(n) (E/C) | Age (E/C) | Disease course (E/C) | Diagnostic criteria | Diagnostic criteria of traditional Chinese medicine | Syndrome types of traditional Chinese medicine | Evaluation Criteria for outcomes | Interventions | | Treatment durtion | Follow-up duration | Adverse event | | Jadad | Outcomes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | E | C | | | E | C | | |
| Luo et al. (43) | 50/50 | 42.3 ± 9.8/ 40.5 ± 10.1 | 3.4 ± 1.5y/ 3.7 ± 1.0y | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Not mentioned | Consensus on TCM Diagnosis and Treatment of Irritable Bowel Syndrome | Acupuncture and acupoint application; 14 sessions over 4 weeks | Trimebutine maleate capsules (0.2g, 3 × /day) | 4 weeks | 6 months | 0 | 0 | 3 | ①②③④⑥ |
| Lin et al. (44) | 60/60 | 40.2 ± 10.8/ 38.5 ± 9.1 | 8.6 ± 3.8y/ 7.3 ± 2.1y | Rome III | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine (Trial) | Not mentioned | Guidelines of Clinical Research of New Drugs of Traditional Chinese Medicine | Acupuncture and acupoint application | Trimebutine maleate capsules (0.2g, 3 × /day) | 4 weeks | NA | NA | NA | 3 | ① |

E, experimental group; C, control group; m, month; y, year.
① total effectiveness rate; ② clinical cure rate; ③ recurrence rate; ④ total symptom score; ⑤ score on irritable bowel syndrome-severity scoring system score questionnaire; ⑥ score on Irritable bowel syndrome-quality of life scale.

presented as effect measures. Forest plots were used to display summary statistics. $I^2$ statistics and the chi-square test method were used to statistically evaluate the heterogeneity of the included studies. $I^2$ statistics < 50% and $p > 0.1$ indicated no significant heterogeneity across the studies. $I^2$ statistics > 50% and $p < 0.1$ indicated significant heterogeneity across the studies. When the results of the $I^2$ statistics and $p$-values were inconsistent, $I^2$ statistics evaluation was selected as the main assessment method. Furthermore, a sensitivity analysis was performed to evaluate the robustness of the primary outcomes. Additionally, subgroup analyses were conducted based on the types of external therapy of TCM to explore whether the results had changed.

# Results

## Search results and study characteristics

A total of 1,710 relevant RCTs were initially retrieved. After gradual screening, we identified 21 studies involving 1,862 subjects, including 983 in the experimental group and 879 in the control group (**Figure 1**). The subjects were 17–71 years of age, and disease duration ranged from 3 months to 20 years. The maximum sample size of the included studies was 231. There were 13 studies on acupuncture therapy, including acupuncture, electroacupuncture, eye acupuncture, and head acupuncture (24–36). Five studies on moxibustion therapy included herb-separated moxibustion, long-snake moxibustion, warming acupuncture moxibustion, and umbilical moxibustion (37–41). Three studies on acupuncture included acupoint application (42–44). Acupuncture and moxibustion therapy are the common external treatment methods used in most of the studies. The treatment methods used for the control group were mainly antispasmodic, antidiarrheal, or adjusted intestinal flora. In terms of treatment duration, most of the studies performed treatment for 4 weeks, and only one study performed treatment for up to 16 weeks (30). Only five studies recorded follow-up durations of 3 or 6 months (25, 26, 40, 41, 43). The basic characteristics of the included trials are detailed in **Table 1**.

## Risk of bias and methodological quality assessment

All the trials reported appropriate random sequence generation methods and were rated as low risk. Four studies recorded adequate information about the methods used for allocation concealment and were rated as low risk (31–33, 38). The allocation concealment of the others was not mentioned and rated as unclear. Because of the particularity of the intervention methods, the participants and personnel were not blinded. Therefore, the performance bias of all the trials was

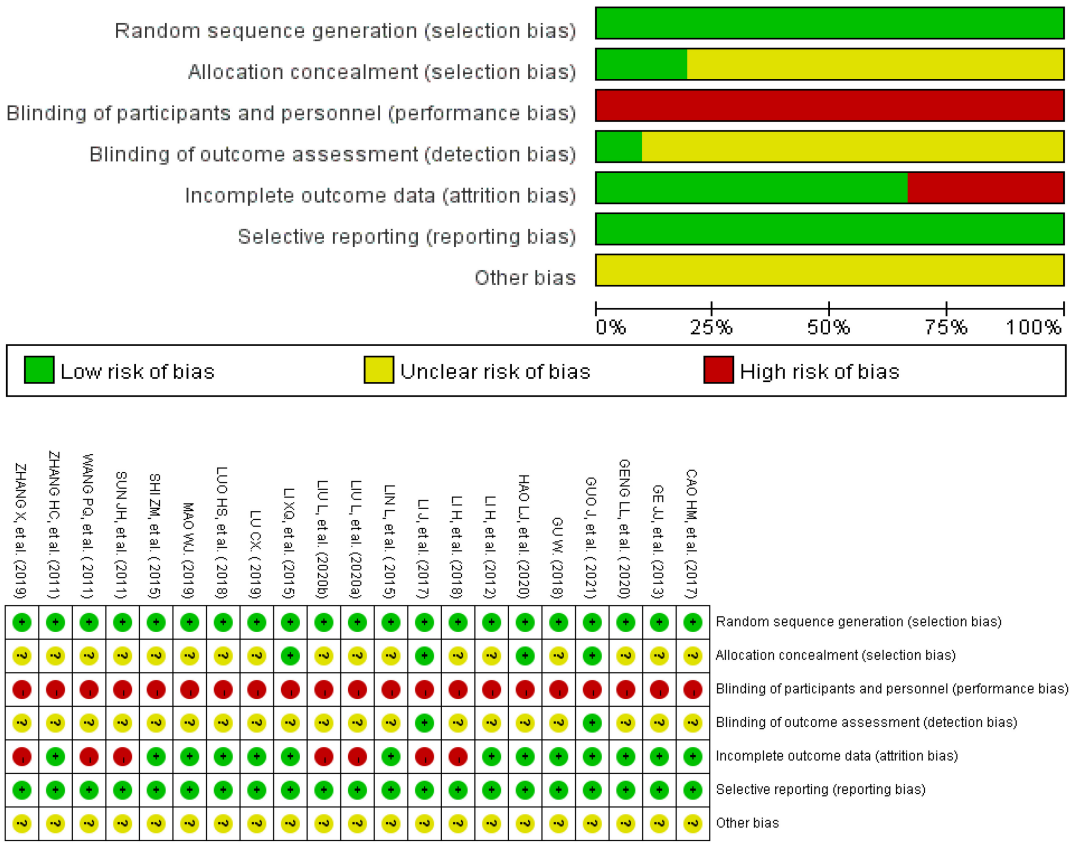


FIGURE 2
Risk of bias graph and summary. red, high risk; green, low risk; yellow, unclear risk.

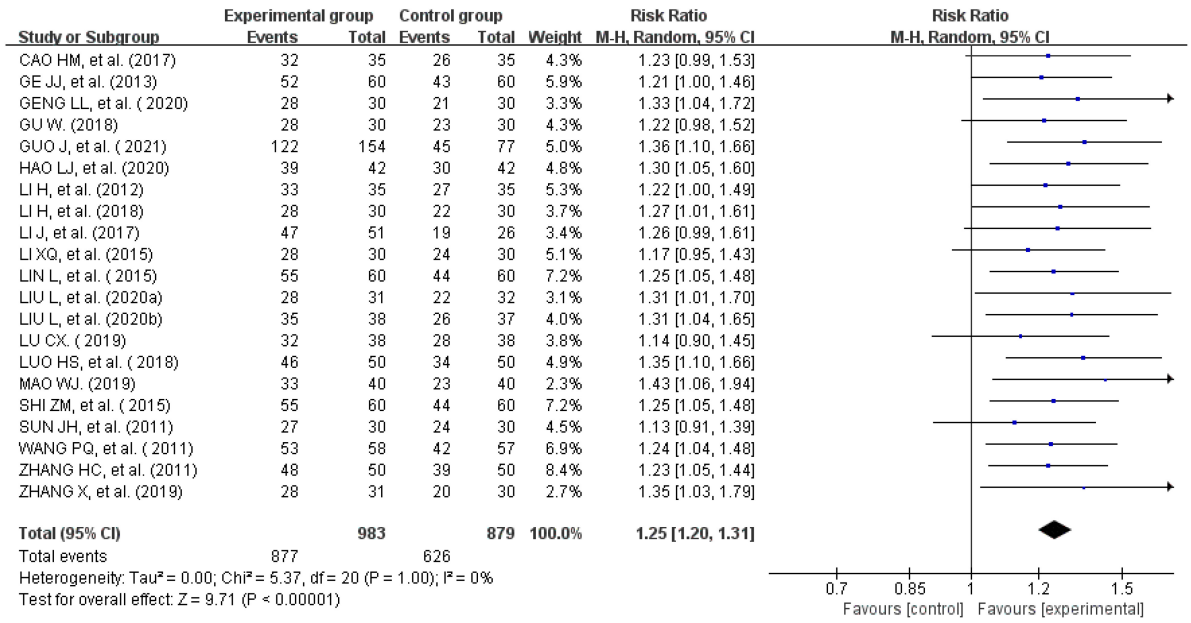


FIGURE 3
Forest plot of the total effectiveness rate.

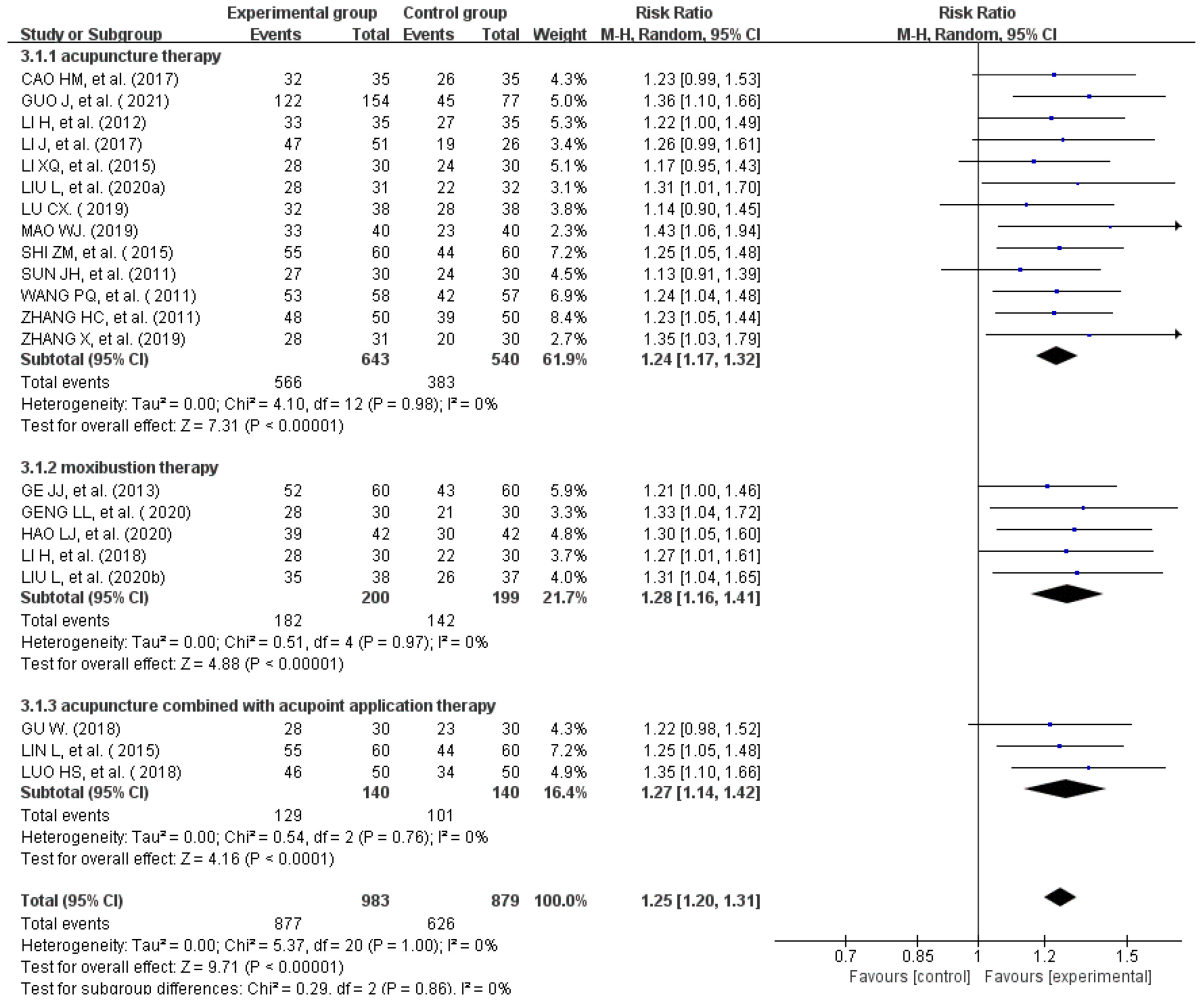


FIGURE 4
Forest plot of the effective rate of subgroups.

rated as high risk. Two trials described the method of blinding of assessors and were rated as low risk (31, 33). Seven studies mentioned that the participants withdrew or dropped out of the trials, and they included abscission data that were not included in the analysis; these were rated as high risk (24, 28, 29, 33, 35, 37, 41). The remaining studies with all data included in the final analysis were rated as low risk. Additionally, it could not be judged whether there was other bias in the 21 studies; therefore, they were rated as unclear. The results of the risk of bias analysis in the included trials are summarized in **Figure 2**.

## Primary outcomes

### Total effectiveness rate

All the 21studies compared the total effectiveness rates of the experimental and control groups. Effectiveness was measured using the scores of the main symptoms. Twelve studies with a CSSI ≥ 30%were evaluated as effective (24, 26, 28, 31, 34–39, 41, 44). One study with a CSSI ≥ 35% was evaluated as effective (27). Additionally, four studies (25, 29, 31, 33) were evaluated using the IBS-SSS score, and four trials (20, 30, 32, 37) were evaluated based on patients' self-reported scores for symptoms such as abdominal pain, abdominal distension, frequency of defecation, and stool form (30, 40, 42, 43). There was no heterogeneity across the trials when tested using $I^2$statistics (df = 20, $I^2$ = 0%). The meta-analysis showed that external therapy with TCM had a significantly higher total effectiveness rate in the experimental group than in the control group, and that the difference was statistically significant ($n$ = 1,862, RR = 1.25, 95% CI [1.2, 1.31], $Z$ = 9.71, $P$ < 0.00001; **Figure 3**). The 21 included studies were further removed individually for the sensitivity analysis, which showed that none of the studies significantly affected the results of this analysis, indicating that it had great reliability and stability. This showed that the total clinical effectiveness rate of external therapy with TCM alone for IBS-D was better

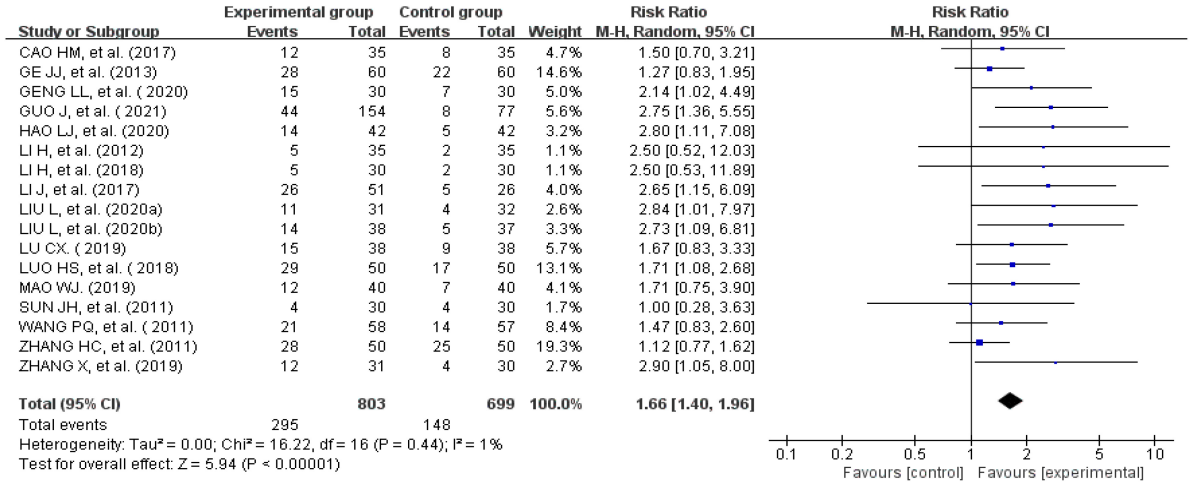


FIGURE 5
Forest plot of the clinical cure rate.

than that of the control group treated with internal Western medicine. Moreover, a subgroup analysis was conducted after various external treatments. The subgroup analysis showed that compared with the control group, acupuncture therapy ($n$ = 1,183, $I^2$ = 0%, RR = 1.24, 95% CI [1.17, 1.32], $Z$ = 7.31, $P < 0.00001$), moxibustion therapy ($n$ = 399, $I^2$ = 0%, RR = 1.28, 95% CI [1.16, 1.41], $Z$ = 4.88, $P < 0.00001$), and acupuncture combined with acupoint application therapy ($n$ = 280, $I^2$ = 0%, RR = 1.27, 95% CI [1.14, 1.42], $Z$ = 4.16, $P < 0.00001$) had greater total effectiveness rates, indicating that the difference was statistically significant (**Figure 4**).

### Clinical cure rate

The clinical cure rate was calculated as follows: number of cured cases/total number × 100%. Seventeen studies reported clinical cure rates (24–31, 33, 35–41, 43). There was no significant heterogeneity across the included studies when tested using $I^2$statistics (df = 16; $I^2$ = 1%). The meta-analysis showed that the experimental group receiving external therapy of TCM had a significantly higher clinical cure rate than the control group; this difference was statistically significant ($n$ = 1,502, RR = 1.66, 95% CI [1.4, 1.96], $Z$ = 5.94, $P < 0.00001$; **Figure 5**). The sensitivity analysis showed that none of the studies significantly interfered with the results of the analysis, indicating that this study had satisfactory reliability and stability.

## Secondary outcomes

### Recurrence rate

Five studies reported recurrence rates (26, 35, 40, 41, 43). There was no heterogeneity across the included trials when tested using $I^2$ statistics (df = 4; $I^2$ = 0%). The meta-analysis showed that the experimental group receiving external therapy of TCM had a significantly lower clinical cure rate than the control group; this difference was statistically significant ($n$ = 260, RR = 0.44, 95% CI [0.34, 0.58], $Z$ = 5.8, $P < 0.00001$; **Figure 6**).

### Total symptom score

Nine studies recorded total symptom scores (24, 26–28, 35, 37, 38, 42, 43). There was significant heterogeneity across the studies when tested using $I^2$statistics ($n$ = 588, $I^2 > 50\%$, MD = -4.9, 95%CI [−7.34, −2.47], $Z$ = 3.95, $P < 0.00001$). A subgroup analysis was performed according to the different weights given to the clinical symptom scores. The results showed that five studies assessed clinical symptom scores using 0, 1, 2, or 3 points ($n$ = 366, $I^2$ = 6%, MD = −1.87, 95% CI [−2.16, −1.59], $Z$ = 12.93, $P < 0.00001$) (26–28, 42, 43). The other four studies evaluated clinical symptom scores using 0, 2, 4, or 6 points ($P < 0.00001$, $I^2$ = 0%), indicating that the heterogeneity was still very high. However, the heterogeneity was significantly reduced after excluding one study ($n$ = 222, $I^2$ = 0%, MD = −9.99, 95% CI [−10.59, −9.39], $Z$ = 32.78, $P < 0.00001$) (35). This means that the different weights given to the clinical symptom scores affected the results of the analysis. A subgroup analysis showed that the improvement in clinical symptom scores for IBS-D treated with external therapy using TCM alone was better than the improvement in clinical symptom scores for IBS-D in the control group (**Figure 7**).

### Irritable bowel syndrome-severity scoring system score

Eight studies reported IBS-SSS questionnaire scores (24, 25, 29, 30, 33, 37–39). One recorded them as the median

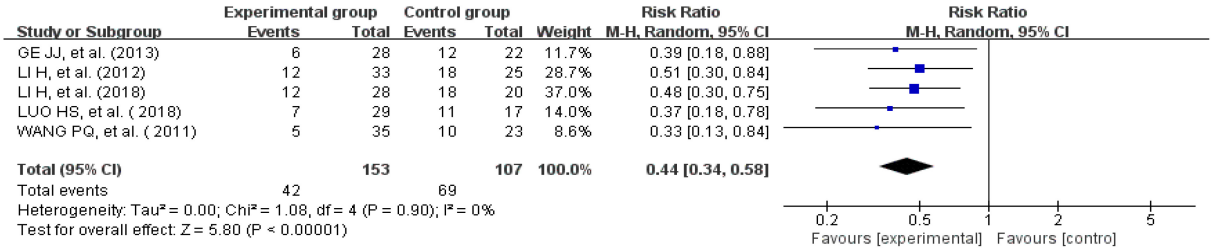


FIGURE 6
Forest plot of the recurrence rate.

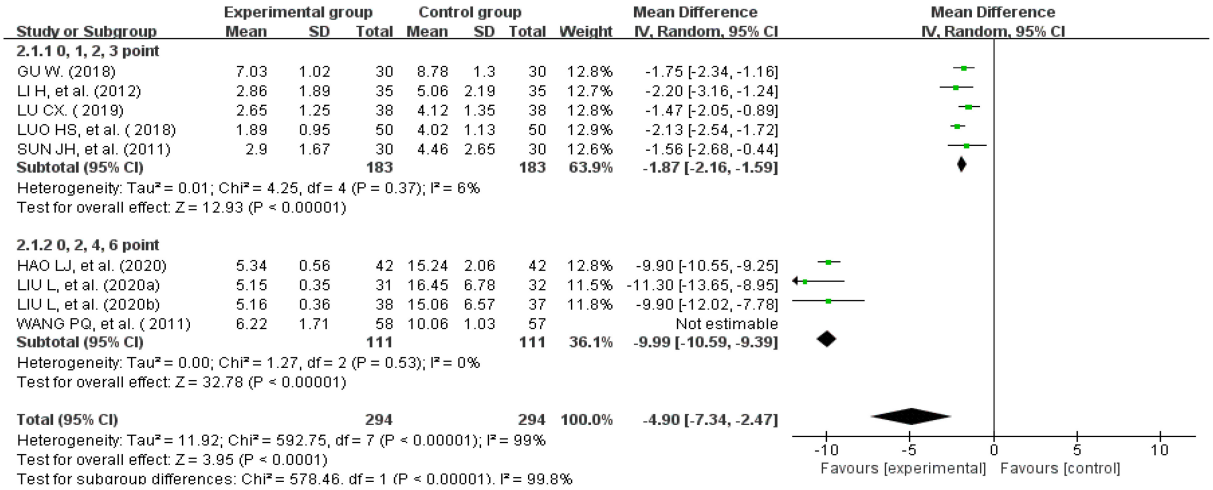


FIGURE 7
Forest plot of the total symptom score.

and quartile; therefore, that study could not be used for this analysis (33). We were unable to consult with the original author to obtain further information. Finally, seven studies were analyzed. There was a significant heterogeneity across the studies when tested using $I^2$ statistics (df = 5, $P < 0.00001$, $I^2 = 98\%$). The heterogeneity was significantly reduced after removing one study ($n = 432$, $I^2 = 30\%$, MD = -52.72, 95% CI [−63.9, −41.53], $Z = 9.23$, $P < 0.00001$) (29). The results showed that the improvement in the IBS-SSS questionnaire scores for external therapy of TCM alone was better than the improvement in the IBS-SSS questionnaire scores of the control group (**Figure 8**).

### Irritable bowel syndrome-quality of life

Five studies recorded IBS-QOL questionnaire scores (25, 26, 28, 33, 43). One of these studies (33) reported IBS-QOL questionnaire scores as the median and quartile; therefore, that study could not be used for this analysis. The difference was statistically significant, because the experimental group had a positive effect on improving the IBS-QOL score ($P < 0.05$). Nevertheless, the heterogeneity was high ($I^2 > 50\%$) (**Figure 9**). A subgroup analysis based on intervention measures and duration showed decreased heterogeneity, but $I^2$ was still > 50%.

## Adverse events

Among the 21 included studies, only six mentioned adverse events; among the six studies, three evaluated them as safe (26, 28, 43). One study recorded one case of anxiety and depression experienced by one patient in the experimental group (25). One study reported four cases of mild nausea and vomiting in the control group. The remaining studies reported seven cases of subcutaneous hemorrhage after acupuncture therapy. Correspondingly, two patients in the control group had a dry mouth, two had dizziness, and one had nausea (33). In particular, one patient developed severe diarrhea after using pinaverium bromide and was withdrawn from the study. In contrast, no adverse reactions were observed in the experimental group (31). All adverse reaction symptoms resolved spontaneously.

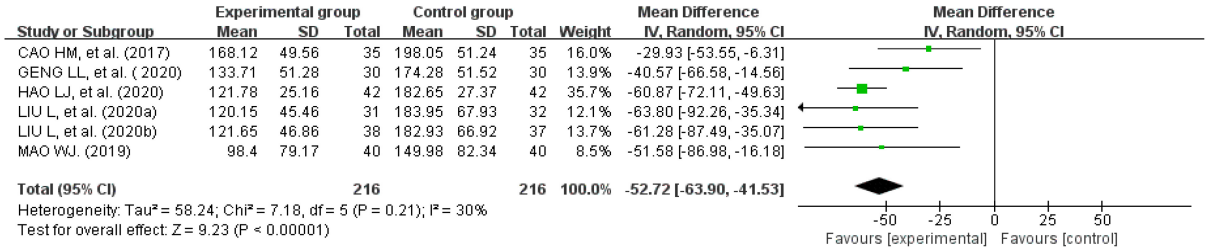


FIGURE 8
Forest plot of the score on irritable bowel syndrome-severity scoring system score questionnaire.

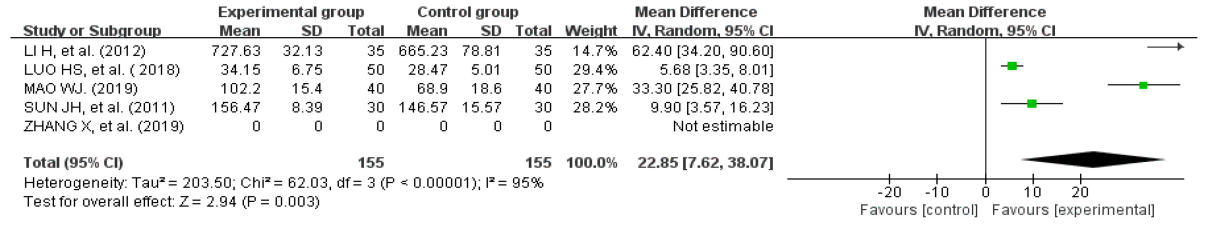


FIGURE 9
Forest plot of the score on Irritable bowel syndrome-quality of life scale.

## Publication bias

To detect a possible publication bias, we analyzed the funnel plot of more than 10 studies. The results showed that the morphological distribution on the left and right sides of the midline of the inverted funnel plot in the included studies was not symmetrical, indicating that the included studies had a potential publication bias (**Figures 10A–C**).

## Grading of recommendations, assessment, development, and evaluations certainty of evidence

The GRADE certainty of evidence for the primary and secondary outcomes is shown in **Supplementary materials**. The certainty of evidence was moderate for the total effectiveness rate and IBS-SSS score, low for the recurrence rate, and very low for the clinical cure rate, total symptom score, and IBS-QOL.

# Discussion

Traditional Chinese medicine, including Chinese herbal medicine and external treatment, can alleviate symptoms, improve cure rate, and reduce the recurrence of IBS-D (45). During this review, the positive effects of 21 external therapies involving TCM on trials involving patients with IBS-D were evaluated. The results showed that external therapy of TCM alone significantly improved the total effectiveness rate and clinical cure rate. Moreover, external therapy of TCM alone can reduce the recurrence rate of and improve both intestinal symptoms and QOL associated with IBS-D, with few side effects. Unlike previous research studies, we comprehensively summarized various external therapies of TCM. Furthermore, all the trials included in this study were high-quality clinical trials with a Jadad score of $\geq$3, which indicated a high quality of evidence.

Conventional Western medicine has a clear therapeutic mechanism and can temporarily improve the symptoms of diarrhea. However, the long-term effects remain unsatisfactory, and the recurrence rate is still quite high (40%) because of complex and unclear pathological mechanisms of IBS (46). External therapy involving TCM is a therapy that acts on the surface of the body or from outside the body to treat disease through corresponding somatic-visceral reactions. It is simple to use, results in few adverse reactions, and has a quick clinical efficacy; therefore, it is widely used to treat diarrhea. Furthermore, the World Health Organization recommends TCM for treatment of IBS (47). The external therapy of TCM during this study focused on acupuncture, moxibustion, and acupoint application; among these, acupuncture therapy and moxibustion therapy are the most commonly studied and mainly used clinical treatments for IBS-D. Based on the theory of somatic-visceral interactions,

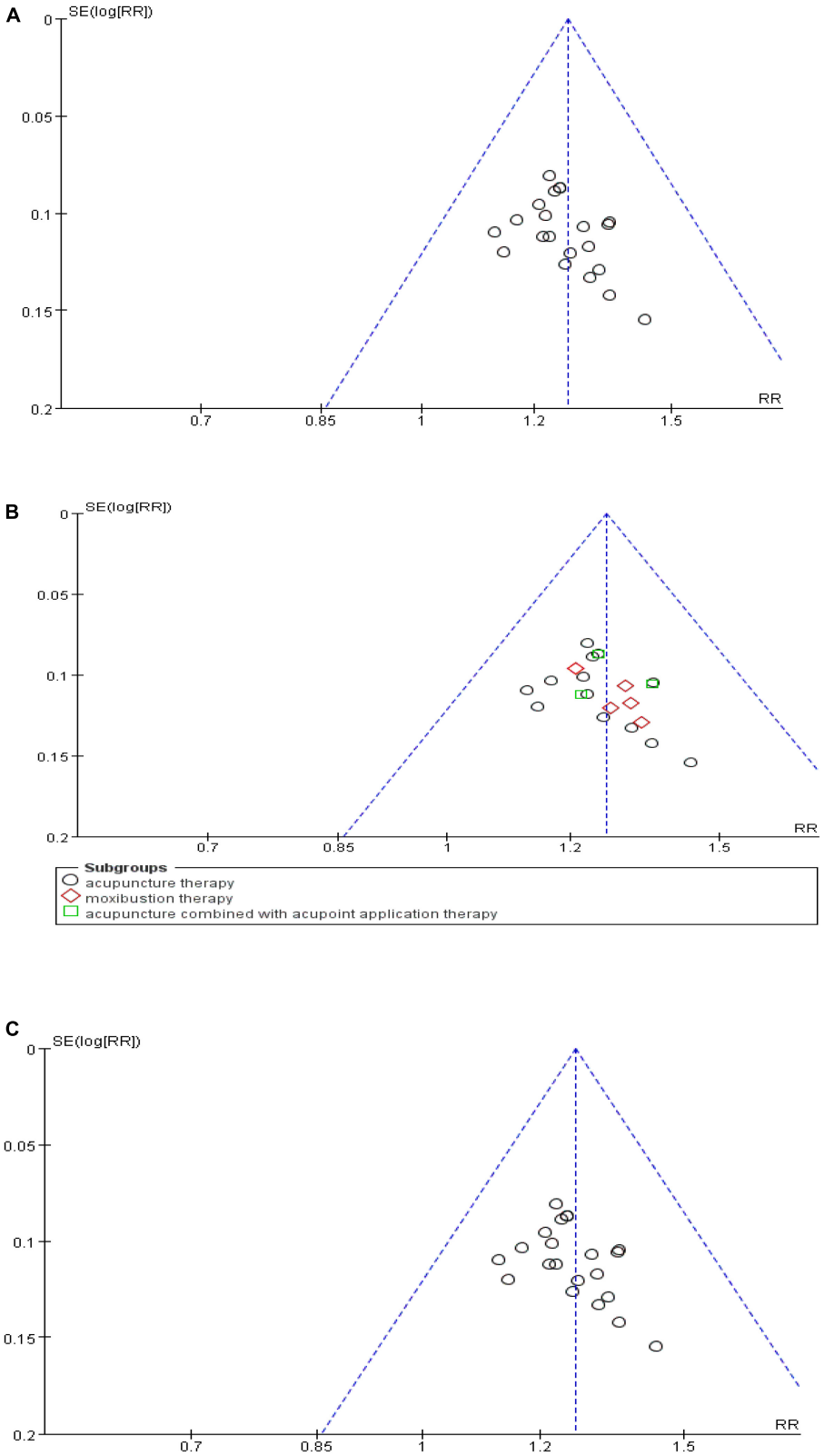


FIGURE 10
Funnel plot of publication bias. **(A)** Funnel plot of the total effective rate. **(B)** Funnel plot of the effective rate of subgroups. **(C)** Funnel plot of the clinical cure rate.

each external therapy with TCM has a similar efficacy when used to treat IBS-D. Additionally, several common clinical external treatment methods emphasize the role of stimulation of specific acupoints, and their mechanisms are also similar; therefore, they have similar effects on intestinal symptoms. Moreover, the sensitivity analysis showed that the total effectiveness and clinical cure rates of IBS-D treated with external therapy using TCM were relatively robust. It is noteworthy that the certainty of evidence was moderate for the total effectiveness rate and IBS-SSS. Similarly, the subgroup analysis revealed that the total clinical efficacy rates of acupuncture and moxibustion for treatment of IBS-D were better than that of Western medicine ($P < 0.0001$). Acupuncture is an important part of external treatment using TCM, which produces somatosensory stimulation at specific acupoints of the human body and releases the whole body to treat the intestinal tract. It induces multifaceted regulation to improve intestinal symptoms through complex mechanisms, such as inhibiting gastrointestinal motility, reducing visceral hypersensitivity, balancing the intestinal-brain axis, and regulating neurotransmitters and the immune system (48). The literature reports no need for acupuncture when the case is suitable for moxibustion. Moxibustion is another commonly used external therapy for patients with IBS-D. It uses the warm and medicinal power of ignited moxa to stimulate acupoints or specific parts of the surface of the body and promote the self-regulation function of the body. Moxibustion regulates intestinal inflammation, alleviates visceral hypersensitivity, and relieves visceral pain to improve functional gastrointestinal disorders (18, 49, 50). Acupoint application is a compound treatment method that integrates acupoints, meridians, and herbs that regulate meridians and improve blood circulation, thus exerting an effect on the intestinal system and improving diarrhea symptoms. Visceral hypersensitivity is the main pathogenesis of abdominal pain and diarrhea for patients with IBS-D; therefore, it has attracted increasing attention (51). Reducing visceral hypersensitivity to alleviate clinical symptoms is an important treatment strategy consistent with the mechanisms of acupuncture and moxibustion.

Unfortunately, the authors of the included studies did not explain the reasons for the lack of current clinical research data. IBS-D is a chronic, recurrent, and functional gastrointestinal disorder with no organic explanation. Its clinical trial is a complex process that involves clinical research and follow-up of participants, which involve high costs. Unfortunately, 20 to 30% of subjects withdrew from study participation (52). This is particularly true of studies with longer trial periods. Lack of long-term follow-up data was one limitation of this study. Another limitation of this study was that the random sequence allocation of the included studies was non-standard according to the summary risk of bias and publication bias graph, which may have caused selection bias. The funnel plot showed a skewed distribution, indicating a publication bias. Moreover, the accuracy of some results may have been affected by differences in the disease, reference standards for efficacy evaluation, and the unequal experience of TCM clinicians. Additionally, because of inevitable problems, such as database permissions, some gray bodies of literature were not retrieved.

External therapy of TCM for the treatment of IBS-D can alleviate abdominal symptoms, improve clinical effectiveness, and reduce recurrence with few side effects. Moreover, external therapy of TCM has a positive effect on improving QOL and can serve as an alternative treatment for IBS-D.

## Conclusion

The current evidence indicates that external therapy of TCM for IBS-D has positive efficacy and high safety. It is also simple, convenient, and low-cost. However, because of the limitations of the follow-up period and publication bias of the included trials, more rigorous clinical studies are necessary to further verify the long-term effects of external therapy of TCM.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

## Author contributions

XW and XT contributed to the conception and design of the study. YoW and YuW searched the databases and extracted the data. XW, XL, and XM evaluated the studies for inclusion and data analysis. XW conducted the statistical analysis of the data and drafted the manuscript. YoW wrote sections of the manuscript. BZ and XT revised the manuscript. All authors read and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.940328/full#supplementary-material

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# Association between grip strength and non-alcoholic fatty liver disease: A systematic review and meta-analysis

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**Background:** The association between grip strength (GS) and non-alcoholic fatty liver disease (NAFLD) has been reported by recent epidemiological studies, however, the results of these studies are inconsistent. This meta-analysis was conducted to collect all available data and estimate the risk of NAFLD among people with low GS, as well as the risk of low GS among patients with NAFLD.

**Methods:** We systematically searched several literature databases including PubMed, Web of Science, Cochrane Library, and Embase from inception to March 2022. These observational studies reported the risk of NAFLD among people with low GS and/or the risk of low GS among patients with NAFLD. Qualitative and quantitative information was extracted, statistical heterogeneity was assessed using the $I^2$ test, and potential for publication bias was assessed qualitatively by a visual estimate of a funnel plot and quantitatively by calculation of the Begg's test and the Egger's test.

**Results:** Of the citations, 10 eligible studies involving 76,676 participants met inclusion criteria. The meta-analysis of seven cross-section studies (69,757 participants) showed that people with low GS had increased risk of NAFLD than those with normal GS (summary OR = 3.32, 95% CI: 1.91–5.75). In addition, the meta-analysis of four studies (14,920 participants) reported that the risk of low GS patients with NAFLD was higher than those in normal people (summary OR = 3.31, 95% CI: 2.45–4.47).

**Conclusion:** In this meta-analysis, we demonstrated a strong relationship between low GS and NAFLD. We found an increased risk of NAFLD among people with low GS, and an increased risk of lower GS among NAFLD patients.

**Systematic review registration:** [www.crd.york.ac.uk/prospero], identifier [CRD42022334687].

KEYWORDS

**NAFLD, grip strength, review, meta-analysis, observational study**

# Introduction

Currently, non-alcoholic fatty liver disease (NAFLD) has become one of the most common causes of chronic liver disease, it is defined by the presence of steatosis in more than 5% of hepatocytes with little or no alcohol consumption (1). NAFLD is characterized by fatty infiltration of the liver without secondary causes of hepatic steatosis (2).

In the United States, approximately 30% of individuals are diagnosed with NAFLD (3). In addition, over 27% of individuals are affected by NAFLD (4). In China, the prevalence of NAFLD was reported to be between 15 and 36% (5, 6). Additionally, as a result of the aging population and obesity, the prevalence of NAFLD is increasing rapidly. However, to date, there is no effective drug for treatment of NAFLD. As shown by a number of compelling studies, NAFLD is associated with some chronic diseases, such as type 2 mellitus (T2DM), cardiovascular disease (CVD), and chronic kidney disease (CKD) (7, 8). Therefore, understanding the pathobiology and risk factors for development of NAFLD is of great importance.

Grip strength (GS) is a measure of the maximum static force that a hand can apply around a dynamometer. GS is often considered an indicator of muscle mass and muscle strength (9). Researches have suggested that low GS is associated with health damage and higher all-cause mortality (10, 11), such as falls, disability and poor quality of life (12, 13). Indeed, previous studies have also shown association between NAFLD and sarcopenia (14). Low muscle strength is used as a principal determinant of sarcopenia over muscle mass (15), and GS is recommended as a substitute measurement of muscle strength (16). Therefore, in clinical practice, people are increasingly aware of the importance of muscle strength.

Non-alcoholic fatty liver disease is a systemic condition that has a bi-directional relationship with the components of metabolic syndrome (17). According to recent studies, muscular strength is inversely related to insulin sensitivity (18) and excessive body and abdominal fat (19), which are independent risk factors for developing NAFLD. Now, several studies have reported that association between GS and NAFLD, therefore, we collected these studies for meta-analysis as a way to explore the relationship between GS and NAFLD.

# Materials and methods

## Protocol and guidance

This meta-analysis followed the Preferred Reports Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline (20). The protocol for this meta-analysis was registered with PROSPERO (CRD42022334687).

## Data sources and searches

Two investigators (LH and SF) independently conducted an electronic literature search using PubMed, Web of Science, Cochrane Library, and Embase, language was restricted to English, from database inception to March 2022. In PubMed, controlled vocabulary terms and the following keywords were used: ("Non-alcoholic Fatty Liver Disease" [Mesh]) OR (Non-alcoholic Fatty Liver Disease) OR (Non-alcoholic Fatty Liver Disease) OR (Fatty Liver, Non-alcoholic) OR (Fatty Livers, Non-alcoholic) OR (Liver, Non-alcoholic Fatty) OR (Livers, Non-alcoholic Fatty) OR (Non-alcoholic Fatty Liver) OR (Non-alcoholic Fatty Livers) OR (Non-alcoholic Steatohepatitis) OR (Non-alcoholic Steatohepatitides) OR (Steatohepatitides, Non-alcoholic) OR (Steatohepatitis, Non-alcoholic) AND ("Hand Strength" [Mesh]) OR (Strength, Hand) OR (Grip Strength) OR (Strength, Grip) OR (Hand Grip Strength) OR (Grip Strength, Hand) OR (Strength, Hand Grip) OR (Grip) OR (Grips) OR (Grasp) OR (Grasps). A similar search strategy was run in other databases. **Supplementary Table 1** presents the search strategy.

The database search revealed 224 articles that could have been included in our meta-analysis, and 43 articles were excluded because they were duplicated. After removing duplicates, all titles and abstracts for potential inclusion were screened by two independent researchers (LH and SF). Based on the inclusion and exclusion criteria, 164 articles were excluded after reading the titles and abstracts. Finally, 17 full texts of these records were selected for detailed assessment. The two researchers extracted the related data according to the inclusion criteria. If the studies were potentially eligible for inclusion, the full text was examined. The two reviewers would discuss with each other any disagreements that may have occurred.

## Study quality assessment

All studies were assessed for selection and measurement biases according to the Newcastle-Ottawa Scale (NOS) (21). The NOS consists of eight items focused on three domains: selection of study groups, ascertainment of the exposure and outcome, and comparability of groups to assess the quality of observational studies. Ratings were based on a star system and studies with a maximum rating of nine. Studies with one to three stars were categorized as low quality, four to six stars categorized as moderate quality, and seven to nine stars categorized as high quality. Each of included studies was assessed for bias by two independent investigators (LH and SF).

## Inclusion criteria

The same two authors evaluated the titles and abstracts of eligible studies and any disagreements were resolved by

consensus. The inclusion criteria are as follows: (1) studies on the association between NAFLD and GS; (2) used a standardized index to diagnose and assess NAFLD and GS; (3) reporting odds ratio (OR) and 95% confidence intervals (95% CI) for GS and NAFLD; (4) the full text of the study could be assessed; and (5) the full text of the study could be assessed.

## Exclusion criteria

The same two authors evaluated the titles and abstracts of eligible studies and any disagreements were resolved by consensus. The exclusion criteria are as follows: (1) did not use clear diagnostic criteria for NAFLD; (2) the measurement of GS is not accurate; (3) the study did not provide the OR of NAFLD and GS; (4) case reports, case series, reviews, posters, and abstracts were excluded; (5) measured only *in vitro* parameters or used animal models; and (6) based on the NOS scores, the low-quality studies were excluded.

## Data collection process

Data collection process two independent researchers (LH and SF) assessed the full texts of included studies and used a standard data extraction form when extracting data. Any disagreements were resolved by discussion until consensus was reached. The data extracted for the analysis involved: (1) the first author's name and publication year; (2) the sample size and number of cases; (3) the mean age and sources of participants; (4) the OR with the corresponding 95% CI; and (5) the scores of NOS in the studies.

## Statistical analysis

The meta-analysis of comparable data was carried out using Review Manager 5.3. OR and their associated 95% CI were used to assess a comparison between outcomes reported by the studies and a *P*-value less than 0.05 was considered to be statistically significant. We collected the summary OR of NAFLD and low GS. The heterogeneity of results between studies was determined by the $I^2$ test (22). For $I^2$, values of 25 to <50% were considered low heterogeneity, 50 to <75% moderate, and 75% highly heterogeneous. If significant heterogeneity was not present ($I^2 < 50\%$), a fixed-effect model was used to pool outcomes, otherwise a random-effect model was applied for the meta-analysis ($I^2 > 50\%$). The publication bias was assessed qualitatively by a visual estimate of the funnel plot and quantitatively by calculation of Begg's test and Egger's test (23).

## Subgroup analyses and sensitivity analyses

Subgroup analyses were performed according to the method of GS ascertainment [GS and relative grip strength (RGS)], diagnosis of NAFLD [ultrasonography and hepatic steatosis index (HSI)], region (China and Korea), mean age (<60 and >60 years old), several participants (<5,000 and >5,000). One-study-removed sensitivity analyses were performed to determine the relative impact of each study on the overall risk estimate.

# Results

## Eligible studies and individual characteristics

Ten articles were included in this analysis (**Figure 1**) (9, 24–32). The selected studies involved 76,676 participants. Among the 10 studies, 7 studies (9, 24–29) reported the odds rate (OR) of NAFLD between low GS group and normal group, 4 studies (26, 30–32) reported the OR of low GS between NAFLD group and normal group and 1 studies (26) involved above the two types of OR. The characteristics of the studies included in our meta-analysis are listed in **Table 1**.

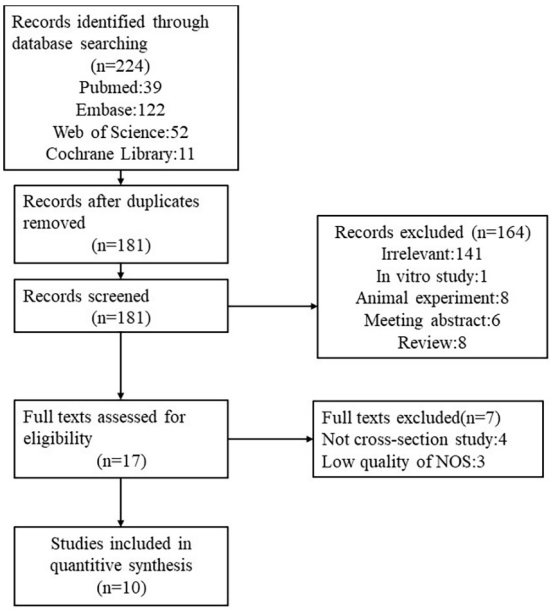


FIGURE 1
Flow chart of literature search for studies investigating the association between grip strength and NAFLD.

TABLE 1 Baseline characteristics of studies included meta-analysis.

| Study | Country | Design | Participants | Mean age | OR | Level of quality |
|---|---|---|---|---|---|---|
| Meng et al. (28) | China | Cross-section | 20,957 | 41.2 | NAFLD | 8 |
| Lee et al. (25) | Korea | Cross-section | 538 | 74.3 | NAFLD | 7 |
| Lee (26) | Korea | Cross-section | 8,001 | 49.9 | NAFLD, LGS | 7 |
| Kim et al. (30) | Korea | Cross-section | 4,103 | 60 | LGS | 7 |
| Gan et al. (24) | China | Cross-section | 3,536 | 53.4 | NAFLD | 8 |
| Hao et al. (32) | China | Cross-section | 1,126 | 36.5 | LGS | 5 |
| Park et al. (29) | Korea | Cross-section | 3,922 | 45.9 | NAFLD | 8 |
| Lee (31) | Korea | Cross-section | 1,690 | 14 | LGS | 6 |
| Cho et al. (9) | Korea | Cross-section | 5,272 | 57 | NAFLD | 6 |
| Lee et al. (27) | Korea | Cross-section | 27,531 | 47 | NAFLD | 7 |

## Quality of the individual studies

The quality level of each study ranged from 5 to 8 stars (Figure 1). The funnel plot (Figures 2, 3) provided a qualitative estimation of publication bias.

## Odds rate of non-alcoholic fatty liver disease between low grip strength group and normal group

In the seven studies of the OR of NAFLD included in this meta-analysis (Table 1), the sample size varied from 538 to 27,531 participants, and the age varied from 18 to 80 years old. As shown in Figure 4, high heterogeneity was present among the seven studies reporting OR ($I^2$=98%), so we chose the random-effects model. Meta-analysis of these studies showed that low GS patients had odds of NAFLD that were 3.32 times as high as normal GS (summary OR = 3.32, 95% CI: 1.91–5.75, Figure 4). The result of Funnel plot analysis is showed in Figure 2, and the result of Begg's test ($P$ = 1) and Egger's test ($P$ = 0.785) suggest that there is no significant publication bias.

Because of the high heterogeneity, we conducted a series of subgroup analyses to identity the heterogeneity source. The subgroup analysis by several participants revealed no significant difference between numbers (Figure 5), the OR = 2.5, 95% CI: 0.99–6.31 for studies conducted in the number of participants less than 5,000, and OR = 3.96, 95% CI: 2.39–6.55 for studies conducted in several participants more than 5,000. There was a significant association between low GS and risk of NAFLD detected in the studies using RGS (Figure 6, OR = 5.11, 95% CI: 4.45–5.86) compared to the using GS (OR = 1.63, 95% CI: 1.46–1.83). Besides, a significantly greater effect size was observed in the studies using HSI (Figure 7, OR = 4.58, 95% CI: 3.44–6.09) than in the ones applying ultrasonography (OR = 1.64, 95% CI: 1.44–1.88). And the subgroup analysis by region revealed stronger association between GS and risk of NAFLD in the studies in Korea (Figure 8, OR = 4.58, 95% CI: 3.44–6.09) than the studies in China (OR = 1.64, 95% CI: 1.44–1.88). Regarding mean age, compared to the studies with mean age more than 60 years old (Figure 9, OR = 1.59, 95% CI: 1.4–1.8), the studies with mean age of fewer than 60 years old (OR = 4.29, 95% CI: 2.82–6.51) were more strongly associated with the risk of NAFLD. Because of the limited number of original articles, the data are only from China and Korea, therefore, we speculate that the high heterogeneity may be due to the regional distribution of the data and the small number of included articles. And all the subgroup analyses are presented in Table 2.

## Odds rate of low grip strength between non-alcoholic fatty liver disease group and normal group

In the four studies of the OR of low GS included in our meta-analysis (Table 1), the sample size varied from 1,126 to 8,001 participants, and the age varied from 10 to 80 years old. As shown in Figure 10, moderate heterogeneity was present among the four studies reporting OR ($I^2$ = 65%). Meta-analysis of these studies showed that patients with NAFLD had odds of low GS that were 3.31 times as high as normal group (summary OR = 3.31, 95% CI: 2.45–4.47, Figure 10). The result of the funnel plot is presented in Figure 3, and the result of Begg's test ($P$ = 0.734) and Egger's test ($P$ = 0.630) suggest that there is no significant publication bias.

Because of the moderate heterogeneity, we conduct a subgroup and meta-regression analyses to identify the heterogeneous source. These four studies, all the shown that NAFLD patients have markedly low GS than the non-NAFLD groups. Because meta-regression was performed to examine possible heterogeneous factors for quantitative variables, we used age as a covariate for meta-regression, but the result ($P$ > 0.05) showed that age may not be the cause of high heterogeneity. Additionally, we conducted a subgroup analysis according to the method of GS ascertainment (GS and RGS), there were significant association between NAFLD and low GS was detected in the studies using RGS (Figure 11,

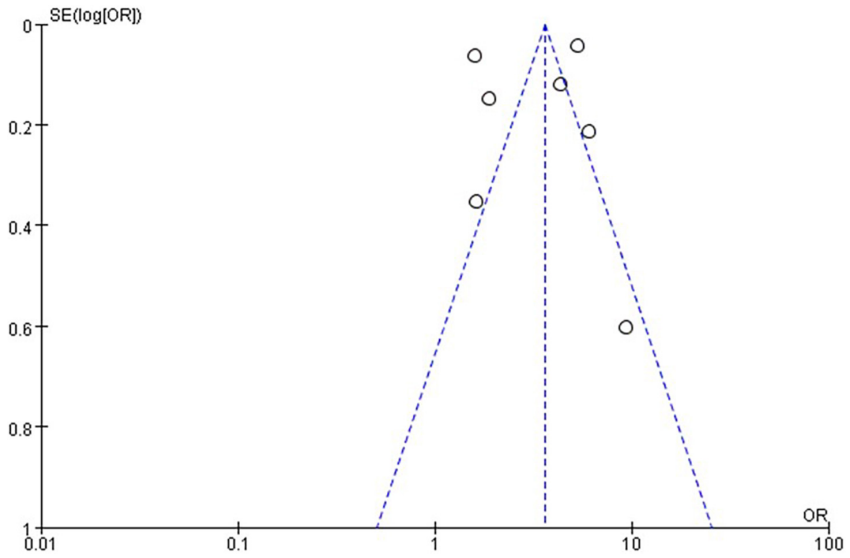


FIGURE 2
Funnel plot with grip strength and risk of NAFLD.

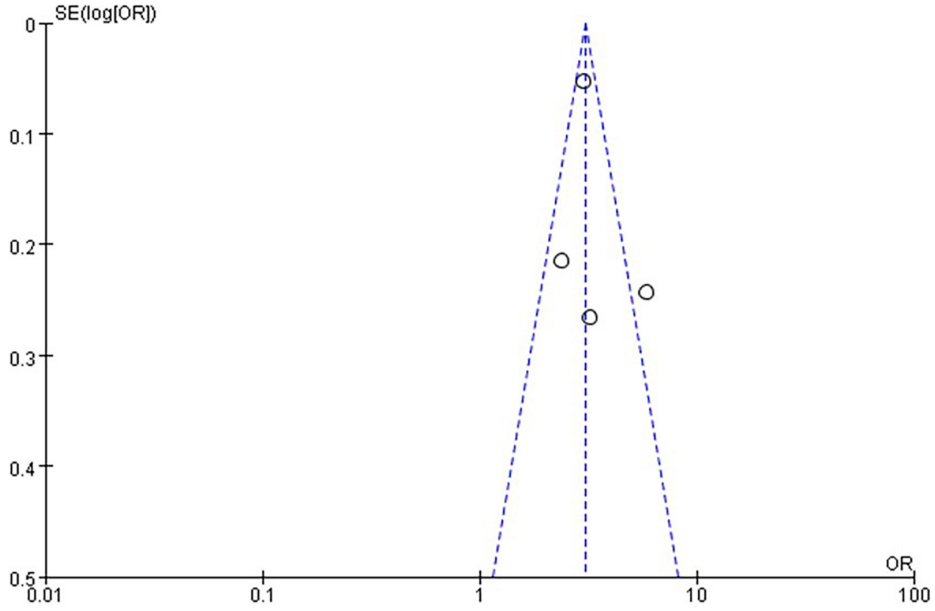


FIGURE 3
Funnel plot with NAFLD and risk of low grip strength.

OR = 4.02, 95% CI: 2.11–7.65) compared to the studies using GS (OR = 2.69, 95% CI: 1.94–3.73).

## Sensitivity analysis

In the two analyses, in one-study-removed sensitivity analyses, we excluded each study and results did not change (**Figures 12**, **13**).

# Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis that summarized available studies regarding the association between GS and NAFLD. In this meta-analysis which included 10 studies with a total of 76,676 participants, we performed two types of meta-analysis, and explored the OR of NAFLD in patients with low GS and the OR of low GS among patients with NAFLD, both results suggest a

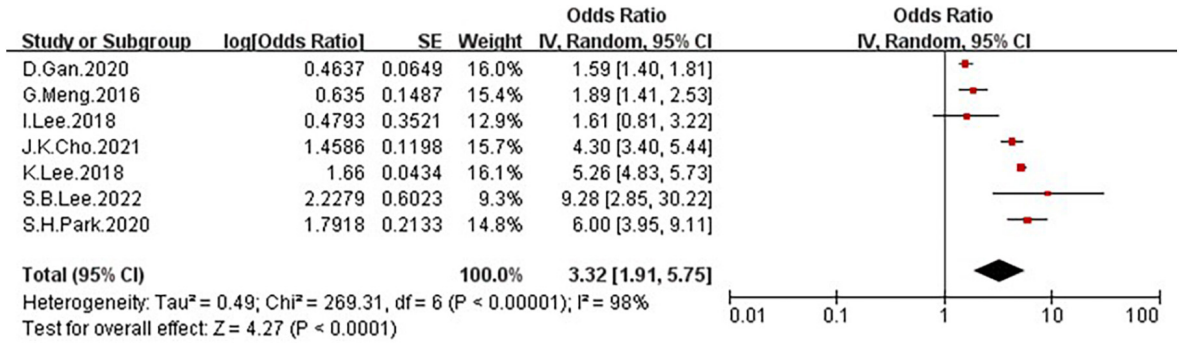


FIGURE 4
Forest plots showing the relation between low grip strength and risk of NAFLD.

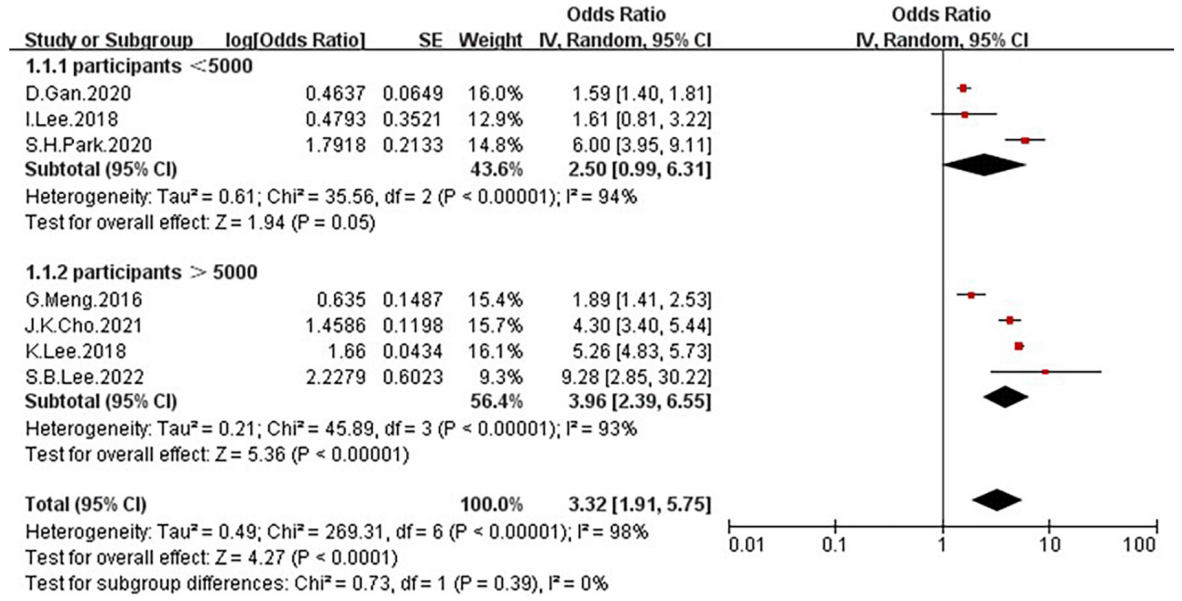


FIGURE 5
Forest plots depicting the association of low grip strength and the risk of NAFLD were subgrouped by several participants.

significant association between the NAFLD and GS: the Figure 4 shown that a significantly increased risk of NAFLD among individuals with low GS with the pooled OR of 3.32 (95% CI: 1.91–5.75). And Figure 10 suggested that NAFLD patients had odds of low GS that were 3.31 times as high as normal people (summary OR = 3.31, 95% CI: 2.45–4.47).

Non-alcoholic fatty liver disease encompasses a wide range of diseases from simple steatosis, non-alcoholic steatohepatitis, fibrosis, and even cirrhosis (33). Skeletal muscle is an insulin-responsive and important endocrine organ, because it secretes myokines that influence metabolic processes in liver and muscle (34). Previously, many reliable studies have found that association between skeletal muscle and NAFLD, Guo et al. (35) reported that skeletal muscle index (SMI) is independently associated with the severity of hepatic steatosis and liver fibrosis of related to NAFLD, and they assessed the association of SMI tertiles with NAFLD and liver fibrosis, individuals with low muscle mass were significantly correlated with NAFLD and liver fibrosis. These findings suggest that NAFLD is affected by skeletal muscle even when people do not have sarcopenia. GS is also an important indicator in the assessment of skeletal muscle and sarcopenia. Previously, several studies have shown a link between sarcopenia and NAFLD, mainly due to a common pathological mechanism, insulin resistance and chronic inflammation have been the most frequently proposed mechanisms, and both are hypothetically plausible (14, 36). Firstly, both the liver and muscle are the target organs for insulin action, and insulin resistance is known as a key factor in the pathophysiology of both NAFLD and sarcopenia (37). With aging, the fat mass in muscle cells increases, which

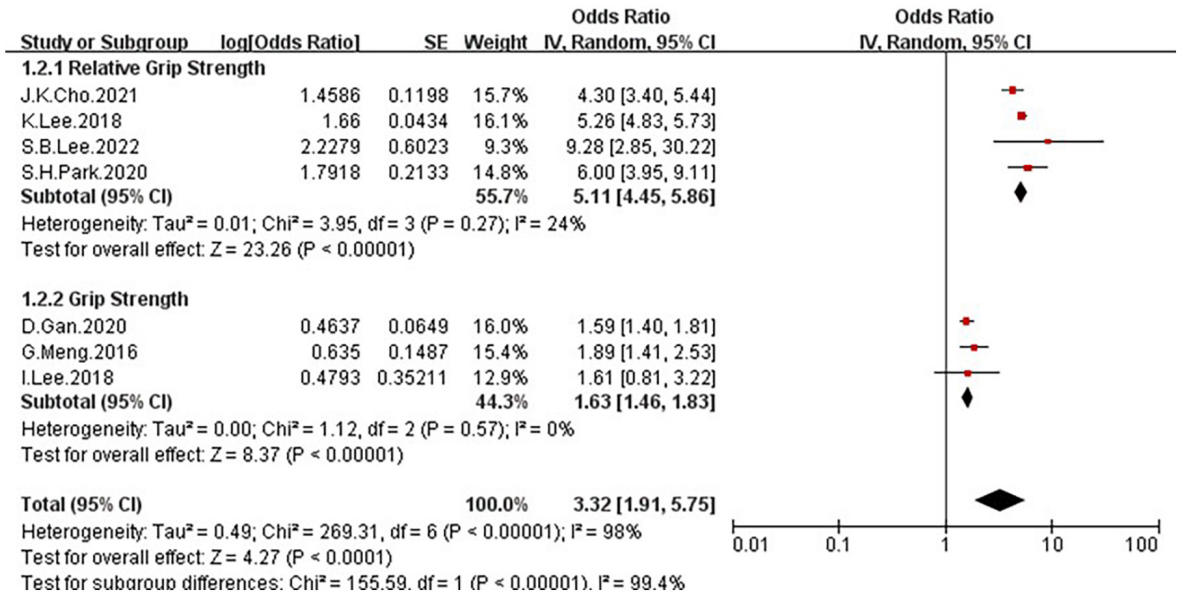


FIGURE 6
Forest plots depicting the association of low grip strength and the risk of NAFLD were subgrouped by the method of grip strength ascertainment.

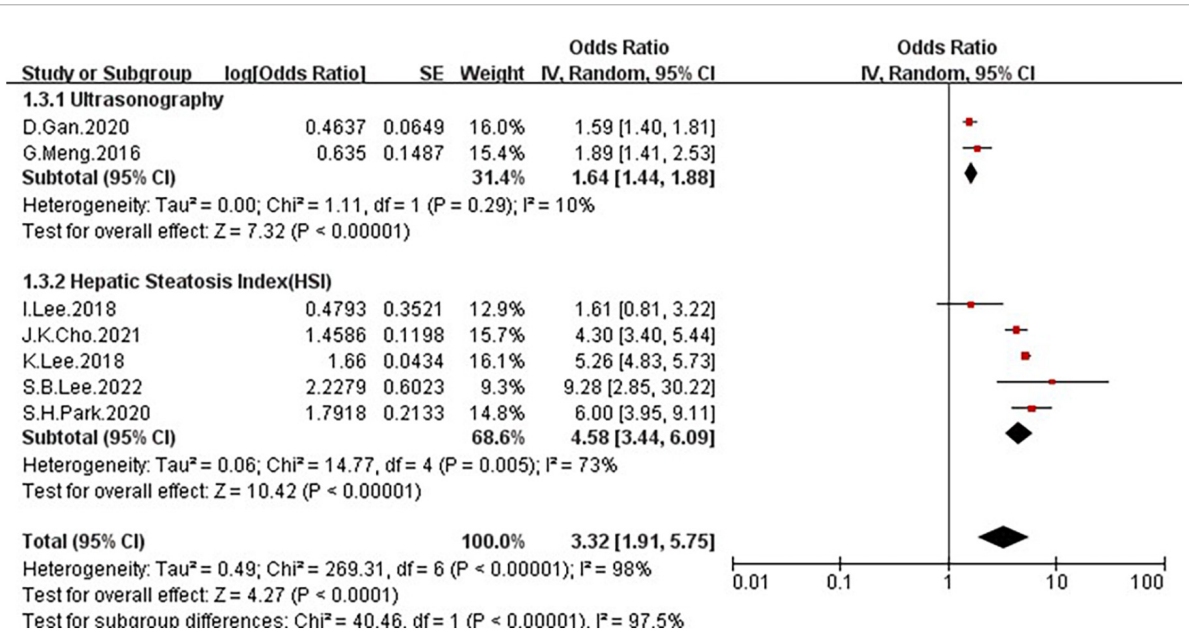


FIGURE 7
Forest plots depicting the association of low grip strength and the risk of NAFLD were subgrouped by the diagnosis of NAFLD.

becomes a risk factor for insulin resistance (38). Furthermore, ectopic fat accumulation in the liver is closely associated with systemic insulin resistance (37). Hong (39) reported that an increased insulin resistance index in subjects with sarcopenia compared to those without sarcopenia, and insulin resistance and SMI showed a significant negative correlation, and they also found a significant relationship between insulin resistance and liver attenuation index (LAI), which reflects fat accumulation in the liver.

On the other hand, chronic inflammation is the other hypothesis most often cited. There are several studies focus on the mediators that link the muscle-liver-adipose tissue axis (40). For example, myostatin, a transforming growth factor (TGF)-β superfamily member, is a regulator of skeletal

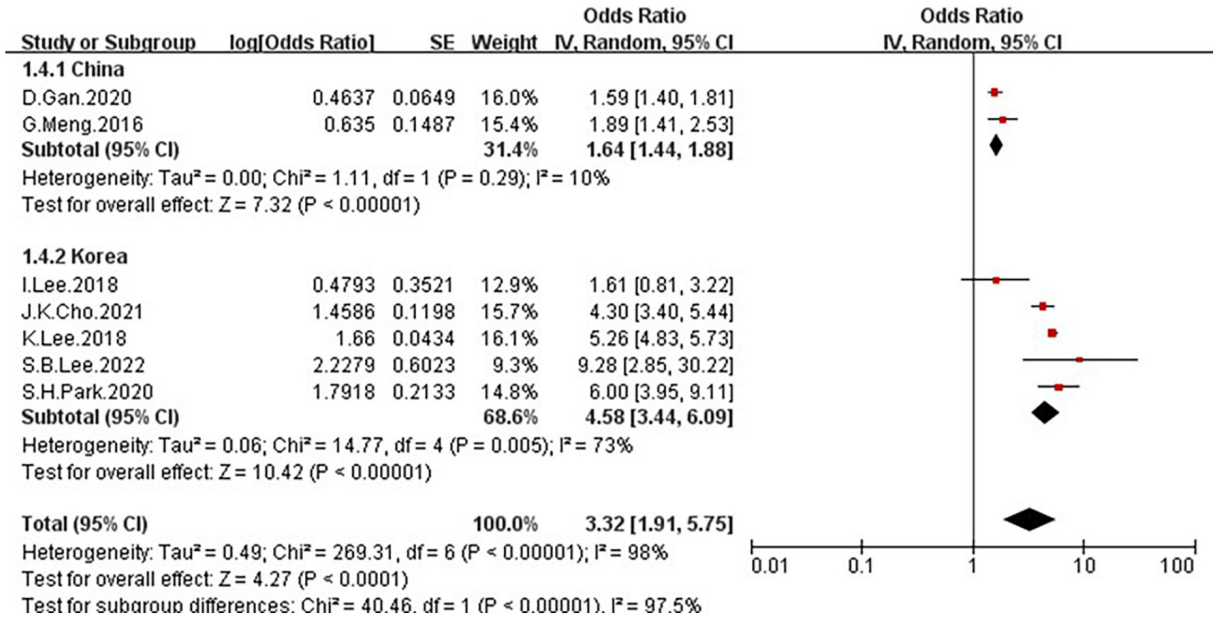


FIGURE 8
Forest plots depicting the association of low grip strength and the risk of NAFLD were subgrouped by the region.

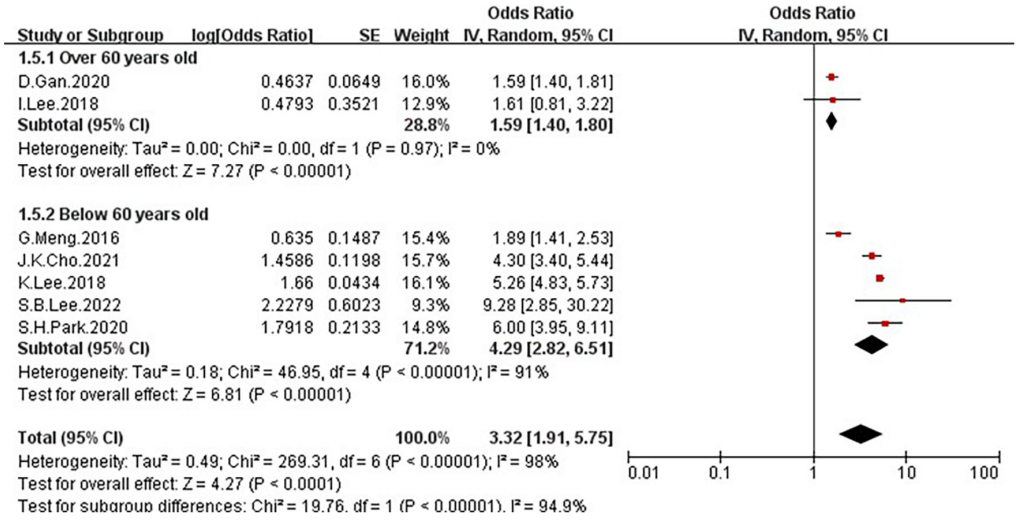


FIGURE 9
Forest plots depicting the association of low grip strength and the risk of NAFLD were subgrouped by the mean age.

muscle mass, and now some animal studies have shown that myostatin has significant hepatic effects by regulating skeletal muscle metabolism, and blocking myostatin not only increases muscle mass but also protects mice from fatty liver and improves insulin resistance (41). It has been demonstrated that oxidative stress and proinflammatory cytokines of chronic inflammation, such as tumor necrosis factor (TNF)-α and Interleukin (IL)-6 can promote fat and muscle metabolism, leading to loss of skeletal muscle (42), various inflammatory factors released from visceral adipocytes can also promote the development of metabolic syndrome (43), and myonectin and irisin have been suggested to contribute to the development of insulin resistance and fatty liver (44, 45), and Hong (39) also found that high-sensitivity C-reactive protein (hsCRP) concentrations were closely correlated with SMI and LAI, which suggests that inflammation may be an important underlying factor associated with both sarcopenia and NAFLD.

TABLE 2 Subgroup analysis of low grip strength and the risk of NAFLD.

| Subgroup title | Number of studies | Summary OR (95% CI) | *P*-for-difference | *P*-for-heterogeneity | $I^2$ (%) |
|---|---|---|---|---|---|
| Overall | 7 | 3.32 (1.91–5.75) | <0.001 | <0.001 | 98 |
| **Region** | | | | | |
| China | 2 | 1.64 (1.44–1.88) | <0.001 | 0.29 | 10 |
| Korea | 5 | 4.58 (3.44–6.09) | | <0.001 | 73 |
| **Number of participants** | | | | | |
| <5,000 | 3 | 2.5 (0.99–6.31) | 0.39 | <0.001 | 94 |
| >5,000 | 4 | 3.96 (2.39–6.55) | | <0.001 | 93 |
| **Mean age** | | | | | |
| <60 | 5 | 4.29 (2.82–6.51) | <0.001 | <0.001 | 91 |
| >60 | 2 | 1.59 (1.4–1.8) | | 0.97 | 0 |
| **Method of GS ascertainment** | | | | | |
| Grip strength | 3 | 1.63 (1.46–1.83) | <0.001 | <0.001 | 0 |
| Relative grip strength | 4 | 5.11 (4.45–5.86) | | 0.27 | 24 |
| **Diagnosis of NAFLD** | | | | | |
| Ultrasonography | 2 | 1.64 (1.44–1.88) | <0.001 | 0.29 | 10 |
| Hepatic steatosis index (HSI) | 5 | 4.58 (3.44–6.09) | | <0.001 | 73 |

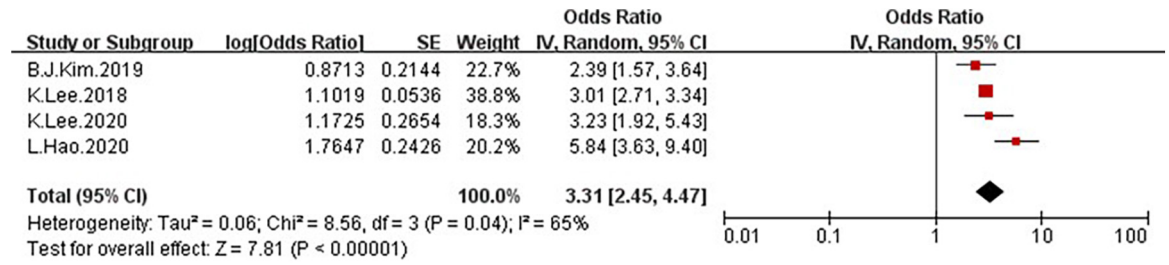


FIGURE 10
Forest plots showing the relation between NAFLD and risk of low grip strength.

Recently, low vitamin D levels have been suggested to be associated with NAFLD and muscle strength. Vitamin D plays an important role in muscle mass and muscle strength. A systematic review revealed that vitamin D supplementation significantly increased muscle strength (46). A separate study also showed that muscle nuclear vitamin D receptor (VDR) was increased by 30% and augmented muscle fiber size by 10% in elderly females taking vitamin D (47). The involvement of vitamin D in mediating several immune-inflammatory (48) and metabolic processes (49) has been demonstrated previously. Roth et al. (50) reported that vitamin D deficiency exacerbates NAFLD through Toll-like receptors (TLR)-activation in a westernized diet rat model, which causes insulin resistance, higher hepatic resistance gene expression, and up-regulation of hepatic inflammatory and oxidative stress. In humans, hepatic VDR expression is inversely correlated with steatosis severity (51). A recent study (52) have shown that liver VDR expression plays an important role in regulating intra-hepatic lipid accumulation.

In addition, gut microbiota is also an important component in the pathological mechanism. The gut microbiota composition is generally shaped in early childhood (53), and by the age of three years old (54), the gut microbiota reaches its mature composition, which is maintained relatively stable over the lifespan, and after age of 65, gut microbiota resilience is generally reduced. In current studies, there is evidence supporting the concept that the gut microbiota composition is moderated by exercise (55), including the animal models (56) and human studies (57). Currently, the most studied putative mediators of the effect of gut microbiota on skeletal muscle function are short-chain fatty acids (SCFA) (58), and the SCFA produced by gut microbiota can enter systemic circulation and be absorbed by skeletal muscle cells, where they act as ligands for free fatty acids receptors 2 and 3 (59), and these receptors have a key role in moderating glucose uptake and metabolism, and in promoting insulin sensitivity (60). In addition, gut microbiota also plays a role in NAFLD. As we all know, increased dietary fat intake, is associated with the development of NAFLD (61), and

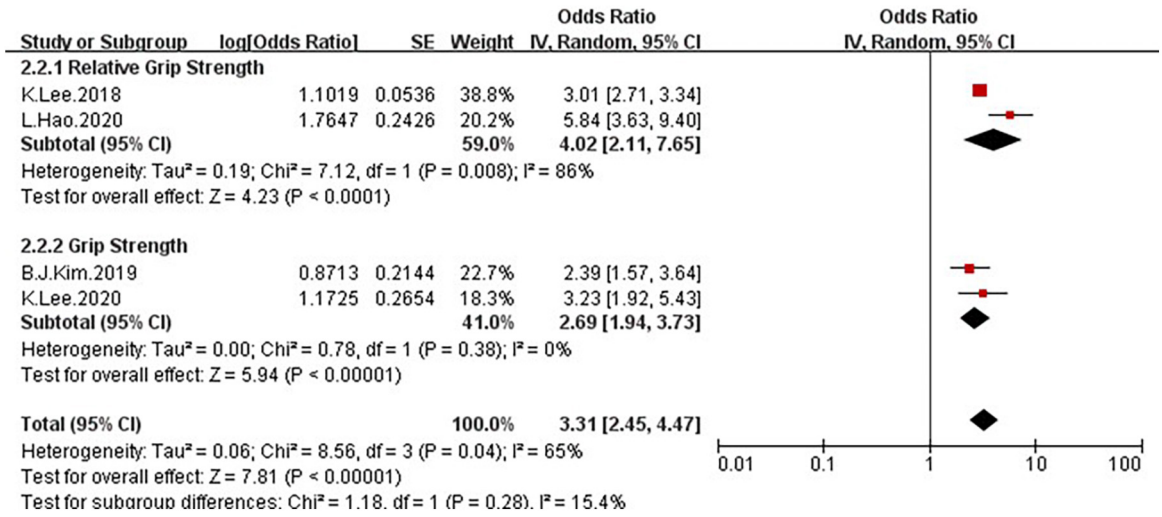


FIGURE 11
Forest plots depicting the association of NAFLD and the risk of low grip strength were subgrouped by the method of grip strength ascertainment.

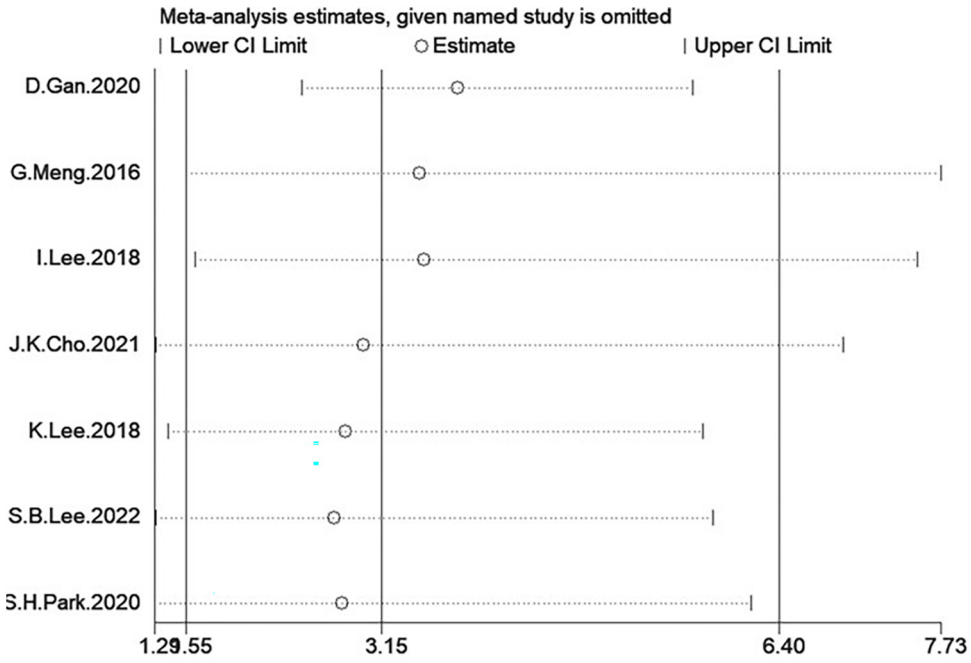


FIGURE 12
Sensitivity analysis of low grip strength group.

the high fat diet can alter the gut microbiota, and favoring gut bacteria associated with the development of NAFLD (62).

This study also has several limitations. Firstly, one analysis has moderate heterogeneity, and another analysis has high heterogeneity, which maybe because of the small number of included studies, the restricted regional distribution of the studies, and age differences in each study. Secondly, in the subgroup analyses, some subgroups only have 2 or 3 studies, which may affect the results. Besides, subgroup analyses are observational by nature and may be subject to confounding by study-level characteristics. Finally, the definition of NAFLD is different in included studies, some studies use ultrasound to examine the NAFLD, and some studies use HSI to diagnose NAFLD, which may affect the study findings.

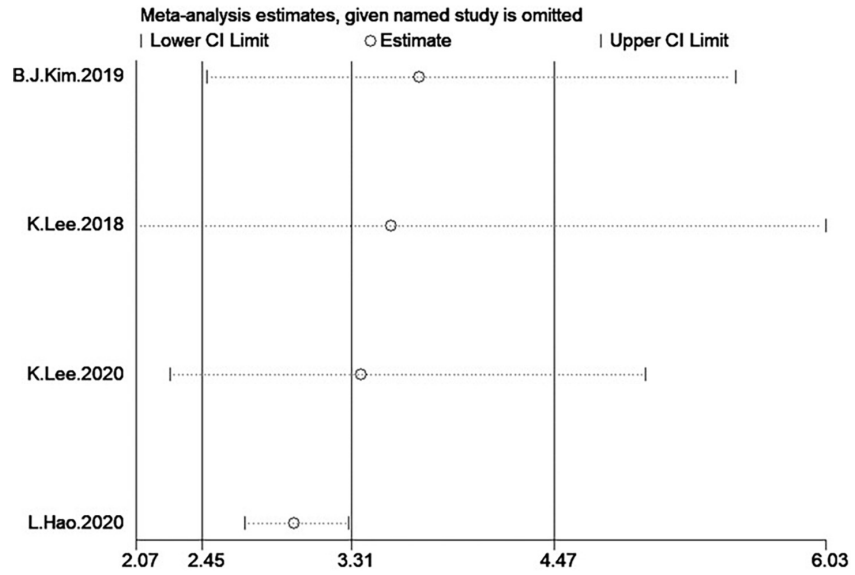


FIGURE 13
Sensitivity analysis of NAFLD group.

Therefore, GS, as an important parameter of sarcopenia and muscle strength, is associated with NAFLD, not only in pathological mechanisms, such as insulin resistance, chronic inflammation, gut microbiota, and regulation of vitamin D, but also in terms of clinical data that people with low GS have a higher risk of NAFLD, and patient with NAFLD have lower GS than normal people.

## Conclusion

In conclusion, there is an association between NAFLD and GS. Compare with the normal group, people with low GS are more likely to develop NAFLD, in addition, GS levels in NAFLD patients are also generally lower than the normal population.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

## Author contributions

LH: study idea, concept and design, data extraction and interpretation of data, drafting of the manuscript, and review of the final manuscript. SF: data extraction and analysis of data, drafting of the manuscript, and review of the final manuscript. JL: drafting of the manuscript, data analysis, and review of the final manuscript. DL: drafting of the manuscript and review of the final manuscript. YT: study idea, concept and design, drafting of the manuscript, and review of the final manuscript. All authors contributed to the article and approved the submitted version.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.988566/full#supplementary-material

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# Cholecystectomy promotes the development of colorectal cancer by the alternation of bile acid metabolism and the gut microbiota

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The incidence and mortality of colorectal cancer (CRC) have been markedly increasing worldwide, causing a tremendous burden to the healthcare system. Therefore, it is crucial to investigate the risk factors and pathogenesis of CRC. Cholecystectomy is a gold standard procedure for treating symptomatic cholelithiasis and gallstone diseases. The rhythm of bile acids entering the intestine is altered after cholecystectomy, which leads to metabolic disorders. Nonetheless, emerging evidence suggests that cholecystectomy might be associated with the development of CRC. It has been reported that alterations in bile acid metabolism and gut microbiota are the two main reasons. However, the potential mechanisms still need to be elucidated. In this review, we mainly discussed how bile acid metabolism, gut microbiota, and the interaction between the two factors influence the development of CRC. Subsequently, we summarized the underlying mechanisms of the alterations in bile acid metabolism after cholecystectomy including cellular level, molecular level, and signaling pathways. The potential mechanisms of the alterations on gut microbiota contain an imbalance of bile acid metabolism, cellular immune abnormality, acid-base imbalance, activation of cancer-related pathways, and induction of toxin, inflammation, and oxidative stress.

KEYWORDS

**colorectal cancer, cholecystectomy, bile acid metabolism, gut microbiota, development**

## Introduction

Colorectal cancer (CRC) is the third most malignancy worldwide for humans (1–3). The incidence and mortality of CRC are terrifyingly high. CRC accounts for over 9% of all cancers incidence (4). It has been estimated that approximately 53,200 deaths projected in 2020 (5) and 3.2 million new CRC cases projected in 2040 (6). CRC exerts a significant geographic difference, more common in the western developed countries (7–9). The incidence rate was 10-fold higher in the highest rate countries than that in the lowest rate countries (10). The incidence of CRC in China is 23.3 per 100,000 (11). In addition, the prevalence of CRC has been growing in the young individuals (12–14), contributing to substantial social and economic burden to the healthcare system (2, 6). Therefore, it is essential to explore the risk factors and pathogenesis of CRC.

Several etiologies have been implicated in the pathogenesis of CRC, including genetic susceptibility and environmental factors, such as consumption of tobacco and alcohol, inflammatory bowel disease (IBD), adenomatous polyps, family history, unhealthy diet, physical inactivity and obesity (8, 15–17). Cholecystectomy is a standard procedure for treatment of symptomatic cholelithiasis and gallstone diseases. The number of this procedure has been increasing. It has been reported that approximately 800,000 cases of cholecystectomy are performed in the United States per year, and the number is also growing in China (18, 19). In the past, cholecystectomy was deemed to be almost harmless. Nevertheless, an increasing body of evidence suggests that cholecystectomy might be associated with the development of CRC (20–25). Alterations of bile acid metabolism and gut microbiota have been demonstrated to play significant roles in CRC. However, the potential specific mechanisms are still unclear. Therefore, in the present study, we described the current knowledge on the association between cholecystectomy and CRC, and summarized the potential mechanisms.

## Epidemiology of colorectal cancer

CRC is the third most common cause of cancer-related mortality worldwide (2), and is also the second most common cause of cancer mortality in the United States (26). It has estimated that more than 1.8 million cases were diagnosed and 881,000 deaths occurred in 2018, accounting for 1 in 10 cancer cases and deaths (11). More recently, over 1.9 million new cases were reported in 2020 (27). Globally, CRC incidence and mortality vary widely across countries, according to GLOBOCAN 2020 data (28). The incidence of CRC is higher in males than in females, and the trend is younger in recent years (12–14). In general, the incidence of proximal colon tumors is the highest, while that of distal colon tumors is the lowest, which is more common in the elderly. The incidence rates increased by 1 and 2% each year among the 50–64 age group and under 50 years of age, respectively (26). The population of CRC patients as a whole is rapidly getting younger as the declining incidence in the older population coincides with the increasing incidence in the younger population, causing huge burden on the healthcare system. It has been well-acknowledged that CRC is associated with several risk factors such as smoking, unhealthy diet, alcohol abuse, physical inactivity and obesity (15, 16, 29). Nevertheless, the risk factors and pathogenesis of CRC still need to be further explored.

## Cholecystectomy

Cholecystectomy is the most common procedure performed in biliary surgery. In most cases, the procedure is relatively standardized and the long-term results after surgery are satisfactory. Cholecystectomy can be performed in two main ways: a laparoscopic or a classic open operation technique. Compared to the classic way, the laparoscopic cholecystectomy is a relatively minimally invasive surgical procedure and has basically replaced the open technique for cholecystectomies since the early 1990s (30). Several advantages have made it a popular procedure over the past few decades, including a short hospital stay, quick return to normal activities, and reduced pain after surgery, more acceptable cosmetic results, less morbidity and less mortality (31–33). The reported short-term complications include postoperative bleeding, biliary leakage biliary peritonitis, subhepatic effusion or subphrenic abscess, postoperative jaundice, postoperative pancreatitis, residual common bile duct stones, and gastrointestinal fistula, and the long-term complications include bile duct stricture, recurrent common bile duct stones, biliary bleeding, post-cholecystectomy syndrome, residual overgrown bile duct syndrome, and increased incidence of CRC.

## Clinical data and characteristics of colorectal cancer patients after cholecystectomy

A meta-analysis of 10 cohort studies described that there was an increased risk for colon cancer up to 30% higher than the non-cholecystectomized group (24). In addition, the study also observed a positive relationship between the female gender and CRC. Moreover, a previous study confirmed that patients who performed cholecystectomy presented a 108% higher risk of developing CRC than the common population. The male and female patients who underwent cholecystectomy were reported to have a 74 and 154% higher risk of CRC, respectively (23). Furthermore, age was also regarded as a risk factor for gastrointestinal cancers in patients with a history of

cholecystectomy (23). The standardized incidence ratio (SIR) was highest in individuals between 40 and 49 years old, followed by those in their ages more than 80 years old (23). The reported median duration from cholecystectomy to the diagnosis of CRC was about 5–15 years or more (34, 35). Additionally, the right colon is more prone to be affected by cholecystectomy. Giovaimucci et al. (34) believed that the proximal and distal colon is related to the different sources of embryos, and they present different sensitivity to carcinogens. The right colon is more sensitive to bile acids, probably due to the high amount of stool fluid in the right colon. Thomas et al. (36) believed that the concentration of secondary bile acids and the activity of 7α-dehydroxylase were higher in the right colon than in the left colon, and there were obvious differences in bile acid metabolism, leading to the susceptibility of CRC in the right colon after cholecystectomy.

# Cholecystectomy promotes the development of colorectal cancer by alternation of bile acid metabolism

## Synthesis, transport and metabolism of bile acids

Bile acids are the main components of bile and are synthesized in the hepatocytes via cytochrome P450-mediated oxidation of cholesterol (37, 38). This process takes place through two biosynthetic pathways: the "classical" and an "alternative" pathway (39). During the "classical" pathway, three cholesterol hydroxylase enzymes cholesterol 7α-hydroxylase (CYP7A1), sterol 12α-hydroxylase (CYP8B1) and mitochondrial sterol 27-hydroxylase (CYP27A1) produce the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) (40, 41). The "alternative" pathway produces CDCA via the hydroxylation of the cholesterol side chain by CYP27A1, and the oxysterol intermediates are then formed by 7α-hydroxylation by CYP7B141 (40). Bile acids can be divided into free bile acids and conjugated bile acids according to their structures. Free bile acids include CA, deoxycholic acid (DCA), CDCA, and lithocholic acid (LCA). The free bile acids are combined with glycine or taurine respectively to form various corresponding conjugated bile acids, including glycocholic acid, taurocholic acid, glycochenodeoxycholic acid and taurochenodeoxycholic acid. Conjugated bile acids are more water-soluble and generally exist in the body as sodium salts, which are more stable than free bile acids. In addition, bile acids can be divided into primary and secondary bile acids according to their sources. Primary bile acids are synthesized directly from cholesterol in hepatocytes including CA and CDCA, while secondary bile acids are formed when primary bile acids are secreted into the intestine and undergo 7-α-hydroxylation by intestinal bacteria including DCA, LCA, ursodeoxycholic acid (UDCA), and tauroursodeoxycholic acid (TUDC). Bile acids are secreted through the tubular membrane into the bile and stored in the gallbladder. After the animal eats, the duodenum secretes cholecystokinin, which stimulates gallbladder contraction, thereby releasing bile acids into the small intestine. In the small intestine, conjugated bile acids specifically activate pancreatic lipases and enhance fat-soluble vitamins solubilization by creating mixed micelles of dietary lipids, sterols, and fat-soluble vitamins. Finally, about 95% bile acids are reabsorbed in the ileum and returned to the liver via the portal vein. However, approximately 5% bile acids escaping from intestinal reabsorption enter the colon, where they are further converted to secondary, more hydrophilic bile acids by the intestinal flora (40, 42, 43).

## Cholecystectomy changes metabolism of bile acids

Under normal conditions, the gallbladder controls the rate and flow of bile into the intestine and enterohepatic circulation of bile acids, which plays a key role in regulating physiological homeostasis (44). However, the rhythm of bile acids entering the intestine is altered after cholecystectomy, which leads to metabolic disorders. The normal bile acid pool is the total amount of bile acids in the enterohepatic circulation, which is about 3 g and consists of 50% CA, 30% CDCA, 20% DCA and very small amounts of other bile acids. Previous studies showed that the bile acid reabsorption and enterohepatic circulation increase due to the sphincter of Oddi disorders after cholecystectomy (45, 46). However, there is also small number of studies finding that the bile acid pool decreases or remains unchanged after cholecystectomy. For example, a previous study observed that the bile acid pool decreased by about 16% three months after cholecystectomy (47). Two animal experiments showed that the total bile acid pool decreased by about 40% two weeks after cholecystectomy, as well as the reduction of circadian rhythm (48, 49). In addition, the total amount of bile acids remained essentially unchanged after five to eight years of cholecystectomy (46). The above findings suggest that the size of bile acid pool decreases in the short-term outcome after cholecystectomy, but there is no significant effect on the long-term outcome. Moreover, it has been confirmed that cholecystectomy increases the bacterial uncoupling and dehydroxylation of bile acids, thereby leading to the high proportion of secondary bile acids (44). Zhang et al. (48) demonstrated that the contents of DCA, LCA and their binding products with taurine were significantly increased in the ileum of mice after cholecystectomy, together with the increasing of fecal bile acid.

## Carcinogenic effects of secondary bile acids on colorectal cancer

Numerous experimental studies have confirmed the tumorigenic potential of bile acids, particularly the secondary bile acids DCA and lesser extent of the LCA (50–55). It is important to note that the bile acids are usually considered as tumor promoters rather than tumor inducers, because the changed bile acid concentrations depend on exposure to carcinogenic chemicals or genetic susceptibility (56). The carcinogenic effects of secondary bile acids on CRC are summarized in **Table 1**.

DCA has been reported to enhance colonic epithelial and colon cancer cell proliferation and/or invasiveness (57–60), promotes dysplasia (61), and disrupts the cell monolayer integrity of intestinal cancer and precancerous cells, increases the production of pro-inflammatory cytokines (51, 62), promotes cell cycle arrest (63), and activate intestinal stem cells and epithelial regeneration (64). In addition, DCA has been demonstrated to inhibit wound healing in wounded colonic epithelial monolayers by impairing cell migration ability (65). Interestingly, DCA exerts pro-apoptotic and anti-apoptotic effects on colon cells (66). It has been revealed that DCA could promote transition from adenoma to carcinoma and resist apoptosis (67), and also induce epithelial-mesenchymal transition (EMT) process, increase vasculogenic mimicry (VM) formation (68). Furthermore, DCA could help cancer cells to escape immune surveillance (69). Besides, DCA was found to cause a redistribution of cholesterol and decrease the fluidity of the membranes (70). As well, previous studies have confirmed that DCA could be converted into a powerful carcinogen 3-methylcholanthrene (3-MC) (71), and also regulate cell junction and increase intestinal permeability (72). Besides, DCA/LCA could increase drug resistance and induce colon carcinogenesis (73).

With respect to the molecular mechanism, DCA and/or LCA was reported to induce expressions of cyclooxygenase (COX)-2 promoter (74) by transactivation of the epidermal growth factor receptor (EGFR) in HCT116, H508 and SNU-C4 human colon cancer cell lines (17, 75), promote the stable and translocated pronucellin entering the nucleus and stimulate the expression of uPA, urokinase-type plasminogen activator receptor (uPAR) and cyclin D1 in SW480 and LoVo cells (57), activate muscarinic receptor (MR) in H508 human colon cancer cells (76), increase the expression of matrix metalloproteases (MMPs) in H508 cells (77), inhibit the effect of microRNA (miR)-199a-5p and/or promote the expression of CDK2 associated cullin domain 1 (CAC1) in HCT-8 cells (78). These above processes were associated with proliferation and invasion of DCA. For the anti-apoptotic characteristics, DCA was shown to upregulate the expression of X-linked inhibitor of apoptosis protein (XIAP) in normal intestinal epithelial cells (IEC-6), while downregulate the expression of p53 in HCT116 cells (79, 80). It is noted that Hu et al. suggested that DCA and/or LCA presented a dual role in modulating cell survival and death by regulating expression of Nur77 and intracellular location in HCT116 and HT29 colon cancer cells (81). Likewise, DCA was revealed to decrease the expression of human leukocyte antigen (HLA) class I antigens on the surface of HT29, SK-CO-l and SW1116 cells to help cancer cells to escape immune surveillance (69). As well, DCA was also described to prompt colonic epithelial cells HCoEpiC into becoming cancer stem cells (CSCs) (73), and form aberrant crypt foci (ACF) and high-grade dysplasia in AKR/J mice (82). Besides, DCA endorses the recruitment of tumor-associated macrophages (TAM) (83), decrease the levels of secretory antibodies of the type IgA (sIgA) and promotes polarization of M2 macrophages in $APC^{min/+}$ mice (51). Interestingly, DCA has also been reported to cause genomic instability including heteroploidy, intrachromosomal instability and gene point mutations (84). The genomic instability appears via several mechanisms, comprising DNA oxidative damage, mitochondria damage, endoplasmic reticulum damage, micronucleus rate increase, disruption of mitosis, and mutations of chromosome aneuploidy (85, 86). DCA-induced DNA oxidative damage is caused after long-term exposure to high concentrations of nitro DCA and oxidation, which can induce apoptosis or DNA damage. Long-term DNA damage leads to mutation and natural selection of mutant cells, and ultimately promotes the development of cancer cells (87). Moreover, DCA can cause abnormal functions of some DNA mismatch repair enzymes by inducing mutations, such as adenomatous polyposis coli (APC) and tumor protein p53 (TP53). Subsequently, the dysfunction of DNA mismatch repair causes genome microsatellite instability (88). Long-term exposure to a high concentration of secondary bile acids can generate reactive oxygen species (ROS), induce oxidative stress (89), active nitrogen species, and cause DNA damage in intestinal epithelial cells, leading to genomic instability and increase gene mutations (90). In contrast, LCA has been reported to promote CRC via promoting expression of MMP-2 in CaCo-2 cells (91), interleukin (IL)-8 in HCT116 cells (92), ATP binding cassette subfamily B member 1 (ABCB1), ATP binding cassette subfamily G member 2 (ABCG2) in HCoEpiC cells (73), and miR-21, and inhibition of PTEN in HCT116 cells (93). As well, LCA induces DNA single-strand breaks (94) and inhibits mammalian DNA polymerase β in rat colon epithelial cells (95).

Several signaling pathways have been reported to be involved in the tumor-promoting effect of DCA on CRC. For example, DCA promotes CRC by activation of EGFR-mitogen activated protein kinase (MAPK), and induction of calcium in HT-29 cells (96) and signal transduction and transcriptional activator (STAT) 3 signaling pathways in HCT116 and HCA-7 cells (97). DCR facilitates proliferation and invasiveness through COX-2 in HT-29, Caco-2, HCA7, and HCT116 cells (98) and/or COX-2/prostaglandin E2 (PGE2) signaling

TABLE 1 The carcinogenic effects of secondary bile acids on CRC.

| Authors (references) | Published year | Country | Cells/Animals | Types of bile acid | Effects, genes, and/or pathways |
|---|---|---|---|---|---|
| Cheng et al. (17) | 2005 | United States | SNU-C4 and H508 | GDCA, DCA | Bile acids enhances CHRM3-dependent cell proliferation by transactivation of EGFR |
| Pai et al. (57) | 2004 | United States | SW480, LoVo | DCA | DCA promotes cell growth and invasiveness by activation of β-catenin signaling |
| Milovic et al. (59) | 2002 | Germany | Caco-2, HT-29 | DC | DC promotes cell proliferation at low-dose, while induces apoptosis at high dose |
| Fu et al. (60) | 2019 | United States | Murine mice, HCT116, Caco2, HT29 | DCA | DCA promotes cancer stem cell proliferation |
| Sorrentino et al. (64) | 2020 | Switzerland | Murine mice | DCA, LCA | Bile acids activate intestinal stem cells and epithelial regeneration via TGR5 |
| Qiao et al. (66) | 2001 | United States | HCT116 | DCA | DCA presents a dual role in apoptosis via the ERK/MAPK pathway |
| Farhana et al. (73) | 2016 | United States | HCoEpiC | DCA, LCA | Bile acids promote colon stemness in colonic epithelial cells via CHRM3 and Wnt/β-catenin signaling |
| Qiao et al. (80) | 2001 | United States | HCT116 | DCA | DCA downregulates p53 via stimulating the ERK signaling pathway |
| Hu et al. (81) | 2015 | United States | HCT116, HT29 | DCA, LCA | Bile acids promote Nur77-mediated cell proliferation and apoptosis |
| Lechner et al. (89) | 2002 | Germany | HT-29 | DCA | DCA causes oxidative stress and increases TR level |
| Halvorsen et al. (91) | 2000 | Norway | CaCo-2 | LCA | LCA increases cell invasion through promoting MMP-2 secretion |
| Nguyen et al. (92) | 2017 | Korea | HCT116 | LCA | LCA induces expression of IL-8 by activating ERK1/2 MAPK and inhibiting STAT3 |
| Centuori et al. (96) | 2016 | United States | HT-29 | DCA | DCA promotes cell viability via activation of EGFR-MAPK pathway |
| Nagathihalli et al. (97) | 2014 | United States | HCT116, HCA-7 | DCA | DCA regulates cell cycle by activation of EGFR, MAPK and STAT3 signaling |
| Zhu et al. (98) | 2012 | United States | HT-29, Caco-2, HCA7, HCT116 | DCA | DCA promotes proliferation and invasiveness by activation of COX-2 signaling |
| Li et al. (100) | 2003 | Japan | HCT116, DLD-1, SW620 | DCA | DCA upregulates EPHA2 via activation of ERK 1/2 cascade |
| Milovic et al. (101) | 2001 | Germany | Caco-2 | DCA | DCA promotes cell migration via PKC |
| Debruyne et al. (102) | 2002 | Debruyne | HCT-8/E11, SRC transformed PCmsrc cells | DCA, LCA, CDCA | Bile acids stimulate cell invasion and haptotaxis via RhoA/Rho-kinase pathway and signaling cascades (PKC, MAPK, and COX-2, etc.) |
| Lee et al. (103) | 2010 | Korea | HM3 | DCA | DCA upregulates MUC2 transcription via activation of EGFR/PKC/Ras/Raf-1/MEK1/ERK/CREB, PI3K/Akt/IKKB/NF-κB and p38/MSK1/CREB and inactivation of JNK/c-Jun/AP-1 pathway |
| Lee et al. (105) | 2004 | Korea | HT-29 | DCA | DCA induces IL-8 expression and exerts anti-apoptotic effect via activation of NF-κB |
| Song et al. (106) | 2005 | United States | LiM6 | DCA, LCA, CDCA | DCA upregulates MUC2 transcription via MAPK, PKC-dependent activation of AP-1 |
| Baek et al. (107) | 2010 | Korea | HT29 and SW620 | LCA | LCA enhances cell invasiveness by increasing expression of uPAR via activation of ERK1/2 and AP-1 pathway |

CRC, colorectal cancer; GDCA, glycodeoxycholic acid; DCA, deoxycholic acid; DC, deoxycholic; LCA, lithocholic acid; CHRM3, cholinergic receptor muscarinic 3; EGFR, epidermal growth factor receptor; TGR5, G protein-coupled bile acid receptor 1; ERK, extracellular signal regulated kinases; MAPK, mitogen activated protein kinase; TR, thioredoxin reductase; MMP2, matrix metalloproteinase 2; IL, interleukin; STAT, signal transduction and transcriptional activator; COX-2, cyclooxygenase 2; EPHA2, EPH receptor A2; PKC, protein kinase C; CREB, cAMP response element binding protein; PI3K, phosphoInositide-3 kinase; IKKB, Ikappa B; NF-κB, nuclear factor kappa-B; MSK1, mitogen and stress-activated protein kinase 1; AP-1, activated protein-1; JNK, c-jun N-terminal kinase; MUC2, mucin 2, oligomeric mucus/gel-forming; uPAR, urokinase-type plasminogen activator receptor.

pathway in human colonic fibroblasts CCD-18Co cells (99). In addition, DCA and/or LCA has been demonstrated to promote CRC by regulating Wnt/β-catenin signaling in SW480, LoVo, or HCoEpiC cells (57, 73), activation of extracellular signal regulated kinases (ERK) 1/2 cascade in HCT116, DLD-1, and SW620 cells (100), protein kinase C (PKC) in Caco-2 cells (101), RhoA/Rho-kinase pathway in HCT-8/E11 and SRC transformed PCmsrc cells (102), EGFR/PKC/Ras/ERK/cAMP response element binding protein (CREB), phosphoInositide-3 kinase (PI3K)/Akt/IkappaB (IKKB)/nuclear factor kappa-B (NF-κB) and p38/mitogen and stress-activated protein kinase 1 (MSK1)/CREB pathways and inactivates c-jun N-terminal kinase (JNK)/c-Jun/activated protein-1 (AP-1) pathway (103) and p53 pathway (80). Moreover, DCA activates JNK 1/2, and AKT signaling pathways that result in selective resistance to apoptosis, angiogenesis, proliferation and oxidative stress (40, 104). Furthermore, DCA is also reported to activate anti-apoptotic effect of NF-κB and induces IL-8 (105) and to upregulate MUC2 transcription via MAPK, PKC-dependent activation of AP-1 pathway in LiM6 cells (106). Meanwhile, LCA induces expression of uPAR and increases cell invasiveness via activation of ERK1/2 MAPK and AP-1 pathway in HT29 and SW620 cells (107) and inactivation of STAT3 and Src/EGFR pathways in HCT116 cells (92, 108, 109).

# Cholecystectomy promotes the development of colorectal cancer by changing the gut microbiota

## Gut microbiota and colorectal cancer

The intestinal flora is a great deal number and diversity of microbial species, which is the most significant micro-ecosystem in the human body. It has been estimated that approximately more than 500 species of bacteria from 30 genera exist in healthy adult intestines (110). These bacteria are composed of aerobes, facultative anaerobes and anaerobes, and most of them are obligate anaerobes or facultative anaerobes. Among the bacteria, 90% of the intestinal flora is *Bacteroidetes* and *Firmicutes* (111). The intestinal flora is a significant contributor in several physiological activities, such as food residue metabolism, micronutrient synthesis, primary bile acid metabolism, secondary bile acid synthesis, and immune response regulation (112). In addition, these bacteria is able to establish a biological barrier in the gut via space-occupying effect, nutrient competition, and some secreted metabolites (113), which can decrease low-grade inflammatory response in the body and maintain the integrity of the intestinal wall. Motivating the intestine to create an effective immune defense system can modulate the absorption and conversion of sugar and fat in the intestinal tract, subsequently ameliorate glucose tolerance and oxidative stress, and lower blood glucose (114). Therefore, the homeostasis of gut microbiota plays significant roles in maintaining human health (115–118). However, dysbiosis of intestinal flora is involved in a wide range of human diseases. A large of human and animal experiments has confirmed that the dysbiosis of gut microbiota shows cancer-promoting effects on gastrointestinal carcinogenesis, especially CRC (119–126).

## Cholecystectomy and gut microbiota

It has been well-acknowledged that cholecystectomy induces tremendous changes in the composition and function of the gut microbiota. For example, previous studies confirmed that after cholecystectomy, the number of *Bifidobacteria* and *Lactobacillus* was significantly decreased, while the number of *Enterococcus*, *Oscillospira*, *Escherichia coli*, *Bacteroidaceae* and *Bacteroidetes* was significantly increased (127–131). A previous study demonstrated that 1 mmol/L of DCA can effectively inhibit the growth of *Clostridium perfringens*, *Bacteroides fragilis*, *Lactobacillus* and *Bifidobacterium* in the intestinal tract (132). In addition, Cao et al. found that DCA significantly upregulated the populations of opportunistic pathogens, including *Ruminococcus*, *Escherichia-Shigella*, *Desulfovibrio*, and *Dorea*. Moreover, they also confirmed that DCA significantly increased the levels of *Clostridium* and *Escherichia-Shigella*, but markedly decreased the abundance of *Lactobacillus_gasseri* and mostly butyrate-producing bacteria, such as *Clostridium leptum Lachnospiraceae bacterium* and *Eubaterium coprostanoligenes* (83). On the contrary, an animal research showed that the population level of *Bacteroides* was increased in the ceca of rats fed with DCA (133). The main potential mechanisms include imbalance of bile acid metabolism, cellular immune abnormality, acid-base imbalance, and activation of cancer-related pathways and induction of toxin, inflammation and oxidative stress.

## Imbalance of bile acid metabolism

Fibroblast growth factors (FGF) are cellular factors that are synthesized by the terminal epithelial cells of the ileum and are involved in the regulation of bile acid metabolism (134). The FGF19 or FGF15 is transported to the liver through the portal vein system to inhibit bile acid synthesis. A previous study showed that the levels of FGF19 mRNA in the epithelial tissues of the gallbladder were 250 times higher than that in the terminal ileal epithelium (135). After cholecystectomy, the balance of bile acid metabolism is disturbed as the expression of FGF19 decreases and the primary bile acid production increases, altering the bidirectional interaction between bile acid and intestinal flora (128). The continuous drainage of

bile into the intestinal lumen continuously stimulates intestinal motility, which increases peristalsis and shortens the total intestinal transit time. The enterohepatic circulation of bile acid is accelerated and the production of secondary bile acids is increased. The hydrophobic nature of secondary bile acids increases their affinity for the phospholipid bilayer of the intestinal bacterial cell membrane, leading to cell membrane damage and bacterial lysis and death (70).

### Cellular immune abnormality

The mucosal epithelium of the gallbladder could synthesize surfactant protein D (SP-D) (136). The SP-D is excreted into the intestinal lumen with bile and facilitates the synthesis of intestinal T cells (137). Intestinal T cells are involved in the regulation of inflammatory responses in the intestine. After cholecystectomy, the lack of SP-D in the gallbladder drastically reduces the number of intestinal T cells and predisposes the intestinal tract to bacterial infection and dysbiosis (137). Gallbladder surface protein D can also inhibit the growth of *Lactobacilli* in the intestinal tract by directly binding to *Lactobacilli* to induce lysis of *Lactobacilli* (137). Although *Lactobacillus* is beneficial to the human body, its excessive growth can affect the growth of other bacteria in the intestinal tract, thus causing dysbiosis of the intestinal flora.

### Acid-base imbalance

Small intestinal fluid is weakly alkaline, with a pH value of 8.0–9.0. Normal bile is weakly acidic, and its pulsatile secretion helps to create a good intestinal microbiological environment and maintain a stable pH value in the intestine. After cholecystectomy, alkaline bile is continuously secreted, which affects the pH balance in the intestine. The optimal pH values for the growth of *Lactobacilli* and *Bifidobacteria* are 5.5–6.0 and 6.5–7.0, respectively (138). Therefore, the increase in pH in the intestine inhibits the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, leading to dysbiosis.

### Activation of cancer-related pathways and induction of toxin, inflammation and oxidative stress

Activation of Wnt/β-catenin pathway has been implicated in the development and progression of CRC (139–141). E-cadherin is a well-known tumor suppressor, which can exert its function through β-catenin (142). The correlation between gut bacteria and E-cadherin/β-catenin has been reported. For example, *Fusobacterium nucleatum* has been demonstrated to attach E-cadherin on epithelial cells via its toxic factor FadA adhesin and stimulate β-catenin signaling pathway, and subsequently induce the gene expression of Wnt pathway (143). Meanwhile, a recent study also confirmed that, in addition to the effect on alteration of gut microbiota, DCA could downregulate the expression of E-cadherin, and increase nuclear β-catenin expression, as well as initiation of the downstream Wnt signaling molecules (83). Furthermore, *Fusobacterium nucleatum* has been reported to release RNA into the host cell cytoplasm, which could be detected by cytosolic retinoic acid-inducible gene 1 (RIG-1), and then activate NF-κB pathway, ultimately induce the expression of inflammatory genes and oncogenes (144, 145). Besides, FadA has been illustrated to bind to vascular endothelial cadherin (VE-cadherin), causing VE-cadherin to relocate and then increasing the permeability of endothelial cells, which enables *Fusobacterium* and other bacteria species to enter into the blood stream (146). *Peptostreptococcus anaerobius* has been identified as a novel microbial promoter of intestinal inflammation and tumor (147, 148). A recent research found that *Peptostreptococcus anaerobius* could interact with toll-like receptors (TLR)-2 and TLR-4 to motivate the generation of reactive oxidative species (ROS), which can stimulate the biosynthesis of cholesterol, leading to colon cell proliferation and dysplasia in mice (149). Additionally, alternation of bile acid has been revealed to induce the growth of pro-inflammatory bacteria, such as *Mogibacterium* and *Sutterella*, which may cause DNA damage and inflammatory response (150). Chronic inflammation could then indorse the event of IBD-associated dysplasia and development of adenoma-carcinoma sequence (151, 152). It has been revealed that *Bacteroides fragilis* could release bacteroides fragilis toxin (BFT), which activates a pro-carcinogenic multi-step inflammatory cascade through IL-17R, NF-κB and STAT3 pathways in colon epithelial cells (153) and contributes the development of polyp-adenoma-CRC (150). *Escherichia coli*, *Bacteroides fragilis*, *Providencia ewing*, *Micromonospora*, and *Peptostreptococcus anaerobius* have been displayed to induce CRC by production of a genotoxin colibactin that could induce DNA damage (154, 155).

## Outcomes of combined bile acid applications

Although DCA and LCA present tumor-promoting effects, UDCA is a therapeutic bile acid and has been reported to have a chemopreventive effect based *in vitro* and *in vivo* (156–160). Recently, UDCA was demonstrated to reduce the risk for advanced colorectal adenoma (161, 162) and CRC (156, 163). In addition, UDCA could modulate the gut microbiome (162). UDCA can inhibit DCA-induced apoptosis via modulation of EGFR/Raf-1/ERK signaling in HCT116 cells (164). Moreover, co-treatment with low-dose celecoxib and UDCA reveals to decrease cell growth in HT-29 colon tumor cells (165). Besides,

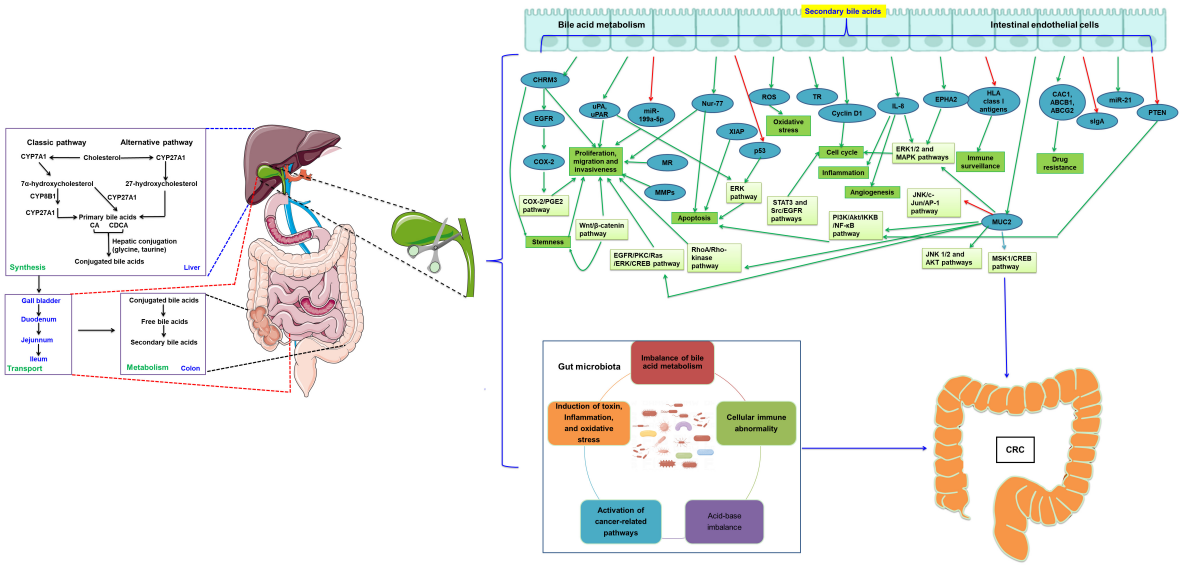


FIGURE 1
Cholecystectomy promotes the development of CRC by the alternation of bile acid metabolism and the gut microbiota. The green arrow indicates the levels are upregulated or the pathway is activated, while the red arrow indicates the levels are downregulated or the pathway is inactivated. CRC, colorectal cancer; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, mitochondrial sterol 27-hydroxylase; CA, cholic acid; CDCA, chenodeoxycholic acid; COX-2, cyclooxygenase 2; EGFR, epidermal growth factor receptor; uPAR, urokinase-type plasminogen activator receptor; MR, muscarinic receptor; MMPs, matrix metalloproteinases; miR, microRNA; MUC2, mucin 2, oligomeric mucus/gel-forming; TR, thioredoxin reductase; IL, interleukin; EPHA2, EPH receptor A2; ABCB1, ATP binding cassette subfamily B member 1; ABCG2, ATP binding cassette subfamily G member 2; HLA, human leukocyte antigen; sIgA, secretory antibodies of the type IgA; XIAP, X-linked inhibitor of apoptosis protein; ROS, reactive oxygen species; PGE2, prostaglandin E2; ERK, extracellular signal regulated kinases; CREB, cAMP response element binding protein; PI3K, phosphoInositide-3 kinase; IKKB, Ikappa B; NF-κB, nuclear factor kappa-B; MAPK, mitogen activated protein kinase; STAT, signal transduction and transcriptional activator; PKC, protein kinase C; MSK1, mitogen and stress-activated protein kinase 1; AP-1, activated protein-1; JNK, c-jun N-terminal kinase.

UDCA inhibits Ras mutations, wild-type Ras activation, and expression of COX-2 in azoxymethane (AOM)-induced colon cancer in rats (166). However, a previous study found that long-term administration of high-dose UDCA was associated with an increased risk of colorectal neoplasia in patients with ulcerative colitis (UC) and primary sclerosing cholangitis (PSC) (167). Currently, there are inadequate data to support the routine application of UDCA for chemoprevention of CRC, either in the common population or among individuals who are at higher risk for CRC.

## Conclusions and prospections

There are many studies on the pathogenesis of CRC. In this paper, we reviewed the recent studies on the effects of cholecystectomy on CRC (Figure 1). The results show that cholecystectomy might promote the development of CRC by alteration of bile acid metabolism and the gut microbiota. The occurrence of CRC is related to changes in bile acid metabolism, the composition and function of the gut microbiota, and/or the interaction between the two factors. General surgeons should strictly grasp the indications of cholecystectomy. Cholecystectomy is necessary for acute and chronic cholecystitis, symptomatic cholelithiasis, biliary tract movement disorders, non-calculous cholecystitis, gallbladder tumors or polyps, and biliary pancreatitis. Gallbladders with good contractile function should be preserved as much as possible, not blindly removed. However, whether other physiological changes after cholecystectomy are associated with intestinal flora, affecting the occurrence and development of CRC, and whether there is a direct correlation between the carcinogenic effect of secondary bile acids and intestinal microorganisms after cholecystectomy are still unclear and need to be further investigated. With the continuous research on the pathogenesis of secondary bile acids-induced CRC, targeted therapies, including targeted bile acid metabolism and intestinal microflora regulation, may be promising treatment strategies for CRC.

## Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

YS conceived the project. XJ and ZJ performed the research. QC and WS collected the background information. XJ, ZJ, QC, WS, and MJ drafted the manuscript. YS revised the manuscript. All authors approved the publication of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Establishment and validation of the survival prediction risk model for appendiceal cancer

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**Objective:** Establishing a risk model of the survival situation of appendix cancer for accurately identifying high-risk patients and developing individualized treatment plans.

**Methods:** A total of 4,691 patients who were diagnosed with primary appendix cancer from 2010 to 2016 were extracted using Surveillance, Epidemiology, and End Results (SEER) * Stat software. The total sample size was divided into 3,283 cases in the modeling set and 1,408 cases in the validation set at a ratio of 7:3. A nomogram model based on independent risk factors that affect the prognosis of appendix cancer was established. Single-factor Cox risk regression, Lasso regression, and multifactor Cox risk regression were used for analyzing the risk factors that affect overall survival (OS) in appendectomy patients. A nomogram model was established based on the independent risk factors that affect appendix cancer prognosis, and the receiver operating characteristic curve (ROC) curve and calibration curve were used for evaluating the model. Survival differences between the high- and low-risk groups were analyzed through Kaplan–Meier survival analysis and the log-rank test. Single-factor Cox risk regression analysis found age, ethnicity, pathological type, pathological stage, surgery, radiotherapy, chemotherapy, number of lymph nodes removed, T stage, N stage, M stage, tumor size, and CEA all to be risk factors for appendiceal OS. At the same time, multifactor Cox risk regression analysis found age, tumor stage, surgery, lymph node removal, T stage, N stage, M stage, and CEA to be independent risk factors for appendiceal OS. A nomogram model was established for the multifactor statistically significant indicators. Further stratified with corresponding probability values based on multifactorial Cox risk regression, Kaplan–Meier survival analysis found the low-risk group of the modeling and validation sets to have a significantly better prognosis than the high-risk group ($p < 0.001$).

**Conclusion:** The established appendix cancer survival model can be used for the prediction of 1-, 3-, and 5-year OS and for the development of personalized treatment options through the identification of high-risk patients.

KEYWORDS

**gastrointestinal surgery direction, appendicular cancer, prognosis, Lasso regression, tumor, treatment**

## Introduction

Appendiceal cancer is a rare malignancy of the digestive tract with an approximate incidence of 0.2–0.5% (1, 2). It has an occult onset, a lack of specificity of early symptoms, and there is difficulty in anatomically locating the right adnexal mass in women (3). At the same time, a lack of understanding of appendix cancer among clinicians often results in misdiagnosis and missed diagnosis, causing great difficulties in terms of clinical treatment and seriously affecting the prognosis of patients. In addition, as a result of the low incidence of appendiceal cancer, there is insufficient clinical attention, and appendix cancer studies relating to prognosis have mainly been based on small sample size, single-center analysis, and a lack of strong evidence, resulting in a certain bias. Therefore, based on the large sample size of the SEER database, this study screens the best variables using Lasso regression, excludes some repeated and unnecessary parameters, and solves the overfitting problem. Finally, the nomogram model is established, and high-risk groups are further stratified by risk factors as a basis for prognosis improvement.

## Materials and methods

### Data collection

The Surveillance, Epidemiology, and End Results (SEER) database is publicly available and covers ∼35% of the US population. Clinical data from SEER *, including patient age, race, sex, pathological type, pathological stage, surgery, radiotherapy, chemotherapy, lymphadenectomy number, insurance status, marital status, T stage, N stage, M stage, tumor size, and CEA from 2010 to 2016 was downloaded using Stat software. A total of 4,691 patients were ultimately included.

### Inclusion criteria

Inclusion criteria were as follows: (1) Pathological diagnosis of primary appendiceal cancer; (2) complete clinicopathological data.

### Exclusion criteria

Exclusion criteria were as follows: (1) Lack of patient follow-up information; (2) unknown or missing general information and data; (3) combined with other malignant or non-primary tumors.

### Statistical treatment

SPSS 25.0 software was used for performing statistical analysis of the data. The count data were expressed as n (%), and a comparison between groups was performed using the $\chi^2$-test. Univariate cox risk regression analysis of risk factors influencing appendiceal cancer overall survival (OS). Based on R3.6.3 the software further screened the best variables through the incorporation of statistically significant single-factor Cox risk regression into Lasso regression and cross-validation and the final selected variables into multifactor Cox risk regression as a means of determining age, pathological stage, surgery, lymph node removal number, T stage, N stage, M stage, and CEA independent risk factors that affect the prognosis of appendix cancer. The multivariate Cox regression index with statistical significance was used for establishing a nomogram model with R software, and the ROC curve and calibration curve were further drawn in order to evaluate model reliability. Finally, probabilistic values were calculated based on multifactorial Cox risk regression, the optimal cut-off that corresponds to the maximum Jordan index of the ROC curve was divided into high-risk and low-risk groups, and the survival differences between the appendix cancer modeling and validation sets were calculated by Kaplan–Meier survival analysis and log-rank assays. $P < 0.05$ was considered to be significant.

### Ethics and consent

The authors were authorized to extract data from the SEER study by the National Cancer Institute. Access to data *via* the SEER database requires no informed patient consent (SEER ID: 13846—Nov2020). This study is a retrospective analysis that is in strict compliance with the Helsinki Declaration of 1964 and subsequent amendments or similar ethical standards.

## Results

### Comparison of the pathological characteristics of patients in the appendiceal cancer modeling and validation sets

A total of 4,691 appendix cancer cases were included in this study, including 3,283 appendix cancer modeling sets and 1,408 appendix cancer validation sets, whereby 2,107 patients were male (44.9%) and 2,584 were female (55.1%). A comparison between the two groups identified significant differences in race, pathological type, and insurance ($p < 0.05$). Age, race, pathological type, pathological stage, surgery, radiotherapy, chemotherapy, number of lymphadenectomies, T stage, N stage,

TABLE 1 Analysis of clinical case data for appendix cancer.

| | Training cohort ($n = 3,283$) | Validation cohort ($n = 1,408$) | $X^2$ | $P$-value |
|---|---|---|---|---|
| **Age** | | | 2.812 | 0.094 |
| <60 | 1,825 (55.6%) | 820 (58.2%) | | |
| ≥60 | 1,458 (44.4%) | 588 (41.8%) | | |
| **Race** | | | | |
| White people | 2,724 (83.0%) | 1,181 (83.9%) | 95.82 | <0.001 |
| Black people | 293 (8.9%) | 204 (14.5%) | | |
| Other | 266 (8.10%) | 23 (1.6%) | | |
| **Sex** | | | 0.445 | 0.505 |
| Man | 1,485 (45.2%) | 622 (44.2%) | | |
| Woman | 1,798 (54.8%) | 786 (55.8%) | | |
| **Pathology type** | | | 10.965 | 0.012 |
| Carcinoid | 720 (21.9%) | 308 (21.9%) | | |
| Cup-shaped cell Carcinoma | 334 (10.2%) | 182 (12.9%) | | |
| Adenocarcinoma | 456 (13.9%) | 214 (15.2%) | | |
| Other | 1,773 (54.0%) | 704 (20.0%) | | |
| **Tumor stage** | | | 0.893 | 0.926 |
| Well-differentiated | 1,222 (37.2%) | 517 (36.7%) | | |
| Moderately differentiated | 896 (27.3%) | 402 (28.6%) | | |
| Poorly differentiated | 467 (14.2%) | 192 (13.6%) | | |
| Undifferentiation | 81 (2.5%) | 34 (2.40%) | | |
| Other | 617 (18.8%) | 263 (18.70%) | | |
| **Operation** | | | 0.039 | 0.843 |
| Yes | 3,191 (97.2%) | 1,370 (97.3%) | | |
| No | 92 (2.80%) | 38 (2.7%) | | |
| **Radiotherapy** | | | 0.885 | 0.347 |
| No | 3,245 (98.8%) | 1,387 (98.5%) | | |
| Yes | 38 (1.2%) | 21 (1.5%) | | |
| **Chemotherapy** | | | 1.399 | 0.237 |
| No | 2,238 (68.20%) | 935 (66.4%) | | |
| Yes | 1,045 (31.8%) | 473 (33.6%) | | |
| **Number of lymph node excision** | | | 0.283 | 0.595 |
| <12 | 1,749 (53.3%) | 762 (54.10%) | | |
| ≥12 | 1,534 (46.7%) | 646 (45.90%) | | |
| **Insurance status** | | | 26.167 | <0.001 |
| No | 137 (4.20%) | 110 (7.80%) | | |
| Yes | 3,146 (95.80%) | 1,298 (92.2%) | | |
| **Marital status** | | | 1.045 | 0.307 |
| Married | 1,920 (58.5%) | 846 (60.10%) | | |
| Unmarried | 1,363 (41.5%) | 562 (39.9%) | | |
| **T stages** | | | 4.066 | 0.254 |
| T1 | 978 (29.8%) | 421 (29.9%) | | |
| T2 | 279 (8.5%) | 98 (7.0%) | | |
| T3 | 843 (25.7%) | 386 (27.4%) | | |
| T4 | 1,183 (36.0%) | 503 (35.7%) | | |
| **N stages** | | | 0.495 | 0.781 |
| N0 | 2,648 (80.7%) | 1,124 (79.8%) | | |
| N1 | 428 (13.0%) | 189 (13.4%) | | |

*(Continued)*

TABLE 1 (Continued)

| | Training cohort ($n = 3,283$) | Validation cohort ($n = 1,408$) | $X^2$ | $P$-value |
|---|---|---|---|---|
| N2 | 207 (6.30%) | 95 (6.7%) | | |
| **M stages** | | | 3.401 | 0.065 |
| M0 | 2,466 (75.10%) | 1,093 (77.6%) | | |
| M1 | 817 (24.9%) | 315 (22.4%) | | |
| **Tumor size (CM)** | | | 0.054 | 0.816 |
| <5 | 1,991 (60.6%) | 1,292 (39.4%) | | |
| ≥5 | 1,292 (39.4%) | 549 (39.0%) | | |
| **CEA** | | | 4.838 | 0.089 |
| Positive | 452 (13.8%) | 174 (12.4%) | | |
| Negative | 449 (13.7%) | 169 (12.0%) | | |
| Other | 2,382 (72.6%) | 1,065 (75.6%) | | |

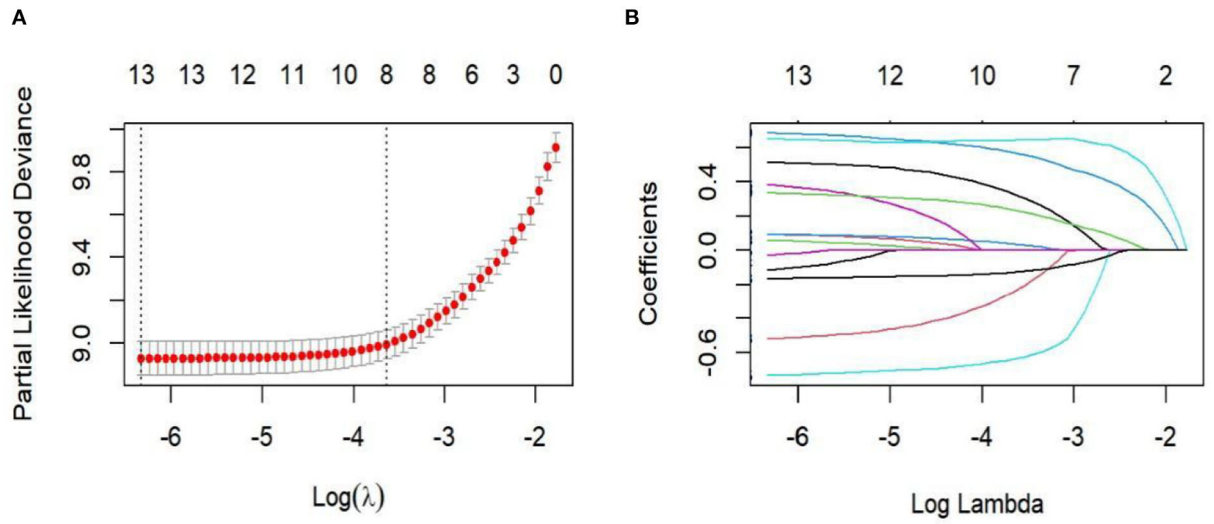


FIGURE 1
Lasso regression for cross-validation and regression analysis. **(A)** Cross-validation; **(B)** Lasso regression path diagram.

M stage, tumor size, and CEA were found to not be significantly different ($p > 0.05$), as shown in Table 1.

## Univariate and multivariate cox regression analysis of the modeling and validation sets

Single-factor Cox regression analysis found there to be statistically significant differences in age, ethnicity, pathological type, pathological stage, surgery, radiotherapy, chemotherapy, lymph node removal, T stage, N stage, M stage, tumor size, and CEA ($p < 0.05$). Lasso regression and cross-validation were performed on 13 statistically significant variables from the aforementioned single-factor Cox regression analysis (Figure 1). The results found race, pathological type, radiotherapy, chemotherapy, and tumor size variables to be excluded. The variables that were finally screened out by Lasso regression—age, pathological stage, surgery, number of lymph node resections, T stage, N stage, M stage, and CEA—were included in the Cox multivariate regression analysis. The results found age, pathological stage, surgery, number of lymph nodes removed, T stage, N stage, M stage, and CEA to be independent risk factors for appendix cancer prognosis ($p < 0.05$; Table 2).

## Establishment and validation of the OS nomogram of appendiceal cancer

The analysis of significant differences in multifactorial Cox regression was incorporated into the R software, and a nomogram model of OS that affects appendiceal cancer was

TABLE 2 Univariate and multivariate analysis of appendiceal cancer prognosis.

| | Univariate analysis | | Multiplicity | |
|---|---|---|---|---|
| | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value |
| **Age** | | <0.001 | | <0.001 |
| <60 | Reference | | Reference | |
| ≥60 | 1.891 (1.638–2.182) | | 1.714 (1.482–1.982) | |
| **Race** | | 0.006 | | |
| White people | Reference | | | |
| Black people | 1.293 (1.029–1.624) | | | |
| Other | 1.366 (1.079–1.729) | | | |
| **Sex** | | 0.056 | | |
| Man | Reference | | | |
| Woman | 0.872 (0.757–1.004) | | | |
| **Pathology type** | | <0.001 | | |
| Carcinoid | Reference | | | |
| Cup-shaped cell Carcinoma | 3.311 (2.076–5.282) | | | |
| Adenocarcinoma | 9.857 (6.534–14.872) | | | |
| Other | 6.598 (4.448–9.788) | | | |
| **Tumor stage** | | <0.001 | | <0.001 |
| Well-differentiated | Reference | | Reference | |
| Moderately differentiated | 2.601 (2.082–5.282) | | 1.966 (1.562–2.474) | |
| Poorly differentiated | 6.818 (5.473–8.493) | | 3.252 (2.552–4.145) | |
| Undifferentiation | 5.427 (3.750–7.855) | | 2.155 (1.462–3.176) | |
| Other | 2.173 (1.697–2.781) | | 1.643 (1.272–2.124) | |
| **Operation** | | <0.001 | | <0.001 |
| Yes | Reference | | Reference | |
| No | 0.204 (0.155–0.267) | | 0.395 (0.292–0.535) | |
| **Radiotherapy** | | <0.001 | | |
| No | Reference | | | |
| Yes | 2.193 (1.407–3.420) | | | |
| **Chemotherapy** | | <0.001 | | |
| No | Reference | | | |
| Yes | 2.542 (2.207–2.928) | | | |
| **Number of lymph node excision** | | <0.001 | | <0.001 |
| <12 | Reference | | Reference | |
| ≥12 | 0.830 (0.720–0.956) | | 0.561 (0.478–0.657) | |
| **Insurance status** | | 0.994 | | |
| No | Reference | | | |
| Yes | 0.999 (0.711–1.402) | | | |
| **Marital status** | | 0.146 | | |
| Married | Reference | | | |
| Unmarried | 1.112 (0.964–1.282) | | | |
| **T stages** | | <0.001 | | <0.001 |
| T1 | Reference | | Reference | |
| T2 | 1.349 (0.880–2.067) | 0.170 | 1.138 (0.739–1.752) | |
| T3 | 2.749 (2.077–3.640) | | 1.613 (1.198–2.171) | |
| T4 | 6.238 (4.824–8.067) | | 2.304 (1.719–3.088) | |
| **N stages** | | <0.001 | | <0.001 |
| N0 | Reference | | Reference | |

*(Continued)*

TABLE 2 (Continued)

| | Univariate analysis | | Multiplicity | |
|---|---|---|---|---|
| | HR (95% CI) | *P*-value | HR (95% CI) | *P*-value |
| N1 | 2.293 (1.915–2.745) | | 2.009 (1.660–2.432) | |
| N2 | 5.911 (4.909–7.118) | | 2.894 (2.324–3.603) | |
| **M stages** | | <0.001 | | <0.001 |
| M0 | Reference | | Reference | |
| M1 | 4.081 (3.543–4.701) | | 1.765 (1.480–2.106) | |
| **Tumor size (CM)** | | <0.001 | | |
| <5 | Reference | | | |
| ≥5 | 1.780 (1.545–2.051) | | | |
| **CEA** | | <0.001 | | 0.002 |
| Positive | Reference | | Reference | |
| Negative | 0.575 (0.465–0.710) | | 0.837 (0.6721.043) | |
| Other | 0.304 (0.257–0.358) | | 0.721 (0.6020.865) | |

established (Figure 2) for predicting 1-, 3-, and 5-year OS in appendiceal cancer patients. The internal validation of the calibration curve found there to be good agreement between 1-, 3-, and 5-year OS predicted by the model and actual OS (Figures 3, 4). In the 1-, 3-, and 5-year modeling sets, the areas under the ROC curve were 0.808 (95% CI: 0.777–0.839), 0.824 (95% CI: 0.804–0.845), and 0.786 (95% CI: 0.759–0.813; Figure 5). The areas under the ROC curve for the 1-, 3-, and 5-year validation sets were 0.823 (95% CI: 0.781–0.864), 0.832 (95% CI: 0.801–0.863), and 0.817 (95% CI: 0.781–0.855; Figure 6). Finally, probabilistic values were calculated based on multifactorial Cox risk regression, and the optimal cut-off values that correspond to the maximum Jordan index of the ROC curve were divided into high-risk and low-risk groups. The Kaplan–Meier survival curve showed the 1-year survival rate in the modeling cohort to be 67.3%, the 3-year specific survival rate was 19.8%, and the 5-year specific survival rate was 3.1%. The validation set had a 1-year survival rate of 64.0%, a 3-year specific survival rate of 17.9%, and a 5-year specific survival rate of 3.4%. The results from the modeling and validation sets were found to be consistent, with the high-risk groups having poor prognoses and the low-risk groups having better prognoses ($p < 0.001$; Figure 7).

## Discussion

Appendiceal cancer is a rare tumor that is found in the digestive tract, and it is often reported on a case-by-case basis. The diagnosis of appendiceal cancer is currently based mostly on postoperative pathology. There are various appendiceal cancer clinical symptoms. During the early stage, no obvious clinical symptoms or pain may be evident at McBurby's point. During the late stage, symptoms including intestinal obstruction and ascites may occur. Appendiceal cancer is often incorrectly clinically diagnosed as acute appendicitis or ovarian adnexal-derived tumors (3). As a preoperative colonoscopy only shows the mucosa of the colorectum, it is impossible to take a biopsy of the appendix mucosa, which is decidedly unhelpful when diagnosing appendix cancer. In the blood biochemical examination, the CEA tumor marker may exhibit an increase, which suggests that it is derived from the digestive tract. Appendiceal cancer advances slowly and good results are generally achieved with surgery. Among domestic and foreign studies, those relevant to appendix cancer are mainly case reports, small sample sizes, and single-center studies. They have low credibility, the selection of variables is mainly subjective, and objective evaluation is lacking. Using data from the SEER database, Lasso regression was used for screening the best variables, establishing a nomogram model, reducing model bias, performing multivariate Cox regression analysis of appendix cancer clinical-pathological data and independent risk factors that affect appendix cancer OS, and establishing an appendix cancer prognosis model for providing a certain clinical basis for appendix cancer survival prognosis.

Decreased pain sensitivity among elderly patients may lead to the promotion of the progression of local cancer tissue and metastasis of distant organs, which results in missed optimal treatment time and reduced survival time (4). The degree of differentiation of appendix cancer cells directly reflects the degree of tumor malignancy through the heterogeneity of tumor cells and mitotic images. According to relevant reports (5), the pathological stage of the tumor is linked to the development of anemia in the body, which affects the patient prognosis. Therefore, this may a reason why the pathotype of highly differentiated appendices has a better prognosis than medium and low-differentiated appendices. This study found that compared to non-surgical patients (HR =

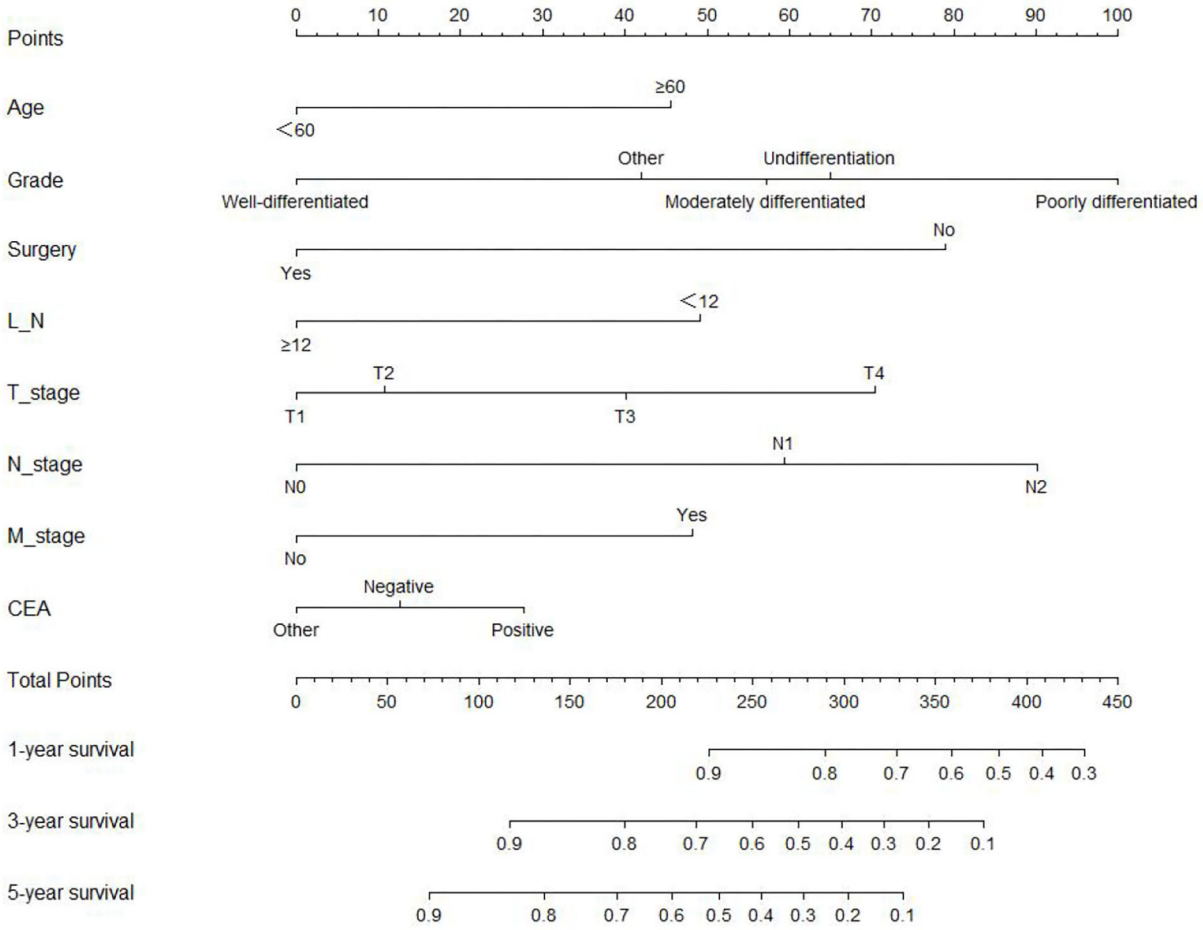


FIGURE 2
Prediction model of the overall survival (OS) nomogram in appendix cancer patients.

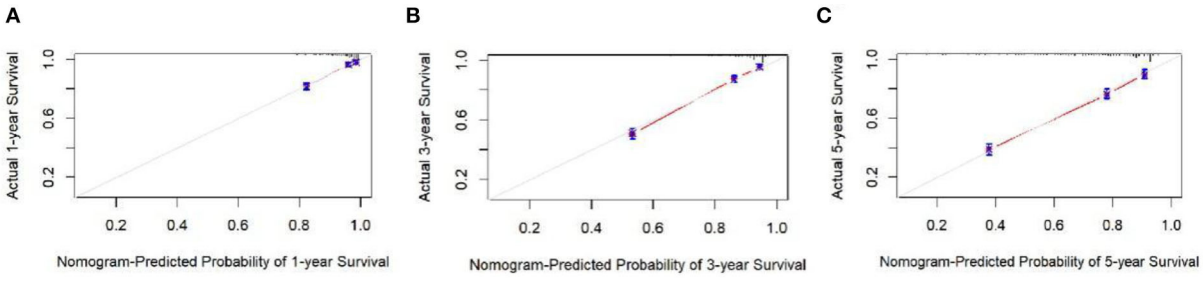


FIGURE 3
Calibration curves at 1, 3, and 5 years in the modeling set. **(A–C)** Are the calibration curves of modeling sets 1, 3, and 5.

0.395 95% CI: 0.292–0.535), the surgical patient prognosis was significantly higher than non-surgical patient prognosis. Therefore, it is recommended that appendix cancer patients actively undergo radical surgical treatment. However, a certain amount of controversy remains regarding surgical treatment methods. It is considered that (6) patients with appendiceal cancer with a tumor >2 cm, late T stage and N stage, and positive suspicious margins should undergo an appendectomy in combination with right hemicolectomy as a means of reducing the local recurrence of the tumor. Another study found that

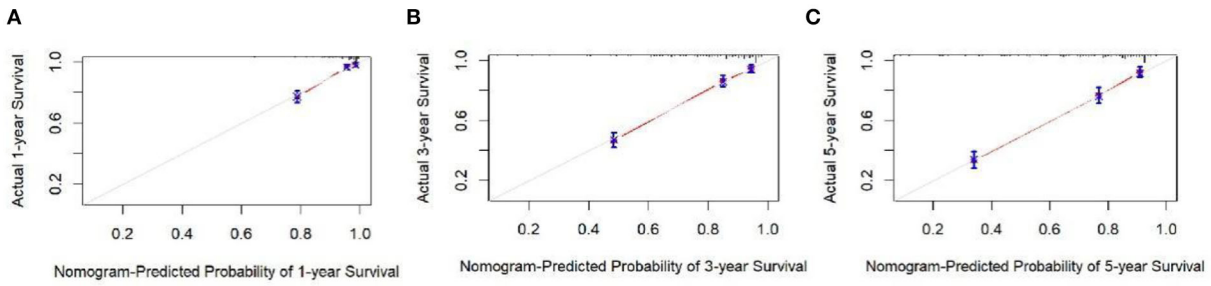


FIGURE 4
Appendix cancer outcome 1-, 3-, and 5-year calibration curves of validation set patients. **(A–C)** Are calibration curves for validation sets 1, 3, and 5.

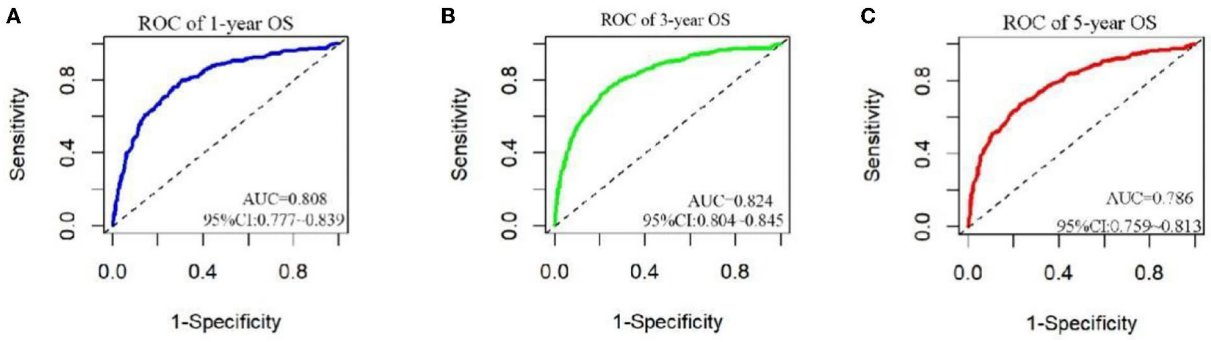


FIGURE 5
ROC curves for 1, 3, and 5 years in patients. **(A–C)** Are ROC curves for modeling sets 1, 3, and 5.

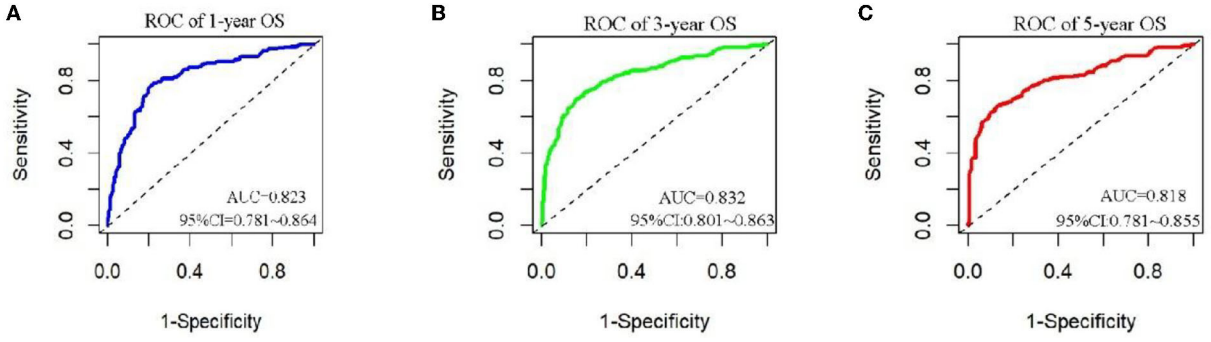


FIGURE 6
ROC curves for 1, 3, and 5 years of appendix cancer prognosis in validation set patients. **(A–C)** Are ROC curves for validation sets 1, 3, and 5.

(7) the removal of the primary lesion of appendix cancer alone can achieve good results, and when combined with right hemicolectomy, the OS time of appendix cancer is not improved. This conclusion still requires further verification through a series of multicenter studies or higher-level META analysis studies. It was determined that extensive resection should be performed for radical treatment purposes, regardless of tumor stage, in order to reduce lymphatic metastasis of appendix cancer and reduce the probability of recurrence following surgery. Lymph node metastasis has been identified as an independent risk factor for the prognosis of the gastric, colon, and other gastrointestinal cancers (8–10). According to a research report (11), ∼38.4% of patients who are diagnosed with appendiceal cancer have metastatic lesions, so a focus on lymph node

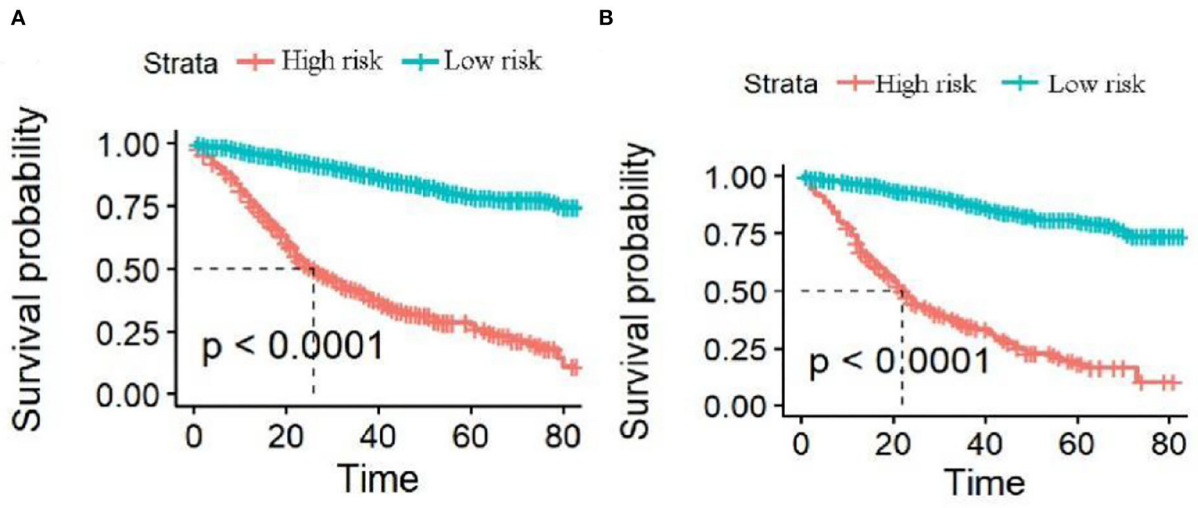


FIGURE 7
Survival curves of high-risk and low-risk groups predicting 1-, 3-, and 5-year overall survival (OS). **(A,B)** Are the 1-, 3-, and 5-year survival curves of the modeling and validation sets, respectively.

metastasis is of the utmost importance for patient prognosis. In this study, it has been shown that the radical surgical removal of ≥12 lymph nodes is a protective factor that affects appendectomy prognosis, which is consistent with the findings of Fleischmann (11). Therefore, the radical surgical removal of ≥12 lymph nodes is recommended for appendectomy patients as a means of improving survival as much as possible. The study suggests (12) that hyperthermic intraperitoneal chemotherapy (HIPEC) is recommended for patients with mucinous adenocarcinoma of the appendix. However, no consensus on neoadjuvant chemotherapy + surgery, surgery + postoperative adjuvant chemotherapy, radical surgery only, or other targeted and immunotherapy currently exists for appendiceal cancer treatment, and further prospective studies are required. TNM staging has long been regarded as a pivotal indicator for the assessment of treatment means and oncological outcomes (13, 14). The results of this study reveal that the later the stage, the higher the risk ratio (HR), and the later the stage indicates that the deeper the tumor invasion of the intestinal wall, the greater the chance of vascular and nerve invasion and the increased probability of metastasis in the distant organs of the tumor cells. CEA is a gastrointestinal cancer marker that is involved in disease diagnosis and the evaluation of disease prognosis (15–17). Several previous studies have found CEA positivity to be a poor prognostic factor in the gastrointestinal tract (18), which is consistent with the results of this study.

The nomogram model quantifies and visualizes the Cox risk regression results of disease prognosis and is currently widely used in liver, breast, and kidney cancer (19–21). Based on the multifactorial Cox risk regression analysis results, a nomogram model was constructed to predict appendiceal cancer 1-, 3-, and 5-year survival rates, and appendiceal cancer patients were classified into high- and low-risk groups with optimal cut-off values. The results found the modeling and validation sets to be consistent, with a difference in survival time for high-risk groups and a better prognosis for low-risk groups. Age, pathological stage, surgery, number of lymph nodes removed, T stage, N stage, M stage, and CEA were also identified as independent risk factors in appendix cancer prognosis. Therefore, attention should be paid to these indicators in clinical practice, high-risk and low-risk patients should be distinguished between, and they should be provided with personalized treatment plans.

In this study, the SEER database was modeled, and an internal verification model was established. ROC curves and calibration curves were used for evaluating the model, and the results were found to be relatively good. However, due to the rarity of appendectomies, an external validation set for better evaluating the reliability of the model could not be established. Furthermore, the database was unable to obtain vascular nerve infiltration and could not be analyzed in more depth, so there are limitations to the study.

In conclusion, age, pathological stage, surgery, lymphadenectomy number, T stage, N stage, M stage, and CEA are independent risk factors that affect appendix cancer prognosis. The nomogram model that is based on these indicators has good predictive value for1-, 3-, and 5-year survival rates. At the same time, the aforementioned factors should be considered for the detection of appendix cancer with early intervention among high-risk groups.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The authors were authorized to extract data from the SEER study by the National Cancer Institute. Access to data *via* the SEER database requires no informed patient consent (SEER ID: 13846—Nov2020). This study is a retrospective analysis that strictly complies with the Helsinki Declaration of 1964 and any subsequent amendments or similar ethical standards.

## Author contributions

TL designed, analyzed, and wrote the paper. JM and YW participated in the statistical analysis and revised the paper. WQ, CW, and ZM participated in arranging the data. CW were responsible for finalizing the manuscript. All authors agree with the above contributions. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Current uses of electro-cautery lumen apposing metal stents in endoscopic ultrasound guided interventions

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The electro-cautery lumen apposing metal stent (EC-LAMS) is a newly developed device that integrates the electro-cautery cyctotome with the one-step metal stent delivery and releasing system in recent years. LAMS was first designed to complete the drainage of pancreatic fluid collection under endoscopic ultrasound guidance, and the technological innovation of EC-LAMS has made more off-labeled indications of endoscopic intervention for gastrointestinal diseases realized, such as abdominal fluid drainage, bile duct, or gallbladder drainage through stomach or duodenum, gastrointestinal anastomosis, and the establishment of fistulous channel for further endoscopic operation when necessary. The unique feature of this metal stent is that it has the design of a saddle shape and a large lumen, and can almost connect the adjacent structures to minimize the risk of perforation and leakage. Compared with traditional LAMS, EC-LAMS, an advanced integrated device, can greatly simplify the endoscopic process, shorten the procedure time and reduce the technical difficulty, thus it can help endoscopists complete more complex endoscopic interventions. In this review, we discuss the state of art with regard to EC-LAMS and its endoscopic process, current indications, outcomes, adverse events, and future application prospects.

KEYWORDS

**electro-cautery lumen apposing metal stents, interventional endoscopic ultrasound, pancreatic fluid collection, endoscopic ultrasound-guided biliary drainage, endoscopic ultrasound-guided gastroenterostomy**

## Introduction

The lumen-apposing metal stent (LAMS) is a saddle shaped metal stent with a large channel, which was first reported by Binmoeller and Shah for transluminal drainage in 2011 (1, 2). It is mainly designed for the drainage of peripancreatic fluid collections (PFCs) and has been applied in recent years. The LAMS contains high patency and provides sufficient fluid drainage, but has the limitation of relatively complicated operational steps of procedures and the use of accessories such as guide wire, catheter, cystotome, or dilation balloons under the guidance of X-ray and endoscopic ultrasound

(EUS). Although the indications of LAMSs are gradually widespread, its complicated operation process and high operation difficulty limit its clinical practice. In recent years, with the progress of technological innovation, different types novel electrocautery LAMS have been developed. The unique design integrates the electro-cautery cyctotome and the metal stent releasing system, which greatly facilitates the operation steps, reduces procedural difficulties, and widely expands the clinical indications.

## Design of EC-LAMS and procedure process

Prior to the introduction of EC-LAMS, several types of conventional non-cautery-based LAMS were widely used (3, 4). The transluminal placement of a cold LAMS requires multiple over-the-wire device exchanges which may result in difficulties for endoscopists to master this technique. During the procedure of releasing a cold LAMS, a 19-G fine-needle is firstly used to enter the target lumen, and the anatomical structure of the lumen is subsequently confirmed for placing the guide wire into the cavity through contrast injection. Then the needle is exchanged for a dilation balloon (or bougie) to expand the transluminal tract to insert a stent delivery catheter and finally a LAMS is placed. Each step of this technique has potential complications. Guidewire access may be lost during instrument exchange. Removing the instrument from the wire can probably leave a step-off between the wire and the tract, which may cause leakage. Inserting an instrument along the guidewire can cause perforation and/or separation of the target and intestinal lumen. Dilation of the transluminal tract may lead to perforation and bleeding (5). For this reason, a novel stent delivery system with simple manipulation and refined procedure steps is needed and the EC-LAMS is consequently developed.

There are two types of EC-LAMS that are currently popular in clinical use: HOT AXIOS stent (5) (Boston Scientific, Marlborough, Mass, US) and HOT SPAXUS stent (6, 7) (Taewoong Medical, Gyeonggi-do, South Korea), and the parameters of them are listed in Table 1.

### HOT AXIOS stent

The HOT AXIOS stent was developed to enable the endoscopist to an immediate release of the stent following an access to the target lumen with a stent-loaded delivery catheter using the electro-cautery tip under endoscopic ultrasound instead of a needle or guidewire insertion or preliminary dilation (3, 5, 8). The operation process of HOT AXIOS stent mainly includes two steps: cyst puncture and stent release. It integrates the cystotome and the stent delivery device together, without the assistance of guide wire or fluoroscopy, and is easy and fast to operate. First, the location of the target lesion to be punctured (such as pancreatic pseudocyst) is identified under EUS and the appropriate depth of cystic lesion is measured to evaluate the puncture length of the catheter. Second, directly puncture into the lumen of lesion under the guidance of EUS through the electro-cautery stent delivery catheter. Third, release the first flange and gently pull it back to make the first flange closely against the cystic wall. Finally, the proximal flange is gradually deployed within the gastrointestinal lumen with the maintain of a certain degree of traction force, so that the metal stent could expand slowly and the drainage channel is established (9) (Figure 1).

### HOT SPAXUS stent

The HOT SPAXUS stent is another EC-LAMS in popular use (6, 7). When using this stent, the target lesion is punctured using a 19-G FNA needle followed by an advancement of a 0.025/0.035-inch guidewire into the lumen. After placement of the guidewire, the transluminal tract is dilated by applying electrocautery. The stent delivery system is then advanced over the guidewire and the two flanges are immediately deployed one after another under the guidance of X-ray or endoscopic ultrasound between the lesion cyst and the gastrointestinal tract.

## Challenges of the deployment of EC-LAMS

The stent release process is critical, and improper operation may cause stent migration. Staudenmann et al. (10) reported

TABLE 1 Current electro-cautery lumen apposing metal stent on market.

| Types of stents | Stent length (mm) | Lumen diameter (mm) | Flange diameter (mm) | Delivery catheter (Fr) |
|---|---|---|---|---|
| HOT AXIOS stent (Boston Scientific, Marlborough, Mass, US) (5) | 8, 10, 15 | 6, 8, 10, 15, 20 | 14, 17, 21, 24, 29 | 9, 10.8 |
| HOT SPAXUS stent (Taewoong Medical, Gyeonggi-do, South Korea) (6, 7) | 20 | 8, 10, 16 | 23, 25, 31 | 10 |

a case of EC-LAMS translocation and dislodgement into the gastric cavity. The stent was retrieved with a biopsy forceps and then placed again by reloading the proximal end of the LAMS into the therapeutic endoscope channel and pushing the biopsy forceps to grasp the distal end of the stent to reintroduce it into the lesion. It is suggested that we should pay much attention to the deployment of the distal flange of EC-LAMS, especially not drag it too hard during the delivery procedure. The proximal flange should be released in the therapeutic channel of endoscope, and then gradually pushed into the gastrointestinal cavity under the direct observation of endoscope view so as to prevent internal leakage caused by early release of the flange.

TABLE 2 Summary of the unique characteristics of EC-LAMS compared with double pigtail plastic stents.

| |
|---|
| The large diameter of EC-LAMS facilitates better drainage of fluids or viscous contents from a cavity or organ. |
| By the virtue of the "apposing" characteristics, the EC-LAMS minimizes the risk of leakage. |
| The large lumen of EC-LAMS acts as a working channel to undertake endoscopic interventions in adjacent structures of the gastrointestinal tract. |
| The integrated single-step delivery system for EC-LAMS simplifies technical steps of the endoscopic procedure. |

# Indications and outcomes of EC-LAMS

## Pancreatic fluid collection (PFC) and wall-off necrosis (WON)

EC-LAMS have become the optimal choice for treatment of PFC or WON primarily related to ease of use and perceived advantage of a large lumen to facilitate drainage and direct endoscopic necrosectomy (9, 11, 12). In a nationwide survey from Italy, 97.2% of endoscopists perform LAMS positioning for PFC (13). The performance of EC-LAMS can reach high technical rate of 97.1%, clinical success rate of 88.8%, and cumulative adverse effects (AE) of 18.3% (7.4% for stent migration, 7.9% for stent occlusion and infection, 2% for major bleeding, and 1% for buried stents) (8). Factors related to higher risks of AEs include pre-procedural evidence of pancreatic duct leak/disruption, vessel alteration, requiring percutaneous drainage, or a multigate technique, and as well hospital volume is significantly associated with improved outcomes (14, 15). When comparing LAMS with plastic stent (PS) for WON drainage, LAMS was more efficacious, with a success rate of 92 vs. 84% for PS, the procedure duration was significantly shorter than PS and rates of unplanned endoscopy and surgery were both lower with LAMS approach that was, however, more costly (20,029 US dallars for LAMS vs. 15,941 US dallars for PS) (16). However, in some cohort study, LAMS was considered to be associated with significantly higher rates of procedure related bleeding and greater need for repeat endoscopic intervention, thus some experts still recommended PS drainage (17).

A recent multicenter study demonstrated that deployment of double-pigtail PSs across EC-LAMS at the time of initial drainage did not have a significant effect on clinical outcomes, adverse events, or need for reinterventions (1-pigtail vs. 2-pigtails, 7 French vs. 10 French pigtail), suggesting application of EC-LAMS alone was enough for PFC drainage (Table 2) (18).

The recommendation time of removal of LAMS is 4 weeks in consensus because of increased possibilities of delayed bleeding and buried stent syndrome, but two recent multicenter studies showed conflicting results in this regard. In an Italian nationwide study from 30 centers, subgroup analysis highlighted no significant differences in terms of AEs according to the LAMS removing time (early <4 weeks and late >4 weeks), and an 18-unit experience from UK and Ireland showed no increased rate of delayed events when the LAMS were removed beyond 4 weeks (7 weeks in average) (19, 20).

## Malignant biliary strictures when ERCP failed

Currently, EC-LAMSs with diameters of 6, 8, and 10 mm are available to simplify the placement in patients with distal malignant biliary strictures (Table 1). EUS-guided choledochoduodenostomy (EUS-CD) with EC-LAMS is usually carried out when ERCP is not possible or failed due to tumor invasion of the papilla or an inaccessible papilla caused by duodenal stenosis or prior duodenal stent placement and unsuccessful biliary cannulation (21, 22). According to the recent multiple-center data from 6 US centers, 7 French centers and 8 UK and Ireland centers, technical success rates ranged from 90.8 to 97.8%, and clinical success rates were ∼93.4–100% with AE rates of 1.6–17.5% (22–24). Duodenal invasion seems to increase the risk of developing EUS-CD dysfunction, potentially representing a relative contraindication for this technique (25). Inserting an axis-orienting stent through the lumen of the LAMS may reduce the need for biliary re-interventions (23).

## Cholecystitis with high risk of surgery

EUS-guided gallbladder drainage (EUS-GBD) has been demonstrated to have similar technical and clinical success with percutaneous transhepatic gallbladder drainage (PT-GBD) for the treatment of cholecystitis in patients with high risks of surgery (2, 26, 27). Patients who undergo EUS-GBD seem to have shorter hospital stays, lower pain scores, and fewer repeated interventions, with a trend toward fewer AEs (26). Dollhopf et al. summarized 75 high-risk surgical patients who

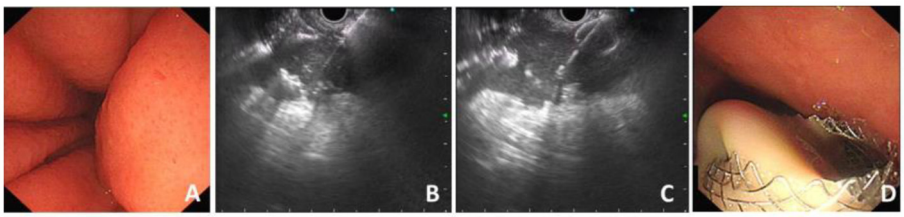


FIGURE 1
Procedure process of EC-LAMS for drainage of PFC. **(A)** Endoscopic view of compression of the posterior wall of the stomach. **(B)** Puncture of PFC with the electrocautery system under the guidance of EUS. **(C)** Release of the distal flange. **(D)** A large amount of necrotic fluid flows out through the deployed EC-LAMS.

underwent EUS-GBD by EC-LAMS, the rates of technical and clinical success were 98.7 and 95.9%, respectively (28). Adverse events were encountered in 10.7% of patients of which 1.3% were intraprocedural and 9.4% were observed at follow up. Three patients without resolution of cholecystitis died, and 1 perforation required surgery. On the other hand, a recent cost-effective analysis showed EUS-GBD had a higher total procedure cost per patient than PT-GBD. The cost of the EC-LAMS accounted for the major cost difference between the two procedures. EUS-GBD saved on the cost in management of AEs, reinterventions, and unplanned readmissions but these did not offset the cost of the stent (29).

## Obstruction of gastrointestinal tract

For patients with gastric outlet obstruction (GOO) or malignant stricture of duodenum who are not candidates of surgeries, endoscopic ultrasound (EUS)-guided gastrointestinal anastomosis with LAMS can be considered when gastrointestinal stents are unsuccessfully placed. This technique was first described in 2012 in a porcine model and was then reported promising results in humans (30, 31). When EC-LAMS was introduced, the delivery system was advanced directly into the adjacent gut lumen over the guidewire (32). EUS-guided gastrointestinal anastomosis with EC-LAMS was preferred for its shorter procedure time when compared with balloon-assisted approach. The technique success rate was reported of 80–94.5%, clinical successful rate was of 72.3–92.7% with AE rates of 6.5–14.3% (32, 33). Its success mainly depends on the distance between the two lumina that are going to be connected by the EC-LAMS and is influenced by the experience of endoscopist. Although this technique was thought to be useful in daily clinical practice, organizational challenges were considered to be the biggest obstacles that affect the diffusion of the procedure in about 55.2% of participants in a recent Italian survey (34).

## Gastric access temporary for endoscopy (GATE)

Another advantage of EC-LAMS is that it can quickly and accurately establish an access between adjacent gastrointestinal tracts. With its wide lumen, it can act as a working channel to allow an endoscope to pass through for further treatments on lesions in the gastrointestinal tract located in a long distance, thus significantly expanding the scope and breadth of endoscopic therapy (35). This technique is more focused on applying in patients who receive endoscopic retrograde cholangiopancreatography (ERCP) treatments with post-surgery anatomical changes like Roux-en-Y gastric bypass. Technical success rates can achieve 96% and persistent fistulas may occur in 11.7% patients, but endoscopic closure seems to be effective (36).

## Drainage of intra-abdominal fluids

As EC-LAMS is mastered by more endoscopists, its indications are also expanding. Drainage of many different types of intra-abdominal fluids can also be achieved by EC-LAMS, such as abdominal abscess (37–39). During these case series, EUS-guided transrectal drainages (EUS-TRD) of pelvic fluid collections with EC-LAMS were successfully performed in all cases and the stents were removed about 2 weeks after the placement without any adverse event or recurrence. Although some meta-analysis showed that EUS guided pelvic abscess drainage proves long-term clinical success with an acceptable rate of complications, the conclusion was drawn without regarding the difference between LAMS and plastic stents (40). Poincloux et al. (41) pointed out that, among the four patients who underwent LAMS for drainage of pelvic abscess, perforation and recurrence of abscess occurred in two patients, respectively, demonstrating LAMS did not achieve a perfect effect. Therefore, more clinical studies are needed to clarify the effectiveness and safety of EC-LAMS in the drainage of pelvic fluids.

# Complications of EC-LAMS

## Bleeding

Resent researches showed EC-LAMS was safe and had low risks in bleeding (42, 43), but there were still some case reports of delayed hemorrhage caused by LAMS (44). Delayed bleeding of LAMS placement when observed mostly due to underlying coagulopathy. One of the rare but life-threatening side effects of LAMS is delayed bleeding due to ruptured pseudoaneurysm (PA) (44). About 43.6% of patients had LAMS placed before PA diagnosis and bleeding from PA induced by erosion of LAMS may occur in the first 2 weeks (45). A possible mechanism for delayed bleeding in LAMS is its double-flange design. The two flanges make the gastric wall tightly close to the pseudocyst wall. After cystogastrostomy, the size of the pseudocyst is decreased because of the fluid drained from the pseudocyst into the gastric cavity. The double-flange design does not allow movement of walls or the stent. Lack of mobility may cause tension in the blood vessel wall and surrounding vessels, leading to PA formation and bleeding. About 4 weeks after LAMS implantation, the size of the cyst decreased significantly, and the possibility of delayed bleeding increased. Bang et al. (46) compared AXIOS and plastic stent for cystogastrostomy, and found that patients using plastic stent did not have delayed bleeding. They proposed that, unlike LAMS, with the collapse of WON, the plastic stent will enter the stomach freely. Brimhall et al. (47) and Lang et al. (17) both reported that patients with LAMS had a higher risk of pseudoaneurysm bleeding than patients with double-pigtail plastic stents in treating PFCs.

## Stent migration

LAMS was originally designed in a saddle shape to tightly connect the gut lumen with the cystic lesion together and minimize the risk of stent migration. However, the migration rate of LAMS had been reported by some studies in a range of 10–19% (48–50). Migration can occur immediately due to improper deployment of the LAMS, but may also occur weeks after stent placement, and also due to subsequent manipulation of the stent during the GATE procedure (50, 51). LAMS can migrate either into the cyst cavity, or back into the gastrointestinal lumen. The management of stent migration into the gastrointestinal lumen is mostly direct endoscopic extraction. Migration into the cyst cavity might lead to tract collapse and procedure failure, which should be managed by urgent endoscopic retrieval or surgery. In patients undergoing EUS-guided choledochoduodenostomy using an EC-LAMS, once the intra-channel release of the proximal flange from the duodenal bulb is not in a precise control, the pylorus could be completely covered by the proximal flange released transpylorically into the stomach, causing a rare complication of pyloric occlusion (52). The proximal flange was pushed in the right position by the gastroscope with a preloaded transparent cap.

## Buried stent

Buried stent refers to the condition that the stent ends pulled in and embedded into the stomach wall. This complication probably mainly occurs in LAMS, whether or not it is electrocautery enhanced, because the flanged edge of the stent is tightly contacted with the gastric and cyst wall. Several previous studies have reported no occurrence of this complication (43, 49, 53), but in other study, the rate of buried stent was reported of nearly 17% (46). The specific cause of this complication is not clear. Most of the buried stents were case reports, but in one recent review concerning complications of LAMS, occurrence rates of buried stent in PFC, bile duct and gallbladder were 0.07%, 0 and 0.59%, respectively (54).

# Application prospect in future

Currently, a porcine pilot study of gastric bypass bariatric surgery assisted by EC-LAMS has been successfully carried out (55). It is believed that this new technique can be applied into clinical practice in the near future, bringing good news to more obese patients.

# Author contributions

HY wrote the review. QL, S-hW, X-dG, and LZ search the related paper. BN gave the idea of this topic and revised the review. SH and BN revised the review. All authors contributed to the article and approved the submitted version.

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# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Global attitudes on and the status of enteral nutrition therapy for pediatric inflammatory bowel disease

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Enteral nutrition (EN) is a diet-remission therapy for inflammatory bowel disease (IBD) that plays a more important role in children than adults. EN includes exclusive enteral nutrition (EEN), partial enteral nutrition (PEN), and maintenance enteral nutrition (MEN). However, EEN remains an unstandardized treatment for pediatric IBD. The types and methods of EN differ around the world. The current study reviewed the EN literature on children with IBD. A total of 12 survey studies were identified that analyzed the current state of EN use, including clinical opinions, implementation methods, treatment course, EEN formula, IBD classification, progress, dietary reintroduction, and patient feedback. The findings revealed that EEN has a strong effect on mild to moderate Crohn's disease (CD). The usage rates of this treatment in different sites were ileum/colon (Paris classification L3) > ileum (L1) > upper digestive tract (L4) > colon (L2) > perianal disease (P) > ulcerative colitis (UC) > extraintestinal lesions. The polymeric formula was the most used EN formulation. New EN diets include a CD exclusion diet (CDED), a specific carbohydrate diet (SCD), and a CD treatment-with-eating (CD-TREAT) diet. Children with IBD responded similarly to EEN administered orally or using a feeding tube. Most guidelines recommended 6–8 weeks of EEN treatment to induce remission. Many clinicians preferred to combine drug medications during EEN and recommended that MEN accounts for at least 25–35% of daily caloric intake. EN remains an unstandardized therapy that requires teamwork across disciplines.

KEYWORDS

**inflammatory bowel disease, enteral nutrition, children, implementation status, questionnaire**

## Introduction

Inflammatory bowel disease (IBD) includes Crohn's disease (CD), ulcerative colitis (UC), and unclassified inflammatory bowel disease (IBD-U). Enteral nutrition (EN), which includes exclusive enteral nutrition (EEN), partial enteral nutrition (PEN), and maintenance enteral nutrition (MEN), is a food-induced therapy for IBD remission. Due to its safety profile, this treatment is commonly used in children. EEN is recommended as the first line of treatment for CD remission, especially among children with active luminal CD (1). This review summarizes the results of 12 survey studies and provides an update on the global status of EN use for pediatric IBD to standardize the treatment.

## Literature search and screening

PubMed, Embase, Cochrane Library, CNKI, and CBM databases were searched for global studies on EN treatment of pediatric IBD. Studies published from the establishment of each database to December 2021 were included in the search. A combination of subject headings and free words, including inflammatory bowel disease, Crohn's disease, ulcerative colitis, enteral nutrition, and children, were used as the search terms. Studies were included if the subjects were children with IBD who were ≤18 years of age, the intervention included EN, the outcome measures included patient attitudes and implementation of EEN among pediatric patients with IBD, and the study was survey-based. Studies were excluded if they were duplicate reports, articles from which the original text could not be obtained, or articles lacking the required information. Two researchers independently screened the literature and cross-checked the data. Disagreements were resolved by discussion or consultation with a third party. Literature screening was performed by first reading the title and excluding irrelevant literature. Further reading of the abstract and full text was then performed to determine whether the study should be included. If necessary, the original authors were contacted by mail or telephone to obtain unidentified information. Several variables were extracted, including research title, first author, publication journal, publication period, survey country or region, and key results. A total of 1,243 relevant studies were obtained during the initial inspection. After a layer-by-layer screening, 12 survey studies were considered highly relevant and selected for further analysis (Study flow diagram in **Figure 1**). These studies reflected the implementation status of pediatric EN, including regional variation, time evolution, clinical opinions, and patient attitudes. Analysis results of the 12 studies (ART12Q) are summarized in **Table 1**.

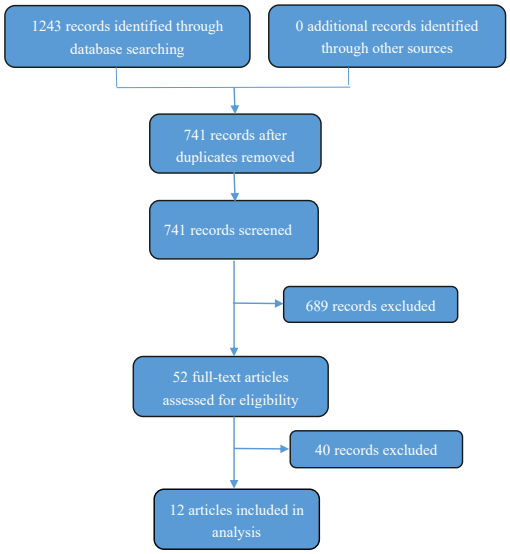


FIGURE 1
Study flow diagram.

## Exclusive enteral nutrition treatment of different inflammatory bowel disease types and lesion sites

Exclusive enteral nutrition is effective at inducing the remission of intraluminal CD (1); however, only a few studies recommend the use of this treatment for active perianal lesions and pediatric UC (2), and the supporting data are insufficient. There is also little evidence to support the use of EEN for isolated extra-gastrointestinal lesions and isolated oral lesions (3). ART12Q found that the use of EEN differed by lesion site, with ileal/colonic lesions > ileal lesions > upper gastrointestinal lesions > colonic lesions > perianal lesions. However, EEN-induced remission is not significantly associated with the lesion site (1), thus it is not necessary to consider this variable in the treatment of children with CD.

## Exclusive enteral nutrition treatment for "induction remission/maintenance remission" and "new onset/recurrence"

Exclusive enteral nutrition is primarily used to induce IBD remission. Some meta-analyses and prospective studies have shown that EEN is as effective as corticosteroids (4, 5) and biologics (infliximab) (6) at promoting pediatric CD remission (7–10), and more effective than corticosteroids at inducing mucosal healing (11, 12). Frivolt et al. reported a 92% response rate after the first course of EEN therapy. In several

TABLE 1 Global questionnaire survey on exclusive enteral nutrition (EEN) implementation of inflammatory bowel disease (IBD) children.

| References | Informant | Investigated area | EEN usage rate | Treatment goal | Disease location | Severity of illness | EEN formula | Dietary supplementation during EEN | Feeding pattern | Treatment course | MEN | Food reintroduction | Drug use during EEN | Factors affecting EEN implementation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (49) | Pediatric gastroenterology center | Australia/New Zealand | No data | No data | No data | No data | Polymeric formula (94%), elemental formula (6.3%) | Flavoring is allowed (44%), small amounts of extra food and drinks (sweets and liquids) are allowed (56%), and only water is allowed (44%) | NG (16%) | 6 W (11%), 6–8 W (50%), 8 W (39%) | Usage rates (88%), the recommended calories are about 24% of total intake | <1 W (17%), 1–4 W gradual introduction (78%), temporary low fiber/low residue diet (78%) | No data | Medical team (100%), family (100%), patient compliance (83.3%), economic cost (72.2%), formulation type (50%), disease severity (55.6%) |
| (16) | Patient/Guardian | USA (Children's Hospital Colorado) | 46.1% | Induced remission (40%), sustained remission (16%), uncertain (2%) | No data | Mild to moderate (68%), severe (32%) | No data | No data | NG (24.5%), colostomy mouth (2%) | <2 W (8%), 2–4 W (4%), 4–6 W (15%), 6–8 W (4%), 8–12 W (20%), >12 W (49%) | No data | No data | No data | Economic cost (33%), social difficulty (27%), formula type (23%), difficulty of tube feeding (18%) |

*(Continued)*



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TABLE 1 (Continued)

| References | Informant | Investigated area | EEN usage rate | Treatment goal | Disease location | Severity of illness | EEN formula | Dietary supplementation during EEN | Feeding pattern | Treatment course | MEN | Food reintroduction | Drug use during EEN | Factors affecting EEN implementation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (15) | PGE | Australia/ New Zealand | 84% | Any time (98%), induced remission (new diagnosis 100%, recurrence 86%) | CD 100% (L2 72%, L3 86%, L4 73%, P 25%) UC 8%, IBD-U 30% | Mild (new diagnosis 73%, recurrence 62%) Moderate (new diagnosis 89%, recurrence 89%) Severe (new diagnosis 87%, recurrence 76%) | Polymeric formula (75%), Semi elemental formula (5.4%), elemental formula (8.1%) | Flavoring is allowed (48.6%) and other liquids besides water are allowed (27%) | No data | 6 w (5%), 6–8 w (95%) | usage rates (51%), the recommended calories are about 30–50% of total intake | Gradual reintroduction (76%), low residue diet first (45%), low allergen diet first (17%) | CS (3%), 5-ASA (16%), AZA (68%), MTX (32%) | Medical team (97%), family (100%), patient compliance (97%), disease site (68%), formulation type (65%), economic cost (46%), disease severity (43%) |
| (17) | Common PGE (65%), PGEIBD (21%), dietician (10%) | 26 countries | 63% | Induced remission (new diagnosis 82%, recurrence 38%) | L1 88%, L2 52%, L3 91%, $P < 31$, UC < 6% | No data | Polymeric formula (88%) | No intake of any food other than water (63%), 31% of common PGE and 26% of PGE-IBD allow intake of small amounts of other foods (candies and liquids) | Po (66%) NG (33%) | 4–6 w (2%), 6 w (31%), 8 w (57%), 8–12 W (7%) | No data | No data | No data | Medical team (21%), economic cost (19%), formula type (58%) |

*(Continued)*





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TABLE 1 (Continued)

| References | Informant | Investigated area | EEN usage rate | Treatment goal | Disease location | Severity of illness | EEN formula | Dietary supplementation during EEN | Feeding pattern | Treatment course | MEN | Food reintroduction | Drug use during EEN | Factors affecting EEN implementation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (18) | Pediatric CD therapist, patient/ guardian | Japan | Doctor 84%, patient 70% | Induced remission (82% new diagnosis, 59.1% recurrence) | L1 (16%), L2 (17%), L3 (48%) | No data | Elemental formula (doctor 85%, patient 98%) | No data | No data | An average of 15.9 d | Usage rates (63.7%) The recommended calories are about 30% of total intake | No data | CS (40%), immunomodulator (36%), 5-ASA (97%), biologics (21%), ABX (12%) | Medical staff and family support are major factors |
| (51) | Patient/ Guardian | UK | No data | Induced remission (new diagnosis 76%, relapse 24%) | No data | No data | No data | No data | Po (45%) NG (55%) | 8 W (79%) | No data | No data | No data | No data |
| (52) | Pediatric gastroenterology unit | Spain | 90% | Induced remission (new diagnosis 70.6%, recurrence 83.3%, nutritional development 96.1%) | Any part of the digestive tract (62.7%), L1 (37%), L3 (37%), L4 (69%), the intestinal outside (50%) | Mild to moderate (100%) | Polymeric formula (70.6%) | Flavoring was allowed (60.8%), only water was allowed (90.2%), and other foods were allowed (9.3%) | Po is preferred | 6 W (19.6%) 6–8 W (22.5%), 8 W (47.1%) | Usage rates (88.4%) | Gradual reintroduction over a variable period of time | CS (20%), immunomodulator (95%), 5-ASA (65%), ABX (69%) | Family (71%), patient compliance (71%), healthcare team (69%), formulation type (30%), economic cost (10%), difficulty in tube feeding (8%) |

*(Continued)*





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TABLE 1 (Continued)

| References | Informant | Investigated area | EEN usage rate | Treatment goal | Disease location | Severity of illness | EEN formula | Dietary supplementation during EEN | Feeding pattern | Treatment course | MEN | Food reintroduction | Drug use during EEN | Factors affecting EEN implementation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (53) | Pediatric center | Europe, North America and Asia Pacific countries | 89% | Induced remission (new diagnosis 94%, recurrence 97%) | No data | No data | Polymeric formula (90%), Semi elemental formula (32%), Elemental formula (48%) | Flavorings are allowed (81%), most allow water and no other liquids (16%) | Po (56%), NG (37%), colostomy mouth (7%) | 4–6 W (3.2%), 6–8 W (81%), 812 w (16.1%) | Usage rates (87%) | The time is 1–12 W. Gradual reintroduction (52%), initial low-fiber diet (26%), specific food recommendations (39%) | 5-ASA (100%), CS (50%), AZA (50%), ABX 50% | No data |
| (54) | PGE | USA, Canada, Mexico | 83% | Induced remission (83%) | CD 83% (L3 79%, L4 76% (P 20%), UC 33% | No data | Polymeric formula (47%), semi elemental formula (55%), elemental formula (47%) | No data | NG (71%) | 6 w (30%), 6–8 w (46%), 8 w (25%), 8–12 w (25%) | Use it often or always (7%) | Go straight back to the regular diet (27%) and gradually reintroduce (57%), low fiber/ residue first (55%), low allergen first (32%) | Overall drug use (USA 63%, Canada 24%), 5-ASA (69%), CS (51%), 6-MP (60%), AZA (40%), infliximab (40%), MTX (12.6%) | Patient compliance (72%) |

*(Continued)*





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TABLE 1 (Continued)

| References | Informant | Investigated area | EEN usage rate | Treatment goal | Disease location | Severity of illness | EEN formula | Dietary supplementation during EEN | Feeding pattern | Treatment course | MEN | Food reintroduction | Drug use during EEN | Factors affecting EEN implementation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (55) | Pediatric unit of IBD | Sweden | 96% | Induced remission (new diagnosis 65%, recurrence 25%) | CD (96%) UC (4%) | Mild to moderate (96%) | Polymeric formulas (54%) | Allow some accompanying food (candy and liquid) (81%) | Po 39%, NG (61%) | 4–6 W (12%), 6 W (52%), 6–8 W (32%) | Usage rates (100%) | No data | CS (69%), immunomodulator (76%), 5-ASA (79%), antiTNF (21%), ABX (62%) | Compliance, discomfort with tube feeding, and psychological and social difficulties |
| (56) | PGE | Australia | 57% | Induced response (100%), maintained response (76%), nutritional development (81%) | CD 100% (L3 75%, L4 67%), UC 19% | No data | Polymeric formulas (92%) | No data | Po (66.7%), NG (33.3%) | 6–8 W (92%) | Usage rates (50%) | Gradual reintroduction (66.7%) | Most doctors do not use EEN in combination with drugs | Compliance of children (95.2%), family (61.9%), medical team (47.6%), cost (28.6%) |
| (57) | Pediatric gastroenterologist | 16 countries | 24.6% (USA 4.3%, Canada 36%, Western Europe 61.8%, Israel 19.2%) | Induced response (100%) | No data | Mild to moderate (59.9%), severe (14.3%) | Polymeric formulation 39% (USA 18%, Canada 20%, Western Europe 79%, Israel 73%) | No data | No data | No data | No data | No data | No data | No data |

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; IBD-U, unclassified inflammatory bowel disease; EEN, exclusive enteral nutrition; PEN, partial enteral nutrition; MEN, maintenance enteral nutrition; CS, corticosteroids; 5-ASA, 5-aminosalicylic acid; anti-TNF, anti-tumor necrosis factor; AZA, azathioprine, 6-MP, 6-mercaptopurine; MTX, methotrexate; ABX, antibiotics; NG, nasogastric tube; PCDAI, pediatric Crohn's disease activity index; ESR, erythrocyte sedimentation rate; PLT, platelet count; PGE, pediatric gastroenterologist. Disease location (Paris classification): L1, distal 1/3 ileum, with or without cecal lesions; L2, colonic lesions; L3, ileocolonic lesions; L4, lesions of the upper digestive tract; P, perianal lesions.





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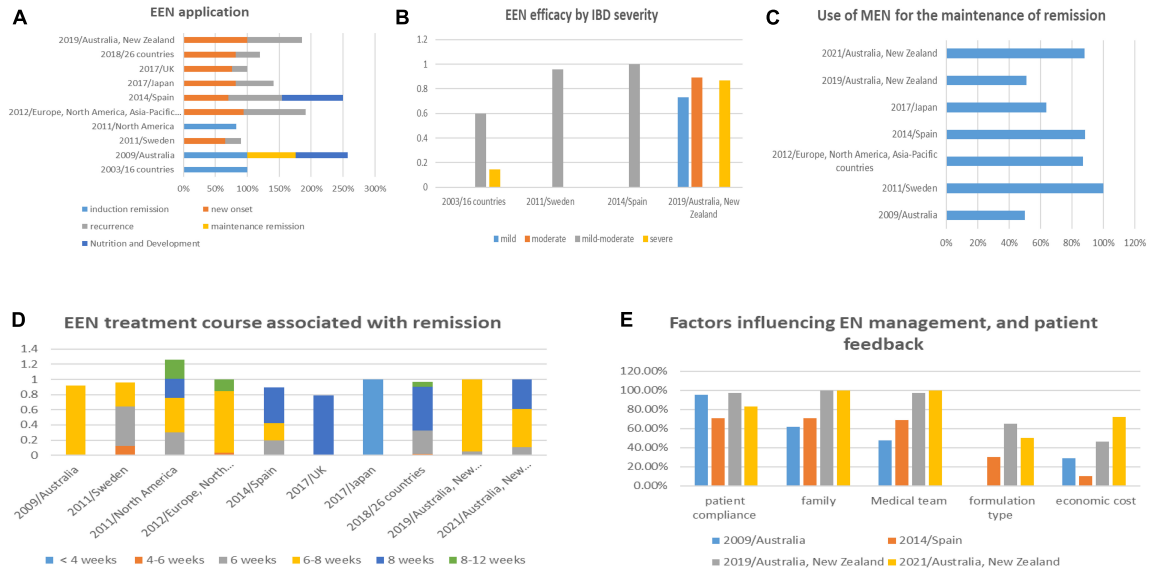


FIGURE 2
Comparison of the application of EN in pediatric IBD in various questionnaires.

retrospective studies, the rate of remission was 58.3–80% after the second course (13). ART12Q found that most clinicians agreed that EEN was effective at inducing the remission of new-onset disease and used this treatment to induce the remission of recurrent cases in some areas (**Figure 2A**). Indeed, the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (14) concluded that EEN treatment can be revisited in cases of recurrence. While EEN is effective at maintaining remission (3), ART12Q found that it was not widely adopted by doctors (**Figure 2A**) and that patient compliance was extremely low. A total of 11 studies reported the rate of recurrence after EEN-induced remission (14), and these values ranged from 2 to 67% within 1 year and 58–68% within 2 years with a median recurrence time of 6.5–12.7 months. Thus, EEN is recommended as the first-line therapy for inducing remission of newly diagnosed CD in children and is suggested for use in treating recurrent cases and maintaining remission as needed.

## Exclusive enteral nutrition efficacy by inflammatory bowel disease severity

Analysis results of the 12 studies found that most pediatricians approved the efficacy of EEN to treat mild-moderate IBD, but showed that EEN efficacy against severe CD was relatively low (**Figure 2B**). ESPGHAN (14) identified that EEN could promote the mucosal healing of pediatric CD and transmural healing in some patients. EEN was also shown to have a partial effect on severe penetrating injury associated with pediatric CD. The use of EEN treatment for severe CD has gradually increased, which may be related to the low compliance of pediatric patients with mild-to-moderate diseases (15) and a change in clinician attitudes toward the treatment.

## Exclusive enteral nutrition treatment course associated with remission

The duration of EEN treatment varied from <2 weeks to >12 weeks in different countries (16), with treatment in North America > Western Europe (17) > Japan, where the duration was only 2 weeks ± (18) (**Figure 2D**). Clinical symptoms usually began to resolve several days after initiating EEN, and the median time to clinical remission was 11 days to 2.5 weeks (19). Inflammatory markers were usually reduced in 1 week (19), while improvement in inflammation and nutrition took several weeks. Endoscopic and histological studies also showed that mucosal healing required about 8 weeks of EEN (9, 20). Thus, the 2020 consensus guideline of the European Crohn's and Colitis Organization (ECC) and ESPGHAN (1) recommended 6–8 weeks of treatment for EEN-induced remission.

## Strictness of exclusive enteral nutrition implementation

Exclusive enteral nutrition is significantly better at relieving symptoms in children with active CD than PEN (6, 21) and is

associated with a stronger decline in the pediatric CD activity index (PCDAI) than PEN (47% of total energy) after 6 weeks of treatment (22). More EN consumption was also associated with a higher remission rate in adults (23, 24). Thus, the stringency of execution correlates with the efficacy of EEN. ART12Q showed that EEN strictness varied by country. Some minor reforms were made to improve the compliance of children, including adding flavorings to reduce taste fatigue, creating high-energy-density formulas with small volumes, and permitting the consumption of small amounts of other beverages.

Partial enteral nutrition is not often used alone to induce remission but can supplement the induction of remission or be used in patients with mild disease and a low risk of recurrence. Sigall-Boneh et al. reported that a 50% PEN diet plus a structured exclusion diet was associated with a 70% remission rate in children with mild-to-moderate CD (25). A retrospective study by Wilschanski et al. found that consumption of a normal diet during the day and PEN at night (through continuous nasogastric tube feeding) could prolong remission and improve linear growth (26). Thus, PEN is a useful substitute for inducing remission in children with mild-to-moderate CD who cannot strictly adhere to EEN (27).

## Exclusive enteral nutrition formulation

Exclusive enteral nutrition formulations include element formula (EF; amino acid type), semi-element formula (SEF; oligopeptide type), and polymeric formula (PF; integral protein type). ART12Q found that PF, at a concentration of 1 kcal/ml, was the most used. While clinicians in Western Europe, Oceania, and Israel all prefer PF, doctors and patient families in Japan are willing to adopt EF. However, meta-analyses (28) and clinical research studies (8, 29) found no difference in the efficacy of EF, SEF, and PE in treating CD. There was also no evidence that dietary protein sources would affect treatment success. Thus, except for special cases, such as patients with a milk protein allergy, standard PF with a moderate fat content is recommended by ESPGHAN (14) due to its palatability and low cost.

## Developments in the exclusive enteral nutrition formula

The exclusive enteral nutrition formula is designed to reduce the complex interaction between diet and host immunity. However, different formulations, including low-fat, high-fat; supplementation with medium-chain triglycerides (MCT) (30), monounsaturated fatty acids (MUFA) (31); or anti-inflammatory substances [glutamine (32), transforming growth factor-β (33), and omega-3 (34)] are not found to cause significant clinical improvements. The addition of probiotics, prebiotics, and dietary fiber also requires further verification using randomized controlled trials (RCT). A specific carbohydrate diet (SCD) (35) was shown to have therapeutic value in treating IBD, but whether excessive carbohydrate levels are beneficial to children remains to be determined. While CDED (36) and CD-TREAT formulations are designed to mimic EEN by excluding certain components found in common foods (37), these are still immature protocols. For example, the low fermentable oligo-, di-, monosaccharide, and polyol (FODMAP) diet was shown to be effective against adult IBD but has not been fully studied in children (38, 39). In addition, the lactose-free diet (LFD) (40) may cause vitamin D deficiency and low calcium. The paleolithic, vegan, gluten-free, and food-specific IgG4 antibody-guided exclusion diets all had some effect on IBD (36), but no substantial progress in their development has been made. Since exclusion/restrictive diets may affect nutrition, psychology, and quality of life, ESPGHA does not recommend them for the treatment of children and adolescents with IBD, unless the potential benefits are higher than the risks. Research on novel formulations is promising, but findings will need to be verified by adequate RCT.

## Methods of exclusive enteral nutrition delivery

Adherence is the biggest issue associated with EEN, especially with the poor-tasting EF and SEF formulas. Feeding through a nasogastric tube (NG) or gastrointestinal stoma is often used to ensure adequate intake. While retrospective studies found no difference in the efficacy of EEN between oral and tube feeding (7), oral intake of <120% of the total daily calorie requirements may affect EEN effectiveness (41). Tube feeding may be more effective in adults because they are less receptive to single-taste diets than children, who still lack experience with rich flavors (7). ART12Q found that most children with IBD choose oral administration, potentially because of taste improvements in EEN formulations. Thus, ESPGHAN has recommended attempting oral administration first and then transitioning to NG feeding if oral intake remains inadequate.

## Drug combination during exclusive enteral nutrition-induced remission

Analysis results of the 12 studies showed that most gastroenterologists believe that combining drugs such as 5-ASA, 6-MP, AZA, CS, or infliximab with EEN achieves

better remission and often prescribe these combinations for their patients. During glucocorticoid-induced remission, the early introduction of immunomodulators is beneficial for the maintenance of remission in patients with moderate to severe CD (42). However, the clinical benefits of early drug combinations during EEN-induced remission have not been confirmed. In addition, side effects, such as nausea, that are associated with immunomodulators may adversely affect EEN treatment.

## Evaluation of exclusive enteral nutrition efficacy

Both invasive and non-invasive methods are used to evaluate the efficacy of EEN to induce remission. Endoscopic evaluation following EEN-induced remission can help achieve mucosal healing, reduce the risk of long-term complications (1), and extend the remission period to 3 years (43). However, ART12Q found that most clinicians still use non-invasive indicators to evaluate EEN efficacy, including clinical PCDAI score, CRP, erythrocyte sedimentation rate (ESR), fecal calprotectin, nutrition score, blood cell count, biochemical indicators, and imaging. Invasive evaluations such as endoscopy and biopsy are only used in about 50% of cases. A comprehensive score combining fecal calprotectin, clinical score, and CRP is currently considered the most suitable non-invasive evaluation method for pediatric CD (1). While an evaluation of EEN induced-remission is typically recommended after 6–8 weeks, many medical centers suggest evaluating its effects after 2–3 weeks.

## Food reintroduction after exclusive enteral nutrition-induced remission

Analysis results of the 12 studies found that most medical centers gradually introduced low-fat, low-fiber, and low-allergen foods after EEN-induced remission. A retrospective study showed that the rate of recurrence and the maintenance of remission at 1 year was similar regardless of whether the food was reintroduced within 5 weeks or 3 days (44). An exclusion diet guided by food-specific antibodies appears to help maintain EEN-induced remission (45). While food intolerance was not common after the reintroduction of conventional foods, the necessity of low-allergen foods was not confirmed (46). Since most EEN formulas do not contain fiber (44), many doctors recommend a short-term low-fiber diet for the reintroduction of food to children; however, there is little evidence to support this. Given the lack of data required to form a standard plan for food reintroduction, ESPGHAN (14) recommends gradually reintroducing regular foods and reducing EEN use within 2–3 weeks. Fiber restriction is not suggested for children with IBD who have no evidence of gastrointestinal stenosis.

## Use of maintenance enteral nutrition for the maintenance of remission

Either MEN or PEN treatment is usually initiated after EEN-induced remission. MEN was developed to maintain remission, improve nutrition, and promote growth and weight gain. ART12Q found that PF was the most used MEN formulation, and almost all dietitians used dietary energy reference values to estimate pediatric energy requirements (47). Gkikas et al. reported that MEN, which accounts for 35% of the daily energy requirement, is sufficient to improve clinical remission (48). ART12Q found that 89% of nutritionists recommend MEN to fulfill 25–30% of their daily energy needs. However, the use of MEN after EEN has not been recommended as a standard protocol, especially in children without malnutrition. The optimal time for MEN treatment is also unclear. Some dietitians suggest using this therapy for as long as possible, while others suggest stopping treatment when maintenance drugs start to show effect, growth is stable and appropriate, and an ideal weight has been reached (49) (**Figure 2C**).

## Exclusive enteral nutrition side effects

While EEN is associated with minimal side effects, nausea, vomiting, diarrhea, abdominal distension, and abdominal discomfort can occur (2). Clinicians need to be aware of the risk of refeeding syndrome in severely malnourished children (50). In this patient population, it is necessary to gradually reduce intake of the normal diet by about 25% of the resting caloric intake needed per day and slowly increase the volume and concentration of EEN over several days until electrolyte levels are balanced (14).

## Factors influencing enteral nutrition management and patient feedback

Analysis results of the 12 studies identified many barriers to the successful implementation of EN, including EEN exclusiveness, compliance of the children and their families, health care resources, and cost-effectiveness (**Figure 2E**). These issues can be resolved by establishing a standardized EN

program, personalizing adjustment, assuring effective doctor–patient communication, and solving social EN restrictions. Most patients and families expect dietary guidance and psychological support to become an integral part of IBD treatment (10, 41). Thus, an ideal EN management model should include education and training as well as a complete management team that includes gastroenterologists, dietitians, psychologists, nursing staff, and social workers. While insurance reimbursement to the national health system and private companies will need to be improved to reduce the burden of IBD on families.

## Conclusion

Enteral nutrition is a safe but underused treatment for children with IBD. However, there are still significant gaps in the global understanding and implementation of EN. This review evaluated recent survey studies and summarized the current status of EN treatment. The findings can be used to develop a standardized EN therapy for children with IBD.

## Author contributions

Y-MX and JL designed the research, performed the research, analyzed the data, and wrote the manuscript. MW, J-GZ, and L-LH analyzed the data. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Surgical treatment of locally advanced right colon cancer invading neighboring organs

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**Purpose:** Invasion of the pancreas and/or duodenum with/without neighboring organs by locally advanced right colon cancer (LARCC) is a very rare clinical phenomenon that is difficult to manage. The purpose of this review is to suggest the most reasonable surgical approach for primary right colon cancer invading neighboring organs such as the pancreas and/or duodenum.

**Methods:** An extensive systematic research was conducted in PubMed, Medline, Embase, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL) using the MeSH terms and keywords. Data were extracted from the patients who underwent en bloc resection and local resection with right hemicolectomy (RHC), the analysis was performed with the survival rate as the outcome parameters.

**Results:** As a result of the analysis of 117 patient data with locally advanced colon cancer (LACC) (73 for males, 39 for females) aged 25–85 years old from 11 articles between 2008 and 2021, the survival rate of en bloc resection was 72% with invasion of the duodenum, 71.43% with invasion of the pancreas, 55.56% with simultaneous invasion of the duodenum and pancreas, and 57.9% with invasion of neighboring organs with/without invasion of duodenum and/or pancreas. These survival results were higher than with local resection of the affected organ plus RHC.

**Conclusion:** When the LARCC has invaded neighboring organs, particularly when duodenum or pancreas are invaded simultaneously or individually, en bloc resection is a reasonable option to increase patient survival after surgery.

KEYWORDS

**advanced colon cancer, en bloc resection, pancreaticoduodenectomy, hemicolectomy, multivisceral resection**

## Highlights

- Invasion of the pancreas and/or duodenum with/without neighboring organs by locally advanced right colon cancer (LARCC) is a very rare clinical phenomenon that is difficult to manage.
- A few studies have attempted to find the reasonable surgical approach to get high survival focusing on en bloc resection when the LARCC invaded neighboring organs.

- The en bloc resection is the gold standard surgical options for LARCC invading neighboring organs when there is no distant metastasis.
- This is important to raise awareness among clinicians and researchers to focus on en bloc resection, to improve patient survivals of LARCC with invading neighboring organs.

# 1. Introduction

The colorectal cancer is the third most common cancer in the world, accounting for more than a third of all cancer cases worldwide, and the mortality rate is usually high (1, 2).

In general, surgery is the first choice for colon cancer, and non-radical resection and blunt mobilization of the attached organs is associated with tumor recurrence and prognosis, particularly when the colon cancer invades neighboring organs, which is defined as "locally advanced colon cancer (LACC)" (3–6). The RCC also occasionally invades the pancreas and/or duodenum in the clinics, and this can cause troubles in the operation because these organs are attached due to an inflammatory or oncologic reaction (7–9).

When LACC invades the several neighboring organs with pancreas and/or duodenum, the first option to consider is performing the multi-organic or extended resection for the more achieving tumor negative margin of the resection (10–12).

Therefore, the en bloc resection is the gold standard surgical options for LACC invading neighboring organs when there is no distant metastasis. The goal of surgical resection of primary colon cancer is complete removal of the tumor, the major vascular pedicles, and the lymphatic drainage basin of the affected colonic segment, and the en bloc resection of contiguous structures is indicated if there is attachment or infiltration of the tumor into a potentially resectable organ or structure (13, 14).

An understanding of these issues may prove important to prolong survival, and surgical options for invading neighboring organs of RCC have been continuously explored over the past several decades (12, 15–23). Unfortunately, their outcomes have the limitations coming from the lack of study samples and designs, and despite of the perfect procedures in the operation, it is still remained unclear which operation is the best options for locally advanced RCC (LARCC) (8, 13, 17, 24–28).

The aim of this study is to provide comprehensive knowledge through the systematic review of fundamental literatures and to find a reasonable surgical approach for LARCC with invading neighboring organs.

# 2. Materials and methods

## 2.1. Research design and criteria

This systematic review was conducted in accordance with the preferred reporting items for systemic review and meta-analysis (PRISMA) statement. The electronic records provided a wealth of rich data and the large sample size required for this study, and the basic data were generated using the appropriate inclusion and exclusion criteria.

### 2.1.1. Inclusion criteria

The randomized controlled trials and non-randomized controlled trials of surgical treatment of LARCC with invasion of adjacent organs such as pancreas and/or duodenum were included in this study.

### 2.1.2. Exclusion criteria

Low quality studies (no detailed explanation pre-operation and surgical process) and studies with poor outcomes (no more than two outcome parameters) were excluded from this study.

### 2.1.3. Patient information

The adult patients (aged 25–85 years old) with LARCC invading neighboring organs without gender specification.

## 2.2. Systematic fundamental literature search

An extensive systematic search was conducted in PubMed, Medline, Embase, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL) using the MeSH terms and keywords: (([All Fields] = (locally advanced*)) AND [All Fields] = (right*) AND [All Fields] = (colon* OR colonic*)) AND [All Fields] = (cancer* OR tumor* OR carcinoma*) AND [All Fields] = (invading* OR affecting*) AND ([All Fields] = (pancreas) OR [All Fields] = (duodenum) OR [All Fields] = (multivisceral*))) OR ((([All Fields] = (right*) AND [All Fields] = (hemicolectomy)) OR [All Fields] = (pancreatoduodenectomy)).

This study was conducted in April 2022 and the search process was performed manually. After collecting records, the three researchers consulted on the database and the very early pre-1950s studies and duplicates were first excluded, then the records were independently sourced according to the inclusion and exclusion criteria by researchers and finally merged into the core database (**Figure 1**).

## 2.3. Data extraction

The data was extracted based on the Cochrane Consumers and Communication Review Group's data extraction template.

## 2.4. Data analysis

We divided LARCC cases into invasion of the duodenum alone, invasion of the pancreas alone, invasion of the duodenum and pancreas simultaneously, or invasion of the pancreas and/or duodenum with other adjacent organs. And the analysis was performed with the survival rate as the outcome parameter.

# 3. Results

## 3.1. The characteristics of included studies

Between 2008 and 2021, 11 studies involving 117 patients with LARCC invasion of neighboring organs were collected from the

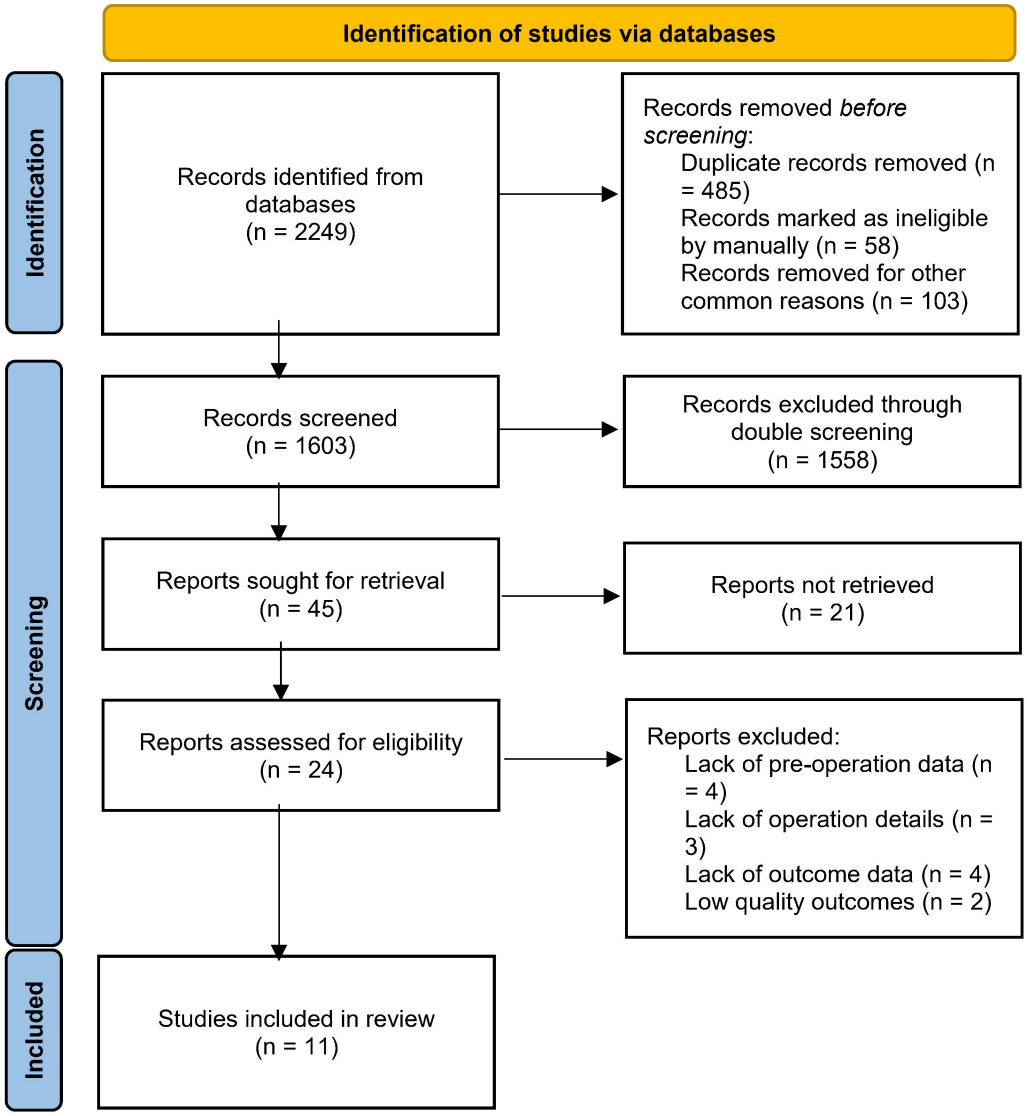


FIGURE 1
Preferred reporting items for systemic review and meta-analysis (PRISMA) flow chart for literature search.

database search (Table 1). Among them, 73 patients (62.4%) were men and 39 patients (33.3%) were women, and the last 5 patients (4.3%) were unreported their gender information. All patients were ranged between 25 and 85 years old, and the primary tumors were located in the right colon, particularly in the hepatic flexion.

## 3.2. Comparison of classic pancreaticoduodenectomy and en bloc resection with right hemicolectomy when RCC invaded duodenum

Patient information, treatments, and outcome data are presented in Table 2. A total of 35 out of 117 patients were diagnosed RCC with invading duodenum, and two surgical methods including classic pylorus preserving pancreaticoduodenectomy (PPPD) and en bloc resection with right hemicolectomy (RHC) were performed. Of 10 patients who performed PPPD and local duodenal resection plus RHC, 5 patients died, including 2 of them with recurrences, while 5 patients are alive, of which 1 had recurrence. The survival rate was 50%. Of the 25 cases of en bloc resection, seven patients died from recurrence. The remaining 18 patients were alive and did not relapse. The survival rate was 72%.

## 3.3. Comparison of classic pancreaticoduodenectomy and en bloc resection with right hemicolectomy when RCC invaded pancreas

All information has been listed in Table 3. RCC with invading pancreas was diagnosed in 14 of 117 patients, and surgical approaches such as local pancreatectomy, classic PPPD, and en bloc resection with RHC were performed. Of seven patients who performed local pancreatectomy or PPPD, four patients died, including 3 of them with recurrences, while three patients were alive and none of them had

TABLE 1 The characteristics of included studies.

| No. | First author | Country | Published year | Number of patients | Diagnosis (with invading neighboring organs) | Operation methods |
|---|---|---|---|---|---|---|
| 1 | A. Saiura | Japan | 2008 | 12 | RCC with invading D and/or P | Eb bloc PD + RHC |
| 2 | E. T. Kimchi | U.S. | 2009 | 14 | RCC with invading D, P, and M | PPPD, PD, RHC, RN |
| 3 | W. S. Lee | South Korea | 2009 | 9 | RCC with invading D and/or P | Eb bloc PD + RHC, PPPD + RHC |
| 4 | S. R. Costa | Brazil | 2010 | 5 | RCC with invading D and/or P and/or S and/or K | Eb bloc PD + RHC, PPPD + RHC with GDP or RN |
| 5 | J. Zhang | China | 2013 | 14 | RCC with invading D and/or P | Eb bloc PD + RHC with RN or PH |
| 6 | Y. Kaneda | Japan | 2017 | 5 | RCC with invading D and/or P and/or S and/or SMV | PD + RHC + SMVR |
| 7 | C. Agalar | Turkey | 2017 | 5 | RCC with invading D and/or P | Eb bloc PD + RHC |
| 8 | X. L. Yan | China | 2021 | 19 | RCC with invading D and/or P and/or S and/or L and/or SMV | Eb bloc PD + RHC with SMVR if necessary |
| 9 | N. Cojocari | Romania | 2021 | 17 | RCC with invading D and/or P | Eb bloc PD + RHC, Du + RHC, Pa + RHC |
| 10 | J. B. Chen | China | 2021 | 11 | RCC with invading D and/or P | PD + RHC, PPPD + RHC |
| 11 | S. S. Uludag | Turkey | 2021 | 6 | RCC with invading D and/or P | Eb bloc PD + RHC |

RCC, right colon cancer; D, duodenum; P, pancreas; M, mesentery; S, stomach; K, kidney; L, liver; SMV, superior mesenteric vein; PPPD, pylorus preserving pancreaticoduodenectomy; PD, pancreaticoduodenectomy; Du, duodenal resection; Pa, pancreatectomy; RHC, right hemicolectomy; GDP, gastroduodenopancreatectomy; RN, right nephrectomy; PH, partial hepatectomy; SMVR, superior mesenteric vein resection.

recurrences. The survival rate was 42.86%. Of the seven cases that underwent en bloc resection, two patients died due to recurrence. The remaining five patients were still alive and had no relapse. The survival rate was 71.43%.

### 3.4. Comparison of classic pancreaticoduodenectomy and en bloc resection with right hemicolectomy when RCC invaded duodenum and pancreas simultaneously

All patient, surgery and outcome information are presented in Table 4. Eighteen of 117 patients were diagnosed with RCC invading the duodenum and pancreas simultaneously and all underwent en bloc resection with RHC. A total of 18 patients who performed en bloc resection, 8 patients died, including 3 with recurrence and 5 without information of recurrence, and the remaining 10 patients are alive and all had no recurrences. The survival rate was 55.56%.

### 3.5. En bloc resection with right hemicolectomy when RCC invaded pancreas and/or duodenum with other neighboring organs

All patient, surgery and outcome information are presented in Table 5. RCC invading the pancreas and/or duodenum with other neighboring organs was diagnosed in 24 of 117 patients, and 19 of them underwent en bloc resection with RHC and 5 patients underwent PPPD with RHC. In addition, local resection of penetrating neighboring organs was performed in all cases. A total of 8 patients out of 19 patients with en bloc resection died, while two out of 5 patients with PPPD died. The survival rate of en bloc resection was is 57.9%.

## 4. Discussion

Right colon cancer is usually treated with a RHC. This surgical treatment has solved many oncological and radical resection problems and is now widely used in clinical practice (29–31). However, if the RCC invades the duodenum and/or pancreas, or if it invades other neighboring organs simultaneously, the treatment is relatively difficult and the post-operative mortality rate is relatively high (8, 15, 28, 32–34). LACC in adjacent organs is a rare phenomenon, recently 5.2–23.6% of all colorectal cancers invade or attach to adjacent organs at this time of presentation (35). This problem had received the substantial interests of colorectal surgeons and they finally produced the significant operation procedure, the RHC with pancreatoduodenectomy which was defined as en bloc resection. This surgical method is considered to be quite difficult surgically in general, and there are relatively many complications including post-operative pancreas and/or gallbladder fistula, and post-operative patient management is relatively difficult and complex while the advantage of this en bloc resection is relatively radical and increases the survival rate of the patient after surgery (3, 15, 36).

The en bloc resection approach was first introduced in 1953 (37). At that time, the post-operative mortality and recurrence rate, and the incidence of post-operative complications were very high due to insufficient surgical equipment and inexperienced surgical technique, especially incomplete resection (38–41). With the development of numerous medical facilities, from laparotomy through laparoscopy to robotic surgery, and the rapid developing of medical science and technology have broken the difficulty of the en bloc resection, greatly increased the possibility of surgery, and lowered the incidence of post-operative complications (42–50). Currently, when RCC has invaded the duodenum and/or pancreas, this en bloc resection is

TABLE 2 The comparison of local pancreaticoduodenectomy and en bloc PD resection with RHC when right colon cancer (RCC) invaded duodenum.

| No. | Gender | Age | Invading location | T stage | N stage | M stage | Operation method | Complication | Status | Disease free (month) | Recurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 75 | D + RC | 4 | 0 | 0 | Du + RHC | No | Dead | 11 | Yes |
| 2 | M | 62 | D + RC | 4 | 2 | 0 | Du + RHC | N/A | Dead | 39 | Yes |
| 3 | F | 76 | D + RC | 4 | 0 | 0 | Du + RHC | N/A | Alive | 24 | No |
| 4 | M | 66 | D + RC | 4 | 0 | 0 | Du + RHC | N/A | Alive | 28 | No |
| 5 | M | 55 | D + RC | 4 | 2 | 0 | Du + RHC | N/A | Dead | 11 | No |
| 6 | M | 52 | D + RC | 4 | 0 | 0 | Du + RHC | N/A | Dead | N/A | N/A |
| 7 | M | 66 | D + RC | N/A | N/A | N/A | Du + RHC | N/A | Alive | 6 | No |
| 8 | M | 68 | D + RC | N/A | N/A | N/A | Du + RHC | N/A | Alive | 43 | No |
| 9 | M | 64 | D + TVC | 4 | 1 | 0 | PPPD + RHC | No | Alive | 11 | Yes |
| 10 | M | 73 | D + RC | N/A | N/A | N/A | PPPD + RHC | N/A | Dead | N/A | N/A |
| 11 | M | 50 | D + TVC | N/A | N/A | N/A | En bloc PD + RHC | No | Alive | 41 | No |
| 12 | M | 83 | D + RC | 4 | 0 | 0 | En bloc PD + RHC | PF, DGE | Alive | 12 | No |
| 13 | M | 46 | D + HFC | 4 | 1 | 0 | En bloc PD + RHC | PF | Alive | 60 | No |
| 14 | F | 76 | D + HFC | 4 | 1 | 0 | En bloc PD + RHC | PF, DGE | Dead | 10 | Yes |
| 15 | F | 48 | D + ASC | 4 | 0 | 0 | En bloc PD + RHC | N/A | Alive | 276 | No |
| 16 | M | 58 | D + HFC | 4 | 1 | 0 | En bloc PD + RHC | DGE | Alive | 18 | No |
| 17 | F | 73 | D + TVC | 4 | 0 | 1 | En bloc PD + RHC | PF | Alive | 77 | No |
| 18 | M | 60 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | N/A | Dead | 16 | Yes |
| 19 | M | 75 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF | Dead | 36 | Yes |
| 20 | M | 62 | D + ASC | N/A | N/A | N/A | En bloc PD + RHC | PF | Dead | 9 | Yes |
| 21 | M | 36 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | No | Alive | 69 | No |
| 22 | F | 65 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF | Alive | 63 | No |
| 23 | M | 45 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF, IAA | Dead | 9 | Yes |
| 24 | M | 73 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | BF | Dead | 41 | Yes |
| 25 | M | 35 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF | Alive | 48 | No |
| 26 | F | 46 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | BF, IAA | Alive | 41 | No |
| 27 | M | 63 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | No | Alive | 23 | No |
| 28 | M | 64 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | No | Alive | 17 | No |
| 29 | F | 66 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF | Alive | 8 | No |
| 30 | F | 65 | D + HFC | N/A | N/A | N/A | En bloc PD + RHC | No | Alive | 5 | No |
| 31 | M | 62 | D + RC | 4 | 1 | 0 | En bloc PD + RHC + PG | No | Alive | 180 | No |
| 32 | F | 77 | D + HFC | N/A | N/A | 0 | En bloc PD + RHC | N/A | Dead | 168 | Yes |
| 33 | F | 51 | D + HFC | N/A | N/A | 0 | En bloc PD + RHC | N/A | Alive | 216 | No |
| 34 | M | 55 | D + HFC | N/A | N/A | 0 | En bloc PD + RHC | N/A | Alive | 60 | No |
| 35 | M | 61 | D + HFC | N/A | N/A | 1 | En bloc PD + RHC | PF | Alive | 154 | No |

M, male; F, female; D, duodenum; RC, right colon; ASC, ascending colon; TVC, transverse colon; RHC, right hemicolectomy; PG, partial gastrectomy; BF, biliary fistula; PF, pancreatic fistula; IAA, intra-abdominal abscess; HFC, hepatic flex colon; DGE, delayed gastric emptying; PPPD, pylorus preserving pancreaticoduodenectomy; Du, duodenal resection; N/A, not reported.





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TABLE 3 The comparison of local pancreaticoduodenectomy and en bloc PD resection with RHC when right colon cancer (RCC) invaded pancreas.

| No. | Gender | Age | Invading location | T stage | N stage | M stage | Operation method | Complication | Status | Disease free (month) | Recurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 63 | P + RC | 2 | 1 | 0 | PPPD + RHC | No | Alive | 19 | No |
| 2 | M | 67 | P + RC | 1 | 1 | 0 | PPPD + RHC | No | Dead | 22 | Yes |
| 3 | M | 85 | P + RC | 2 | 1 | 0 | PPPD + RHC | No | Dead | 16 | N/A |
| 4 | F | 64 | P + RC | 3 | 1 | 0 | PPPD + RHC | No | Dead | 13 | Yes |
| 5 | F | 65 | P + RC | 1 | 0 | 0 | PPPD + RHC | N/A | Alive | 11 | No |
| 6 | M | 71 | P + HFC | 4 | 1 | 0 | PPPD + RHC | DGE | Alive | 27 | No |
| 7 | F | 74 | P + RC | 4 | 1 | 0 | Pa + RHC | N/A | Dead | 7 | Yes |
| 8 | M | 64 | P + RC | 3 | 1 | 0 | En bloc PD + RHC | DGE | Alive | 9 | No |
| 9 | F | 83 | P + RC | 4 | 1 | 0 | En bloc PD + RHC | SSI | Dead | 5 | Yes |
| 10 | F | 76 | P + RC | 3 | 1 | 0 | En bloc PD + RHC | POB | Dead | 20 | Yes |
| 11 | M | 39 | P + HFC | 4 | 0 | 0 | En bloc PD + RHC | PF | Alive | 48 | No |
| 12 | M | 69 | P + HFC | 4 | 2 | 0 | En bloc PD + RHC | IAA | Alive | N/A | No |
| 13 | M | 25 | P + RC | 4 | 0 | 0 | En bloc PD + RHC | No | Alive | 96 | No |
| 14 | M | 62 | P + HFC | N/A | N/A | 0 | En bloc PD + RHC | No | Alive | 42 | No |

M, male; F, female; P, pancreas; RC, right colon; Pa, pancreatectomy; PPPD, pylorus preserving pancreaticoduodenectomy; PD, pancreaticoduodenectomy; RHC, right hemicolectomy; SSI, surgery site infection; POB, post-operation bleeding; DGE, delayed gastric emptying; HFC, hepatic flex colon; PF, pancreatic fistula; IAA, intra-abdominal abscess; N/A, not reported.

the most reasonable surgical treatment option and several studies reported the long-term outcomes of this procedure (51–57).

In Saiura et al. (19) reported that pancreaticoduodenectomy for the advanced RCC invading the duodenum and/or pancreas was beneficial with a 5-year survival rate of 55%. In Costa et al. (58) reported that pancreatoduodenectomy plus RHC for LARCC could provide the long-term survival rates, while there were sample size limitations in five T4 patients. In Cojocari et al. (20) reported that four patients underwent RHC with duodenectomy and they had no recurrence at 11–39 months, and this surgical method could be a good choice for right-sided colon cancer invading neighboring organs.

In Curley et al. (51) reported the resection for cure of carcinoma of the colon directly invading the duodenum or pancreatic head, and the survival rate with en bloc resection is relatively high than local resection, especially in invading pancreatic head cases. They concluded long-term survival could be achieved by en bloc resection in patients with locally advanced carcinoma of the colon involving the duodenum or pancreatic head. In Sasson et al. (42) reported the en bloc resection for locally advanced pancreatic cancer. In their study, 13 of 116 patients required partial colectomy to completely remove the lesion because extensive involvement of the mesentery of the transverse and right colon resulted in significant shortening of the mesentery. However, they could not find any differences in outcomes for patients with locally advanced cancer requiring en bloc resection compared to patients with standard pancreatectomy, and only suggested that en bloc resection involving surrounding structures to completely remove all macroscopic disease in selected patients with locally advanced disease might be beneficial, particularly when combined with pre-operative chemoradiotherapy. The results like these can be found in papers published by other research groups (59–64).

In our study, the survival rate with en bloc resection was relatively higher than with RHC plus local duodenectomy, RHC plus local pancreatectomy, and RHC plus PPPD. This result was consistent with previous reports in several studies and it was because this operation could achieve the tumor clearance in patients with an adherent but not penetrating right colon carcinoma when RCC invades duodenum or pancreas alone or simultaneously (16, 18, 23, 65–67).

As we have already discussed, RCC is more advanced and the most commonly affected organs are the duodenum and pancreas. In some cases, however, abdominal organs such as the stomach, liver, gallbladder, and kidneys, etc., are affected singly or in combination, and there are also cases of invasion of the peritoneum and/or mesentery (68–71). In these cases, the partial excision of the affected organ and the RHC are performed simultaneously (16, 20, 23, 72). Unfortunately, in these cases there are many post-operative complications with a high rate of recurrence and a low survival rate. In combination with metastatic comorbidities, the prognosis is not so good (73, 74).

We also summarized that en bloc resection with RHC when RCC invaded pancreas and/or duodenum with other neighboring organs, and the survival rate was is 57.9%. This result still tells us that the mortality in these cases is relatively high and that the advanced study is needed to increase the patient's survival.

This research has some shortcomings. It is a method of collecting English-only documents within the scope of collecting data, which does not guarantee the wide range of the research scope. It is revealed that en bloc resection is a very beneficial option only in terms of the surgical method without revealing the factors that have a great

TABLE 4 The comparison of local pancreaticoduodenectomy and en bloc PD resection with RHC when right colon cancer (RCC) invaded pancreas and duodenum.

| No. | Gender | Age | Invading location | T stage | N stage | M stage | Operation method | Complication | Status | Disease free (month) | Recurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 62 | D + P + HFC | 4 | 1 | 0 | En bloc PD + RHC | BF, DGE | Dead | 12 | Yes |
| 2 | F | 45 | D + P + HFC | 4 | 0 | 0 | En bloc PD + RHC | None | Alive | 24 | No |
| 3 | M | 47 | D + P + ASC | 4 | 0 | 0 | En bloc PD + RHC | PF, IAA | Dead | N/A | N/A |
| 4 | M | 54 | D + P + HFC | 4 | 0 | 0 | En bloc PD + RHC | PF, DGE | Alive | 12 | No |
| 5 | M | 70 | D + P + ASC | 4 | 0 | 0 | En bloc PD + RHC | PF, DGE | Alive | 95 | No |
| 6 | M | 48 | D + P + ASC | N/A | N/A | N/A | En bloc PD + RHC | PF, IAA | Dead | 25 | Yes |
| 7 | M | 65 | D + P + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF | Alive | 47 | No |
| 8 | F | 42 | D + P + HFC | N/A | N/A | N/A | En bloc PD + RHC | PF | Alive | 20 | No |
| 9 | M | 61 | D + P + RC | 4 | 1 | 0 | En bloc PD + RHC | N/A | Dead | N/A | N/A |
| 10 | M | 52 | D + P + RC | 4 | 1 | 0 | En bloc PD + RHC | N/A | Dead | 12 | Yes |
| 11 | M | 65 | D + P + RC | 4 | 1 | 0 | En bloc PD + RHC | N/A | Alive | 24 | No |
| 12 | M | 69 | D + P + RC | 4 | 1 | 0 | En bloc PD + RHC | N/A | Alive | 31 | No |
| 13 | F | 59 | D + P + HFC | N/A | 1 | N/A | En bloc PD + RHC | DF | Dead | 1 | N/A |
| 14 | M | 49 | D + P + HFC | N/A | 1 | N/A | En bloc PD + RHC | N/A | Dead | 39 | N/A |
| 15 | M | 68 | D + P + HFC | N/A | 1 | N/A | En bloc PD + RHC | N/A | Dead | 10 | N/A |
| 16 | M | 65 | D + P + RC | N/A | N/A | N/A | En bloc PD + RHC | N/A | Alive | 40 | No |
| 17 | M | 57 | D + P + RC | N/A | N/A | N/A | En bloc PD + RHC | N/A | Alive | 31 | No |
| 18 | M | 73 | D + P + RC | N/A | N/A | N/A | En bloc PD + RHC | N/A | Alive | 26 | No |

M, male; F, female; D, duodenum; P, pancreas; RC, right colon; ASC, ascending colon; HFC, hepatic flex colon; PD, pancreaticoduodenectomy; RHC, right hemicolectomy; BF, biliary fistula; DF, duodenum fistula; PF, pancreatic fistula; DGE, delayed gastric emptying; IAA, intra-abdominal abscess; N/A, not reported.





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TABLE 5 En bloc PD with RHC when right colon cancer (RCC) invaded pancreas and/or duodenum with other neighboring organs.

| No | Gender | Age | Invading location | T stage | N stage | M stage | Operation method | Complication | Status | Disease free (month) | Recurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 63 | P + RC + Mes | 2 | 1 | 0 | PPPD + RHC | None | Alive | 19 | N/A |
| 2 | M | 67 | P + RC + Mes | 1 | 1 | 0 | En bloc PD + RHC | None | Dead | 22 | N/A |
| 3 | M | 85 | P + RC + Mes | 2 | 1 | 0 | En bloc PD + RHC | None | Dead | 16 | N/A |
| 4 | F | 64 | P + RC + Mes | 3 | 1 | 0 | En bloc PD + RHC | N/A | Dead | 13 | N/A |
| 5 | F | 76 | P + RC + Mes | 3 | 1 | 0 | PPPD + RHC | POB | Dead | 20 | N/A |
| 6 | F | 65 | P + RC + Mes | 1 | 0 | 0 | PPPD + RHC + SMVR | None | Alive | 11 | No |
| 7 | M | 64 | P + RC + Mes | 3 | 1 | 0 | En bloc PD + RHC | DGE | Alive | 9 | No |
| 8 | M | 64 | D + RC + Mes | 4 | 1 | 0 | PPPD + RHC | None | Dead | 11 | Yes |
| 9 | F | 56 | D + P + K + HFC | 4 | 0 | 0 | En bloc PD + RHC | PF, DGE | Alive | 63 | No |
| 10 | M | 46 | D + Gb + L + HFC | 4 | 2 | 0 | En bloc PD + RHC + PH | DGE | Alive | 49 | No |
| 11 | M | 48 | D + L + HFC | 4 | 0 | 0 | En bloc PD + RHC | PF, DGE, IAA | Alive | 4 | N/A |
| 12 | F | 54 | D + K + ASC | 4 | 2 | 0 | En bloc PD + RHC + Ne | None | Alive | 13 | No |
| 13 | F | 74 | D + P + S + ASC | 4 | 1 | 1 | En bloc PD + RHC + SMVR | PF | Dead | 11 | Yes |
| 14 | F | 57 | D + P + S + SMV + HFC | 4 | 0 | 0 | En bloc PD + RHC + PG + SMVR | PF | Alive | 85 | No |
| 15 | M | 47 | D + P + SMV + ASC | 4 | 1 | 1 | En bloc PD + RHC + SMVR | PF | Dead | 11 | Yes |
| 16 | F | 44 | D + P + SMV + HFC | N/A | N/A | N/A | En bloc PD + RHC + SMVR | None | Alive | 112 | No |
| 17 | M | 48 | D + L + HFC | N/A | N/A | N/A | En bloc PD + RHC + PH | PF, IAA | Alive | 103 | No |
| 18 | M | 54 | D + L + HFC | N/A | N/A | N/A | En bloc PD + RHC | Ileus | Alive | 30 | No |
| 19 | M | 65 | D + P + K + RC | 4 | 2 | 0 | En bloc PD + RHC + Ne | N/A | Dead | N/A | N/A |
| 20 | F | 64 | D + P + K + RC | 4 | 0 | 0 | PPPD + RHC + Ne | N/A | Alive | 36 | N/A |
| 21 | M | 50 | P + S + HFC | N/A | 0 | N/A | En bloc PD + RHC | GF | Dead | 25 | N/A |
| 22 | M | 34 | P + S + TVC | N/A | 0 | N/A | En bloc PD + RHC | GF | Alive | 75 | N/A |
| 23 | M | 69 | D + H + ASC | N/A | 1 | N/A | En bloc PD + RHC | DF | Dead | 15 | N/A |
| 24 | F | 62 | D + S + TVC | N/A | 0 | N/A | En bloc PD + RHC | None | Alive | 3 | N/A |

M, male; F, female; P, pancreas; K, kidney; S, stomach; Gb, gallbladder; L, liver; RC, right colon; Mes, mesentery; ASC, ascending colon; RHC, right hemicolectomy; TVC, transverse colon; PPPD, pylorus preserving pancreaticoduodenectomy; PH, partial hepatectomy; PG, partial gastrectomy; POB, post-operation bleeding; DGE, delayed gastric emptying; DF, duodenum fistula; GF, gastric fistula; SMVR, superior mesenteric vein resection; PF, pancreatic fistula; IAA, intra-abdominal abscess; Ne, nephrectomy; N/A, not reported.





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influence on the survival rate of post-operative patients, such as post-operative chemotherapy and lymph node-negative status, which were announced in some studies. In addition, the time interval in this study is relatively long, from 2008 to 2021. This may lead to a possible bias in the results of the study, but in practice, LARCC invading neighboring organs is very rare and there is insufficient data to avoid this bias by shortening the time interval. In this regard, we hope that readers will read the study results with caution. In the future, we plan to further supplement and deepen the related contents in future research.

## 5. Conclusion

This systematic review concludes that when LARCC has invaded neighboring organs, en bloc resection is a reasonable option to prolong patient survival after surgery.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

HR, XC, and HK: conceptualization. ZX, ZG, HJ, and BA: investigation. YX, YR, and WZ: data curation. HR, HK, and XC: writing—original draft preparation. ZX, ZG, HJ, YX, YR, and WZ: writing—review and editing. HR, XC, and BA: supervision and project administration. All authors have read and agreed to the submitted version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# The progress to establish optimal animal models for the study of acute-on-chronic liver failure

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Acute-on-chronic liver failure (ACLF) defines a complicated and multifaceted syndrome characterized by acute liver dysfunction following an acute insult on the basis of chronic liver diseases. It is usually concurrent with bacterial infection and multi-organ failure resulting in high short-term mortality. Based on the cohort studies in ACLF worldwide, the clinical course of ACLF was demonstrated to comprise three major stages including chronic liver injury, acute hepatic/extrahepatic insult, and systemic inflammatory response caused by over-reactive immune system especially bacterial infection. However, due to the lack of optimal experimental animal models for ACLF, the progress of basic study on ACLF is limping. Though several experimental ACLF models were established, none of them can recapitulate and simulate the whole pathological process of ACLF patients. Recently, we have developed a novel mouse model for ACLF combining chronic liver injury [injection of carbon tetrachloride ($CCl_4$) for 8 weeks], acute hepatic insult (injection of a double dose $CCl_4$), and bacterial infection (intraperitoneal injection of *Klebsiella pneumoniae*), which could recapitulate the major clinical features of patients with ACLF worsened by bacterial infection.

KEYWORDS

**ACLF, animal model, hepatotoxic reagents, DAMPs, PAMPs**

## 1. Introduction

Acute-on-chronic liver failure (ACLF) is a clinical syndrome, defined by an acute hepatic/extrahepatic insult and subsequent rapid deterioration of liver function in patients with pre-existing chronic liver diseases or cirrhosis. This complicated syndrome is usually concurrent with bacterial infection and multi-organ failure resulting in high short-term mortality (1–4), and is becoming a major threat to those with chronic liver diseases (5).

Though the definitions and diagnostic criterion of ACLF vary worldwide, the main pre-existing chronic liver diseases are alcoholic liver disease (ALD) in the West and chronic hepatitis B (CHB) in the East, and the most common acute insults usually include excessive alcoholic consumption, hepatitis B virus (HBV) reactivation and drug-induced liver injury (DILI) (5). Bacterial infections are nearly inevitable events in ACLF patients according to the cohort with 1,343 consecutive patients from European Association for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium revealing up to two-thirds detected (1, 6). Based on

the cohort studies in ACLF worldwide (1, 7), the clinical course of ACLF could be divided into three major stages including chronic liver injury, acute hepatic/extrahepatic insult, and systemic inflammatory response caused by over-reactive immune system which worsened by bacterial infections.

Experimental animal model is pivotal for the study of ACLF (8). Several experimental ACLF models were established *via* combination of chronic and acute liver injury (9–13), including mice, rats or rabbits, however, none of them can recapitulate and simulate the whole pathological process of ACLF patients. Injection of carbon tetrachloride ($CCl_4$) or bile duct ligation (BDL) surgery is the most commonly used way to mimic chronic liver injury in animal models, whereas injection of D-galactosamine (D-GalN) or lipopolysaccharide (LPS) is often used as acute injury. The combination of these chronic and acute liver injuries could lead to considerable mortality, but the mean survival period is too short after acute insult to applicate preclinical interventions. Moreover, bacterial infection could not be fully simulated *via* LPS injection and no viable bacterial infection is applicated in the above-mentioned models. This scenario surely hinders the investigations of mechanism research and drug screening in ACLF field.

Recently, we have developed a novel mouse model for ACLF combining chronic liver injury [injection of carbon tetrachloride ($CCl_4$) for 8 weeks], acute hepatic insult (injection of a double dose $CCl_4$), and bacterial infection [intraperitoneal injection of *Klebsiella pneumoniae* (*K.P.*)], which could recapitulate the major clinical features of patients with ACLF worsened by bacterial infection (14). This model could not only mimic the major three stages of ACLF, but also prolong the animal survival period with longer observation and intervention time for screening drugs and mechanism studies. In this review, the merits and demerits of emerging animal models are summarized, aiming to provide thoughts for researchers who focused on ACLF.

## 2. Current understanding of the mechanism of ACLF

The mechanism of ACLF is multifactorial and multifaceted (15). The most commonly underlying liver disease of ACLF is cirrhosis resulting from viral hepatitis or alcohol hepatitis. Progression of cirrhotic clinical course can be divided into three stages including pre-cirrhotic diseases, compensated cirrhosis and decompensated cirrhosis (16).

According to the triggers, ACLF can be categorized into two types. Clinical identifiable inducers include pathogen-associated molecular patterns (PAMPs) (4, 8, 17) such as bacterial components, and damage-associated molecular patterns (DAMPs) (18) such as pieces of necrotic or apoptotic cells. Besides, sepsis-induced ACLF also accounts for a large proportion, of which the most common are spontaneous bacterial peritonitis (SBP) (1) and severe alcoholic hepatitis (SAH), represents nearly 25% of ACLF cases (4, 19). Sepsis-induced ACLF is mainly caused by the dysfunctional immune response. According to the current reports about ACLF, there was an opinion that sepsis acting as an extrahepatic trigger, usually participated in the progression of ACLF (20). Except for those identifiable triggers, there are also some cases of ACLF with no obvious triggers that accounted for 40–50%. Till now there were three hypotheses that may account for this situation including the dysregulation of gut microbiota, translocation of PAMPs such as LPS, and DAMPs released by cell necrosis or apoptosis.

The proposition and confirmation of the systemic inflammation (SI) hypothesis in ACLF field is a big milestone for further understanding the mechanism of ACLF (21–23). In the pathophysiological mechanism of ACLF, systemic inflammation usually plays a pivotal role. ACLF patients with severe systemic inflammation, mostly accompanied with increased levels of pro-inflammatory cytokines, chemokines, growth factors, bioactive lipid mediators, such as IL-6, IL-8, and IL-1β (24). Excessive systemic inflammation will lead to "cytokine storm" in final, which is a critical factor causing immune-mediated tissue damage and organ injury (22, 23, 25–28). Systemic inflammation is mainly associated with PAMPs and DAMPs. Bacteria released PAMPs are recognized by pattern-recognition receptors (PRRs), and farther trigger the cascade amplification reaction. The most typical paradigm of these signaling pathways is LPS-Toll-like receptor 4 (TLR4), which contributes to the releasing of pro-inflammatory cytokines and type 1 interferons (IFNs). Apart from this, systemic inflammation can also occur in the absence of bacteria or virus infection, called sterile inflammation, mainly caused by DAMPs. DAMPs which expressed by broken cells, are also recognized by PRRs. Different forms of liver injury have different underlying mechanisms, respectively. Such severe systemic inflammation may result in several outcomes like tissue hypoperfusion, immune-mediated tissue damage and mitochondrial dysfunction (29). Among them, mitochondrial dysfunction serves a link in the progression of ACLF. There is a decreased oxidative phosphorylation and adenosine triphosphate (ATP) production in ACLF patients, which may exacerbate organ failures. Excessive pro-inflammation cytokines release consumes quantity of energy, combined with obstructed energy production, will finally result in immune paralysis (15). This suppression of immune system will increase the risk of secondary infection (30) and lead to higher mortality compared with those who remain free of immune suppression. MER tyrosine kinase (MERTK) also inhibits the immune system of ACLF patients (31). The number of MERTK expressing monocytes and macrophages is increased while the sensitivity toward LPS is decreased (31). Besides, Prostaglandin E2 (PGE2) and IL-10 also suppress immune system by reducing sensitivity of innate immune response and upregulation of regulatory immune cells (32, 33). It was also reported that the level of $CD14^+$ monocytes and $CD14^+CD15^-$HLA-DR-myeloid-derived suppressor cells is higher in ACLF patients, which will suppress the immune response to bacterial PAMPs (31, 32).

## 3. Methods for inducing chronic or acute liver injury

According to current understanding of the clinical course and pathological mechanism of ACLF, the clinical course of ACLF could be divided into three major stages: chronic liver injury, acute hepatic/extrahepatic insult, and bacterial infection.

The principle of inducing liver fibrosis is the transformation of quiescent hepatic stellate cells (HSCs) to activated type expressing α-smooth muscle actin (α-SMA) and other extracellular matrixes. The first step to develop an animal model for ACLF is the

induction of liver fibrosis/cirrhosis *via* some kinds of chronic liver injuries. Hepatotoxic chemical drugs induced liver injury and immune responses mediated liver injury are the most commonly used ways for chronic or acute liver damage (14). Hepatotoxic chemical drugs usually include $CCl_4$, D-GalN, acetaminophen (APAP), concanavalin A (Con A), and thioacetamide (TAA). Heterologous serum or serum constituent, such as human serum albumin (HSA) and porcine serum (PS), are always used for immune responses mediated liver injury. In addition, surgical procedures induced liver injury is also adopted, such as common BDL surgery, hepatic ischemia/reperfusion and partial hepatectomy (HPx). The following summarizes the most recognized methods for inducing liver injury.

## 3.1. Carbon tetrachloride ($CCl_4$)

Carbon tetrachloride is a powerful hepatotoxin which is used to induce liver fibrosis/cirrhosis through oral administration or injection (34, 35). $CCl_4$ induced liver fibrosis can be reproduced in both rats and mice, even in rabbits and dogs. Liver injury caused by repeated injection of hepatoxic reagents, such as $CCl_4$, would lead to the regeneration of hepatocytes, formation of fibrosis, and collapse of reticulin, and finally, result in liver architectural distortion and cirrhosis (34, 36). Besides, $CCl_4$ is relevant with cell metabolism, the dysregulation of cations such as $Ca^{2+}$, $Na^{+}$, and $K^{+}$ in cells and the activation of cytochrome 450 (CYP450), which also plays an important role in inducing liver steatosis (37). Single injection of $CCl_4$ would result in acute hepatocytes damage and centrilobular necrosis (36, 38), which can be used to mimic acute hepatic insult for ACLF animal model. $CCl_4$ can be given in several different routes including subcutaneous, intramuscular or intraperitoneal injections, oral administration and inhalation (39–43).

Carbon tetrachloride is the most commonly used reagent to induce acute liver injury and liver fibrosis due to its convenience and low cost. $CCl_4$ induced liver fibrosis in mice can be developed in 6–8 weeks with continuous injection and is similar to clinical patients in pathophysiology. However, the hepatic fibrosis in mice induced by $CCl_4$ is easily to reverse, and $CCl_4$ would definitely cause damage to other organs. In addition, considering the toxicity and volatility of $CCl_4$, this reagent should be carefully used in fume cupboard.

## 3.2. D-galactosamine (D-GalN)

D-Galactosamine is a powerful hepatotoxic reagent. Interfering with the uridine pool in the cell is the underlining mechanism of D-GalN in inducing liver injury. It induces lethal liver injury at large dose and would enhance the sensitivity of liver to LPS, an agonist of TLRs, playing synergetic liver damaging effects. Thus, D-GalN is widely used in combination with LPS in acute liver failure or endotoxemia animal models (44).

## 3.3. Thioacetamide (TAA)

Thioacetamide, an indirect hepatotoxin, exerts toxic effect *via* a two-step biotransformation mediated mainly by CYP450 2E1 to thioacetamide sulfoxide and further to thioacetamide sulfur dioxide ($TASO_2$). $TASO_2$, the dominating reactive metabolite of TAA, leads to hepatic cellular damage, apoptosis and necrosis *via* oxidative stress and downregulation of catabolism enzymes (45). TAA is applied to induce acute or chronic liver disease in experimental animal models (46). It is reported that the main features of clinical chronic liver disease, such as hepatic encephalopathy, metabolic acidosis, elevated transaminases, abnormal coagulopathy, and centrilobular necrosis, could be induced after TAA administration (47). However, the carcinogenicity of TAA to humans (class 2B rating) limits its extensive use.

## 3.4. Acetaminophen (APAP)

Acetaminophen N-acetyl-*p*-APAP, the most widely used antipyretic and analgesic drug, would cause severe liver injury even acute liver failure in the case of overdose in human (48). In mice, acute liver injury or failure can be induced following APAP overdose. Generally, at therapeutic dose, the majority of APAP will be metabolized in the liver to non-toxic metabolites (APAP-sulfate or APAP-glucuronide) and excreted *via* the bile and urine, whereas at toxic dose, the excess APAP will be oxidized in hepatocytes by CYP450 isoforms to highly toxic metabolite N-acetyl-*p*-benzoquinone imine (NAPQI) (49). The accumulation of NAPQI that causes hepatocellular necrosis and subsequent DAMPs secreted by damaged hepatocytes that activate innate inflammatory response eventually leads to acute liver injury/failure (50).

## 3.5. Concanavalin A (Con A)

Concanavalin A is a lectin isolated from Jack beans (also called Canavalia ensiformis). Lectins are proteins that bind to carbohydrates, and the specific binding structures for Con A are α-Mannose and α-Galactose structures found in sugars, glycoproteins and glycolipids (51). Con A is a well-known T cell mitogen that can activate the immune system, recruit lymphocytes and elicit cytokine production (52). Unlike the hepatoxic reagents, Con A induced acute liver injury in mice is mainly based on the activation of CD4 + T cells and the subsequent secretion of proinflammatory cytokines, mainly IFN-γ and TNF. The mouse model of Con A induced liver injury is commonly adopted for investigating the mechanisms of autoimmune hepatitis (AIH) (53).

## 3.6. Human serum albumin (HSA)

Human serum albumin, the most abundant serum protein in blood with a half-life of 19 days in humans (54), is a typical constituent of heterologous serum for murine. HSA is often used to develop the immunologic reaction induced chronic liver injury models in rats and mice (13, 55, 56). Immune mediated chronic liver injury induced by repeated administration of HSA would lead to typical liver fibrosis in mice or cirrhosis in rats. Subsequently, D-GalN plus LPS are administrated to establish ACLF model (56, 57). However, it is reported that the mortality of HSA administration during chronic liver injury or fibrosis-induction period is relatively high at 23% (56). The high mortality limits the application of HSA in establishing chronic liver fibrosis models.

### 3.7. Porcine serum (PS)

Immune-mediated hepatic injury models are easily developed *via* the administration of heterologous serum constituent such as HSA. But the high mortality during the period of HSA induced chronic liver injury in murine models impels the usage of other kinds of heterologous serum. Porcine serum has been used to induce hepatic fibrosis for a long time, but the mechanism is uncertain until 1996. In order to investigate whether the hepatic fibrosis is caused by immune responses, Bhunchet et al. (58) divided rats into two groups, the porcine serum tolerant group and control group. Rats in the tolerant group had been injected with porcine serum peritoneally from the day of birth for 18 weeks while 8 weeks old rats in the control group received porcine serum injection for 10 weeks peritoneally. And antibody against porcine albumin level in the tolerant group is extremely lower than the control group, which suggests that no immune responses exist in tolerant group. Besides, no rats in the tolerant groups developed hepatic fibrosis. Based on this study, the mechanism of porcine serum induced hepatic fibrosis can be verified. Porcine serum is a suitable candidate for inducing the immune mediated liver injury models because of the low mortality reported (9). Compared with $CCl_4$, immune metabolism disorder is the basis of PS induced liver fibrosis, which mainly used to mimic the chronic liver injury caused by HBV infection or autoimmune liver diseases mediated cirrhosis (59–62). From histological perspective, the infiltration of monocytes and the formation of fibrosis around portal vein are the remarkable features of this model (60). PS induced immune mediated chronic liver cirrhosis demonstrates great popularity due to its economic efficiency and practicability.

### 3.8. Bile duct ligation (BDL)

Bile duct ligation is a typical surgical approach established since 1930s to simulate extrahepatic biliary obstruction that leads to biliary cirrhosis in rats or mice (63–65). The core procedure for BDL surgery is that rats or mice are subjected to double ligation of the common bile duct with section between the two ligatures, then hyperbilirubinemia would be mimicked in these BDL rats (64, 65). In BDL models, acute obstructive jaundice occurs and the expression of pro-inflammatory cytokines (such as TNF, IL-6, and IL-17) and pro-fibrotic proteins (such as collagen-α1, MMP-2, and TIMP-1) are induced in portal areas, which would progress to cirrhosis (66–68). Though liver inflammation and fibrosis are well displayed in the BDL models, the surgical procedures are difficult to handle that limits its wide application.

## 4. Methods for mimicking bacterial infection

### 4.1. Bacterial component: Lipopolysaccharide (LPS)

Lipopolysaccharide is the main component of the outer membrane of all Gram-negative bacteria, which is mainly consist of three parts, the lipid A (or endotoxin), a core phosphorylated oligosaccharide, and a variable specific long polysaccharide chain composed of repeating oligosaccharide (or O-antigen) (69, 70). LPS, one of the classical PAMPs, is a powerful mediator of systemic inflammation and septic shock *via* activating the PRRs-TLR4/TLR2 signaling pathways (4, 71–73). Normally, LPS first binds to LPS-binding protein to form an activated receptor complex with myeloid differentiation factor 2 (MD2), the CD14, and TLR4. Signals are transduced to intracellular proteins (MyD88, IRAKs, TRAFs, and NIK) by the activated receptor complex, generating an intricate network of cellular responses, activation of the NF-kB pathway, and secretion of a large amount of pro-inflammatory cytokines (74). Usually, LPS is co-administrated with D-GalN to induce acute liver injury models or fibrosis models in rats or mice which has been widely used and extensively studied (75, 76).

### 4.2. Polymicrobial infection: Cecal ligation and puncture (CLP)

In order to investigate sepsis and sepsis-associated multiorgan failure, several experimental animal models with polymicrobial infection have been established to mimic the pathophysiological changes in septic patients (77). Cecal ligation and puncture (CLP) in murine is the most widely used and typical model for experimental sepsis which has been developed more than 30 years. Moreover, the CLP model is considered to be an ideal model for the induction of polymicrobial sepsis (77, 78). The surgical procedure features of CLP include midline laparotomy, ligation below the ileocecal valve, and needle puncture of the cecum (79). The severity of CLP model can be tailored via the ligation length of cecum and the needle puncture size.

### 4.3. Polymicrobial infection: Cecal slurry (CS)

Since the major problem for CLP-based polymicrobial sepsis model is consistency of the surgery, cecal slurry (CS) injection based polymicrobial peritoneal sepsis model is developed to solve the consistency problem and simplify the surgical procedure (80). CS-induced sepsis model is an infectious model with bacterial colonization, systemic inflammation and dose-dependent mortality without surgery, which is widely accepted and now considered as the "gold standard" model for murine neonatal sepsis study (80, 81). The advantages of CS-induced polymicrobial sepsis are no surgical procedures, a single CS donor can be administrated in a large number of animals, and easy to perform.

### 4.4. Single bacterial infection: *Klebsiella pneumoniae* (*K.P.*)/*Escherichia coli* (*E. Coli*)/*Salmonella typhimurium* (*S. Typhimurium*)

To study the role of liver during bacterial infection in different organs, several experimental bacterial animal models have been developed. For systemic single bacterial infection model, mice or rats are injected intraperitoneally with a certain dose [colony-forming unites, (CFU)] of *K.P.* or *Escherichia coli* (*E. Coli*) or *Salmonella typhimurium* (*S. Typhimurium*) directly (82–84). For the

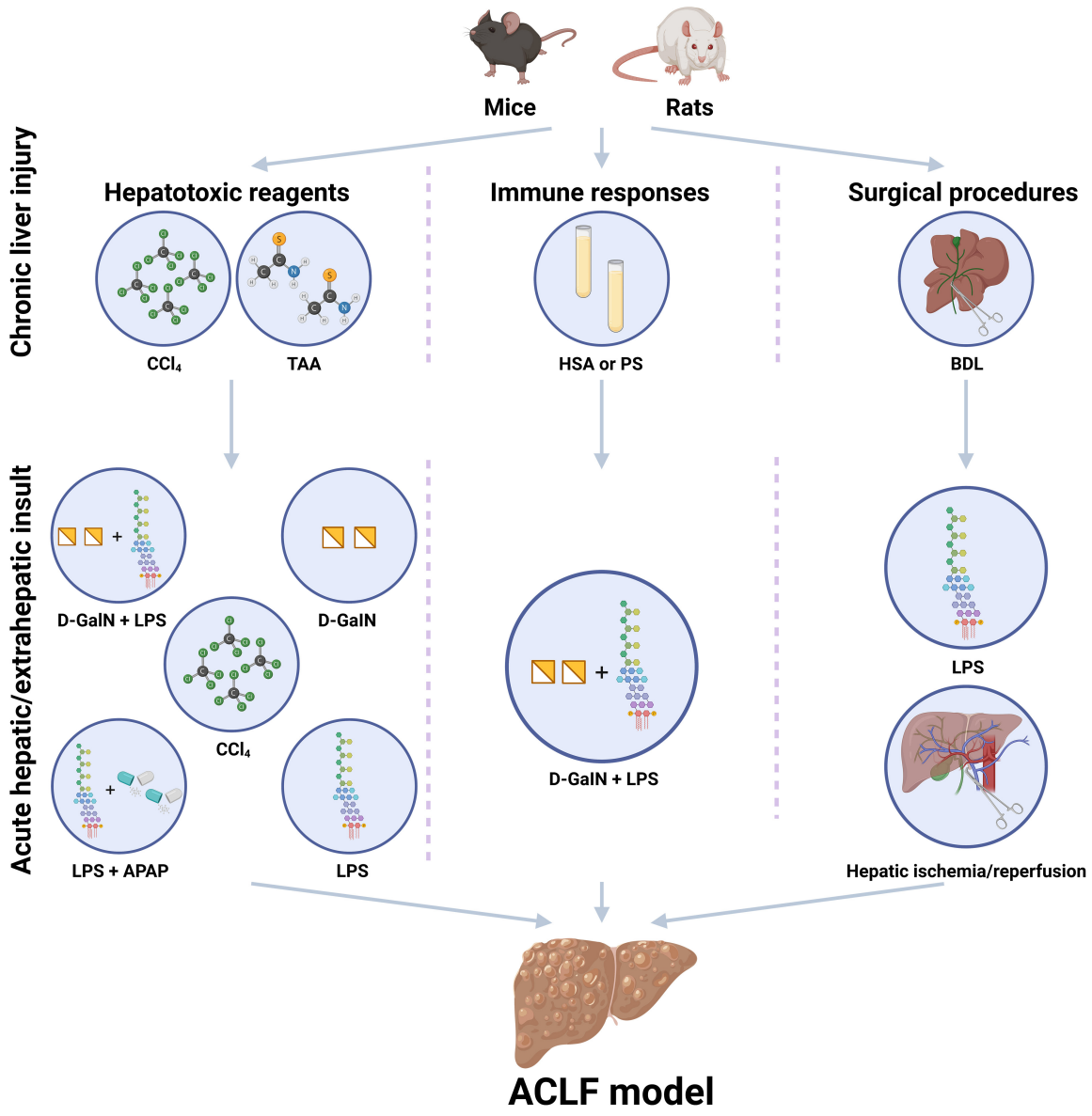


FIGURE 1
Existing experimental animal models for the study of acute-on-chronic liver failure (ACLF). The existing experimental animal models for ACLF usually contain the steps of chronic liver injury and acute hepatic/extrahepatic insult and could be classified into three major patterns, including ACLF models induced by hepatotoxic reagents, immune responses, or surgical procedures. Hepatotoxic reagents usually include carbon tetrachloride ($CCl_4$), D-galactosamine (D-GalN), acetaminophen (APAP), thioacetamide (TAA) concanavalin A (Con A), and lipopolysaccharide (LPS). Immune responses induced ACLF models are usually based on heterologous serum or serum constituent, such as human serum albumin (HSA) and porcine serum (PS). Surgical procedures induced liver injury includes common bile duct ligation (BDL) surgery, partial hepatectomy (HPx) and hepatic ischemia/reperfusion. D-GalN and LPS are always used as acute insults. $CCl_4$, carbon tetrachloride; PS, porcine serum; HSA, human serum albumin; BDL, bile duct ligation; TAA, thioacetamide administration; LPS, lipopolysaccharide; D-GalN, D-galactosamine; APAP, acetaminophen. (Created with BioRender.com).

lung bacterial infection model, animals are given *K.P.* through a non-invasive intratracheal intubation (85).

## 5. Existing experimental animal models for ACLF

Currently, the existing experimental animal models for ACLF could be classified into three major types, including ACLF models induced by hepatotoxic reagents, immune responses, or surgical procedures respectively, (Figure 1), which are created *via* the combination of the above-mentioned methods sequentially to simulate the pathogenic course of this devastating disease. The following displays the principles and methods used in the existing experimental animal models for ACLF (Table 1).

### 5.1. Hepatotoxic reagents induced ACLF models

Hepatotoxic reagents induced ACLF models are the most commonly used models and suitable for mimicking most clinical cases.

### 5.1.1. $CCl_4$ + D-GalN/LPS

The combination of repeated $CCl_4$ administration and subsequent D-GalN/LPS could perfectly mimic the chronic liver injury and acute insult of ACLF. Repeated treatments of $CCl_4$ result in chronic liver injury which would lead to fibrosis in mice or cirrhosis in rats. Moreover, the systemic inflammatory response caused by bacterial infection are also simulated by LPS, one of the typical PAMPs secreted by Gram-negative bacteria.

As illustrated in **Table 1**, $CCl_4$ is the most frequently used method to establish ACLF models. Normally, rats are selected to administrate $CCl_4$ *via* intraperitoneal injection, subcutaneous injection, intragastric gavage or inhalation for 6–8 or 8–12 weeks to induce the chronic liver injury with a fibrotic or cirrhotic state (12, 86–88), then D-GalN alone or D-GalN plus LPS are administrated (86, 89). Meanwhile, ACLF models can also be established in mice treated with $CCl_4$ for 6–8 weeks to a fibrotic state then following the D-GalN/LPS administration (90–94).

Ni et al. (95) dissolved $CCl_4$ in peanut oil [(volume, 1: 1) 1.5 ml/kg in the first month, 2.0 ml/kg weight in the second month], and further injected with LPS (80 μg/kg) and D-GalN (500 mg/kg) to induce ACLF, aiming to illustrate the mechanism of degradation of regulatory T cells. Tripathi et al. (12) summarized three ACLF models including BDL, $CCl_4$, and TAA induced liver cirrhosis, respectively, to verify the protective efficacy of Simvastatin. In this study, $CCl_4$ group received $CCl_4$ inhalation 3 times weekly for 10 weeks combined with phenobarbital in drinking water (0.3 g/L) in order to short the period to form liver cirrhosis.

The combination of $CCl_4$ and D-GalN/LPS for inducing ACLF models is easily to perform and suitable for mimicking most clinical cases. The key defect of these models is that the mean survival periods after treating with D-GalN/LPS are too short to conduct preclinical interventional studies.

### 5.1.2. TAA + LPS

It is reported that chronic liver injury induced by TAA in rats can lead to cirrhosis with typical features such as hepatic encephalopathy, abnormal coagulopathy and centrilobular necrosis (46, 47). Tripathi et al. (12) developed three chronic liver injury models in rats through $CCl_4$ inhalation, BDL, and TAA administration, respectively, followed by intraperitoneal or intravenous administration of LPS to mimic ACLF. It was confirmed that LPS administration in these cirrhotic rats could recapitulate the features of ACLF syndrome in some extent. For the TAA model, male Sprague–Dawley (SD) rats were treated intraperitoneally with TAA (250 mg/kg) twice a week for 10 weeks (96, 97) and then treated with LPS (1 mg/kg) to develop ACLF model (12). Though the combination of TAA and LPS is also easy to perform, reports about TAA plus LPS induced ACLF model are rare and details of this model need further studies to display and elucidate.

### 5.1.3. $CCl_4$ + APAP/LPS

It is theoretically possible that co-administration of APAP and LPS in $CCl_4$ induced chronic liver injury mice would develop a kind of experimental ACLF model. However, there is only one group has reported the establishment of ACLF model in this kind until 2021 (98). Nautiyal et al. (98) have confirmed that APAP plus LPS can be served as a hepatic insult for constructing ACLF model. In their study, mice were intraperitoneally administrated of $CCl_4$ (0.1–0.5 ml/kg) twice a week for 10 weeks, followed by APAP (350 mg/kg) and LPS (50 μg/kg) injection intraperitoneally (98). It is reported that progressive hepatocyte necrosis, liver failure, impaired regeneration, development of portal hypertension and multi-organ dysfunction were demonstrated in this new ACLF model after 11 days (98). This model showed a prolonged survival period after hepatic insult, which would surely provide us a choice to carry out interventional studies, whereas the high short-term mortality feature of ACLF patients was not showed in this study. It is worth trying to do further studies on this ACLF model in order to accumulate more evidence and details.

## 5.2. Immune responses induced ACLF models

Immune responses induced ACLF models are mostly used to imitate autoimmune liver cirrhosis or hepatitis virus induced liver cirrhosis. The occurrence of autoimmune disease mainly due to the dysregulation of immune response, which will result in the breakdown of immune tolerance, and further, lead to the immune mediated organ or tissue damage caused by host itself. The mechanism of autoimmune hepatitis induced liver cirrhosis is still unclear, but there are several hypotheses may account for it. Molecular mimicry is a process that immune system responses to self-components which are similar to external pathogens such as HBV or hepatitis C virus (HCV). Autoantibody like anti-nuclear Antibody (ANA) and smooth muscle antibody (SMA) can be found in these patients, indicating that HBV and HCV may play an important role in autoimmune hepatitis induced liver cirrhosis (99). Besides, genetic factors may also participate in the occurrence of autoimmune hepatitis. Donaldson (100) revealed that major histocompatibility complex (MHC) is associated with autoimmune hepatitis to a large degree. However, no matter what the trigger is, a mass of activated inflammatory cells, especially the CD4 + T helper/inducer cells such as Th1, Th2, and Th17 cells, should be responsible for this immune mediated organ or tissue damage via the secretion of IL-2, IL-6, IFN-γ, and TGF-β .

### 5.2.1. HSA + D-GalN/LPS

Human serum albumin, a heterologous serum constituent for murine, is usually used to mimic immune response or autoimmune disorder induced chronic liver cirrhosis in rats or fibrosis in mice. The combination of HSA and D-GalN/LPS to establish an ACLF model has a wide application in rats (56, 57, 101, 102). Lots of studies of ACLF are based on this model (57, 101–105). The major limitation of this model is the high mortality during the induction of chronic liver injury and the short survival period after acute hepatic insult like the D-GalN/LPS based models (56). Hu et al. (106) have reported that the mortality of rats during cirrhosis induction was 20% after 2 weeks and 60% after 3 weeks.

### 5.2.2. PS + D-GalN/LPS

To decrease the mortality during the period of HSA induced liver injury, other heterologous serum such as PS is selected as an alternative because of the low mortality (9).

Acute-on-chronic liver failure models of this combination are established *via* the administration of PS (0.5 ml) twice a week for 11 weeks or 8 weeks intraperitoneally, followed by injection of LPS (50–100 μg/kg) intravenously and D-GalN (600 mg/kg) intraperitoneally (9, 107–109). Recently, Hassan et al. (110) have

TABLE 1 The animal models used for the study of acute-on-chronic liver failure (ACLF).

| Animal | Chronic liver injury/Fibrosis | Acute liver injury/Insult | Bacterial infection | Mortality during chronic injury | Mean survival time after acute insult | References |
|---|---|---|---|---|---|---|
| Wistar | 20% HSA to induce liver injury/Fibrosis.<br>First subcutaneous injection of HSA 4 mg for 24 days.<br>Second intravenous injection of HSA 2.5–4 mg for 2 months | D-GalN 400 mg/kg.<br>LPS 100 μg/kg.<br>Injected intraperitoneally. | None | 20–30% | 16.1 ± 3.7 h.<br>Less than 1 day | Liu et al. (102) |
| Sprague–Dawley | Dissolution of $CCl_4$ in peanut oil (volume, 1: 1).<br>Injection of $CCl_4$ at a dose of 1.5 mL/kg in the 1st month and 2.0 mL/kg in the 2nd month once every 3 days.<br>Injected intraperitoneally | D-GalN 500 mg/kg.<br>LPS 80 μg/kg.<br>Injected intraperitoneally. | None | 11.25% | Less than 1 day | Ni et al. (95) |
| Sprague–Dawley | Dissolution of $CCl_4$ in peanut oil (10%).<br>Doses are modified according to liver function and body weight of rats.<br>Injected intraperitoneally | D-GalN 700 mg/kg.<br>Injected intraperitoneally | None | Not described | 2–3 days | Zhang et al. (86) |
| Wistar | $CCl_4$ inhalation 3 times a week for 15–16 weeks and received phenobarbital (0.3 g/l) in drinking water | LPS 1 mg/kg.<br>Injected intraperitoneally | None | Not described | Less than 1 day | Tripathi et al. (12) |
| Wistar | Injection of porcine serum at a dose of 0.5 mL twice per week for 11 weeks.<br>Injected intraperitoneally | LPS 50 μg/kg.<br>Injected intravenously.<br>D-GalN 600 mg/kg.<br>Injected intraperitoneally | None | Not described | Less than 1 day | Li et al. (9) |
| Sprague–Dawley | BDL | LPS 1 mg/kg.<br>Injected intraperitoneally | None | 10–20% | Not described | Shah et al. (113)<br>Balasubramaniyan et al. (11) |
| Wistar | BDL | Hepatic ischemia/Reperfusion | None | Not described | Not described | Hu et al. (106) |
| Sprague-Dawley | Dissolution of TAA in saline (250 mg/kg).<br>Injected intraperitoneally.<br>Twice a week, for 10 weeks | LPS 1 mg/kg<br>Injected intraperitoneally or intravenously | None | Not described | Less than 1 day | Tripathi et al. (12) |

*(Continued)*





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TABLE 1 (Continued)

| Animal | Chronic liver injury/Fibrosis | Acute liver injury/Insult | Bacterial infection | Mortality during chronic injury | Mean survival time after acute insult | References |
|---|---|---|---|---|---|---|
| BALB/c | Dissolution of $CCl_4$ dissolved in mineral oil (20%).<br>Injection of $CCl_4$ at a dose of 2 uL/g.<br>Injected intraperitoneally twice a week for 6 weeks | D-G alN 500 μg/g.<br>LPS 10 ng/g.<br>Injected intraperitoneally | None | Not described | Not described | Bai et al. (94) |
| C57BL/6 | Dissolution of $CCl_4$ in olive oil.<br>Injection of $CCl_4$ at a dose of 0.5 mL/kg.<br>Gavage twice weekly for 6 weeks | LPS 4 mg/kg.<br>Injected intraperitoneally | None | Not described | Not described | Engelmann et al. (91) |
| C57BL/6 | Dissolution of $CCl_4$ in olive oil.<br>Injection of $CCl_4$ at a dose of 0.5 mL/kg.<br>Gavage twice weekly for 6 weeks | GalN 1,000 mg/kg.<br>Injected intraperitoneally | None | Not described | Not described | Kondo et al. (92) |
| C57BL/6 | Dissolution of $CCl_4$ in olive oil.<br>Injection of $CCl_4$ at a dose of 0.1 mL/kg for first 3 weeks;<br>0.2 mL/kg for next 3 weeks; 0.5 mL/kg for last 4 weeks.<br>Injected intraperitoneally twice a week for 10 weeks | LPS (50 μg/kg) + APAP (350 mg/kg).<br>Injected intraperitoneally | None | Not described | Not described | Nautiyal et al. (98) |
| BALB/c | Dissolution of $CCl_4$ in olive oil (10%) 10% $CCl_4$ (5 mL/kg)<br>twice a week for 8 weeks.<br>Injected intraperitoneally | 50% $CCl_4$ 4 mL/kg.<br>Injected intraperitoneally | None | Not described | Not described | Zhang et al. (93) |
| C57BL/6J | Dissolution of $CCl_4$ in olive oil (volume, 1: 9).<br>0.2 mL/kg twice a week for 8 weeks.<br>Injected intraperitoneally | $CCl_4$ 0.4 mL/kg on the 1st day of week 9.<br>*Klebsiella pneumonia* 1,000 CFU/mouse.<br>Injected intraperitoneally | Yes | None | 3–5 days | Xiang et al. (14) |
| C57BL/6J | Dissolution of $CCl_4$ in olive oil (volume, 1: 9).<br>Injection of $CCl_4$ at a dose of 0.2 mL/kg twice a week for<br>8 weeks.<br>Injected intraperitoneally. | $CCl_4$ 0.4 mL/kg on the 1st day of week 9.<br>Injected intraperitoneally.<br>Cecal ligation and puncture (CLP) surgery on<br>the 2nd day. | Yes | None | 3–5 days | Xiang et al. (14) |
| C57BL/6J | Dissolution of $CCl_4$ in olive oil (volume, 1: 9).<br>0.2 mL/kg twice a week for 8 weeks.<br>Injected intraperitoneally. | $CCl_4$ 0.4 mL/kg on the 1st day of week 9.<br>*Salmonella typhimurium* 8,000 CFU/mouse.<br>Injected intraperitoneally. | Yes | None | 3–5 days | Zhang et al. (84) |





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optimized this combination to develop an ACLF rat model with PS administration (2 ml/kg, twice a week) for 12 consecutive weeks and LPS (100 μg/kg) plus D-GalN (800 mg/kg), demonstrating the classic features of ACLF. ACLF model in this combination has its own advantages in investigating ACLF based on immune mediated chronic liver diseases.

## 5.3. Surgical procedures induced ACLF models

Surgical procedure such as bile duct ligation is appropriate to mimic clinical cases suffer from cholestasis.

### 5.3.1. BDL + LPS

The combination of surgical procedure with chemical drugs to develop an ideal ACLF model is always an important research direction, and BDL is one of the most commonly used surgery (111). Rats or mice that endure BDL surgery would have an obstructive jaundice to reproduce the hyperbilirubinemia (64, 65). Cirrhosis or fibrosis is confirmed in these rats or mice with cholestasis. Subsequently, a single dose of LPS (11, 12, 91, 92, 112–117) would make the ACLF model established, and this model is widely used in recent years.

Nevertheless, high mortality in early phase after BDL surgery is frequently occurred because the surgical operation would certainly concurrent with tissue damage and high risk of infection especially in mice. Therefore, modified surgical procedures are created, such as reversible BDL (118) and partial BDL (119).

### 5.3.2. BDL + hepatic ischemia/reperfusion

The combination of BDL surgery and other operations which would cause liver damage to develop ACLF models is a feasible strategy. Surgical based models with liver injury include partial hepatectomy (PHx), hepatic ischemia/reperfusion, and CLP. There are no reports on the combination of BDL or CLP so far. Hu et al. (106) reported an ACLF model combining BDL and hepatic ischemia/reperfusion surgeries in rats to reflect the characteristics of patients progressed to ACLF after liver resection. A reduced-size hepatic ischemia/reperfusion injury procedure was used in this model (120), as well as partial hepatectomy (106). This ACLF model mimics the pathophysiological process, histological characteristics and surgical treatment process well, however, the surgical procedures are too complicated to perform which would limit its application.

## 5.4. The search for an optimal mouse model for ACLF

According to the current understanding and findings on the mechanism ACLF, the clinical course of ACLF could divide into three major stages, including chronic liver injury, acute hepatic/extrahepatic insult, and the excessive systemic inflammatory response caused by over-reactive immune system especially bacterial infection (14). However, due to the lack of optimal experimental animal model for ACLF, the progress of basic study on ACLF is limping. Though the above-mentioned experimental ACLF models were established, none of them can recapitulate and simulate the whole pathological process of ACLF patients.

Recently, we have developed a novel mouse model for ACLF combining chronic liver injury (injection of $CCl_4$ for 8 weeks, 0.2 ml/kg), acute hepatic insult (injection of a double dose $CCl_4$, 0.4 ml/kg), and bacterial infection (intraperitoneal injection of a single dose *K.P.*, 1,000 CFU/mouse) (**Figure 2**), recapitulating the major clinical features of patients with ACLF worsened by bacterial infection (14). Moreover, this ACLF model includes chronic liver injury, acute hepatic insult, bacterial infection, renal injury, high short-term mortality, which could simulate the major pathological course of ACLF patients (14). To our knowledge, we introduced for the first time an easy double dose of $CCl_4$ injection as acute hepatic insult and a single dose of viable *K.P.* injection to mimic bacterial infection that occurred in most ACLF patients. In addition, systemic inflammatory responses induced by both PAMPs and DAMPs were fully simulated in this model. Importantly, the survival period of this ACLF model has been prolonged to 5–7 days after acute insult, which provide appropriate time for preclinical interventional researches, such as drug screening.

The establish process of this ACLF model was not go well in the beginning. In brief, we first combined $CCl_4$ injection with CLP surgery to test if an ACLF model could be developed. Repeated $CCl_4$ injection combined with CLP surgery could generate a model with high short-term mortality and sepsis like symptoms, however, no ALT

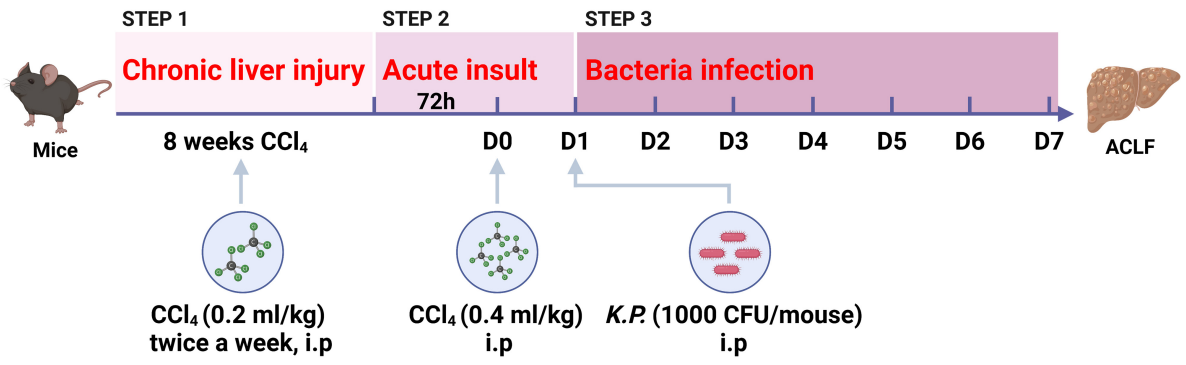


FIGURE 2
Schematic timeline of the three-step mouse model of acute-on-chronic liver failure (ACLF). Mice were administrated with carbon tetrachloride ($CCl_4$) for 8 weeks to induce chronic liver injury, followed by double dosage of $CCl_4$ (0.4 ml/kg) injection to induce acute hepatic insult, and *Klebsiella pneumoniae* (*K.P.*) were injected intraperitoneally to induce bacterial infection at 24 h post-acute insult. (Created with BioRender.com).

or AST elevation was found even the mice died. Thus, when a double dose of $CCl_4$ was added as the acute hepatic insult in the chronic $CCl_4$ treated mice, followed by CLP surgery, an ideal ACLF model was established with the three major stages, including chronic liver injury (0.2 ml/kg, $CCl_4$ injection twice a week), acute hepatic (a double dose of $CCl_4$ injection) insult and polymicrobial infection (CLP surgery) (14). However, the defects of CLP-based ACLF model are obvious. First, it is hard to accurately control the spillage of cecal contents into the peritoneal cavity. Second, the surgical wounding has influence on the pathogenesis of the end stage liver failure. Third, the CLP surgical procedure is more time consuming to some extent.

To improve the CLP-based ACLF model, CS injection was adopted for the substitution of CLP induced polymicrobial infection. We prepared the CS solution according to a recently published protocol and administrated a suggested high dose of 200 μl/mouse in mice (121). However, no mortality was observed in mice injected with CS though the blood cultures showed positive results of bacteriotoxemia (122).

Subsequently, we turned to use single bacterial infection to replace the CLP or CS induced polymicrobial infection. Clinically, *Escherichia coli* (*E. Coli*) and *K.P.* are in the top rank of pathogens in ACLF patients (123). Different doses of *E. Coli* were first sent to the test. Surprisingly, mortality in mice was able to be observed after *E. Coli* injection till at a dose of $10^8$ CFU/mouse (14), which is too high to apply. Subsequently, different doses of *K.P.* were tested and an optimal mortality with appropriate survival period was found at a dose of 1,000 CFU/mouse (14). Meanwhile, *Salmonella* at a dose of 8,000 CFU/mouse was found similar results like *K.P.* (84). Therefore, a three-step ACLF model has been developed in mice, which could not only recapitulate the major three stages of ACLF, but also prolong the animal survival period with longer observation and interventional time for screening drugs and mechanism studies (**Figure 2**).

## 6. Prospect and conclusion

The efforts for developing an optimal animal model for the study of ACLF are far from over. Along with the further understanding for pathophysiological mechanism of ACLF, more precise and perfect animal models would be established in the near future. To date, there are three main patterns of ACLF experimental models worldwide, which were induced via hepatotoxic reagents, immune responses, and surgical procedures, respectively. Generally, each pattern of ACLF model always reflects a particular aspect of ACLF patients clinically, and it is very difficult to construct a single model to meet all the aspects for the study of ACLF. Similar like the controversy in the definitions and criteria of ACLF among APASL, ESAL, and AASLD, animal models for ACLF would not be consistent till there is a universal agreement on the mechanism and definition of ACLF globally. At present, on the way to further reveal and elucidate the pathogenesis of ACLF, the optimal animal model of ACLF should be selected by the purpose of the study.

## Author contributions

XX, HZ, and JZ wrote the manuscript. XX, DS, and CZ reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Baclofen as a therapeutic option for gastroesophageal reflux disease: A systematic review of clinical trials

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**Background:** The main components of gastroesophageal reflux disease (GERD) management include a combination of medications and lifestyle modifications; Nevertheless, based on the severity of symptoms and their response to medications, other treatments could be considered. Baclofen has been demonstrated in studies to relieve GERD symptoms. The current study aimed to precisely address the effects of baclofen on the treatment of GERD and its characteristics.

**Methods:** A systematic search was carried out in Pubmed/Medline, Cochrane CENTRAL, Scopus, Google Scholar, Web of Science, and clinicaltrials.gov up to December 10, 2021. The search terms included baclofen, GABA agonists, GERD, and reflux.

**Results:** We selected 26 papers that matched the inclusion criteria after examining 727 records. Studies were classified into four categories based on the study population and reported outcomes: (1) adults, (2) children, (3) patients with gastroesophageal reflux-induced chronic cough, (4) hiatal hernia patients. The results revealed that baclofen can significantly improve reflux symptoms and pH-monitoring and manometry findings to different degrees in all four mentioned categories; although its effect on pH-monitoring parameters seems less significant than the other parameters. Mild neurological and mental status deterioration were the most reported side effects. However, side effects occurred in a portion of less than 5% of short-term users and nearly 20% of long-term users.

**Conclusion:** In PPI-resistant patients, a trial of adding baclofen to the PPI may be helpful. Baclofen therapies may be more beneficial for symptomatic GERD patients who also report concurrent conditions including alcohol use disorder, non-acid reflux, or obesity.

**Systematic review registration:** https://clinicaltrials.gov/.

KEYWORDS

**baclofen (PubChem CID: 2284), gastroesophageal reflux disease (GERD), reflux, GABA agonist B, refractory, benign esophageal disease**

# 1. Introduction

Gastroesophageal reflux disease (GERD) is a clinical condition caused by the chronic retrograde reflux of acidic contents of the stomach into the esophagus with discomforting symptoms or complications or both (1). Gastroesophageal reflux (GER) is a physiological condition during infancy and childhood and may not require treatment. In children, GERD is the symptomatic reflux of gastric contents into the esophagus and should be treated according to the severity of symptoms (2, 3). Global surveys in 2017 estimated an 18.1% increase in the total prevalence of GERD cases and a 67.1% increase in the years lived with disability (YLD) compared to 1990 (4). These findings suggest GERD as a public health concern with a considerable socioeconomic burden in the near future.

Diagnosis of GERD is based on clinical symptoms (heartburn, regurgitation, and non-cardiac chest pain) and response to empiric proton pump inhibitors (PPIs). Nonetheless, studies have shown limitations of non-objective diagnosis; as a result, diagnostic evaluation such as upper endoscopy is recommended based on the clinical setting, especially in patients with red flags like dysphagia (5).

The main components of GERD management are lifestyle modification and PPIs. Nevertheless, based on the severity of symptoms and their response to PPIs, H2blockers, baclofen, antacids, sucralfate, prokinetic agents, and invasive anti-reflux procedures (such as surgeries, sphincter augmentation, and endoscopic therapy) are considered in combination with other treatments or as the replacement therapy for patients. Consideration is required based on efficacy and tolerability profile of each treatment option (5–7).

The pathophysiology of GERD is multifactorial and is explained by natural anti-reflux barrier. Studies suggest following mechanisms for GERD: the hypotonic lower esophageal sphincter (LES), hiatal hernia, Gubaroff valve failure, and thoraco-abdominal pressure (8, 9). Also, studies have shown that baclofen has been highly beneficial in the treatment of refractory GERD. Baclofen is an FDA-approved agonist of the gamma-aminobutyric acid (GABA) receptor, which is generally used for the relaxation of pathologic spasms originating from the central nervous system (CNS). Its mechanism of action on GERD is by inhibition of LES relaxation induced *via* vasovagal reflexes. Baclofen inhibits these reflexes through $GABA_B$ receptor activation (10, 11).

Although previous studies proved effectiveness of baclofen on GERD (12), to the date, there are no systematic reviews regarding outcomes and side effects in different patient (grouped by: age, and comorbidities); A systematic review in that regard might resolve probable hesitancies in prescribing baclofen. Therefore, we will conduct this systematic review, aiming to facilitate informed decisions in managing GERD using baclofen. To achieve this goal we will review efficacy, side effects, and response predictors in different patients grouped by: age (adult vs. pediatrics), comorbidities (hiatal hernia and GERD-related chronic cough), and some other factors.

Abbreviations: GABA, gamma-aminobutyric acid; GER, gastroesophageal reflux; GERC, gastroesophageal reflux-induced chronic cough; GERD, gastroesophageal reflux disease; HH, hiatal hernia; LES, lower esophageal sphincter; PPI, proton pump inhibitor; TLESR, transient lower esophageal sphincter relaxation.

# 2. Materials and methods

This study was conducted and reported according to the Preferred Reported Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (13).

## 2.1. Search strategy

We searched Pubmed/Medline, Cochrane CENTRAL, Scopus, Google Scholar, Web of Science, and Clinicaltrials.gov for studies reporting the efficacy/effectiveness of baclofen in patients with GERD, published up to December 10, 2021. The search terms were baclofen, GABA agonists, GERD, and reflux. No language restrictions were imposed.

## 2.2. Study selection

The records found through database searching were merged, and the duplicates were removed using EndNote X9. Two authors independently screened the records by title/abstract and full-texts to exclude those unrelated to the study topic. Included studies met the following inclusion criteria: (i) patients were diagnosed with GERD based on a defined criterion; (ii) patients were treated with baclofen; and (iii) treatment outcomes were recorded. Conference abstracts, reviews, experimental studies on animal models, and articles that their full-text or original data were not available were excluded.

## 2.3. Data extraction

Two authors designed a data extraction form. These reviewers extracted the following items from all eligible studies: first author's name, year of publication, country/ies where the research was conducted, type of epidemiological study, demographics, treatment protocols, adverse effects, and outcomes. Data was inserted into an excel sheet, and differences were resolved by consensus.

## 2.4. Quality assessment

The checklists provided by the National Institute of Health (NIH) for controlled intervention and before-after (pre-post) studies with no control group were used to perform the quality assessment (14).

# 3. Results

We investigated a total of 952 records found in the systematic search; after removing duplicates and full-text reviews, 26 were chosen. Studies included and excluded through the review process are summarized in **Figure 1** and **Supplementary Table 1**. Among the included studies, there were 9 crossover RCTs, 8 RCTs, and 9 single-arm clinical trials. The studies originated from twelve

countries: United States ($n$ = 6), China ($n$ = 4), Iran ($n$ = 3), Australia, Belgium, Italy, Sweden ($n$ = 2, for each one), Switzerland, the Netherlands, Mexico, Japan, and Germany ($n$ = 1, for each one) (Table 1). Two of the studies had two separate parts (15, 16), so we looked at these parts separately and overviewed 28 studies as a whole. All studies assessed baclofen efficacy based on clinical status, pH monitoring, or manometry findings. Additionally, in most trials, the safety of treatment was evaluated by the occurrence of adverse events or side effects.

## 3.1. Quality of included studies

Based on the NIH checklists for controlled intervention and before-after (pre-post) studies with no control group, the included studies had a low risk of bias (Supplementary Tables 2, 3).

## 3.2. Patient characteristics

Except for one study that did not report the number of patients (17), the remaining 27 trials included 785 patients who got baclofen and 358 who received control medication. The baclofen groups included individuals aged 7.1 months (infants) to 58 years (adults). The most frequently utilized methods for diagnosing GERD were, in order, history, pH monitoring, manometry, and endoscopy (Table 2).

## 3.3. Intervention characteristics

In twenty-two studies, the treatment regimen consisted only of baclofen, whereas in the six remaining studies, the treatment regimen consisted of baclofen and a PPI (18–23). The majority of studies used baclofen for at least 1 week; however, some used shorter treatment periods to investigate baclofen's acute effects. In all studies, treatments were administered orally, except one that also administered enteric baclofen (24) (Table 3).

## 3.4. Outcomes

### 3.4.1. Safety and side effects

Among 28 studies, four studies did not investigate treatment side effects (17, 25–27). No treatment-related severe adverse events were observed in the remaining 24 studies. The adverse effects noted were somnolence among 14.2% of participants, dizziness (10.0%), fatigue (4.9%), nausea (1.6%), gastrointestinal symptoms (0.9%), headache (0.7%), anxiety (0.2%), and a slight reduction in muscular tone (0.2%). The most frequently reported adverse effects were neurological and mental status deterioration (particularly dizziness and somnolence). However, some of these events were not induced by baclofen; rather, they were caused by the other underlying comorbidities. The side effects occurred in a portion of less than 5% of short-term users (less than 4 weeks) and nearly 20% of long-term users (more than 4 weeks), and also occurred during placebo therapy in certain studies. Baclofen had no adverse effects in seven studies, and was well tolerated (11, 16, 18, 21, 28–30) (Table 4).

### 3.4.2. Efficacy

Studies were classified into four categories based on the study population and reported outcomes: (1) adults, (2) children, (3) patients with gastroesophageal reflux-induced chronic cough, and (4) hiatal hernia patients.

#### 3.4.2.1. Outcomes in adults

This category contained seventeen studies. These publications evaluated baclofen's efficacy using changes in clinical status, acid reflux time, TLESR incidence, GER incidence, LES pressure, and some other parameters.

Eleven trials reported changes in clinical status. Baclofen significantly improved clinical symptoms in seven studies. However, Bajbouj et al. (19) and Zhang et al. (11) discovered no significant improvement following baclofen treatment. According to Abbasinazari et al. (18) baclofen alleviated esophageal symptoms (heartburn and regurgitation) but had no significant effect on extra-esophageal symptoms (chest pain and hoarseness). Baclofen reduced belching with no effect on other reflux symptoms in Cange et al.'s (31) trial.

Changes in acid reflux time were documented in eleven studies. Baclofen significantly decreased acid reflux time in five studies. However, four studies found that baclofen has no significant effect on acid reflux time (11, 15, 19, 20). Orr et al. (30) reported that baclofen reduced recumbent acid contact time by 50% compared to placebo, although the difference was not statistically significant. In Curcic et al.'s (28) study, baclofen increased reflux duration by a small but significant degree.

Four studies reported changes in TLESR incidence. All of them mentioned that baclofen has a significant reducing effect on TLESR incidence (11, 32–34).

Thirteen studies reported changes in GER incidence. All of them declared baclofen had a significant effect on reducing GER incidence, except for two studies: (a) Koek et al. (20) reported that after adding baclofen, the number of acid reflux episodes remained unchanged, but the number of duodenal reflux episodes and the number of duodenal reflux episodes lasting longer than 5 min decreased significantly. (b) Bajbouj et al. (19) observed no significant changes in reflux episodes, before and after adding baclofen. Additionally, two trials examined the effect of body posture on efficacy of baclofen (15, 25). Both of them suggested that baclofen decreased reflux episodes in the upright position significantly, while reflux episodes in the supine position did not change significantly.

Six studies investigated changes in LES pressure. Baclofen significantly elevated LES pressure in four studies (11, 28, 32, 33). Cossentino et al. (25) and van Herwaarden et al. (34) on the other hand, found no difference in LES pressure between the baclofen and placebo groups. All outcomes are present in more detail in Table 5.

#### 3.4.2.2. Outcomes in children

Six studies were included in this category. The mean age of the participants ranged between 7.1 months and 10.0 years.

Four studies reported changes in clinical status. All of them confirmed baclofen's significant efficacy in the improvement of clinical status by symptom remission, weight gain, or reduction in crying and restlessness.

Three studies evaluated the efficacy of baclofen in children using invasive GI procedures (pH monitoring or esophageal

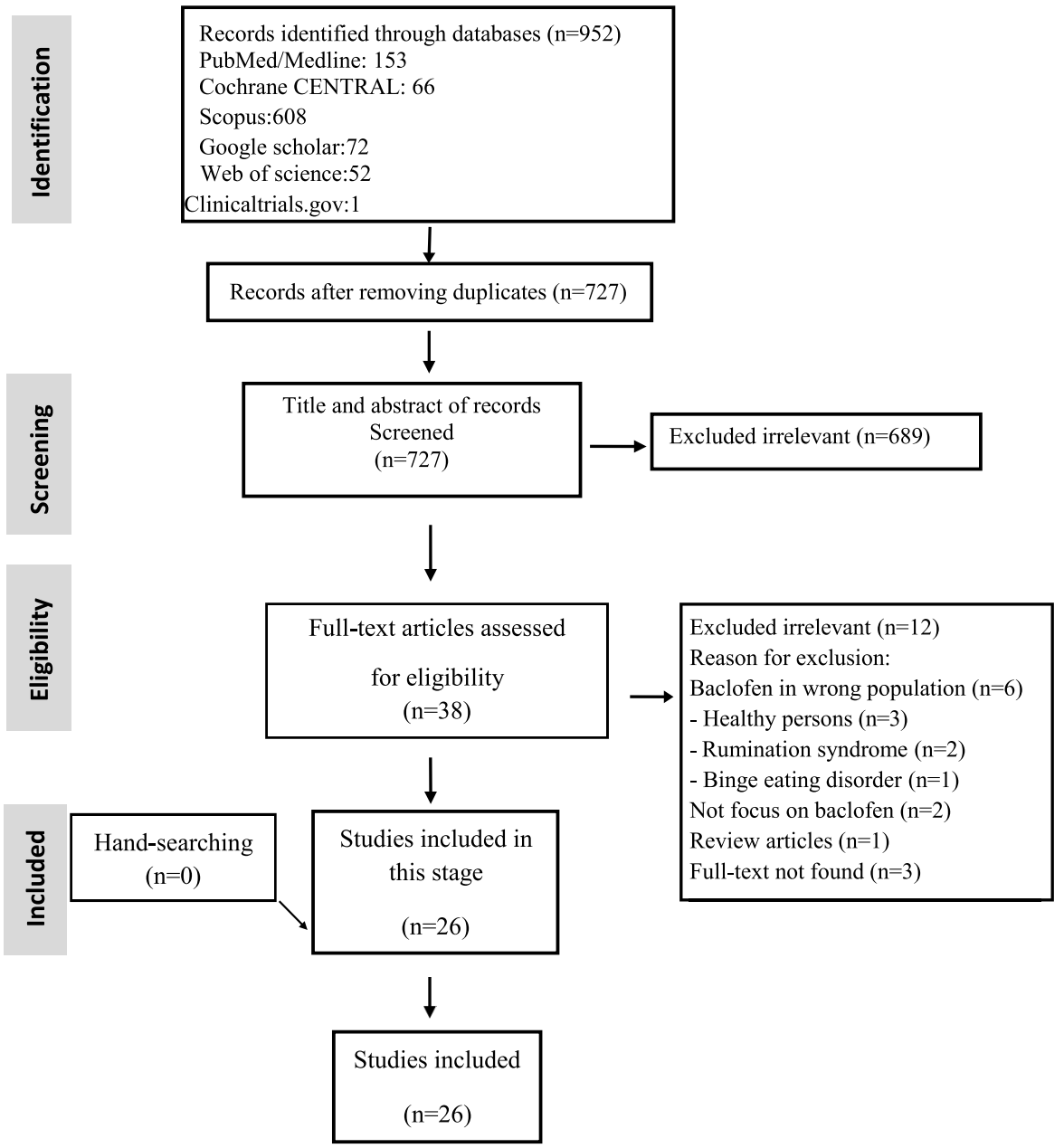


FIGURE 1
Flow chart of study selection for inclusion in the systematic review and meta-analysis.

manometry). Omari et al. (35) and Dibner (17) found that children receiving baclofen had considerably lower TLESR and acid GER compared to children receiving a placebo. In Kawai et al.'s (24) study, the total number of acid reflux events was reduced significantly during the postprandial and entire 24-h periods. However, they found no significant changes in total acid exposure time, the percentage of time with esophageal pH < 4 and the duration of the longest acid reflux resulting from baclofen (24) (**Table 6**).

#### 3.4.2.3. Outcomes in patients with GERC

In this category, four studies were included. Cough period ranged from 12.6 to 36 months on average. The daytime cough symptom score was greater (3, 4) than the night time cough symptom score (1, 2). Overall, baclofen was effective in treating GERC in 122 of 214 (57.0%) individuals. Outcomes are available in more detail in **Table 7**.

#### 3.4.2.4. Outcomes in patients with HH

Three studies were included in this category because they featured a subgroup of patients with HH. Cange et al. (31) reported a significant reduction in acid reflux time and reflux episodes after receiving baclofen, compared to placebo. Beaumont et al. (15) found no significant changes in total acid exposure time and the percentage of time with pH < 4 in patients with HH after administration of baclofen and placebo. However, they observed

TABLE 1 Characteristics of included studies.

| References | Country | Study design | Purpose |
|---|---|---|---|
| Curcic et al. (28) | Switzerland | RCT, crossover | Effects of baclofen on the functional anatomy of the OGJ and proximal stomach in adult GERD patients |
| Abbasinazari et al. (18) | Iran | RCT | Effect of co-administration of omeprazole plus baclofen compared to omeprazole plus placebo on alleviation of symptoms in adult patients with GERD |
| Ciccaglione et al. (16) | Italy | RCT | Effects of acute and chronic administration of baclofen on 24 h esophageal and gastric pH patterns in adult patients with GERD |
| Beaumont et al. (15) | Netherlands | RCT, crossover | Effect of baclofen during PPI treatment on gastroesophageal reflux in GERD patients with no hiatal hernia compared to those with a large hiatal hernia |
| Cange et al. (31) | Sweden | RCT, crossover | Effect of baclofen on esophageal acid exposure in adult patients with GERD |
| Cossentino et al. (25) | USA | RCT | Effect and tolerability of baclofen in adult GERD patients over 2 weeks |
| Dibner et al. (17) | Mexico | RCT | Effect of baclofen on TLESR, gastroesophageal reflux and gastric emptying in children with GERD |
| Gerson et al. (54) | USA | RCT, crossover | Efficacy and safety of Arbaclofen Placarbil for decreasing meal-induced reflux episodes in adult patients with GERD |
| Grossi et al. (32) | Italy | RCT | Effect of baclofen on 24-h esophageal and LES motility in a group of GERD patients after multiple oral doses of the drug |
| Omari et al. (35) | Australia | RCT | Effect of baclofen on the rates of TLESR, gastroesophageal reflux, and gastric emptying in children with GERD. |
| van Herwaarden et al. (34) | Sweden | RCT, crossover | Effect of baclofen on reflux symptoms, esophageal pH and lower esophageal sphincter manometry in GERD patients. |
| Vela et al. (27) | USA | RCT, crossover | Compare the frequencies of postprandial GER and associated symptoms after treatment with placebo and baclofen in heartburn patients |
| Scarpellini et al. (33) | Belgium | RCT, crossover | Investigate the effect of baclofen on the presence and extension of an acid pocket in naive GERD patients with heartburn as the predominant symptom |
| Zhang et al. (11) | Australia | RCT, crossover | Effect of baclofen on TLESRs and postprandial gastro-esophageal reflux in patients with reflux disease. |
| Orr et al. (30) | USA | RCT, crossover | To determine if baclofen would significantly reduce reflux during sleep, and also improve objective and subjective measures of sleep |
| Sobhani Shahmirzadi et al. (21) | Iran | RCT | Effect of baclofen in pediatric GERD |
| Vakil et al. (26) | USA | RCT | Efficacy and safety of arbaclofen placarbil over 4 weeks in symptomatic GERD patients |
| Vadlamudi et al. (55) | USA | Single-arm clinical trial | Evaluate the efficacy of baclofen on symptoms in children with refractory GERD |
| Xu et al. (56) | China | Single-arm clinical trial | efficacy of baclofen in treating patients with refractory chronic cough induced by gastroesophageal reflux resistant to PPIs |
| Xu et al. (23) | China | Single-arm clinical trial | Efficacy and safety of baclofen for the treatment of refractory gastroesophageal reflux-induced chronic cough unresponsive to standard anti-reflux therapy |
| Xu et al. (22) | China | Single-arm clinical trial | Efficacy of baclofen for treating refractory gastroesophageal reflux-induced chronic cough unresponsive to omeprazole and ranitidine |
| Zhu et al. (42) | China | Single-arm clinical trial | Baclofen effect on pressure and length of the lower esophageal sphincter as predictive indicators of therapeutic efficacy for refractory gastroesophageal reflux-induced chronic cough |
| Kawai et al. (24) | Japan | Single-arm clinical trial | Effects of baclofen on GERD in neurologically impaired children |
| Khodadad et al. (29) | Iran | Single-arm clinical trial | Efficacy of baclofen on lower esophageal sphincter in infants |
| Koek et al. (20) | Belgium | Single-arm clinical trial | Effect of baclofen in patients with persistent non-acid duodenal reflux during PPI therapy |
| Bajbouj et al. (19) | Germany | Single-arm clinical trial | Influence of an anti-reflux therapy with 80 mg esomeprazole plus baclofen for the treatment of refractory GERD in adult patients |

GERD, gastroesophageal reflux disease; LES, lower esophageal sphincter; OGJ, oesophagogastric junction; PPI, proton pump inhibitor; RCT, randomized controlled trial; TLESR, transient lower esophageal sphincter relaxation.

that baclofen reduced the total number of reflux episodes compared to placebo. In addition, they made a comparison between patients with and without HH and found: (I) patients with a large HH did not show a significantly more proximal reflux compared to patients without HH. No correlation was found between the size of the HH and the proximal extent of the reflux ($r = 0.1$; $P = 0.75$); (II).

TABLE 2 Patients characteristics.

| References | Study design | Case definition | Control definition | Case population (M/F) | Control population (M/F) | Age (case/control) (yr) | Way of reflux diagnosis |
|---|---|---|---|---|---|---|---|
| Curcic et al. (28) | RCT, crossover | GERD patients with erosive esophagitis or pathological esophageal acid exposure who received baclofen | GERD patients with erosive esophagitis or pathological esophageal acid exposure who received placebo | 12 (7/5) | 12 (7/5) | 37 (37/37) | Endoscopy based on LA-classification and/or pathological findings on pH monitoring |
| Abbasinazari et al. (18) | RCT | Patients with a diagnosis of GERD who received baclofen in addition to omeprazole as treatment | Patients with a diagnosis of GERD who received placebo in addition to omeprazole as treatment | 25 (11/14) | 28 (13/15) | NR(41.0/36.8) | History (mayo gastroesophageal reflux questionnaire) |
| Ciccaglione et al. (16) (1) | RCT | Patients chose based on clinical symptoms indicating GERD for at least 3 months before enrollment who received baclofen for 1 day | Patients chose based on clinical symptoms indicating GERD for at least 3 months before enrollment who received placebo for 1 day | 15 | 13 | 40 | History (clinical symptoms) |
| Ciccaglione et al. (16) (2) | RCT | Patients chose based on clinical symptoms indicating GERD for at least 3 months before enrollment who received baclofen for 4 weeks | Patients chose based on clinical symptoms indicating GERD for at least 3 months before enrollment who received placebo for 4 weeks | 10 | 6 | 45 | History (clinical symptoms) |
| Beaumont et al. (15) (1) | RCT, crossover | GERD patients with no hiatal hernia taking a PPI for at least 3 months before the study who received baclofen (treatment with PPI was continued) | GERD patients with no hiatal hernia taking a PPI for at least 3 months before the study who received placebo (treatment with PPI was continued) | 16 (8/8) | 16 (8/8) | 54 | History |
| Beaumont et al. (15) (2) | RCT, crossover | Patients with a large hiatal hernia (≥3 cm) taking a PPI for at least 3 months before the study who received baclofen (treatment with PPI was continued) | Patients with a large hiatal hernia (≥3 cm) taking a PPI for at least 3 months before the study who received baclofen (treatment with PPI was continued) | 11 (7/4) | 11 (7/4) | 58 | History |
| Cange et al. (31) | RCT, crossover | GERD patients with a history of esophagitis and/or time of esophageal acid exposure (pH < 4) of more than 5% on 24-h pH monitoring who received baclofen | GERD patients with a history of esophagitis and/or time of esophageal acid exposure (pH < 4) of more than 5% on 24-h pH monitoring who received placebo | 20 (15/5) | 20 (15/5) | 41.2 (41.2/41.2) | History, Los Angeles classification grade A–C or pH monitoring |
| Cossentino et al. (25) | RCT | Symptomatic GERD patients with the evidence of upright or supine reflux on 24-h pH testing who received baclofen | Symptomatic GERD patients with the evidence of upright or supine reflux on 24-h pH testing who received placebo | 23 (17/6) | 20 (10/10) | 49 (47.2/50.3) | History (questionnaires to assess GERD symptoms), esophageal manometry, and 24-h pH monitoring |
| Dibner et al. (17) | RCT | Neurologically healthy children with GERD with failure of conventional treatment who received baclofen | Neurologically healthy children with GERD with failure of conventional treatment who received placebo | NR | NR | NR(range: 2.6 to 17.4 yr) | NR |
| Gerson et al. (54) | RCT, crossover | Patients with reported GERD symptoms occurring at least 3 times a week and 20 reflux episodes on impedance-pH monitoring over a period of 2 h who received baclofen | Patients with reported GERD symptoms occurring at least 3 times a week and 20 reflux episodes on impedance-pH monitoring over a period of 2 h who received placebo | 44 (22/22) | 44 (22/22) | 41 (41/41) | History (clinical symptoms) and pH monitoring |
| Grossi et al. (32) | RCT | Symptomatic GERD patients who received baclofen | Symptomatic GERD patients who received placebo | 14 | 7 | 43 | Endoscopy and manometric exam |
| Omari et al. (35) | RCT | Children with severe GERD who had failed to improve after routine therapeutic measures that received baclofen | Children with severe GERD who had failed to improve after routine therapeutic measures that received placebo | 15 (9/6) | 15 (8/7) | 10.0 (9.1/11.0) | History (clinical symptoms) |
| van Herwaarden et al. (34) | RCT, crossover | Symptomatic GERD patients who received baclofen | Symptomatic GERD patients who received placebo | 20 (12/8) | 20 (12/8) | 45.1 | History (clinical symptoms) |

*(Continued)*





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TABLE 2 (Continued)

| References | Study design | Case definition | Control definition | Case population (M/F) | Control population (M/F) | Age (case/control) (yr) | Way of reflux diagnosis |
|---|---|---|---|---|---|---|---|
| Vela et al. (27) | RCT, crossover | Heartburn patients receiving baclofen | Heartburn patients who received placebo | 9(6/3) | 9(6/3) | 36 (36/36) | History (clinical symptoms) |
| Scarpellini et al. (33) | RCT, crossover | Adult GERD patients with heartburn as predominant symptom who received baclofen as treatment | Adult GERD patients with heartburn as predominant symptom who received placebo | 13 (6/7) | 13 (6/7) | 29.8 | History (questionnaires) |
| Zhang et al. (11) | RCT, crossover | Symptomatic GERD patients with evidence of esophagitis on endoscopy who received baclofen | Symptomatic GERD patients with evidence of esophagitis on endoscopy who received placebo | 20(15/5) | 20(15/5) | 56.5 (56.5/56.5) | History (clinical symptoms) and endoscopy (hetzel grading) |
| Orr et al. (30) | RCT, crossover | Individuals with complaints of nighttime heartburn or regurgitation at least twice per week and a carlsson GERD score of at least 5 who received baclofen | Individuals with complaints of nighttime heartburn or regurgitation at least twice per week and a carlsson GERD score of at least 5 who received placebo | 21(9/12) | 21(9/12) | 43 (43/43) | History (carlsson GERD questionnaire) |
| Sobhani Shahmirzadi et al. (21) | RCT | 6 months to 12 years old children with GERD who received baclofen + PPI | 6 months to 12 years old children with GERD who received PPI | 54(27/27) | 58(20/38) | 6.61 | NR |
| Vakil et al. (26) | RCT | Patients with heartburn and/or regurgitation ≥3 days a week and receiving arbaclofen placarbil | patients with heartburn and/or regurgitation ≥3 days a week who received placebo | 125(56/69) | 31(15/16) | 41.8 (41.8/41.8) | History (clinical symptoms) |
| Vadlamudi et al. (55) | Single-arm clinical trial | Children ages 1 to 18 years with a known diagnosis of GERD receiving baclofen | NA | 53(34/19) | NA | 6.1 | NR |
| Xu et al. (56) | Single-arm clinical trial | Patients with GERC resistance to proton pump inhibitors who received baclofen | NA | 26-year-old male/42-year-old male/63-year-old female | NA | 46.3 | History (clinical symptoms) and pH monitoring |
| Xu et al. (23) | Single-arm clinical trial | Patients with suspected refractory GERC who received baclofen | NA | 16(9/7) | NA | 47.8 | History (clinical symptoms) and pH monitoring |
| Xu et al. (22) | Single-arm clinical trial | Patients with suspected refractory GERC unresponsive to omeprazole and ranitidine who received baclofen | NA | 57 | NA | NR | History (clinical symptoms) and pH monitoring |
| Zhu et al. (42) | Single-arm clinical trial | Patients with suspected refractory GERC unresponsive to standard anti-reflux therapy who received baclofen | NA | 138(66/72) | NA | 51.4 | Esophageal manometry and multichannel intraluminal impedance-pH monitoring |
| Kawai et al. (24) | Single-arm clinical trial | Neurologically impaired children with GERD who received baclofen | NA | 8 | NA | 3 | 24-h esophageal pH monitoring |
| Khodadad et al. (29) | Single-arm clinical trial | Infants with GERD (diagnosed by specific clinical criteria) for at least 1 month who received baclofen. | NA | 30(17/13) | NA | 7.1 months | History (clinical symptoms) |
| Koek et al. (20) | Single-arm clinical trial | GERD patients with persistent heartburn or regurgitation that treated for at least 3 months with omeprazole 20 mg twice daily who received baclofen. | NA | 16 (5/11) | NA | 46.2 | History (standardized questionnaire for symptoms), endoscopy, and esophageal pH monitoring |
| Bajbouj et al. (19) | Single-arm clinical trial | Adult GERD patients with persistent pathological pH/MII results despite receiving esomeprazole who were treated with baclofen + esomeprazol | NA | 7 | NA | NR | History (standardized questionnaire for symptoms) and pH monitoring |

GERC, gastroesophageal reflux-induced chronic cough; GERD, gastroesophageal reflux disease; LA, Los Angeles; NA, not applicable; NR, not reported; PPI, proton pump inhibitor; RCT, randomized controlled trial.





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TABLE 3 Intervention characteristics.

| References | Case treatment regimen | Treatment duration | Route of delivery |
|---|---|---|---|
| Curcic et al. (28) | Single dose baclofen (400 mg suspension) | Single dose (7 days washout time) | Oral |
| Abbasinazari et al. (18) | Sustained release baclofen 10 mg tablet BD with omeprazole 20 mg OD | 2 weeks | Oral |
| Ciccaglione et al. (16) (1) | Baclofen 10 mg QID | 1 day | Oral |
| Ciccaglione et al. (16) (2) | Baclofen 10 mg TDS for the first week, and then 10 mg QID for the next 3 weeks | 4 weeks | Oral |
| Beaumont et al. (15) (1) | Baclofen 5 mg TDS, gradually reached 20 mg TDS after 10 days | 12 days (at least 7 days washout time) | Oral |
| Beaumont et al. (15) (2) | Baclofen 5 mg TDS, gradually reached 20 mg TDS after 10 days | 12 days (at least 7 days washout time) | Oral |
| Cange et al. (31) | Baclofen 40 mg single dose | Single dose (at least 4 weeks washout time) | Oral |
| Cossentino et al. (25) | Baclofen 10 mg TDS, gradually reached 20 mg TDS after 6 days | 2 weeks | Oral |
| Dibner et al. (17) | Baclofen 0.5 mg/kg | Single dose | Oral |
| Gerson et al. (54) | Baclofen 10, 20, 40, or 60 mg single dose | Single dose (3–7 days washout time) | Oral |
| Grossi et al. (32) | Baclofen 10 mg QID | 1 day | Oral |
| Omari et al. (35) | 0.5 mg/kg baclofen (up to a maximum of 40 mg) single dose | Single dose | Oral |
| van Herwaarden et al. (34) | Baclofen 40 mg single dose | Single dose (3–10 days washout time) | Oral |
| Vela et al. (27) | Baclofen 40 mg single dose | Single dose (2–7 days washout time) | Oral |
| Scarpellini et al. (33) | Baclofen 40 mg single dose | Single dose (1 week washout time) | Oral |
| Zhang et al. (11) | Baclofen 40 mg TDS | 1 day (1 week washout time) | Oral |
| Orr et al. (30) | Baclofen 40 mg OD | 2 days (1 week washout time) | NR |
| Sobhani Shahmirzadi et al. (21) | Baclofen 0.25 mg/kg divided into two daily doses and omeprazole 1mg/kg | 1 month | Oral |
| Vakil et al. (26) | Baclofen 20, 40, or 60 mg once daily; or 30 mg twice daily | 4 weeks | Oral |
| Vadlamudi et al. (55) | Baclofen 0.5 mg/kg divided into 3 daily doses (maximum daily dose was 30 mg/day) | NR | Oral |
| Xu et al. (56) | Baclofen 20 mg TDS | 8 weeks | Oral |
| Xu et al. (23) | Baclofen 20 mg TDS + omeprazole 20 mg BD | 8 weeks | Oral |
| Xu et al. (22) | Baclofen 20 mg TDS + omeprazole 20 mg BD | 10.96 weeks (median time) | Oral |
| Zhu et al. (42) | Baclofen 10 mg TDS, gradually reached 20 mg TDS after 9 days | NR | Oral |
| Kawai et al. (24) | Baclofen 0.7 mg/kg/day in 3 divided doses | 7 days | Enteral formula *via* NGT for 7, oral for 1 |
| Khodadad et al. (29) | 0.25 mg/kg/day baclofen in 2 divided doses | 3 months | Oral |
| Koek et al. (20) | Baclofen 5 mg TDS, gradually reached 20 mg TDS after 10 days + omeprazole 20 mg BD | 14 days | Oral |
| Bajbouj et al. (19) | Baclofen 5 mg TDS, gradually reached 20 mg TDS after 4 weeks + esomeprazole 40 mg BD | 3 months | Oral |

BD, twice a day; NGT, nasogastric tube; NR, not reported; OD, once daily; QID, four times a day; RCT, randomized controlled trial; TDS, three times a day.

The total number of reflux episodes of placebo was significantly higher in patients with HH and remained significantly higher compared to in patients without HH ($P < 0.05$). No correlation ($r = 0.01$; $P = 0.95$) was found between the size of the HH and the number of acid reflux episodes (15). In Scarpellini et al.'s (33) study, preprandial LES pressures following baclofen were significantly lower in patients with HH compared to those without HH ($P < 0.05$), and did not change significantly in the postprandial period (Table 8).

### 3.4.3. Feasibility of using baclofen in the treatment of GERD

Table 9 summarizes the evidence from the included studies regarding the feasibility of using baclofen in the treatment of GERD.

## 4. Discussion

### 4.1. Summary of the main results

We conducted this study to review the effects of baclofen on the treatment of GERD along with its advantages and disadvantages. Most included studies we reviewed diagnosed GERD based on clinical presentation and only a few diagnosed on pH-monitoring results. The results showed that baclofen is a relatively safe choice that may significantly improve reflux symptoms and pH-monitoring and manometry findings, although its effect on pH-monitoring parameters seems less significant than the other parameters. Baclofen showed effective to different degrees in all of four assessed categories (including adults, children, patients with GERC, and patients with HH).

TABLE 4 Treatment safety and side effects.

| References | Treatment side effects |
|---|---|
| Curcic et al. (28) | None |
| Abbasinazari et al. (18) | None |
| Ciccaglione et al. (16) (1) | None |
| Ciccaglione et al. (16) (2) | 2/12 patients withdrew from the study after 10 days of treatment with baclofen, one because of nocturnal anxiety with sleepiness, and the other due to low blood pressure with dizziness. Baclofen was well tolerated in all other patients. |
| Beaumont et al. (15) (1 and 2) | Somnolence (6/27), dizziness (3/27), and nausea (2/27) occurred with baclofen. One of 27 patients withdrew from the study prematurely because of dizziness. No AEs occurred during the placebo. |
| Cange et al. (31) | Tiredness and/or mild vertigo (8/20), headache (1/20), GI symptoms (1/20) occurred with baclofen. tiredness and/or mild vertigo (1/20), headache (3/20), GI symptoms (2/20) occurred with placebo. |
| Cossentino et al. (25) | NR |
| Dibner et al. (17) | NR |
| Gerson et al. (54) | Abdominal pain (1/44), constipation (0), nausea (0), diarrhea (1/44), vomiting (0/44), somnolence (1/44), fatigue (1/44), headache (2/44) occurred with baclofen. Abdominal pain (1/44), constipation (2/44), nausea (3/44), Diarrhea (1/44), vomiting (2/44), somnolence (1/44), fatigue (1/44), and headache (3/44) occurred with placebo. |
| Grossi et al. (32) | Headache (1/14) and dizziness (1/14) occurred with baclofen. No AEs occurred with placebo |
| Omari et al. (35) | Breathlessness ($n = 2$; 1 placebo group, 1 baclofen group); tiredness ($n = 2$; 1 placebo group, 1 baclofen group) and nausea ($n = 1$; baclofen group). none of these events was considered significant. These symptoms were judged to most likely be reflux disease itself or discomfort caused by the invasive GI procedures rather than baclofen |
| van Herwaarden et al. (34) | 80% of baclofen and 35% of the placebo group experienced mental/neurological AEs ($P = 0.00001$). Other AEs occurred in both groups but were statistically not significant |
| Vela et al. (27) | NR |
| Scarpellini et al. (33) | 9/13 patients reported mild dizziness and sleepiness with baclofen |
| Zhang et al. (11) | None |
| Orr et al. (30) | None |
| Sobhani Shahmirzadi et al. (21) | None |
| Vakil et al. (26) | NR |
| Vadlamudi et al. (55) | 3/53 patients withdrew from the study because of side effects. Dose related drowsiness reported from 4 of 50 remained patients |
| Xu et al. (56) | (1/3) slight dizziness and (1/3) sleepiness occurred with baclofen |
| Xu et al. (23) | Somnolence (5/16), dizziness(2/16), fatigue (3/16), nausea (1/16), diarrhea (1/16) occurred with baclofen |
| Xu et al. (22) | Somnolence ($n = 21/57$), dizziness ($n = 7/57$), and drowsiness ($n = 12/57$) occurred with baclofen |
| Zhu et al. (42) | Somnolence ($n = 49/138$), dizziness ($n = 33/138$), fatigue ($n = 24/138$), nausea ($n = 4/138$), and diarrhea ($n = 1/138$) occurred with baclofen |
| Kawai et al. (24) | A slight reduction in muscle tone in 1 of 8 patients |
| Khodadad et al. (29) | None |
| Koek et al. (20) | Transient nausea in 2 and drowsiness in 3 of 16 patients |
| Bajbouj et al. (19) | 2/7 patients had to discontinue study because of drowsiness |

AE, adverse event; GI, gastrointestinal; NR, not reported.

The mechanisms, safety, and efficacy of baclofen in the GERD management will be discussed in the following sections.

## 4.2. Mechanisms

Baclofen is a GABA agonist that works primarily in the spinal cord by binding to $GABA_B$ receptors and inhibits the release of substance P and excitatory neurotransmitters; so, baclofen alleviates muscle spasms and discomfort (36). Although the spinal cord is the principal site of action for baclofen, its receptors are also found in the brain. Studies suggest that baclofen interacts with serotonin, dopamine, and other neurotransmitters, an off-label treatment for post-traumatic stress disorder and anxiety (37).

However, how does baclofen work to treat gastroesophageal reflux? The molecular and neural mechanisms of action of baclofen in reflux disease are still unclear. The vasovagal reflex relaxes the LES as food enters the stomach, but predisposes to acid and food reflux to occur. As a result, the TLESR is the most likely cause for the gastroesophageal reflux disease. Number of neurotransmitters play a role in this reflex, but $GABA_B$ receptor agonists have received the most attention for drug intervention. A low basal pressure of the LES is another proposed mechanism for reflux disease (38, 39). All included clinical trials reported the significant effect of baclofen on TLESRs and LES pressure, except for Cossentino et al. (25) and van Herwaarden et al. (34) who found no significant change in LES pressure between the baclofen and placebo groups. They prescribed baclofen for a period of 2 weeks and 1 day, respectively. Their result does not seem to be attributable to the period of baclofen administration, since there are some studies reporting the efficacy of baclofen on LES pressure during the same administration period (11, 28, 32, 33).

TABLE 5 Outcomes in adults.

| References | Baclofen on | | | | | |
|---|---|---|---|---|---|---|
| | Clinical status | Acid reflux time | TLESR incidence | GER incidence | LES pressure | Others |
| Curcic et al. (28) | NR | Baclofen increased reflux duration in patients with GERD by a small but significant degree ($P < 0.0001$) | NR | Baclofen reduced the number of reflux events from 3 to 2 in GERD patients resulting in a 40% reduction ($P < 0.0001$) | Treatment increased LES pressure in patients with GERD by $4.50 \pm 1.49$ mmHg ($P < 0.003$) and intra-abdominal LES length by $0.35 \pm 0.06$ cm ($P < 0.0001$) | Gastric emptying was faster during baclofen treatment; nevertheless, measurement variability was high and this change was not statistically significance. Baclofen reduced the esophagogastric insertion in GERD patients by $4.09° \pm 1.82°$ ($P = 0.025$). Baclofen had no effect on the change in proximal gastric curvature |
| Abbasinazari et al. (18) | Significant differences were observed between the two groups in the prevalence of heartburn ($p < 0.0001$) and regurgitation ($p < 0.0001$); baclofen had no significant effect on chest pain ($p = 0.35$) or hoarseness ($p = 0.93$) compared to placebo | NR | NR | NR | NR | The total GERD score (the sum of the scores for all four symptoms; heartburn, acid regurgitation, chest pain, and hoarseness) was significantly affected by baclofen ($p < 0.0001$) |
| Ciccaglione et al. (16) (1) | NR | In baclofen group, there was a highly significant reduction in percent time with pH < 4 (−57.81%). During placebo, no significant change was observed for percent time pH < 4 ($p$ = NS) | NR | In all GERD patients, baclofen reduced the number of reflux episodes (−51.01%). During placebo, there was no significant change in the number of reflux episodes ($p$ = NS). In the placebo group, patients had a statistically significant higher average number of reflux episodes compared to the patients in the baclofen group ($p < 0.003$). | NR | A statistically significant increase in mean gastric pH value in the 24-h period was observed 15 GERD patients who received baclofen ($p < 0.0004$). No change was observed in the placebo ($p$ = NS). |
| Ciccaglione et al. (16) (2) | The intensity and frequency of symptoms were significantly improved in patients after receiving baclofen, while in the placebo group the total symptom scores was not changed. The number of antacid tablets consumed per week was 7 before placebo, 8 during placebo (NS), 8 before baclofen, and 2 during baclofen ($p < 0.01$). | The percent time pH < 4 was significantly decreased after the treatment with baclofen compared to the values reported at the beginning of the treatment (−53.45%). The percent time with pH < 4 in GERD patients treated with placebo was not significantly changed ($p$ = NS) | NR | The number of reflux episodes was significantly decreased after the treatment with baclofen compared to the values reported at the beginning of the treatment (−76.36%). The median number of reflux episodes in GERD patients receiving placebo was not significantly changed ($p$ = NS) | NR | The number of reflux episodes longer than 5 min was assessed in five patients treated with baclofen and in three patients treated with placebo. In baclofen group, a significant reduction was noted ($p < 0.002$) while no change was observed in placebo group ($p$ = NS). |
| Beaumont et al. (15) (1) | NR | The total acid exposure time and the percentage of time pH < 4 after baclofen treatment, both not significantly changed compared with the placebo. No significant changes in the percentage of time with pH < 4 were noted for the upright, postprandial, and supine periods compared with placebo. Acid clearance time was not changed significantly after baclofen ($p$ = NS). | NR | After Baclofen, the total amount of reflux episodes was significantly reduced ($P < 0.01$). Reflux in the upright position and postprandial reflux were significantly decreased [($P < 0.02$) and ($P < 0.02$), respectively]. Number of reflux episodes in the supine position was not significantly changed after treatment baclofen. The number of acid reflux episodes was reduced by 36.6%, however this change was not statistically significant compared to placebo. Bolus clearance was not affected by baclofen ($p$ = NS) | NR | The total number of reflux episodes extending to the most proximal impedance electrodes was significantly reduced after treatment with baclofen ($P < 0.05$) |

*(Continued)*





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TABLE 5 (Continued)

| References | Baclofen on | | | | | |
|---|---|---|---|---|---|---|
| | Clinical status | Acid reflux time | TLESR incidence | GER incidence | LES pressure | Others |
| Cange et al. (31) | The total amount of belching was significantly decreased ($P < 0.01$), but no effect was observed on heartburn or other symptoms associated with GERD. | A significant reduction was observed in the fraction of time pH < 4 during the first 4 h after dosing with baclofen ($P = 0.0019$), postprandially ($P = 0.0083$) and for the whole 12-h period ($P < 0.039$). However, during the 4–8 and 8–12 h periods, no significant reduction was noted. | NR | After baclofen, a significant reduction in the number of acid reflux episodes, both during the first 4 h after dosing (48%) and during the total 0–12 h recording period ($P < 0.0001$) was recorded. | NR | Baclofen had no significant effect on esophageal clearance. |
| Cossentino et al. (25) | Baclofen significantly decreased overall symptom score ($P = 0.004$) and symptom scores including belching ($P = 0.036$), regurgitation ($P = 0.036$). No improvement in heartburn score ($P = 0.186$) or chest pain score ($P = 0.249$) and no significant increase in drowsiness after baclofen treatment ($P = 0.064$) was noted. | Significant improvement in 24-h pH score ($P = 0.020$) after treatment with baclofen was noted. | NR | After baclofen, the number of postprandial reflux episodes ($P = 0.045$) and the percent total postprandial reflux decreased significantly ($P = 0.003$). Significant improvements in several reflux parameters including percent upright reflux ($P = 0.016$), percent total reflux ($P = 0.003$), number of reflux episodes ($P = 0.018$) and number of reflux episodes > 5 min ($P = 0.016$) were observed after baclofen treatment. No significant differences in percent supine reflux ($p = 0.057$) or the longest episode of reflux ($p = 0.063$) were observed. | No significant changes in LES pressure after treatment with baclofen ($P = 0.590$) were observed. | NR |
| Gerson et al. (54) | The mean number of heartburn events associated with acid reflux episodes or non-acid reflux episodes for all dose groups combined was significantly reduced. | NR | NR | There was a statistically significant reduction in reflux episodes during treatment with AP compared with placebo treatment. | NR | NR |
| Grossi et al. (32) | NR | NR | The number of TLESRs was significantly reduced [basal 57.5; baclofen 41.5 ($P = 0.01$); placebo 55 ($P$ = NS)] | NR | A significant increase in the basal LES tone was observed [basal 20.5 mmHg; baclofen: 24.8 mmHg ($P = 0.02$); placebo 21.5 mmHg ($P$ = NS)] | The number of swallows was reduced significantly after baclofen compared to the baseline ($P = 0.02$) |
| van Herwaarden et al. (34) | NR | Baclofen significantly reduced the acid reflux time ($P = 0.029$). In contrast, baclofen had no effect on the mean duration of reflux episodes ($P$ = NS). | The incidence of TLESR was significantly reduced after the treatment with baclofen compared to placebo ($P < 0.0001$); However, baclofen had no effect on the percentage of TLESR associated with acid reflux ($P$ = NS) | Baclofen significantly reduced the number of reflux episodes ($P = 0.004$) | Baclofen had no significant effect on LES pressure ($P$ = NS) | NR |

*(Continued)*





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TABLE 5 (Continued)

| References | Baclofen on | | | | | |
|---|---|---|---|---|---|---|
| | Clinical status | Acid reflux time | TLESR incidence | GER incidence | LES pressure | Others |
| Vela et al. (27) | Treatment with baclofen resulted in a significant reduction in the median number of total ($P = 0.004$), acid-related ($P = 0.008$) and non-acid-related ($P = 0.04$) symptoms. | NR | NR | Baclofen significantly reduced the median number of episodes of acid reflux ($P = 0.004$), re-reflux ($P = 0.02$), non-acid reflux ($P = 0.003$), and all reflux combined ($P = 0.004$) | NR | NR |
| Scarpellini et al. (33) | Baclofen significantly decreased epigastric burning, retrosternal cramps, and abdominal pain in the preprandial period compared to placebo ($P < 0.05$). In the postprandial period, baclofen significantly decreased belching and epigastric burning compared to placebo ($P < 0.05$). | NR | Baclofen did not change the number of TLESRs preprandially but significantly prevented TLESRs from increasing in postprandial period, resulting in a reduction in the occurrence of postprandial TLESRs after baclofen compared with placebo | NR | After placebo LES pressure reduced from $32.7 \pm 6.1$ before meal to $24.5 \pm 3.1$ mm Hg at 60 min postprandially, $P < 0.05$). In contrast, baclofen prevented the postprandial drop in LES pressure ($25.4 \pm 7.0$ preprandially vs. $29.4 \pm 4.9$ mm Hg at 60, $P$ = NS). | The proximal extent of acid pockets detected postprandially was not affected by baclofen or placebo ($P$ = NS). |
| Zhang et al. (11) | No overall significant differences in changes in symptom scores after baclofen administration compared to placebo were noted. | Although reflux episodes were significantly reduced, esophageal acid exposure was not significantly affected by baclofen. The duration of time that esophageal pH was <4 was not changed during baclofen compared to that during placebo ($p$ = NS). | The rate of TLESRs per 3 h was significantly reduced ($p < 0.0002$) after baclofen. Baclofen had no effect on the likelihood of reflux occurring during a TLESR. Reflux occurred with 53.8% (40.9–67.3%) of TLESRs during baclofen compared to 41.5% (25.0–75.5%) during placebo. | After baclofen administration, 101 acid reflux episodes happened compared to 174 during placebo. For the postprandial period as a whole, the rate of reflux episodes was reduced by 43% from 7.0 episodes per 3 h to 4.0 episodes per 3 h after baclofen ($p < 0.02$). | LES pressure in fasting basal and first 90 min postprandial after baclofen was similar to placebo; however, basal LES pressure during baclofen was significantly higher in 90–180 min postprandial, compared to placebo ($p < 0.005$), and overall mean postprandial basal LES pressure was significantly higher during baclofen compared to placebo ($p < 0.02$). | Rate of swallowing decreased significantly per 3 h ($p < 0.05$). Fasting or postprandial esophageal peristalsis was not affected by baclofen. Baclofen had no effect on the success rates of both primary and secondary peristalsis and distal esophageal wave amplitude. |
| Orr et al. (30) | NR | The recumbent acid contact time was decreased by approximately 50% in baclofen compared with placebo, although this deacrease was not statistically significant. | NR | Reflux events occurred during wakefulness and sleep but the number of reflux events were significantly lower during sleep ($P < 0.05$). Baclofen reduced reflux events associated with wakefulness and sleep compared with placebo ($P < 0.05$). | NR | Baclofen significantly improved objective and subjective measures of sleep compared to placebo. Baclofen increased total sleep time ($P < 0.001$) and sleep efficiency ($P < 0.001$), decreased stage 1 sleep ($P < 0.01$) and wake time after sleep onset ($P < 0.001$). Baclofen also significantly improved the subjective number of awakenings ($P < 0.05$) and quality of sleep ($P < 0.01$) compared to placebo. Onset latency, the percent of REM sleep or slow-wave sleep was not affected by baclofen compared to placebo. |

*(Continued)*





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TABLE 5 (Continued)

| References | Baclofen on | | | | | |
|---|---|---|---|---|---|---|
| | Clinical status | Acid reflux time | TLESR incidence | GER incidence | LES pressure | Others |
| Vakil et al. (26) | Change from baseline in weekly heartburn events in baclofen compared to placebo was not statistically significant; however, a significant interaction was noted between prior PPI use and response to baclofen treatment. In the PPI-responsive subgroup, percent reductions from baseline in weekly heartburn events were higher for each baclofen dose vs. placebo ($P < 0.05$) and the percentage of subjects who reported complete resolution of heartburn during week 4 was higher in each baclofen treatment group (21, 28, 30, and 50% for baclofen 20, 40, 60 mg daily, and 30 mg twice daily, respectively) compared to placebo (6%) | NR | NR | NR | NR | NR |
| Koek et al. (20) | After adding baclofen to the treatment, overall symptom severity was significantly reduced ($p < 0.01$). The severity of heartburn, odynophagia, and choking was significantly reduced ($p < 0.05$) and a borderline reduction in the severity of throatache was noted ($p = 0.07$). | Under combination therapy with omeprazole 20 mg BD and baclofen 20 mg TDS, esophageal acid exposure was unchanged but distal esophageal exposure duodenal reflux was significantly reduced ($p < 0.05$). | NR | The number of acid reflux episodes was unchanged after addition of baclofen. The number of duodenal reflux episodes and the number of duodenal reflux episodes lasting longer than 5 min were significantly reduced. Duodenal reflux exposure during treatment with baclofen was decreased both in the upright and supine positions. | NR | NR |
| Bajbouj et al. (19) | None of the patients experienced a significant resolution of symptoms after adding baclofen to the treatment. | No significant changes were noted before and after adding baclofen. | NR | No significant changes were noted before and after adding baclofen. | NR | NR |

BD, twice daily; GER, gastroesophageal reflux; GERD, gastroesophageal reflux disease; LES, lower esophageal sphincter; NR, not reported; NS, not significant; PPI, proton pump inhibitor; TDS, three times a day; TLESR, transient lower esophageal sphincter relaxation; vs, versus.



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TABLE 6 Outcomes in children.

| References | Age (case/control) | Baclofen effect on | |
|---|---|---|---|
| | | Clinical status | Others |
| Dibner et al. (17) | Range: 2.6–17.4 years | NR | Children receiving baclofen had significantly less TLESR and acid GER in the test duration than in the control duration and for the placebo group, no differences were detected. Children receiving baclofen had faster gastric emptying and a higher frequency of normal gastric emptying than those who received the placebo. |
| Omari et al. (35) | 10.0 (9.1/11.0) years | NR | In the control period, the average number of acid GER episodes and the proportion of TLESR-associated acid GER episodes in the placebo group were significantly lower than in the baclofen group. Children receiving baclofen 1 h before the second drink, recorded significantly fewer TLESRs and acid GER episodes during the test in comparison with the control period. For children receiving the placebo, no significant differences between the test and control periods for the frequency of TLESRs and reflux were recorded. |
| Sobhani Shahmirzadi et al. (21) | 6.61 years | 85.2% of cases in the baclofen treatment group and 55.2% of cases in the non-baclofen treatment group had moderate to full remission. Weight gain in the baclofen-treated group was significantly higher than in the non-baclofen group ($p = 0.0001$) | NR |
| Vadlamudi et al. (55) | 6.1 years | 66% of patients showed a significant reduction in clinical symptoms at their first follow-up visit. Baclofen was stopped in the remaining 34% of patients because of either no response (28%) or adverse events (6%). A total of 27 patients continued treatment and were assessed for long-term response. Of those 81% had a sustained response to baclofen at 12 months, whereas 19% lost response. | NR |
| Kawai et al. (24) | 3 years | The emesis score was significantly decreased ($P = 0.03$) | The incidence of acid refluxes was significantly decreased during the entire 24-h period ($P = 0.01$) and the postprandial period ($P = 0.049$). The number of long acid refluxes (>5 min) was significantly decreased during the 24-h period ($P = 0.02$), but there was no significant difference during the postprandial period ($p = 0.21$). The percentage of total time with esophageal pH < 4.0, the duration of the longest acid reflux, and esophageal acid clearance time had no significant change with therapy either during the 24-h period or during the postprandial period ($p > 0.05$) |
| Khodadad et al. (29) | 7.1 months | The average weight gain of patients was significantly increased ($p < 0.0001$). Crying and instability were significantly decreased ($p < 0.0001$). Vomiting was significantly decreased ($p < 0.0001$). The feeding frequency was significantly increased ($p < 0.001$). | NR |

GER, gastroesophageal reflux; NR, not reported; TLESR, transient lower esophageal sphincter relaxation.

There are still many questions about the mechanism of baclofen in GERD. Although the manometry findings confirm the effect of baclofen on the LES, baclofen has been less effective on pH-monitoring findings. Unlike PPIs, baclofen has no known effect on gastric acid secretion, but theoretically, it may reduce acid exposure time secondary to increased LES pressure. However, most of the studies reported no significant improvement in acid exposure time with baclofen. Furthermore, in some of these studies, despite no significant reduction in acid exposure time, GERD symptoms were significantly improved. This can suggest other mechanisms rather than the effect on the LES; for example, baclofen may have a role in suppressing esophageal sensory neurons, as a result, despite the reflux of the acid, patient feels no heartburn. Taken together, it seems that the increased LES pressure cannot be the only mechanism of action of baclofen in GERD; more studies are needed in this field.

## 4.3. Safety and side effects

One of the most critical considerations in any treatment is the patient's safety. Baclofen's side effects have caused hesitancies in prescribing for the treatment of GERD.

As previously mentioned, baclofen is a $GABA_B$ agonist which justifies its side effects by this mechanism. CNS side effects may include dizziness, drowsiness, confusion, sedation, asthenia, and nausea. These side effects are dose-dependent and related to the pharmacologic action of binding to the presynaptic $GABA_B$ receptors within the brain stem, dorsal horn of the spinal cord, and other CNS parts while reducing the release of excitatory neurotransmitters. Taking oral doses of more than 60 mg per day and severe renal impairment (eGFR less than 30 ml/minute/1.73 $m^2$) are the major predictors for CNS side effects. Patients who are concurrently taking other CNS depressants

TABLE 7 Outcomes in patients with GERC.

| References | Cough duration (months) | SAP for acid reflux (%) | SAP for non-acid reflux (%) | Cough symptom score (daytime/ nighttime) | Demeester score | Baclofen effect on cough |
|---|---|---|---|---|---|---|
| Xu et al. (56) | Patient 1: 42, patient 2: 24, patient 3: 25 | Patient 1: 0.0, patient 2: 97.3, patient 3: 84.4 | Patient 1: 95.2, patient 2: 0.0, patient 3: 0.0 | Patient 1:(3/1), patient 2:(4/2), patient 3:(3/2) | Patient 1: 0.7, patient 2: 168.1, patient 3: 20.2 | Cough reduced and waned in all 3 patients (approved for improvement of cough symptom score and cough reflex sensitivity to capsaicin) |
| Xu et al. (23) | 36 | 73.1 | 71.2 | (3/1) | 33.1 | The overall therapeutic efficacy of baclofen was 56.3% (9/16). In the remaining 7 patients who withdrew baclofen therapy ($n = 4$) or were resistant to treatment ($n = 3$), the cough was resolved by subsequent therapies of the double dose of omeprazole in 5 patients and a double dose of omeprazole combined with ranitidine in 2 patients. |
| Xu et al. (22) | NR | 75.3 | 69.1 | NR | 29.9 | Baclofen was effective in cough resolution 66.7% of the patients with refractory GERC who failed to respond to high-dose omeprazole and ranitidine |
| Zhu et al. (42) | 12.6 | 52.3 | 78 | (3/1) | NR | The overall therapeutic successful rate of baclofen was 52.2% (72/138). For 66 patients who either withdrew from baclofen therapy ($n = 10$) or were unresponsive to baclofen treatment ($n = 56$), the cough was resolved by a double dose of omeprazole in 57 patients or by the consequent therapies combining gabapentin with omeprazole in 9 patients |

GERC, gastroesophageal reflux-induced chronic cough; NR, not reported; SAP, symptom associated probability.

TABLE 8 Outcomes in patients with HH.

| References | Hiatal hernia length | Baclofen effect on | | |
|---|---|---|---|---|
| | | Acid reflux time | GER incidence | Others |
| Beaumont et al. (15) | >3 cm | During baclofen, the total acid exposure time and the percentage of time esophageal pH < 4, both showed no significant difference compared with the placebo ($p$ = NS). During baclofen, no significant changes in the percentage of time with pH < 4 were observed for the upright, postprandial and supine period compared with the placebo. Baclofen had no significant effect on acid clearance time. | A significant reduction in the total amount of reflux episodes was recorded ($P = 0.003$, corresponding with a reduction of 43.3%), but the number of acid reflux episodes had no significant reduction by baclofen. Reflux in the upright position was significantly lower ($P = 0.003$), but reflux in the supine position showed no significant reduction by baclofen. Baclofen significantly reduced the amount of mixed ($P = 0.003$) and pure liquid ($P < 0.02$) reflux episodes. | The amount of most proximal reflux episodes was significantly reduced after baclofen ($P = 0.005$, corresponding with a reduction of 57.1%). The proportion of all reflux episodes that reached the most proximal extent was not significantly changed by baclofen (placebo: 21.8%; baclofen: 22.8%) |
| Cange et al. (31) | NR | In hiatal hernia patients ($n = 13$) a significant reduction was found for the fraction of time pH < 4 during the first 4 h after dosing ($P = 0.0215$) and post-prandially, both during the first and second 4-h periods ($P < 0.05$). | In patients with a verified hiatus hernia ($n = 13$), a significant reduction was found in the number of reflux episodes for the whole registration time ($P < 0.0002$) as well as for each 4-h period ($P < 0.05$). | NR |
| Scarpellini et al. (33) | >2 cm | NR | NR | Preprandial LES pressures after baclofen were significantly reduced in patients with HH compared with those without HH ($P < 0.05$), and had no significant change in the postprandial period. |

GER, gastroesophageal reflux; HH, hiatal hernia; LES, lower esophageal sphincter; NR, not reported.

(for example, benzodiazepines or opioids) are more susceptible to these side effects (40, 41).

As expected, these side effects were also observed in the clinical trials. These side effects were well explained both by the agonistic effects of baclofen on $GABA_B$ receptors in the central and peripheral nervous systems and the normal postprandial symptoms of GERD. The duration of the treatment and other factors may have an impact on its safety. We review that in short -term use the

TABLE 9 Feasibility of using baclofen in the treatment of GERD.

| References | Does the study recommend baclofen for GERD management? | Baclofen suggested for (or not suggested for) |
|---|---|---|
| Curcic et al. (28) | Yes, reduces the frequency of TLESRs and reflux events after meals and also reduces the esophagogastric insertion angle maintains the intra-abdominal LES segment which may suppress reflux | GERD patients |
| Abbasinazari et al. (18) | Yes, reduces GERD related symptoms | GERD patients |
| Ciccaglione et al. (16) | Yes, baclofen in multiple doses reduces gastroesophageal acid reflux for a 24-h period in terms of the number and period of acid exposure in the esophagus, also improves reflux parameters and symptoms related to GER in long term therapy | GERD patients |
| Beaumont et al. (15) | Yes, reduces the number of reflux episodes | GERD patients with or without hiatal hernia and incomplete response to acid suppression |
| Cange et al. (31) | Yes, reduces the number of reflux episodes and the fraction of time with esophageal pH $< 4$ | GERD patients |
| Cossentino et al. (25) | Yes, improves pH parameters and symptoms | GERD patients, may be more effective in patients with predominantly upright reflux and belching |
| Dibner et al. (17) | Yes, decreases GER by inhibiting TLESRs and accelerating gastric emptying | Children with GERD |
| Gerson et al. (54) | Yes, reduces reflux episodes and associated heartburn symptoms. | GERD patients |
| Grossi et al. (32) | Yes, decreases the frequency of TLESRs and elicits a greater basal LES tone | GERD patients |
| Omari et al. (35) | Yes, reduces GER by inhibiting the triggering of TLESR and accelerating Gastric emptying | Children with GERD |
| van Herwaarden et al. (34) | May, reduce acid reflux time but couldn't decrease symptoms. Baclofen decreases post-prandial acid reflux by reducing the incidence of transient lower esophageal sphincter relaxations | GERD patients |
| Vela et al. (27) | Yes, reduces post-prandial acid and non-acid reflux and their associated symptoms | GERD patients with heartburn as the predominant symptom |
| Scarpellini et al. (33) | Yes, although baclofen was able to significantly improve upper GI symptoms both preprandially and postprandially, the effect on symptoms does not depend on a change in the extent of the acid pocket | Heartburn-prevalent GERD patients |
| Zhang et al. (11) | May, baclofen significantly inhibits gastroesophageal reflux episodes by inhibition of TLESRs | GERD patients |
| Orr et al. (30) | Yes, reduces the number of reflux events during sleep and significantly improves several measures of sleep. Therefore, could be considered as a useful adjunct therapy to PPIs | GERD patients with sleep disturbances or nighttime heartburn resistant to PPI therapy |
| Sobhani Shahmirzadi et al. (21) | Yes, along with routine gastroesophageal reflux treatments in children can help reduce or improve symptoms of the disease | Children 6 months to 12 years old with GERD |
| Vakil et al. (26) | Yes, but just if taken with PPI | GERD patients with prior use of PPIs |
| Vadlamudi et al. (55) | Yes, reduces reflux events by inhibiting TLESRs and can be used as supplemental therapy to PPI | Children with refractory GERD in combination with PPIs |
| Xu et al. (56) | Yes, decreases cough symptom score and cough reflex sensitivity to capsaicin | GERC patients resistant to PPIs |
| Xu et al. (23) | May, when a standard therapy for GERC fails, baclofen can at least be considered as a treatment option, even though its therapeutic efficacy is suboptimal | GERC patients unresponsive to standard anti-reflux therapy |
| Xu et al. (22) | Yes, decreases cough symptom score and cough reflex sensitivity to capsaicin | GERD patients unresponsive to PPI plus H2-blocker |
| Zhu et al. (42) | No, may not be strong enough to allow a routine clinical use | (Patients with refractory GERC) |
| Kawai et al. (24) | Yes, Repetitive administration of baclofen reduces the frequency of emesis and the total number of acid refluxes | Neurologically impaired children with GERD |
| Khodadad et al. (29) | Yes, controls the occurrence of vomiting and reduces instability of infants, and causes gaining weight and improvement of nutrition. It could be used as a replacement for prokinetics to treat GERD. | Infants with GERD |
| Koek et al. (20) | Yes, improves duodenal reflux and associated reflux symptoms that persist during PPI therapy, inhibits the number of TLESRs, and decreases reflux events and duodenal reflux exposure | GERD patients with reflux that persisted during PPI treatment as add-on therapy |
| Bajbouj et al. (19) | No, baclofen administration as an add-on therapy showed inconclusive results concerning reflux events. | (GERD patients with persistent GER despite therapy with PPI at the standard dose and double dose) |

GER, gastroesophageal reflux; GERC, gastroesophageal reflex-induced chronic cough; GERD, gastroesophageal reflux disease; H2-blocker, histamine-2 blocker; LES, lower esophageal sphincter; PPI, proton pump inhibitor; TLESR, transient lower esophageal sphincter relaxation.

overall adverse effects of baclofen in GERD patients are negligible, yet in long-term use side effects are more prominent. However, Sobhani Shahmirzadi et al. (21) and Khodad et al. (29) administered baclofen for a period of 1 month and 3 months, respectively; and no significant adverse event was observed despite long-term use of baclofen.

Li et al. (12) conducted a meta-analysis of nine RCTs to examine the safety of baclofen in reflux therapy. Baclofen- and placebo-treated participants did not have a statistically significant difference in the frequency of overall adverse events (OR = 1.62; 95% CI: 1.03, 2.54; $P = 0.04$). Neurological/psychiatric symptoms were the most reported side effects. All reported adverse events were mild to moderate in intensity (12).

Some clinicians administer low dose baclofen and increment the dosage as a precaution against baclofen adverse effects. This approach was utilized in six of our trials (15, 16, 19, 20, 25, 42). However, results are inconclusive due to lack of studies comparing high-dose baclofen at the beginning of treatment with the incremental dosing mentioned above in adverse effects.

This review concludes an acceptable safety and tolerability profile for baclofen in GERD, yet Caution should be taken in the long-term use of baclofen, as 20% of long-term users experienced neurological and mental side effects. We noted that some trials did not report side effects, and some had restricted criteria toward side effects; therefore, side effects might not be reported in these studies. Furthermore, the populations of the included studies were heterogeneous. Thus, we recommend that cautions should be considered when administering baclofen to susceptible populations. In general, to limit the risk of baclofen side effects, we recommend: (i) starting with a low dose and gradually increasing it; (ii) prescribing no more than 60 mg of baclofen per day; and (iii) obtaining a comprehensive history of the patient, including comorbidities, medications (particularly CNS depressants) and a previous history of dizziness, somnolence and other baclofen side effects.

## 4.4. Efficacy

In our included trials, the efficacy of baclofen was evaluated through changes in clinical status, acid reflux time, TLESR incidence, GER incidence, LES pressure, and several other factors. Due to heterogeneity of the included studies, a meta-analysis of the data was not possible. Nonetheless, the effects of baclofen on GERD from the results are noticeable. Li et al. (12) conducted a meta-analysis on nine RCTs to determine the efficacy of baclofen on reflux statistically (eight studies administered baclofen in GERD patients and one administered baclofen in normal healthy subjects). The results revealed a statistically significant difference between baclofen-treated and placebo-treated subjects in reduction of GER incidence [standardized mean difference (SMD): −0.65; 95% CI: −0.94, −0.36; $P = 0.00001$], acid reflux time (SMD: −1.14; 95% CI: −1.72, −0.56; $P = 00001$) and TLESR incidence (SMD: −3.56; 95% CI: −4.30, −3.00; $P < 0.00001$) (12).

Baclofen may benefit a diverse population including: adults, infants with refractory regurgitation, neurologically impaired children with GERD, patients with GERC, and patients with hiatal hernia may benefit from baclofen. Of the twenty-six included, only Bajbouj et al. (19) and Zhu et al. (42) advised against using baclofen for the treatment of reflux disease.

The study by Bajbouj et al. (19) involved seven patients with GERD who did not respond to PPI. They added 15 mg of baclofen to the 80 mg of esomeprazole daily, which was increased to 60 mg after 1 month. They maintained this regimen for a period of 2 months. They found no significant change in patients' clinical status, acid reflux time and GER incidence after 3 months of treatment. Also, two patients discontinued the study because of drowsiness. Therefore, Bajbouj et al. (19) recommended against the use of baclofen in patients who did not respond to PPI (19). However, in all other studies, baclofen was significantly more effective than placebo in treating GERD patients who did not respond to PPI; we cannot independently explain Bajbouj et al.'s (19) study results.

Among PPI-resistant patients, when deciding to prescribe baclofen, how to distinguish responders from non-responders? The study by Zhu et al. (42) included 138 patients with refractory GERC. In contrast to Bajbouj et al.'s (19) study, baclofen was effective in alleviating symptoms, with 72 of 138 (52.2%) GERC patients receiving a successful treatment. Nonetheless, a significant proportion of patients experienced CNS side effects as a result of long-term baclofen use, and the improvement in cough was not satisfactory. So, Zhu et al. (42) recommended against using baclofen because of its unsatisfactory efficacy and side effects. They discovered that LES pressure with a cut-off point of 11 mmHg (with a sensitivity of 83.1% and a specificity of 79.1%) and LES length with a cut-off point of 2.35 cm (with a sensitivity of 81.6% and a specificity of 72.1%) are the independent predictors of baclofen efficacy in reflux disease (42). Although the population of the study was limited to patients with refractory GERC, considering the mechanism of baclofen on GERD, it may be possible to use these cut-off points for all patients with refractory GERD; but more studies are needed to determine these cut-offs. However, using manometry to assess response to baclofen is impractical, as it is used in a limited portion of GERD patients.

Is baclofen a proper choice in patients with GERC? 64% of individuals in this category were the patients of Zhu et al.'s (42) study. The issue that is remarkable about their study is that they prescribed baclofen as monotherapy without PPI. 52.2% of patients were treated with baclofen and 86.3% of baclofen non-responders were treated with double dose of PPI (42). On the other hand, Xu et al.'s (23) reported that adding baclofen to the PPI was effective in cough resolution of 66.7% of patients with GERC who failed to response high-dose PPI and H2blocker. On the whole, the heterogeneity of the studies in this category prevents us from making a correct judgment, and it seems that baclofen lacks the potency for standard clinical use in patients with GERC, apart from the fact that the risk of side effects is also higher in these patients due to the long-term use.

Does HH reduce the efficacy of baclofen during PPI add-on therapy? Beamount et al. (15) studied 27 GERD patients, including 16 patients without HH and 11 without a large HH. The total number of reflux episodes decreased by 36% in patients without HH and 43% in patients with HH, but the number of acid reflux episodes and total acid exposure time did not change. They reported that baclofen may also be effective in patients with a large HH but findings are not satisfactory (15).

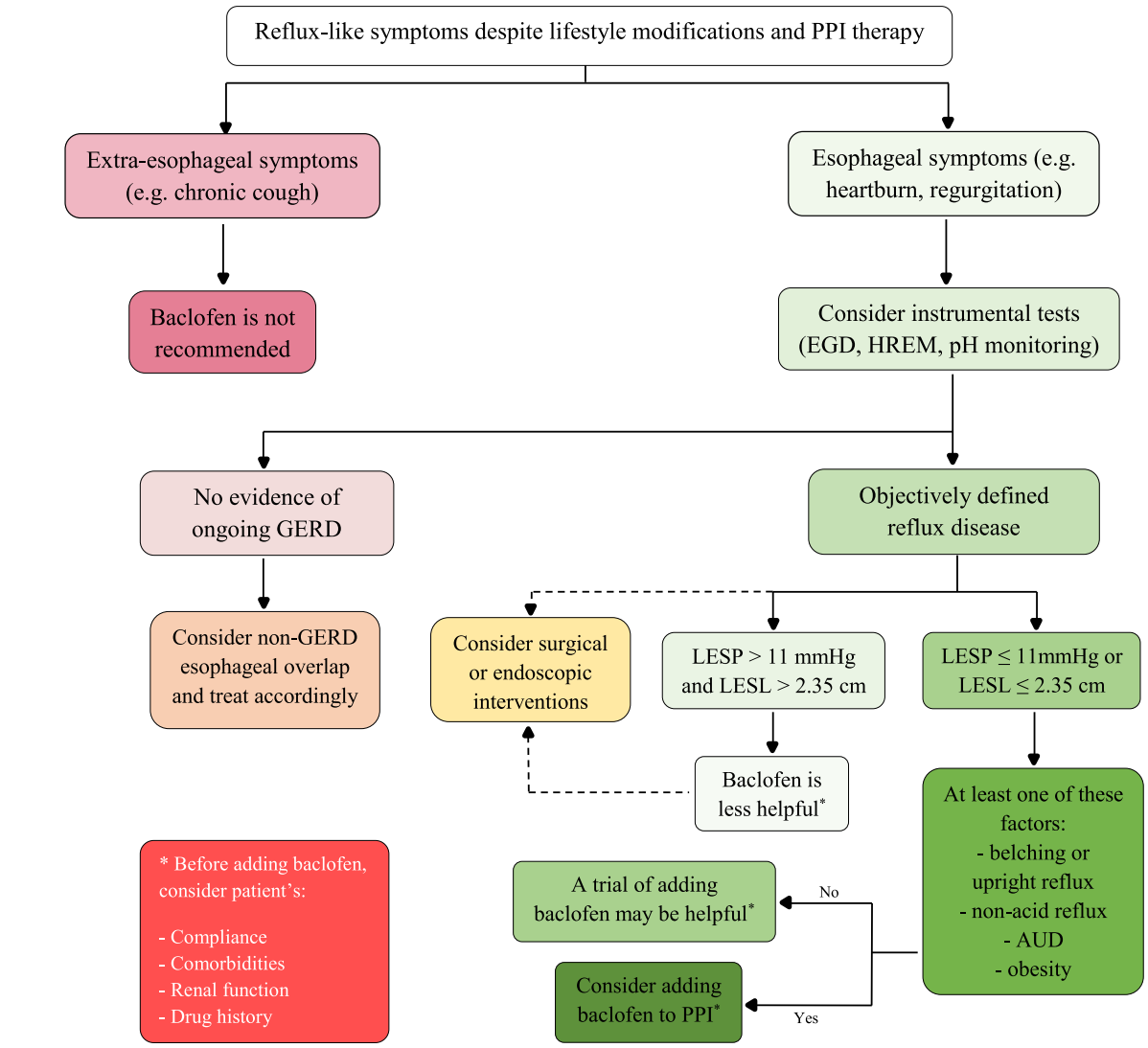


FIGURE 2
Suggested algorithm for administration of baclofen for refractory reflux disease. AUD, alcohol use disorder; EGD, esophagogastroduodenoscopy; GERD, gastroesophageal reflux disease; HREM, high resolution esophageal manometry; LESL, lower esophageal sphincter length; LESP, lower esophageal sphincter pressure; PPI, proton pump inhibitor. *Means to pay attention to the issues that are listed in red rectangle.

Is baclofen effective as a stand-alone treatment for GERD? Twenty-two studies used baclofen as monotherapy and six used it as a combination therapy with PPI. Baclofen improved symptoms in both treatment groups significantly, but due to the heterogeneity of studies, it is impossible to compare these two groups. Despite the fact that baclofen monotherapy is effective in treating GERD, it is not recommended to use it as the first-line treatment without PPI; especially for long-term use, in which case its side effects are more pronounced. We recommend that, if high-dose PPI treatment fails to improve GERD, baclofen can be added to PPI to benefit the synergistic effects. In PPI-resistant patients, baclofen could be used as a replacement for prokinetics such as domperidone; particularly in patients who have heart diseases, as prokinetics prolong the QT interval and increase the risk of torsades de pointes and fatal arrhythmias (43).

Does body posture affect the efficacy of baclofen? Due to the strain of abdominal organs, the LES pressure is significantly higher in the supine position than the upright position and a low basal pressure of the LES is one of the important mechanisms of the GERD (44). Theoretically, baclofen, which increases the pressure of the LES, will be more effective in upright reflux; because of the lower LES pressure in this posture. Two studies evaluated the effect and reported that baclofen significantly decreased reflux episodes only in the upright position (15, 25). Thus, it seems that baclofen is not a good choice in patients with reflux in supine position (e.g., patients with nocturnal reflux). In contrast, Orr et al. (30) evaluated the efficacy of baclofen in reducing reflux during sleep (supine position). They reported that baclofen can not only significantly reduce reflux events during sleep, but also improve objective and subjective measures of sleep (30). So, it seems that the knowledge about the efficacy of baclofen in upright and supine positions are still paradoxical and more studies are needed to provide an answer.

Adding baclofen or suggesting anti-reflux surgery? After inadequate response from PPIs, the management of GERD

is complex (45). Spechler et al. (46) compared the efficacy of medical treatment versus surgical treatment for refractory heartburn. 25 patients received baclofen plus omeprazole and 27 patients underwent nissen fundoplication surgery. The treatment success rate with surgery was significantly higher than medical treatment (67% against 28%, $p$-value = 0.007). Although the study evaluated heartburn (which is not specific for GERD, and also is not the only symptom of GERD), baclofen appears to be a less effective alternative for surgery candidates (46).

## 4.5. Other considerations

Baclofen may be more effective in some populations when other comorbidities of patients with GERD are considered. In addition to patients with muscle spasms, baclofen can be a priority in GERD patients with the following disorders:

### 4.5.1. Alcohol use disorder (AUD)

Alcohol is one of the substances that can relax the LES and exacerbates reflux symptoms. Patients with symptomatic GERD are frequently advised to abstain from alcohol (47). Moreover, studies have shown that 30–80 mg of baclofen per day could be effective in quitting alcohol or preventing relapse: an off-label indication (48). In symptomatic GERD patients with AUD, baclofen not only reduces symptoms by decreasing TLESR episodes but also aids in alcohol cessation as a lifestyle modification.

### 4.5.2. Non-acid reflux and rumination syndrome

Antacid medications (including histamine-2 blockers and PPIs) are the first-line treatment for GERD. However, in patients with non-acid reflux or rumination syndrome, the antacid approach does not relieve symptoms. In these patients, increasing LES pressure and minimizing TLESR episodes may be the best option (49, 50). Thus, if pH monitoring reveals non-acid reflux, baclofen could be the treatment of choice.

### 4.5.3. Obesity

Studies have shown that central obesity is associated with symptomatic GERD. The mechanism is thought to be associated with increasing the gastroesophageal pressure gradient and shortening of the lower esophageal sphincter, which baclofen can resolve the latter. Moreover, obese patients are at higher risk of long-standing GERD complications, including erosive esophagitis, Barrett esophagus, and esophageal adenocarcinoma (51). A pilot study shows the positive effects of baclofen on weight reduction in obese patients (52). Thus, in obese GERD patients, baclofen could reduce both the GERD symptoms and body weight, which is one of the risk factors for symptomatic GERD.

## 4.6. Finally, when and how?

Management of refractory GERD can be very challenging. In PPI non-responders, the American College of Gastroenterology recommends against adding medications other than PPI to the regiment (53); Sometimes it is inevitably necessary to add other medications. Although trials confirm the efficacy of baclofen as a stand-alone treatment for GERD, we do not recommend it as a mono-therapy. In PPI-resistant patients, a trial of adding baclofen to the PPI may be helpful under special circumstances. This can help reduce symptoms (regurgitation, heartburn, and belching) and may decrease the dose of PPI. We recommend against using baclofen in patients with extra-esophageal reflux symptoms (e.g., GERC); as the efficacy of baclofen is low and the side effects are more frequent and severe. Also, we recommend against using baclofen for maintenance therapy or long-term use. In patients with symptomatic reflux who are candidates for anti-reflux surgery but refuse, baclofen can be a modest alternative. However, these patients may experience more side effects of baclofen as well. To reduce side effects, starting baclofen with a dose of 5–10 mg and incrementing to a maximum dose of 60 mg is recommended. Symptomatic GERD patients (especially those with belching or upright reflux) with an AUD, non-acid reflux, or obesity may benefit more from baclofen. An esophageal manometry (measures LES pressure and length) can help selecting refractory GERD patients who may respond appropriately to baclofen. Suggested algorithm for administration of baclofen for refractory reflux disease is illustrated in **Figure 2**.

## 4.7. Limitations

Some limitations of this study should be taken into consideration. First, most of the studies diagnosed GERD (before and after intervention) by symptoms (not pH-monitoring) what is uncertain. Second, the relatively small number of trials in some outcome categories. This may diminish the persuasiveness of the conclusions. Third, the potential influence of the preexisting conditions and the severity of the reflux disease could not be investigated because of the limited information obtained from the reviewed articles. Fourth, as with any systematic review, limitations associated with potential publication bias should be considered. Furthermore, trials' variability, different patients' characteristics, and a wide range of outcome measures were other limitations.

# 5. Conclusion

The present study, to the best of our knowledge, is the first study that systematically addresses various aspects of baclofen administration in the spectrum of GERD patients. A trial of adding baclofen to the PPI may be helpful in PPI-resistant patients. Baclofen therapies may be more beneficial for symptomatic GERD patients who suffer AUD, non-acid reflux, or obesity. To reduce the side effects, we recommend starting baclofen with a low dose and increasing it gradually, avoiding prescribing more than 60 mg of baclofen per day, and paying close attention to the patients' history.

## Data availability statement

The original contributions presented in this study are included in this article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

## Author contributions

EA and MAbde designed the study. EA, SK, AA, and NT performed the search, study selection, and data extraction. EA and SK wrote the first draft of the manuscript. EA, MAbdi, DA, and MAbde revised the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.997440/full#supplementary-material

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