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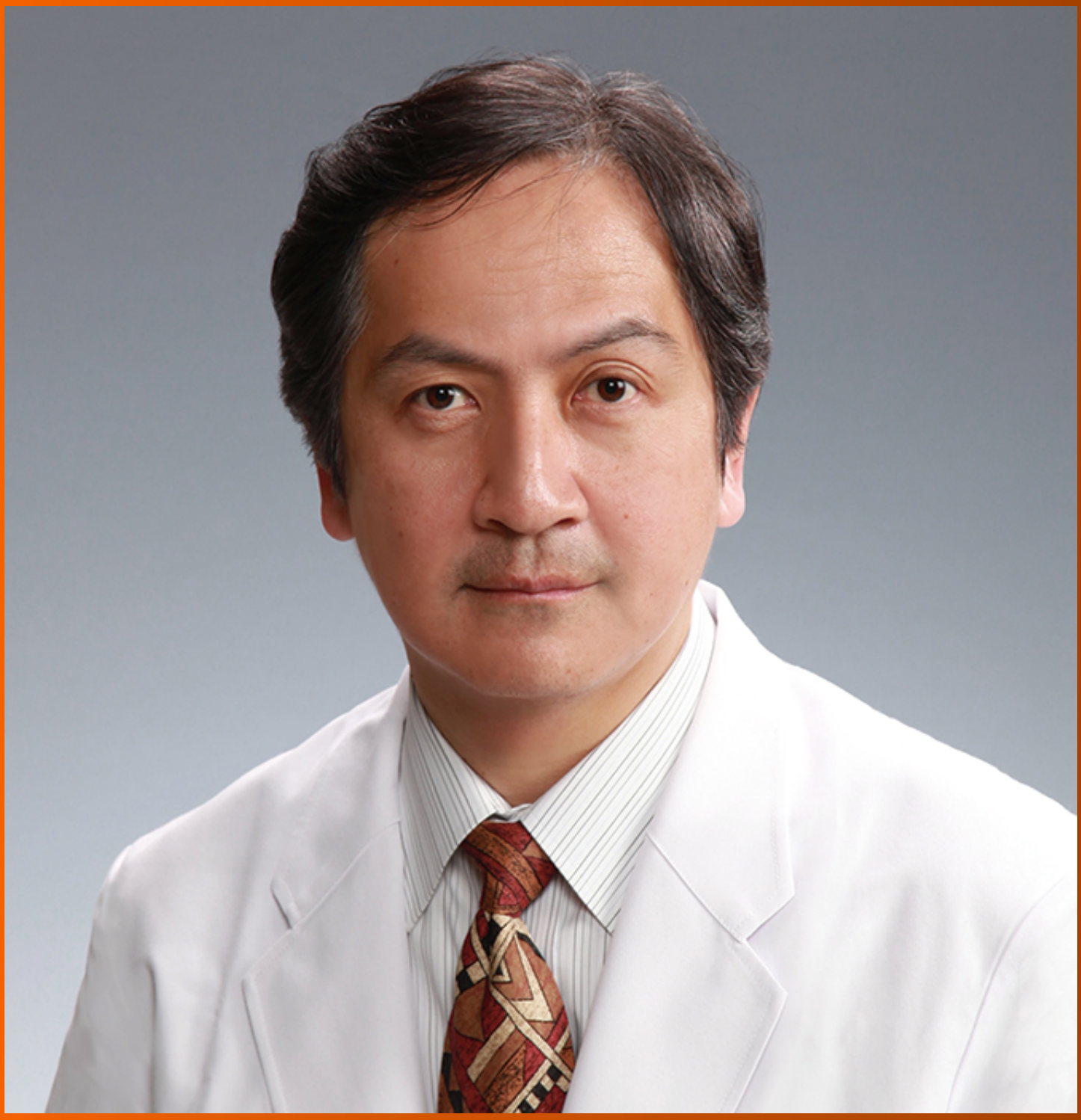

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REVIEW

Gastrointestinal and liver disease in patients with schizophrenia: A narrative review

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Abstract

Schizophrenia is a severe mental illness which can have a devastating impact on an individual's quality of life. Comorbidities are high amongst patients and life expectancy is approximately 15 years less than the general population. Despite the well-known increased mortality, little is known about the impact of gastrointestinal and liver disease on patients with schizophrenia. We aimed to review the literature and to make recommendations regarding future care. Literature searches were performed on PubMed to identify studies related to gastrointestinal and liver disease in patients with schizophrenia. High rates of chronic liver disease were reported, with Non-Alcoholic Fatty Liver Disease being of particular concern; antipsychotics and metabolic syndrome were contributing factors. Rates of acute liver failure were low but have been associated with antipsychotic use and paracetamol overdose. Coeliac disease has historically been linked to schizophrenia; however, recent research suggests that a causal link is yet to be proven. Evidence is emerging regarding the relationships between schizophrenia and peptic ulcer disease, inflammatory bowel disease and irritable bowel syndrome; clinical vigilance regarding these conditions should be high. Patients with schizophrenia poorly engage with bowel cancer screening programmes, leading to late diagnosis and increased mortality. Clozapine induced constipation is a significant issue for many patients and requires close monitoring. There is a significant burden of gastrointestinal and liver disease amongst patients with schizophrenia. Better levels of support from all members of the medical team are essential to ensure that appropriate, timely care is provided.

Key Words: Schizophrenia; Gastrointestinal disease; Liver disease; Mental health

Core Tip: We report significant rates of liver disease, particularly Non-alcoholic Fatty Liver Disease, in patients with schizophrenia, in addition to emerging evidence regarding the prevalence of lower gastrointestinal disease and peptic ulcer disease. We have clearly demonstrated the importance of a multidisciplinary approach to management and propose recommendations to ameliorate future care of this vulnerable group of patients.

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INTRODUCTION

Schizophrenia is a complex and frequently devastating illness, worldwide prevalence of which is estimated to be at 0.32%[1]. It is well established that patients with schizophrenia often suffer from a large number of comorbidities, with high rates of alcohol and substance misuse[2]. Life expectancy amongst patients has also been demonstrated to be approximately 15 years shorter than that of the general population[3].

Although the general rate of comorbidities is acknowledged to be high, no comprehensive review currently exists regarding the burden and distribution of gastrointestinal and liver disease amongst this cohort. This is particularly relevant in light of the coronavirus disease 2019 pandemic, as already marginalised patients may have become more isolated from services and only present when seriously unwell. This subject is of importance to gastroenterologists and hepatologists, in addition to general medical physicians, in order to better understand the particular risks associated with these patients and to help ensure that optimal inpatient and outpatient care is provided. It is also relevant to community and inpatient psychiatric teams to highlight when prompt referrals to specialists may be required.

In this review we summarise the available evidence regarding different aspects of gastrointestinal and liver disease in patients with schizophrenia (Figure 1) in a clear and systematic manner, highlighting key findings, and suggesting recommendations (Table 1) regarding the management of each illness to better inform the future care of this vulnerable group of patients. All articles referenced in this review were identified following a PubMed search for literature specifically considering both gastrointestinal and/or liver disease in patients who had a diagnosis of schizophrenia.

CHRONIC LIVER DISEASE

Chronic liver disease (CLD) has consistently been demonstrated to have a higher prevalence amongst those with schizophrenia than amongst the general population. Carney *et al*[2] reported that people with schizophrenia were 4.42 times more likely than healthy controls to have a diagnosis of CLD; amongst a population of veterans, Fuller *et al*[4] reported an eightfold (8.73) increased likelihood of CLD, and Hsu *et al*[5] have also noted a significantly higher prevalence and incidence of CLD than in the general population. Furthermore, mild and severe liver disease were reported in a Danish study[6] (along with dementia) to have the highest incidence rate ratio amongst nineteen somatic chronic diseases in a cohort of 16,079 patients with schizophrenia who were hospitalised between 1995-2007. Only one study[7] has recorded no elevated risk of liver disease, however the results were adjusted for substance use disorders, which undoubtedly will have had a significant effect on results.

Given such findings, it is essential to reflect on the potential aetiology of the reported results. CLD primarily comprises non-alcoholic fatty liver disease (NAFLD), chronic viral hepatitis and alcohol related liver disease; each will presently be considered and their individual relevance to patients with schizophrenia reviewed.

Non-alcoholic fatty liver disease

Metabolic syndrome: Prior to focusing specifically on NAFLD, the importance of the broader role of metabolic syndrome must be acknowledged. Metabolic syndrome is defined as insulin resistance, impaired glucose tolerance, abdominal obesity, reduced high-density lipoprotein cholesterol levels, elevated triglycerides, and hypertension[8]. A significant association has been widely reported[9,10]

Table 1 Key recommendations

Gastrointestinal/liver disease	Recommendations
NAFLD	Regular monitoring of body mass index and triglycerides
	Encouragement of lifestyle modification
	Liver function tests performed and Fibrosis-4 scores calculated in all psychiatric inpatients and outpatients
Viral hepatitis	Routine screening for viral hepatitis during contact with mental health services
	Integrated approach between mental health, sexual health, substance misuse and specialist hepatology services
ALD	Integrated approach between mental health, substance misuse and hepatology
	Long term studies prospective studies required to fully comprehend burden of ALD
ALF	Clinicians prescribing clozapine should be aware of the potential risk of ALF and have a low threshold for checking liver enzymes and prothrombin time
	Psychiatric review of all patients presenting with POD
	For patients presenting with POD who have a pre-existing psychiatric diagnosis prompt communication must be made with community mental health teams, and appropriate follow-up arranged for those with a new diagnosis
	Decisions regarding liver transplant in patients with schizophrenia must involve the multidisciplinary team and be made on a patient-by-patient basis
PUD	Encouragement of lifestyle modification, particular smoking cessation and alcohol reduction
	Physicians to have a high suspicion for *Helicobacter pylori* infection
Coeliac disease	Large-scale studies amongst diverse population required
	Diagnosis to be considered in patients with schizophrenia presenting with malabsorption
Colorectal cancer	Supporting patients to participate in screening programmes and to attend follow-up appointments is key
IBD	Increased vigilance amongst clinicians regarding a potential diagnosis of IBD is central in enabling prompt diagnosis and maintenance of remission
IBS	Patients should be directly questioned concerning IBS symptoms when undergoing physical health review as many cases may go unrecognised
Clozapine induced constipation	Lifestyle advice to reduce risk
	Regular screening and escalation to GP/secondary care as appropriate
	Physicians to be aware of clozapine as potential cause of constipation and to discuss with psychiatry if considering dose adjustment/cessation

NAFLD: Non-alcoholic fatty liver disease; ALD: Alcohol related liver disease; ALF: Acute liver failure; POD: Paracetamol overdose; PUD: Peptic ulcer disease; IBD: Inflammatory bowel disease; IBS: Irritable bowel syndrome; GP: General practitioner.

between major psychiatric disorders and metabolic syndrome; a prevalence of 32.5% was recorded in a 2013 meta-analysis[11] of patients with schizophrenia and related disorders. An unhealthy lifestyle[12] and use of antipsychotics[13] have been cited as contributing factors to its development in this group.

Definition: The development of NAFLD is associated with obesity, insulin resistance, and type 2 diabetes mellitus and it therefore may be viewed as the hepatic manifestation of metabolic syndrome. NAFLD is characterised by excessive hepatic fat accumulation, which is defined as the presence of steatosis in > 5% of hepatocytes[9]. For a diagnosis of NAFLD to be made, steatosis secondary to factors including hepatitis C, alcohol use, genetics, medications and toxins must first be excluded. NAFLD is reported to have a prevalence amongst the general population of 20% to 30%[14].

Role of antipsychotic medications: The metabolic side effects of antipsychotic medications are well-established, with clozapine and olanzapine in particular being related to weight gain, hyperglycaemia and dyslipidaemia[15]. Understandably, therefore, much of the research into NAFLD and schizophrenia has focused on the potential role of these medications in its development.

A 2016 prospective Spanish study[16] involving 191 patients with a first episode of schizophrenia (94.2% of which were antipsychotic naïve), reported that 25.1% (48/191) of patients demonstrated a fatty liver index (FLI) result suggestive of the presence of steatosis after three years of antipsychotic treatment; none of the included patients had steatosis at index abdominal ultrasound scan (AUSS). The patients in the study were randomised to aripiprazole, risperidone, quetiapine and ziprasidone; the

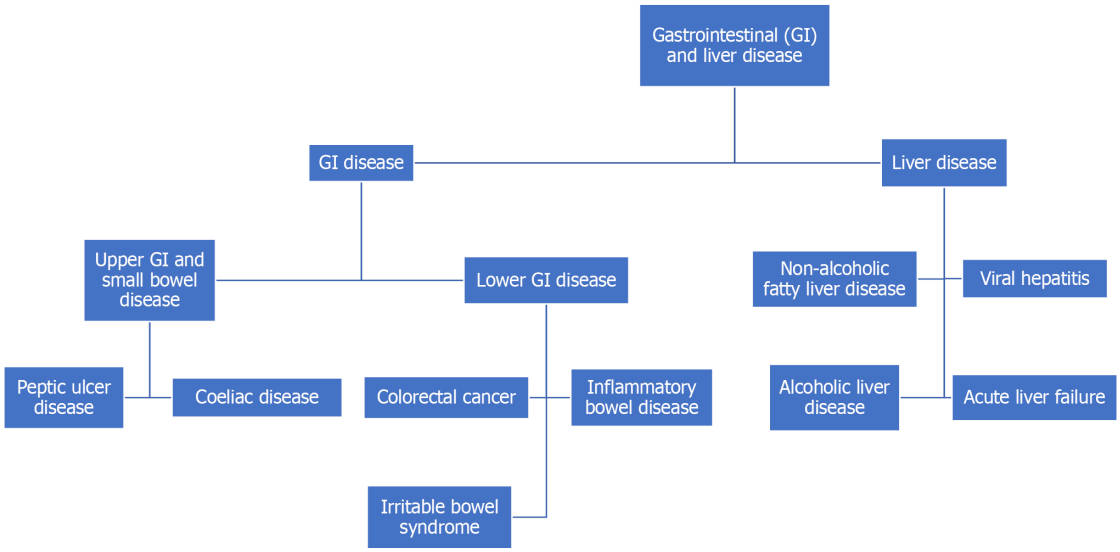

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Figure 1 Summary of gastrointestinal and liver disease in patients with schizophrenia. GI: Gastrointestinal.

dose and type of antipsychotic changed throughout the follow-up period and results were not stratified according to medications. There was no control group. This study is unique in that it prospectively demonstrated the development of NAFLD following the initiation of antipsychotics; the other studies considered hereafter have focused on NAFLD in patients already established on treatment regimes.

In a cohort of 253 patients with schizophrenia, Koreki *et al*[17] reported that 42.7% (108/253) were found to have steatosis on AUSS. All patients included in this study were on at least one antipsychotic treatment (duration was not specified); on univariate analysis total antipsychotic dose (all daily doses were calculated based on chlorpromazine equivalents) was noted to be significantly associated with the presence of steatosis (P = 0.009). On regression analysis, doses of antipsychotic medications recognised as carrying an increased risk of metabolic syndrome and hyperprolactinaemia were significantly associated with NAFLD (P = 0.049 and P = 0.041 respectively). A comparably high prevalence of NAFLD was also reported in a 2017 Chinese study[18] which compared 202 male patients aged 18-35 with schizophrenia and on antipsychotics for at least one month to 149 controls. 49.5% (100/202) of the schizophrenia group had steatosis on AUSS, compared with 20.1% (30/149) of the control group. Although NAFLD prevalence amongst inpatients with schizophrenia was not as high (22.44%) as in the aforementioned studies, in another 2021 Chinese study[19] comprising 66,273 patients with "mental disorders", it was still higher than amongst those with bipolar affective disorder (17.89%), depressive disorder (12.62%) and other mental disorders (12.99%) and was demonstrated to be associated with antipsychotic treatment.

The highest reported prevalence of NAFLD was recorded in a 2021 Taiwanese study[20] which considered 182 patients with schizophrenia who had been hospitalised for at least two years and been on antipsychotic treatment for at least one year. 70.8% (129/182) were diagnosed with steatosis on AUSS; results were stratified according to antipsychotic generation and cumulative dose (using chlorpromazine equivalents), however no significant association between these and NAFLD were demonstrated. Given the reported increased association of NAFLD with second generation antipsychotics[15], the authors attribute the disparity in their results to population demographics and sample size. Interestingly, antipsychotic treatment duration, potentially the biggest contributor to the reported results in this cohort, was not reported in the results.

Rates of NAFLD have also been reported in studies not specifically considering antipsychotic therapies. In a population of veterans in the United States[4], those with a diagnosis of schizophrenia were significantly more likely to be diagnosed with NAFLD ($P \leq 0.001$) and in a cross-sectional Danish study[21] 21.4% (31/145) were diagnosed with moderate-severe steatosis on non-enhanced CT. In these studies, significant associations were however demonstrated with metabolic syndrome, suggesting (although not confirming due to lack of data) that antipsychotics may also be potentially playing a role in these reported results.

Lifestyle and negative symptoms: While research has predominantly focused on the close links between antipsychotics and NAFLD, it is also important to briefly consider potential alternative aetiologies that have been proposed.

Patients with schizophrenia are acknowledged as being at higher risk of an unhealthy lifestyle compared to the general population[17]. They may have an unbalanced diet, poor self-care, avoid certain foods due to paranoid delusions or hallucinations, and withdraw from physical and social

activities, leading to a lack of exercise[22]. Yan *et al*[18] in their 2017 study emphasised the role of negative symptoms in young men with schizophrenia and NAFLD; a negative factor score in the Positive and Negative Syndrome Scale (PANSS) was significantly associated with a NAFLD diagnosis ($P = 0.025$). It is not challenging to understand how the negative symptoms of anhedonia, poor attention, low motivation, apathy and social withdrawal may lead to obesity and the associated sequelae of metabolic syndrome.

Recommendations: It is evident that a diagnosis of schizophrenia carries with it a significant risk of developing NAFLD, both due to treatment with antipsychotics and also due to the intrinsic nature of the illness itself. In order to prevent progression to cirrhosis and its associated complications it is necessary to increase awareness of this risk and consider how it may be ameliorated. At the very least, body mass index (BMI) and triglycerides should be monitored regularly in all patients with schizophrenia and psychiatrists and general medical physicians should make every effort to support patients in modifying their lifestyles. Furthermore, all patients admitted to psychiatric hospitals and attending outpatient psychiatry appointments ought to have liver function tests performed and Fibrosis-4 (Fib-4) scores calculated to determine if a referral to hepatology is necessary.

Chronic viral hepatitis-hepatitis B and hepatitis C

People with serious mental illness (including schizophrenia) have been shown to be at increased risk of blood borne viruses (BBVs)[23]. A significant minority of patients with schizophrenia engage in risky behaviour (unprotected sex with multiple partners, sex work, and intravenous drug use) putting them at risk of infection; they are also more likely to live in shared accommodation and may share personal equipment such as razors and toothbrushes, carrying a potential increased risk of hepatitis B and C transmission[23]. Patients with hepatitis B and C have an increased risk of developing hepatocellular carcinoma[24], therefore adequate treatment and monitoring of the conditions is vital.

In 2017 a Swedish study[25] considered the prevalence and risk factors for BBVs amongst people with severe mental illness; when compared with healthy controls, those with schizophrenia were significantly more likely to have either a diagnosis of hepatitis B ($P \leq 0.001$) or hepatitis C ($P \leq 0.001$). A history of substance misuse was noted as having contributed the most to the risk of developing a BBV ($P \leq 0.001$). Increased prevalence of hepatitis C was also shown in a study of veterans[4] diagnosed with schizophrenia when compared to controls ($P \leq 0.001$). Substance use disorder and alcohol use disorder were identified as significant risk factors for development of schizophrenia ($P \leq 0.001$ and $P \leq 0.001$ respectively). A small study performed in Boston[26] identified eight patients with hepatitis C out of a total cohort of 98 patients (8.2%) treated with clozapine; one was a confirmed intravenous drug user, three had a history of polysubstance misuse and in the remaining four patients no risk factors were identified. A further small Canadian study[27] reported a hepatitis C rate of 2.7% (3/110) of clozapine-treated patients. Two of the three patients diagnosed with hepatitis C had a history of intravenous drug use.

Outcomes in patients with schizophrenia diagnosed with hepatitis C and hepatitis B were considered in a 2021 Chinese study[24]. From a total cohort of 15,914 patients with newly diagnosed schizophrenia between 2007 and 2013, 614 patients were identified with viral hepatitis and were matched with 1228 controls. The primary outcome measure was the incidence and risk of "severe hepatic outcomes" (SHOs), including liver cancer, failure and decompensation. The viral hepatitis group were significantly more likely to develop SHOs ($P \leq 0.001$) than the control group; a total of 26 patients with viral hepatitis developed SHOs (20/26 Liver decompensation, 3/26 Liver failure, and 3/26 Liver cancer).

Recommendations: While the risk of viral hepatitis amongst people with schizophrenia cannot be disputed, optimal management of it is challenging. Routine screening for viral hepatitis during contact with mental health services would allow an opportunity for discussion about safe sex and drug use, it may also prompt consent to other tests (such as HIV), leading to the discovery of potentially treatable illnesses, and, crucially, a positive result may allow prevention of further liver damage[26]. If diagnosed, encouragement of treatment adherence in this population may be difficult, therefore an integrated approach between mental health, sexual health, substance misuse[25] and specialist hepatology services would be required to aid optimal patient outcomes.

Alcohol related liver disease

A high prevalence of alcohol use disorder (AUD) has been demonstrated in patients with schizophrenia, with a recent meta-analysis reporting a lifetime prevalence of AUD of 24.3%[28]. A number of hypotheses have been put forward to explain this association, including genetic polymorphisms, poor cognitive development, poor social functioning, the effects of poverty, poor social environments and "self-medication" to gain relief from distressing symptoms[29].

Despite such a high prevalence of AUD in patients with schizophrenia, research into alcohol related liver disease (ALD) in this cohort is relatively limited with variable results. The most common manifestations of ALD are alcoholic fatty liver, acute alcoholic hepatitis and alcohol related cirrhosis of the liver. In 2014 Hsu *et al*[5] reported an increased risk of alcohol related fatty liver in patients with schizophrenia when compared with the general population ($P = 0.007$); risk of alcoholic hepatitis and cirrhosis

did not vary significantly between the two cohorts. In a 2022 Scottish study[30] of patients with schizophrenia admitted to a general hospital under the care of gastroenterology, 42.5% (17/40) of patients had a background of cirrhosis (88.2% of which had a background of alcohol excess) and 60.0% (24/40) of all patients had a history of alcohol excess. Interestingly, Fuller *et al*[4], in their 2011 study, reported schizophrenia being a protective factor (odds ratio = 0.53) in the diagnosis of alcohol related cirrhosis when a group of veterans with schizophrenia were compared to those without.

Recommendations: In common with viral hepatitis, an integrated approach between mental health, substance misuse services and specialist hepatology services is key in supporting patients with schizophrenia and alcohol use disorder. Further research, including long term prospective studies, is also required to better comprehend the burden of ALD amongst patients with schizophrenia.

ACUTE LIVER FAILURE

Acute liver failure (ALF) is a rare clinical syndrome in which the onset of liver injury, with coagulopathy and hepatic encephalopathy, occurs in a patient with no underlying CLD and previously normal liver function. The associations between ALF and schizophrenia can be considered under the following categories: ALF arising as a result of pharmacological treatment for schizophrenia, ALF arising as a consequence of mental state alteration in patients with schizophrenia and finally the implications of a diagnosis of schizophrenia on decision making around emergency liver transplantation for ALF.

Pharmacological treatments

In patients with treatment resistant schizophrenia (*i.e.*, following the failure of at least two antipsychotics, including a second-generation antipsychotic) prompt initiation of clozapine, the only remaining evidence-based treatment, is required to reduce symptoms and risk of relapse[31,32]. Clozapine acts as a dopamine and serotonin receptor antagonist. The most recognised and feared side effect of clozapine is agranulocytosis, however there are now case reports of clozapine induced ALF[33,34]. The mechanism of liver injury in this scenario is unclear, but it is idiosyncratic. A modest elevation in liver enzymes occurs in up to two thirds of patients treated with clozapine, and often resolves spontaneously[35]. Clinically apparent liver injury (with jaundice) occurs in approximately 1 in 2000 patients treated with clozapine[35] and progression to ALF (and death, or the need for emergency liver transplant) is now reported. ALF secondary to clozapine is extremely rare however, with less than five reported cases in the literature[33,34,36]. ALF has also been reported as a rare side effect of quetiapine therapy[37]; no cases of ALF have been reported with olanzapine or risperidone. Most treatment guidelines do not give clear recommendations on monitoring liver function tests in patients on clozapine and other antipsychotics, in part due to the fact that the vast majority of liver enzyme abnormalities secondary to these drugs do resolve spontaneously. Clinicians prescribing clozapine should be aware of the risk of ALF and could consider checking liver enzymes and prothrombin time when performing blood work monitoring for agranulocytosis.

Paracetamol overdose

Schizophrenia is associated with suicidal ideation[38]. In the United Kingdom, intentional paracetamol overdose is the most frequent cause of ALF. There is no clear evidence that schizophrenia is associated with an increased risk of paracetamol overdose resulting in ALF compared with the general population or patients with other psychiatric diagnoses. Scottish data have shown that of 472 patients with paracetamol overdose requiring admission to the Scottish Liver Transplant Unit who underwent formal inpatient assessment by psychiatry, schizophrenia was diagnosed or recorded in 2.8% of patients [Personal communication: Dr Roger Smyth, Consultant Liaison Psychiatrist, Royal Infirmary of Edinburgh]. In comparison, affective disorders were recorded in 16.1% and personality disorders in 6.9% of patients. One Danish study assessed the association between paracetamol (and other weak analgesic) poisoning and the subsequent diagnosis of a psychiatric disorder[39]. The risk of an admission with schizophrenia increased 3.9-fold after paracetamol poisoning and 2-fold after non-paracetamol poisoning compared with matched population controls. Patients with a previous psychiatric admission were excluded, but it is possible that patients with schizophrenia who had not required prior hospitalisation (*i.e.*, but who already had an established diagnosis) were included in the study. Furthermore, the admission with poisoning is likely to have prompted a psychiatric review and subsequent diagnosis. These data suggest that poisoning is a risk marker for a psychiatric disorder, rather than there being a causative association. Clinicians assessing patients presenting with paracetamol overdose should be aware of the risk of underlying psychiatric disorder and ensure appropriate assessments are arranged.

Interestingly, in addition to its role in treatment of paracetamol overdose, there is ongoing research into the reduction of negative symptoms and anti-suicidal properties of N-acetyl cysteine (NAC) in the treatment of schizophrenia. Chen *et al*[40] identified two placebo-controlled, double-blind, randomised

clinical trials of NAC in schizophrenia and reported that NAC may be efficacious in reducing the negative and general symptoms of schizophrenia. A meta-analysis by Zheng *et al*[41], which included three randomized control trials with 307 (N-acetylcysteine: 153, placebo: 154) participants, showed that NAC significantly improved total symptom scores in schizophrenia. Other related systematic reviews, including a Cochrane review on antioxidant treatment for schizophrenia, have also found NAC to be a promising add-on treatment for schizophrenia[42].

Emergency liver transplantation

Emergency liver transplantation may be required in patients with ALF if spontaneous recovery of sufficient liver function is deemed unlikely. Psychiatric disorders can be relative or absolute contraindications to liver transplantation, particularly if the disorder is deemed "untreatable" and may be graft threatening. A diagnosis of a psychiatric condition such as schizophrenia is relevant if it affects the prospect of survival post-transplant or affects compliance with medication and clinic follow-up. In a Scottish cohort of patients with ALF secondary to paracetamol overdose, 56.6% of patients who were rejected for listing for liver transplantation were rejected on the basis of psychiatric contraindications [43]. As described above, schizophrenia is associated with an increased risk of suicide, and this must be taken into account when considering the role of transplantation in an illness potentially arising from self-harm or parasuicide such as paracetamol overdose; the consequences of further suicidal intent must be reviewed. Specific to severe psychiatric disorders such as schizophrenia, absolute contraindications to liver transplantation include chronic, severe illness with a poor prognosis, especially if refractory to appropriate treatment. The potential impact of a diagnosis of schizophrenia on substance misuse, compliance with immunosuppression and clinic follow-up must be considered. Therefore, patients with schizophrenia being considered for emergency liver transplantation for ALF should be assessed by the wider multi-disciplinary team and decisions made on a highly individualised basis.

Recommendations: While cases of ALF are rare in patients treated with clozapine, prescribing clinicians should be aware of the potential risk and have a low threshold for checking liver enzymes and prothrombin time, particularly in patients presenting with possible symptoms of liver dysfunction such as nausea, vomiting and/or anorexia[44].

Regarding paracetamol overdose, it is essential that treating physicians are aware of the risk of a diagnosis of a psychiatric disorder and ensure patients are reviewed by psychiatric teams during their admission. For patients who have a pre-existing diagnosis prompt communication must be made with community mental health teams (CMHT), and appropriate follow-up arranged for those with a new diagnosis. The use of NAC in the treatment of schizophrenia is an emerging area of research and further evidence is required regarded its potential.

The assessment of patients with schizophrenia for liver transplant is complex. Decisions must involve the multidisciplinary team (MDT) and be made on a patient-by-patient basis.

UPPER GASTROINTESTINAL AND SMALL BOWEL DISEASE

Peptic ulcer disease and upper gastrointestinal bleeding

Upper gastrointestinal bleeding (UGIB) is the consequence of often readily treatable digestive conditions such as gastroduodenal ulcers and reflux oesophagitis progressing to a potentially fatal event; in patients with schizophrenia the risk may be heightened due to high rates of alcohol misuse, smoking and non-steroidal anti-inflammatory drug use, in addition to reluctance to present to medical services. There are relatively limited studies which have considered incidence of peptic ulcer disease (PUD) and UGI bleeds in this cohort, with evidence being inconclusive, and, at times, contradictory.

Helicobacter pylori (*H. pylori*) infection is recognised[45] as a major contributor to PUD and consequently UGIBs. Following a 1997 study by De Hert *et al*[46] and a 2005 study by Yilmaz *et al*[47] (both of which reported high rates of *H. pylori* infection in patients with schizophrenia), a Turkish group [47] discussed the role of *H. pylori* as a possible environmental contributor to the pathogenesis of schizophrenia in genetically predisposed individuals. The authors identified four mechanisms to support this theory: (1) Dopaminergic dysfunction; (2) inflammation; (3) polyunsaturated fatty acids; and (4) hyperhomocysteinaemia. With regard to dopaminergic dysfunction specifically, the authors propose that dopamine antagonists (such as commonly used antipsychotics) promote ulcer formation favouring the growth of *H. pylori*; this is a theory which is supported by Ozdemir[48] in their 2007 paper in which they propose that the lesser prevalence of PUD in their cohort of patients with schizophrenia was due to the over expression (and subsequent protective effect) of dopamine.

The reduced prevalence of PUD in patients with schizophrenia reported by Ozdemir *et al*[48] is in contrast to results reported in other studies. In 1968 Hussar[49] proposed that the incidence of PUD was at least as high amongst long term institutionalised patients with schizophrenia compared with the general population and, more recently, in 2014, Liao *et al*[50] reported an incidence of PUD that was 1.27 times higher in patients with schizophrenia than in the general population. An American study in 2014 [51] of 224,361 patients with schizophrenia, reported an incidence of bleeding ulcers amongst this cohort

as being 1.4 per 1000 person years, compared to 1.2 per 1000 person years in those without schizophrenia. Similarly increased risk of UGIB was also noted in a 2018 Danish study[52] of 39,998 patients with schizophrenia; the authors acknowledge the role that increased rates of *H. pylori* infectivity amongst patients with schizophrenia may have played in the observed results.

Recommendations: It is challenging to draw definitive conclusions regarding biological links between the aetiology of PUD and schizophrenia and more research is required before such links are established. However, it important to acknowledge the increased risk of PUD those patients with schizophrenia has due to lifestyle factors, and, as a result, ensures that help is given to aid modification. Suspicion regarding the presence of *H. pylori* should also be high, particularly as it may have the potential to contribute to mortality through a catastrophic UGIB or the development of gastric cancer[53].

Coeliac disease

Coeliac disease is an autoimmune disease which is triggered by gluten peptides in wheat, rye, barley and other grains. Histologically it is characterised by villous atrophy, hypertrophy of intestinal crypts and increased lymphocytes in the epithelium and lamina propria[54]. This results in malabsorption and the associated bloating, pain and diarrhoea in sufferers. Current prevalence of coeliac disease is estimated at 1% of the general population[54].

Historical perspective: A potential association between coeliac disease (or gluten intolerance) and schizophrenia is one which has long since interested researchers[55], with Bender[56] first reporting in 1953 that children with schizophrenia were more likely to have coeliac disease. This was then followed by the publication of a case series on five patients with schizophrenia with coeliac disease who were admitted to a psychiatric hospital during the same year[57]. Dohan was particularly prolific in the publication of papers regarding coeliac disease and schizophrenia, with results variably showing reduced prevalence of schizophrenia in areas of low grain consumption and an improvement in the symptoms of schizophrenia following the initiation of a gluten free diet[58-62].

Current status: More recent research has focused on the risk of patients with coeliac disease developing schizophrenia as opposed to prevalence of coeliac disease amongst those with schizophrenia; nevertheless, the strength of an association between the two conditions may be extrapolated from the available evidence. A 2018 meta-analysis[63] of the four most recent epidemiological studies[64-67] reported a significantly higher risk (two-fold) of schizophrenia in those with coeliac disease when compared with controls. Of the four studies, three[64,66,67] reported a significant association and one [65] did not (in common with an earlier British study[68]). The authors of the meta-analysis acknowledge that the mechanism underlying this association is unclear, however propose the following potential explanations: (1) There is high prevalence of folate deficiency in individuals with coeliac disease, given the important role of folate in DNA methylation, aberrant methylation may contribute to the development of schizophrenia; (2) schizophrenia may share a genetic diathesis with coeliac disease; and (3) diagnoses of schizophrenia in patients with coeliac disease may be resulting from surveillance bias as those with coeliac will present more often to physicians due to their chronic illness requiring regular follow-up. Since the publication of the meta-analysis, a small study[69] of 16 patients with IgG antigliadin antibodies (raised in coeliac disease) has also demonstrated an improvement in psychiatric patients with schizophrenia or schizoaffective disorder with a gluten free diet; a larger scale clinical trial is planned.

Recommendations: While research into a possible link between coeliac disease and schizophrenia has previously relied on relatively small case reports and series, more recently trial evidence has emerged to strengthen the association. Proof of a causal relationship between the two remains unclear and further large-scale studies, amongst more diverse populations, are required. Nevertheless, current evidence does serve to increase awareness of the likelihood of a diagnosis of coeliac disease amongst patients with schizophrenia presenting with symptoms of malabsorption.

LOWER GASTROINTESTINAL DISEASE

Colorectal cancer

Evidence regarding rates of cancer mortality in patients with schizophrenia has historically been contradictory, with some studies reporting lower or similar risk of cancer mortality compared to the general population[70-72], while others have reported higher rates[73-75]. Regarding colorectal cancer, however, a recent systematic review and meta-analysis[76] found that patients with schizophrenia had a significantly higher risk of mortality, with male patients having a relative risk of 1.90 and female patients 2.42. Increased risk of colorectal cancer[77] was also reported in a 2007 study. As in the general population, screening is vital in promoting earlier diagnosis and reducing mortality; a 2018 Japanese study[78] reported a rate of participation in colorectal cancer screening of 13.4% in patients with schizophrenia (in contrast to 47.8% of men and 40.9% of women in the general population[79]). The benefit of

case management interventions, in the form of three counselling sessions to support adherence to screening, was demonstrated in a 2021 Japanese study[79], which showed a 35.3% increase in participation amongst the case management group.

Recommendations: As reported in the 2021 study[79], participation in screening programmes is key to reducing mortality from colorectal cancer amongst patients with schizophrenia to be in line with the general population. Supporting patients to participate in screening and attend potential follow-up procedures is essential to address the disparity between colorectal cancer mortality in this cohort when compared to the general population.

Inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) are the two main types of inflammatory bowel disease (IBD) and are characterised by chronic inflammation - in the case of UC it is limited to the mucosa of colon and rectum, and, in CD, it is transmural and extends potentially to the whole GI tract. Symptoms of both conditions include diarrhoea, abdominal pain and extreme fatigue. Prevalence of IBD is increasing and is projected to reach 1% by 2028[80].

The relationship between IBD and schizophrenia has been explored in a small number of studies, with variable results. Bernstein *et al*[81] reported an increased incidence of schizophrenia amongst patients with CD but not with UC, whereas no increased risk in either form of IBD was demonstrated by West *et al*[68]; a Swedish study actually noted a diminished risk of schizophrenia in those with IBD[82].

In contrast to earlier studies which focused on risk of schizophrenia in IBD patients, a recent study from Taiwan[83] considered risk of a new IBD diagnosis amongst patients with a diagnosis of schizophrenia. It included 116,164 patients with schizophrenia and 464,656 matched controls and concluded that overall incidence of IBD was significantly higher amongst the schizophrenia cohort (1.14% *vs* 0.25%); risk was also higher amongst those with more severe schizophrenia. These findings are potentially supportive of research suggestive of shared genetic susceptibility between the two conditions[84,85], although the role of diet and microbiota remain important confounding factors.

Recommendations: CD and UC are challenging conditions to manage, this risk is heightened in vulnerable patients with schizophrenia as engagement with health services is essential for positive outcomes. As recommended by the authors of the recent Taiwanese study[83], increased vigilance amongst clinicians and other members of the MDT regarding potential cases of IBD may prove vital in enabling prompt diagnoses and maintenance of remission.

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterised by abdominal pain, bloating and a change in bowel habit [either constipation (IBS-C), or, more commonly, diarrhoea (IBS-D)]; diagnosis is made based on the Rome IV criteria. Current prevalence of IBS in the United Kingdom is estimated to be at 14%[86].

The first study to consider IBS in patients with schizophrenia was published in 1997[87]. The authors reported that 19% of the patients with schizophrenia met the diagnostic criteria for IBS, compared with 2.5% of those in the control group. It must however be acknowledged that the study only included 87 patients in total. Interestingly, it was noted that the patients with schizophrenia did not complain of symptoms of IBS, unlike when the authors considered a group of patients with anxiety and depressive disorder; the authors attributed this to a preoccupation with delusions and hallucinations or being due to apathy resulting from negative symptoms.

A further study[88] comprising 134 patients with schizophrenia reported IBS in 48.8%; the study also reported higher rates of IBS-D in patients presenting with positive symptoms of schizophrenia, IBS-C and alternating IBS-D/C were more common in those with negative symptoms. The group was not compared to a control, which makes conclusions limited.

Recommendations: Research suggests that there may be an under-reporting of IBS symptoms amongst patients with schizophrenia. Consideration should therefore be given to IBS symptomatology in patients with schizophrenia undergoing physical health review as many cases may go unrecognized unless patients are directly questioned. Addressing diet and lifestyle factors are also important in improving the quality of life in patients with schizophrenia and IBS symptoms.

Clozapine induced constipation

Whilst recognising it as a side effect of clozapine treatment as opposed to a disease in itself, it is necessary to acknowledge the risk of clozapine induced constipation in patients with schizophrenia. Risk of constipation is greatest in the first four months following treatment initiation, however may occur at any time[89]. A 2016 systematic review and meta-analysis[90] of 11 studies reported a prevalence of clozapine induced constipation of 31.2%. The authors attribute increased risk of constipation to the anti-muscarinic and anti-histaminergic effects of the drug, as well as to a sedentary lifestyle, dehydration, obesity and a diet which is low in fibre. Complications of constipation such as bowel obstruction, ileus (a Danish study[91] reported a 0.8% incidence of ileus in clozapine treated

patients) and toxic megacolon may be life threatening; in severe constipation the case fatality rate has been documented at approximately 20%-30%[89]. Following the aforementioned systematic review and meta-analysis[90], subsequent studies and case reports[92-96] have also reported similarly high incidence of clozapine induced constipation and gastrointestinal hypomotility, highlighting the ongoing significance of this issue.

Recommendations: Given the high prevalence of clozapine-induced constipation, all patients on clozapine should be given lifestyle advice to help prevent the development of constipation. It is crucial to screen for its presence during CMHT review (regular attendance at clozapine blood monitoring may help to facilitate this) and it is particularly important to ask directly about it as incidence may be underreported by patients. Patients may be referred to their general practitioner (GP) for prescription of laxatives and ongoing monitoring if constipation is present, in addition to plasma clozapine levels being reviewed by the CMHT; if there are features concerning for obstruction prompt referral to secondary care is required.

Caution should be exercised in the prescription of anti-cholinergics for hypersalivation (a known side effect of clozapine) due to their potential to contribute to constipation; other regular medications with constipating side effects should also be kept under close review. For secondary care physicians it is important to be aware of clozapine as a potential cause of constipation and essential to seek advice from colleagues in psychiatry if dose adjustment or treatment cessation is being considered.

GUT MICROBIOME AND SCHIZOPHRENIA

In consideration of gastrointestinal disease in schizophrenia it is important lastly to acknowledge an emerging area of active research - the gut microbiome. The gut microbiome in humans comprises a diverse population of microbes, the most numerous of which are reported to be Bacteroidetes and Firmicutes[97]. Factors such as diet, smoking and social circumstances have been suggested to influence the composition of an individual's developing microbiome. While each microbiome is unique, when the microbial composition differs significantly from controls, it is referred to as dysbiosis. Alterations in gut microbiota have been demonstrated to be implicated in several psychiatric illnesses, including depression, addiction and eating disorders. Evidence is now emerging regarding the potential role of gut dysbiosis in the aetiology of schizophrenia and the use of pre and probiotics in treatment pathways is also being explored.

In 2020 Szeligowski *et al*[98] performed a narrative review of research considering the differences in microbiome between healthy controls and patients with schizophrenia; six studies were identified. The authors reported only one consistent finding between the studies-that patients with schizophrenia had significantly elevated Lactobacilli, which also correlated with symptom severity. As Lactobacilli are typically thought to be beneficial for gut health, this finding was attributed to the existence of different subtypes. The authors conclude that different exclusion criteria, stage of illness and treatments make definitive conclusions regarding the role of dysbiosis in schizophrenia challenging and further larger scale prospective studies are required.

Pre and probiotics are also being investigated for their ability to reduce the effect of antipsychotic medications on the microbiome which can lead to potentially life- threatening constipation and significant weight gain. Results of small studies which have been reported in reviews of the literature [98-101] are encouraging, for example co-administration of prebiotics to olanzapine treated rats led to attenuation of weight gain[102]. As with the role of dysbiosis in the aetiology of schizophrenia, more detailed studies in human subjects are of course needed but early results do suggest this may be a promising area for future treatment.

CONCLUSION

Gastrointestinal and liver disease have been shown to have a profound impact on patients with schizophrenia. The scale of liver disease, and metabolic syndrome in particular, in this population, is perhaps the most significant finding upon review of the literature. Optimal management of these conditions deserves close consideration from psychiatrists, hepatologists and all members of the MDT involved in the care of patients with schizophrenia. A recurring theme from consideration of all gastrointestinal and liver diseases is that patients with schizophrenia need appropriate additional levels of support from care providers to ensure that their physical health receives the attention it requires and which they, as individuals, deserve.

FOOTNOTES

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MINIREVIEWS

Ultrasound-based artificial intelligence in gastroenterology and hepatology

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Abstract

Artificial intelligence (AI), especially deep learning, is gaining extensive attention for its excellent performance in medical image analysis. It can automatically make a quantitative assessment of complex medical images and help doctors to make more accurate diagnoses. In recent years, AI based on ultrasound has been shown to be very helpful in diffuse liver diseases and focal liver lesions, such as analyzing the severity of nonalcoholic fatty liver and the stage of liver fibrosis, identifying benign and malignant liver lesions, predicting the microvascular invasion of hepatocellular carcinoma, curative transarterial chemoembolization effect, and prognoses after thermal ablation. Moreover, AI based on endoscopic ultrasonography has been applied in some gastrointestinal diseases, such as distinguishing gastric mesenchymal tumors, detection of pancreatic cancer and intraductal papillary mucinous neoplasms, and predicting the preoperative tumor deposits in rectal cancer. This review focused on the basic technical knowledge about AI and the clinical application of AI in ultrasound of liver and gastroenterology diseases. Lastly, we discuss the challenges and future perspectives of AI.

Key Words: Artificial intelligence; Ultrasound; Liver; Gastroenterology; Deep learning

Core Tip: Artificial intelligence (AI) based on ultrasound has been confirmed to be helpful in diagnosing diffuse liver diseases and focal liver lesions, such as analyzing the severity of nonalcoholic fatty liver and the stage of liver fibrosis, identifying benign and malignant liver lesions, predicting microvascular invasion of hepatocellular carcinoma, curative transarterial chemoembolization effect, and prognoses after thermal ablation. AI based on endoscopic ultrasonography has been applied in some gastrointestinal diseases. We focused on basic technical knowledge about AI and the aforementioned clinical application in the ultrasound of liver and gastroenterology. Additionally, we discuss the challenges and future perspectives of AI.

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INTRODUCTION

Liver disease causes two million deaths per year in the world among which cirrhosis is the 11th leading cause of death in the world and liver cancer is the 16th leading cause of death[1]. The prevalence of nonalcoholic fatty liver disease (NAFLD) is 25.0% and is estimated to be 33.5% by 2030[2]. Gastrointestinal diseases affect an estimated 60 to 70 million American citizens annually. It is reported that pancreatic cancer (PC) is one of the top five causes of death from cancer, and colorectal cancer accounts for 8.5% of cancer-related deaths[3-5]. Therefore, it is of great importance to pay attention to these diseases.

In clinical practice, many imaging techniques such as X-ray, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound have played a vital role in the detection and treatment of diseases[6]. Ultrasound, a noninvasive and real-time diagnostic technique, is the most commonly used method for detecting and diagnosing human digestive diseases[7]. However, the interpretation and analysis of ultrasound images depend deeply on the subjective judgment and experience of human experts. Radiologists may make mistakes due to exhaustion when dealing with a large number of images[8].

Artificial intelligence (AI) is defined as computer algorithms created by humans and improved with analogs of the thoughts, judgments, and reactions that take place in the human brain. In recent years, radiologists have increasingly embraced the aid of AI-powered diagnoses. AI can make a quantitative analysis by recognizing the information of images automatically and is widely applied in the medical images of ultrasound in diffuse liver diseases, focal liver lesions, PC, and colorectal cancer. In this review, we described the development of AI-based ultrasound in the aforementioned applications. In addition, we also discussed the future opportunities and challenges of AI-based ultrasound.

AI

Currently, the algorithms of AI used in medical images mainly include traditional machine learning algorithms and deep learning.

Machine learning

Machine learning is described as a kind of data science that offers computers with the capacity to study without being programmed with specific rules[9]. It focuses on computer algorithms that are studied from the training model and give predictions on another model[10]. Machine learning depends primarily on the predefined characteristics that display the regular patterns inherent in models acquired from regions of interest with explicit parameters on the basis of expert experience. Then, other medical image features, such as various mass shape, size, and echo, can be quantified.

Radiomics, which belongs to traditional machine learning, is a popular field of study related to the acquisition and assessment of patterns within medical images, including CT, MRI, and ultrasound. These patterns include complicated patterns that are difficult to recognize or analyze by the human eye [11].

Deep learning

Deep learning is at the leading edge of AI and is developing rapidly. Deep learning is described as a group of artificial neural network (ANN) algorithms, which include many hidden layers. Namely, deep learning depends on a subset of algorithms that try to model high-level abstractions[12].

Recently, convolutional neural networks (CNNs) are the preferred type of deep learning architecture in the assessment of medical images[13]. CNNs consist of an input layer, multiple hidden layers, and an output layer (Figure 1). The hidden layers include convolutional layers, pooling layers, connected layers, and normalization layers. Convolutional layers and pooling layers can complete feature extraction and aggregation[9].

APPLICATION OF ULTRASOUND-BASED AI IN HEPATOLOGY

Diffuse liver diseases

Diffuse liver diseases display a failure in the metabolic and synthesis processes of the liver[14]. Liver biopsy is the gold standard for the diagnosis of fibrosis and NAFLD. However, liver biopsy is an invasive process that has many complications such as hemorrhage, biliary peritonitis, and pneumothorax[15]. In addition, liver biopsy is not feasible for the long-term management of patients with chronic liver diseases. Noninvasive liver imaging methods such as CT, MRI, and ultrasound have been extensively studied. Ultrasound is one of most common methods to diagnose liver diseases due to its noninvasiveness, inexpensive price, and real-time ability. Machine learning algorithms based on ultrasound have been applied for analysis of steatosis and the staging of liver fibrosis. Table 1 shows the application of ultrasound-based AI in diffuse liver disease.

Fatty liver diseases: An excess amount of fat in the liver cells is found in fatty liver diseases (FLD). The main causes of FLD include obesity, alcoholism, diabetes, nonalcoholic steatohepatitis, drugs, and toxins [16,17]. FLD is related to the growing risk of cirrhosis and liver cancer. The most common cause of FLD is NAFLD, which ranges in prevalence from 25% to 45%[18]. Several noninvasive imaging methods such as CT, MRI, and ultrasound can diagnose NAFLD[19]. Ultrasound is the cheapest diagnostic method with 93% sensitivity, while hepatic steatosis is greater than 33%[18].

Conventional ultrasound is commonly used for NAFLD evaluation, but its qualitative nature, doctor dependency, and unsatisfactory accuracy limits the application. Moreover, the ultrasound images of fatty liver and early cirrhosis have many common features, making it hard to distinguish the two diseases by the human eye[20].

In recent years, ultrasound-based AI has demonstrated high accuracy for detection of steatosis and represents excellent reproducibility and reliability.

Byra *et al*[21] created a CNN model to acquire features from B-mode ultrasound image. It was reported that they could assess the amount of steatosis present in the liver with the area under the receiver operating characteristic curve (AUC) of 0.98, and their approach may assist the doctors in automatically assessing the amount of fat in the liver clinically[21].

Biswas *et al*[22] revealed that a deep learning-based algorithm reached a superior performance for FLD identification and risk stratification with 100% accuracy and AUC of 1.0 when compared with a conventional machine learning system support vector machine (SVM) (accuracy: 82%, AUC: 0.79) and extreme learning machine (accuracy: 92%, AUC: 0.92).

Deep learning has also been applied to quantitatively evaluate NAFLD. The radiofrequency data of ultrasound displays much more information of hepatic microstructure than that of gray-scale B-mode images[23]. Han *et al*[24] developed a deep learning algorithm that used radiofrequency data for NAFLD assessment. The results revealed that the sensitivity, specificity, and positive predictive value (PPV) for NAFLD diagnosis were 97%, 94%, and 97%, respectively. They confirmed that the quantitative analysis of raw radiofrequency ultrasound signals showed the potential of identifying NAFLD and quantifying hepatic fat fraction[24].

Liver fibrosis and cirrhosis: Patients with chronic liver disease may have no clinical symptoms for an extended period, or it may develop to fibrosis and cirrhosis[25]. The activation of the resting hepatic stellate cell into an activated myofibroblast plays an important role in the progression of liver fibrosis. The activated myofibroblast expresses abundant a-smooth muscle actin and collagen[26].

Cirrhosis, which consists of various nodules and is harder than the normal liver, is the advanced period of fibrosis[27]. Liver fibrosis and early cirrhosis are confirmed to be partly reversible. Therefore, the precise diagnosis of liver fibrosis is vital for the treatment and management of chronic liver disease patients.

In clinical practice, liver biopsy is the gold standard for the diagnosis of liver fibrosis. Various noninvasive modalities such as ultrasound and elastography have been used as alternatives to liver biopsy. Some studies suggest that AI models based on ultrasound and elastography have great potential for the classification of liver fibrosis.

Table 1 Application of ultrasound-based artificial intelligence in diffuse liver diseases

Ref.	Diseases: number of cases	Type of ultrasound	Algorithm of AI	Performance
Byra *et al*[21]	Severely obese patients: 55	B-mode	CNN	Sensitivity: 100%
				Specificity: 88%
				Accuracy: 96%
	Fatty liver disease: 38			AUC: 0.98
Biswas *et al*[22]	Normal patients: 27	B-mode	Deep learning	Accuracy: 100%
	Fatty liver disease: 36			AUC: 1.0
Han *et al*[24]	NAFLD: 140	B-mode	CNN	Sensitivity: 97%
				Specificity: 94%
	Control: 64			Accuracy: 96%
				AUC: 0.98
Yeh *et al*[28]	Postsurgical human liver samples: 20	B-mode	SVM	F2 accuracy: 91%
				F3 accuracy: 85%
				F4 accuracy: 81%
				F6 accuracy: 72%
Zhang *et al*[29]	Liver fibrosis or cirrhosis: 239	Duplex	ANN	Sensitivity: 95%
	Training group: 179			Specificity: 85%
	Validation group: 60			Accuracy: 88%
Gao *et al*[30]	S0: 4	B-mode	ANN	S0 accuracy: 100%
	S1: 16			S1 accuracy: 90%
				S2 accuracy: 70%
				S3 accuracy: 90%
	S2: 8			S4 accuracy: 100%
	S3: 5			
	S4: 4			
Lee *et al*[31]	Patients: 3446	B-mode	CNN	AUC: 0.86
	Internal validation set: 263			
	Internal test set: 266			
	External test set: 572			
Gatos *et al*[34,35]	Chronic liver disease: 70	Shear-wave elastography	SVM	Sensitivity: 94%
	Healthy: 56			Specificity: 81%
				Accuracy: 87%
Wang *et al*[36]	Liver fibrosis: 398	Shear-wave elastography	Deep learning radiomic	F4 AUC: 0.97
	Training group: 266			
	Validation group: 132			F3 AUC: 0.98
				F2 AUC: 0.85
Xue *et al*[38]	Liver fibrosis: 401	Elastography	CNN by TL radiomics	S2 AUC: 0.95
	Patient without fibrosis: 65			S3 AUC: 0.93
				S4 AUC: 0.93

AI: Artificial intelligence; ANN: Artificial neural network; AUC: Area under the receiver operating characteristic curve; CNN: Convolutional neural network; NAFLD: Nonalcoholic fatty liver disease; SVM: Support vector machine; TL: Transfer learning.

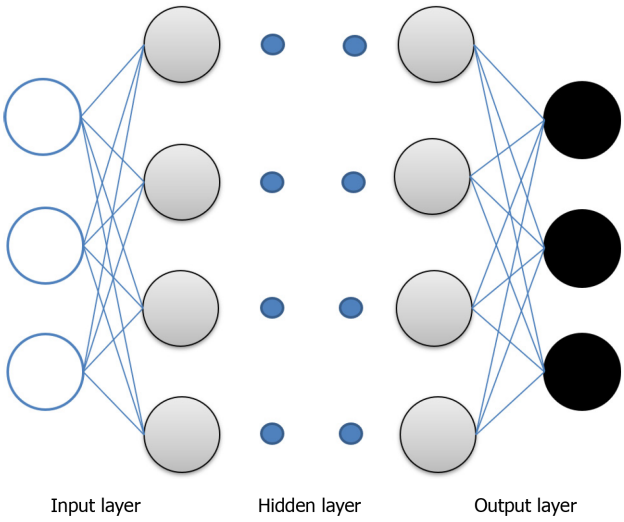

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Figure 1 Framework of convolutional neural networks. Blue dots represent multiple hidden layers.

AI based on conventional ultrasound: AI based on conventional ultrasound has been applied to improve their performance for the diagnosis and grading of liver fibrosis.

Yeh *et al*[28] built an SVM model to analyze liver fibrosis. B-mode images of 20 fresh postsurgical human livers were used to assess ultrasound capacity in evaluating the stage of fibrosis. The study indicated the best classification accuracy of two, three, four, and six classes were 91%, 85%, 81%, and 72%, respectively[28]. The results confirmed that the SVM model may be suggested to assess diverse liver fibrosis stage.

Other than the B-mode ultrasound, duplex ultrasound has also been applied to diagnose liver fibrosis. Using an ANN model based on duplex ultrasound, Zhang *et al*[29] demonstrated that their model reached the accuracy, sensitivity, and specificity were 88.3%, 95.0%, and 85.0%, respectively. The ANN model included five ultrasonographic parameters: thickness of spleen, liver vein waveform, the hepatic parenchyma, liver artery pulsatile index, and hepatic damping index. The study suggested that their ANN model has the potential to diagnose liver fibrosis noninvasively[29].

Studies confirmed that radiomics show great performance in the grading of liver fibrosis. By the use of texture analysis to analyze ultrasound liver images, the study found the accuracies of S0-S4 were 100%, 90%, 70%, 90%, and 100%, respectively[30].

It was reported that deep learning has great potential for liver fibrosis evaluation. Lee *et al*[31] built a deep CNN and trained a four-class model (F0 *vs* F1 *vs* F23 *vs* F4) to predict METAVIR scores. They used 13608 ultrasound images of 3446 patients who accepted surgery, liver biopsy, or transient elastography to train the deep CNN model. The model achieved a higher AUC of 0.857 for the classification of cirrhosis compared with five radiologists (AUC range, 0.656-0.816; $P < 0.05$) using the external test set [31].

AI based on ultrasound elastography: ultrasound elastography has been performed to acquire quantitative assessment of liver tissue stiffness, which is related to the grades of fibrosis. These technologies include strain elastography and shear wave elastography (SWE)[32]. Recently, some studies confirmed that the AI based on SWE has great value to identify and stage liver fibrosis.

Compared to conventional radiomics, a multiparametric ultrasonic model using machine learning algorithms demonstrated better manifestation in fibrosis assessment[33]. By quantifying color information from SWE images, Gatos *et al*[34,35] created an SVM model that could differentiate patients with liver diseases from controls with accuracy, sensitivity, and specificity of 87.3%, 93.5% and 81.2%, respectively.

Deep learning has also been applied in the assessment of liver fibrosis. A multicenter study used deep learning radiomics on 2D-SWE ultrasound images for the classification of liver fibrosis[36]. 2D-SWE ultrasound images had higher AUCs of 0.97 for F4, 0.98 for ≥ F3, and 0.85 for ≥ F2 fibrosis when compared with standard 2D-SWE.

It is necessary to contain a large training dataset for deep learning. However, it is difficult and expensive to get abundant medical images in clinics. One method to solve this problem is the employment of transfer learning (TL), which can enhance the performance by TL from other areas to the ultrasound area[37]. A study developed a CNN model by TL radiomics to assess ultrasound images of gray-scale modality and elastogram modality for the grade of accurate liver fibrosis. TL in gray-scale modality and elastogram modality revealed much higher diagnostic accuracy of AUCs compared with non-TL. Multimodal gray-scale modality + elastogram modality was confirmed to be the most precise diagnostic model with AUCs of 0.930, 0.932, and 0.950 for classifying ≥ S2, ≥ S3, and S4, respectively. It

was suggested that this TL model had excellent performance in liver fibrosis staging in clinical applications[38].

Focal liver lesion

Focal liver lesions (FLLs) are described as an abnormal part of the liver mainly coming from hepatocytes, biliary epithelium, and mesenchymal tissue[39]. Due to its cheap price, noninvasiveness, and real-time imaging, ultrasound is the preferred method for the diagnosis of FLLs. Based on this trend, the AI models using ultrasound images have more advantages over CT and MRI in routine clinical applications[40]. Table 2 shows the application of ultrasound-based AI in FLLs.

The application of AI in the diagnosis of benign and malignant FLLs: Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and accounts for the second leading cause of cancer-related deaths[41]. It is vital to identify benign and malignant FLLs for patients in the early stage.

AI based on conventional ultrasound: deep learning based on B-mode ultrasound has been demonstrated to be helpful in the diagnosis of benign and malignant FLLs. A CNN model was used to distinguish benign and malignant FLLs and achieved a higher accuracy than two experts[42]. Yang *et al*[43] developed a multicenter study to improve the B-mode ultrasound diagnostic performance for FLLs. The CNN of ultrasound performed high sensitivity and specificity in detecting FLLs, and it may be helpful for less-experienced doctors to enhance their judgment in liver cancer diagnosis.

AI based on B-mode ultrasound images has also been applied for the diagnosis of primary or secondary malignant liver tumors. A study proposed machine learning for discriminating HCC and metastatic liver tumors using SVM. The results revealed a classification accuracy of 91.6% with a sensitivity of 90.0% for HCCs and 93.3% for metastatic liver tumors[44].

AI based on contrast-enhanced ultrasound (CEUS): Recently, CEUS has become a commonly used ultrasound modality for the detection of FLLs[45]. Many studies have indicated that CEUS images had better sensitivity and specificity for the differentiation of malignant and benign tumors compared with B-mode images. One of the advantages of CEUS is that the images can be analyzed quantitatively. Time intensity curve (TIC) is a common quantitative analysis tool for CEUS[46]. Recently, AI based on CEUS images was reported to have great performance for the discrimination of FLLs.

Gatos *et al*[47] created a pretrained SVM algorithm to distinguish benign and malignant FLLs. In this model, a complex segmentation method based on TIC was used to detect lesions and process contours of 52 CEUS images. The accuracy, sensitivity, and specificity were 90.3%, 93.1%, and 86.9%, respectively [47]. Another study using SVM revealed that the sensitivity, specificity, and accuracy of benign and malignant grading were 94.0%, 87.1%, and 91.8%, respectively, while the classification accuracy of HCC, metastatic liver tumor, and benign were 85.7%, 87.7%, and 84.4%, respectively[46].

In addition to TIC, extracting features except TICs from a region of interest on CEUS images and videos was also applied in AI. A two-stage multiview learning framework, which was the integration of deep canonical correlation analysis and multiple kernel learning for CEUS-based computer-aided diagnosis, was proposed to identify liver tumors. The deep canonical correlation analysis-multiple kernel learning framework achieved performance for discriminating benign from malignant liver tumors with the accuracy, sensitivity, and specificity of 90.4%, 93.6%, and 86.8%, respectively[48].

The application of AI for the differential diagnosis of FLLs: With the development of AI, AI based on B-mode ultrasound images has great performance on the diagnosis of different FLLs. Hwang *et al*[49] extracted hybrid textural features from ultrasound images and used an ANN to diagnose FLLs. They indicated that the model revealed enormous potential with the diagnosis accuracy of over 96% among all FLLs groups (hemangioma *vs* malignant, cyst *vs* hemangioma, and cyst *vs* malignant)[49].

Deep learning was also applied in the distinction of different FLLs. Schmauch *et al*[50] created an algorithm that simultaneously detected and characterized FLLs. Although the amount of training data was relatively small, the average AUC of FLL detection and characterization was 0.935 and 0.916, respectively.

A CNN model was developed and validated for tumor detection and 6-class discrimination (HCC, focal fatty sparing, focal fatty infiltration, hemangiomas, and cysts)[51]. This model reached 87.0% detection rate, 83.9% sensitivity, and 97.1% specificity in the internal evaluation. In external validation groups, the model achieved 75.0% detection rate, 84.9% sensitivity, and 97.1% specificity.

CEUS also had excellent potential for AI to distinguish different FLLs. An ANN was applied to study the role of TIC analysis parameters of 4-class discrimination of liver tumors. The neural network had 94.45% training accuracy and 87.12% testing accuracy. The automatic classification process registered 93.2% sensitivity and 89.7% specificity[52].

Căleanu *et al*[53] reported the 5-class classification of liver tumors using deep neural networks with an accuracy of 88%. In this study, deep neural network algorithms were compared with state-of-the-art architectures, and a novel leave-one-patient-out evaluation procedure was presented.

All these studies indicated that AI based on conventional ultrasound and CEUS played a vital role in the detection and distinction of FLLs.

The application of AI in the management of HCC patients: Because of the development of new treatments, the management of HCC patients has become much more complicated. Radiomics can offer

Table 2 Application of ultrasound-based artificial intelligence in focal liver lesions

Ref.	Diseases: number of cases	Type of ultrasound	Algorithm of AI	Performance
Xi *et al*[42]	Benign lesions: 300	B-mode	CNN	All lesions
				Accuracy: 84%
				Uncertain set of lesions
	Malignant lesions: 296			Accuracy: 79%
Yang *et al*[43]	Benign tumor: 427	B-mode	CNN	AUC for EV: 0.924
	Malignant tumor: 1786			Sensitivity: 86.5%
				Specificity: 85.5%
Virmani *et al*[44]	HCC: 27	B-mode	SVM	Accuracy of HCC: 91.6%
				Sensitivity
	Metastatic liver tumor: 24			HCC: 90%
				Metastatic liver tumor: 93.3%
Hwang *et al*[49]	Cyst: 29	B-mode	ANN	Accuracy: 96%
				Cyst *vs* hemangioma
	Hemangioma: 37			Cyst *vs* malignant
	Malignant: 33			Hemangioma *vs* malignant
Schmauch *et al*[50]	Non-tumorous liver: 258	B-mode	CNN	AUC
	Hemangioma: 17			FLL detection: 0.935
	Metastasis: 48			
	HCC: 6			FLL discrimination: 0.916
	Cyst: 30			
	FNH: 8			
Tiyarattanachai *et al*[51]	HCC: 2414	B-mode	CNN	Detection rate: 87.0%
	Cyst: 6600			Sensitivity: 83.9%
	Hemangioma: 5374			Specificity: 97.1%
	Focal fatty sparing: 5110			
	Focal fatty infiltration: 934			
Gatos *et al*[47]	Benign FLL: 30	CEUS	SVM	Accuracy: 90.3%
				Sensitivity: 93.1%
	Malignant FLL: 22			Specificity: 86.9%
Kondo *et al*[46]	Benign FLL: 31	CEUS	SVM	Benign *vs* malignant
				Accuracy: 91.8%
				Sensitivity: 94%
				Specificity: 87.1%
	Malignant FLL: 67			Accuracy
				Benign: 84.4%
				HCC: 87.7%
				Metastatic liver tumor: 85.7%
Guo *et al*[48]	Benign FLL: 46	CEUS	Deep canonical correlation analysis and multiple kernel learning	Accuracy: 90.4%
				Sensitivity: 93.6%

	Malignant FLL: 47			Specificity: 86.8%
Streba *et al*[52]	HCC: 41	CEUS	ANN	Training accuracy: 94.5%
	Hypervascular liver metastasis: 20			Testing accuracy: 87.1%
	Hypovascular liver metastasis: 12			Sensitivity: 93.2%
	Hemangioma: 16			Specificity: 89.7%
	Focal fatty changes: 23			
Căleanu *et al*[53]	HCC: 30	CEUS	Deep neural network	Accuracy: 88%
	Hypervascular liver metastasis: 11			
	Hypovascular liver metastasis: 11			
	Hemangioma: 23			
	FNH: 16			
Dong *et al*[56]	HCC: 322	B-mode	Radiomics	AUC: 0.81
Hu *et al*[57]	HCC: 482	CEUS	Radiomics	AUC: 0.731
	Training cohort: 341			
	Validation cohort: 141			
Zhang *et al*[58]	HCC: 313	CEUS	Radiomics	AUC
	Primary cohort: 192			Primary dataset: 0.849
	Validation cohort: 121			Validation dataset: 0.788
Liu *et al*[63]	HCC: 130	CEUS	Deep learning radiomics	AUC: 0.93
	Training cohort: 89			
	Validation cohort: 41			
Ma *et al*[66]	HCC: 318	CEUS	Radiomics	AUC: 0.89
	Training cohort: 255			
	Validation cohort: 63			
Liu *et al*[69]	HCC: 419	CEUS	Deep learning radiomics	C-index
	RFA: 214			RFA: 0.726
	SR: 205			SR: 0.741

AI: Artificial intelligence; ANN: Artificial neural network; AUC: Area under the receiver operating characteristic curve; CEUS: Contrast-enhanced ultrasound; CNN: Convolutional neural network; EV: External validation; HCC: Hepatocellular carcinoma; FNH: Focal nodular hyperplasia; FLL: Focal liver lesion; RFA: Radiofrequency ablation; SR: Surgical resection; SVM: Support vector machine.

accurate assessment of great numbers of image features from medical images. These features that are difficult to detect by the human eye can be detected by machine learning or deep learning. AI models based on radiomics has also been reported to be applicable for the management of HCC, such as the prediction of microvascular invasion (MVI), curative transarterial chemoembolization (TACE) effect, recurrence after thermal ablation, and prognosis.

Predicting MVI: MVI is described as the invasion of tumor cells within a vascular space lined by endothelium. It has been proven that MVI is a predictor of early recurrence of HCC and poor survival outcomes[54]. The only way to confirm MVI is *via* histopathology after surgery. Patients with HCC can receive a great benefit when MVI is identified noninvasively and accurately before surgery[55]. The application of AI based on gray-scale ultrasound images and CEUS indicated good performance in predicting preoperative MVI.

A study indicated that the radiological features of gray-scale ultrasound images of gross tumoral area predicted preoperative MVI of HCC with an AUC of 0.81[56]. A CEUS-based radiomics score was built for preoperative prediction of MVI in HCC[57]. The radiomics nomogram revealed great potential in the detection of MVI with an AUC of 0.731 compared with the clinical nomogram with an AUC of 0.634. It

was indicated that the radiomics data based on ultrasound was a single predictor of MVI in HCC. Our group created a radiomics model based on CEUS to evaluate MVI of HCC patients before surgery. The model revealed a better detection in the primary group with an AUC of 0.849 *vs* 0.690 as well as the validation group with an AUC of 0.788 *vs* 0.661 when compared with the clinical model. We confirmed that the portal venous phase, delay phase, tumor size, rad-score, and alpha-fetoprotein level were single predictors related to MVI[58].

Predicting curative TACE effect: Pathways participating in important cancer-related progression, such as cell proliferation and angiogenesis, are major goals for the treatment of HCC patients. Additionally, transcription factors and cell cycle regulators are also considered to be interesting for anti-HCC drugs[59].

TACE is a widely used first-line therapy for HCC patients diagnosed at the intermediate stage. The tumor response to the first TACE treatment is highly different and obviously related to the subsequent therapies as well as the patients' survival[60]. Hence, the exact prediction of HCC responses after the first TACE treatment is vital for patients.

The prediction of tumor responses to TACE heavily depends on MRI and serological biomarkers[61,62]. But these methods achieved unsatisfactory accuracy of prediction. The application of AI based on both B-mode ultrasound and CEUS demonstrated better prediction efficacy.

An AI-based radiomics was established and validated to predict the personalized responses of HCC to the first TACE session. The deep learning radiomics-based CEUS model showed better performance compared with the machine learning radiomics-based B-mode model and machine learning radiomics-based time intensity curve of CEUS model with AUCs of 0.93, 0.80, and 0.81, respectively[63]. They suggested that the deep learning-based radiomics could benefit TACE candidates in clinical work.

Predicting recurrence after thermal ablation: Thermal ablation has been confirmed to be an available therapy for early-stage HCC patients who are unsuitable for operation or recurrence after surgery[64]. In addition, the recent 2-year recurrence rates of HCC patients who underwent thermal ablation were reported as 2%-18%[65]. The accurate preoperative prediction of thermal ablation outcomes is of great importance for HCC patients. Compared with other imaging modalities, CEUS is radiation-free and has better temporal resolution when revealing the blood supply of the tumor. The application of AI based on CEUS could be performed for the preoperative prediction of thermal ablation outcomes.

A radiomics model was created to predict the early and late recurrence of HCC patients who accepted thermal ablation[66]. The combined model including CEUS, ultrasound radiomics, and clinical factors showed better performance for early recurrence with an AUC of 0.89 and for late recurrence prediction with a C-index of 0.77.

Predicting the prognoses: Surgical resection (SR) and radiofrequency ablation (RFA) are common curative strategies for HCC patients diagnosed at the early stage[64]. Some studies have compared the long-term survival of RFA and SR for early-stage HCC patients[67,68]. However, the conclusions were sharply different. Hence, it is necessary to find useful predictive means to select the optimal patients who are suitable for RFA or SR before surgery. AI models based on CEUS had great performance for the prediction of progression-free survival (PFS).

A deep learning-based radiomics from CEUS images was built to predict the PFS of SR and RFA for HCC patients. Both SR and RFA models achieved high prediction accuracy of 2-year PFS. They also identified that a higher average probability of 2-year PFS may be acquired while some RFA and SR patients exchange their choices[69]. By utilizing conventional ultrasound images and CEUS, these AI prediction models can be applied in the individualized management of HCC patients.

APPLICATION OF ULTRASOUND-BASED AI IN UPPER GASTROINTESTINAL DISEASE

Gastric mesenchymal tumors

The majority of gastric mesenchymal tumors are occasionally found during routine esophagogastroduodenoscopy examinations. The incidence of gastric mesenchymal tumors is uncertain, but the prevalence of subepithelial tumors identified under endoscopy in Korea was reported as 1.7%[70]. Most gastric mesenchymal tumors are gastrointestinal stromal tumors (GISTs), which may metastasize to the liver and peritoneum after surgery[71,72]. Hence, distinguishing GISTs from benign mesenchymal tumors such as leiomyomas or schwannomas is of great importance in clinic practice. Endoscopic ultrasonography (EUS) is a common method to assess gastric mesenchymal tumors. It helps doctors evaluate the detailed size, shape, origin, and border of the lesions[73-75]. But the interpretation of EUS images by endoscopists is subjective and has poor interobserver agreement. Recently, EUS image interpretation using AI has developed rapidly and is applied to distinguish GISTs from benign mesenchymal tumors.

A convolutional neural network computer-aided diagnosis (CNN-CAD) model based on EUS images was developed to assess gastric mesenchymal tumors. They reported the model distinguished GISTs from non-GIST tumors with 83.0% sensitivity, 75.5% specificity, and 79.2% accuracy[76]. The CNN-CAD model had the potential to provide diagnostic assistance to endoscopists in the future.

Pancreatic diseases

EUS is currently a common tool to diagnose pancreatic diseases in clinical practice. However, the specificity for the diagnosis of pancreatic diseases using EUS images is low and deeply depends on the subjective judgment of endoscopists. Studies have confirmed that AI based on EUS improves their performance for the diagnosis of pancreatic diseases. Recently, AI using EUS images has been applied in the differential diagnosis of PC, distinguishing intraductal papillary mucinous neoplasms (IPMNs) and detecting pancreatic segmentation.

Pancreatic cancer: PC is relatively uncommon, with an incidence of 8-12 per 100000 per year. PC is attributed to hereditary germline or somatic acquired mutations in some genes such as tumor suppressor genes and cell cycle genes. These mutations are also associated with the progression and metastasis of PC. Moreover, shortened telomerase, cell turnover, and genomic instability have an important role in the development of PC[77].

The early diagnosis and surgery of PC, especially for lesions less than 1 cm, can achieve long-term prognoses with a 5-year survival rate of 80.4%[78]. However, PC is most frequently detected at an advanced stage, and the 5-year survival rate remains as low as 3%-15%[79]. Hence, early detection is vital for the treatment of PC patients. Studies have reported that AI based on EUS has great performance for the diagnosis of PC.

AI based on B-mode EUS: AI models based on B-mode EUS have been applied to improve their performance for the diagnosis of PC. Norton *et al*[80] first reported the use of CAD utilizing EUS images in pancreatic diseases in 2001. The study included 14 patients with focal chronic pancreatitis and 21 patients with PC. They showed the diagnostic sensitivity of the two diseases was 89%, and the overall accuracy was 80%[80]. However, this study cannot be referred to as AI-CAD in current applications as the number of patients was limited and the resolution of images were very low.

With the development of AI, ANN and SVM presented good performance in the diagnosis of PC[81-83]. Das *et al*[81] developed an ANN model to distinguish chronic pancreatitis from PC. The results achieved 93% sensitivity, 92% specificity, 87% PPV, 96% negative predictive value (NPV), and 0.93 AUC [81]. By using a multilayered neural network, the study confirmed the first machine learning results for the EUS images of the pancreas. But the sample size was small and lacked pathological evidence in the chronic pancreatitis and normal pancreas groups.

By selecting better texture features that included multifractal dimensional features, a quantitative measure of fractality (self-similarity), and complexity from EUS images, a SVM prediction model was created to identify PC and non-PC patients[83]. The model reached 97.98% accuracy, 94.32% sensitivity, 99.45% specificity, 98.65% PPV, and 97.77% NPV. The study demonstrated that SVM using EUS images is a useful tool for diagnosing PC and pancreatic diseases.

It was reported that AI was also applied for the age-dependent pancreatic changes on EUS images of PC cases. Ozkan *et al*[84] suggested a high-performance CAD model applying ANN to discriminate PC and noncancer patients in three age groups. In the under 40-year-old group, the accuracy, sensitivity and specificity were 92.0%, 87.5%, and 94.1%, respectively. In the 40-year-old to 60-year-old group, the accuracy, sensitivity, and specificity were 88.5%, 85.7%, and 91.7%, respectively. In the > 60-year-old group, the accuracy, sensitivity, and specificity were 91.7%, 93.3%, and 88.9%, respectively. The total performance of this model showed the accuracy, sensitivity, and specificity were 87.5%, 83.3%, and 93.3%, respectively.

Besides machine learning, deep learning has been applied to B-mode EUS images for analysis of PC. A CNN model using EUS images was developed for the detection of PC[85]. The sensitivity, specificity, PPV, and NPV were 90.2%, 74.9%, 80.1%, and 88.7%, respectively. The CNN model included six normalization layers, seven convolution layers, four max-pooling layers, and six activation layers. The EUS-CNN application was first reported to have the potential to detect PC from EUS images.

AI based on EUS elastography: Real-time EUS elastography can provide more information about the features of pancreatic masses by the use of strain assessment. It was reported that EUS elastography has been applied in the differential diagnosis of pancreatic lesions. However, the accuracy and reproducibility were unstable[86,87].

The application of AI improves their performance in the diagnosis of PC. A prospective, blinded, multicentric study using EUS elastography by ANN was performed in focal pancreatic lesions[88]. They demonstrated the sensitivity, specificity, PPV, and NPV values for the diagnosis of PC were 87.59%, 82.94%, 96.25%, and 57.22%, respectively. The study suggested that the ANN model may provide fast and accurate diagnoses in the clinical.

AI based on contrast-enhanced EUS: Contrast-enhanced EUS has been used to enhance the detection of pancreatic lesions[89]. AI based on contrast-enhanced EUS has great performance for the diagnosis of PC. An ANN model based on the TIC analysis from contrast-enhanced EUS images was designed to diagnose PC and chronic pancreatitis. The study reached 94.64% sensitivity, 94.44% specificity, 97.24% PPV, and 89.47% NPV[90]. The study suggested that the model could provide additional diagnostic value to CEUS interpretation and EUS fine needle aspiration results.

IPMNs: IPMNs are considered to be precursor lesions of pancreatic adenocarcinoma. Early surgical resection of IPMNs can provide a survival benefit for patients[91]. EUS is often used to assess the

malignancy of IPMNs in clinics. Several predictive techniques were used to diagnose the malignancy of IPMNs with no satisfactory results (70%-80%)[92,93].

Compared with human diagnosis and conventional EUS features, AI *via* deep learning algorithms was confirmed to be a more exact and objective way for the differential diagnosis of malignant IPMNs. Kuwahara *et al*[94] performed a predictive CNN model using EUS images to detect malignant IPMNs. The model reached 95.7% sensitivity, 94.0% accuracy, and 92.6% specificity. The accuracy was higher compared with the diagnosis of a radiologist (56.0%). The author suggested that the application of AI can evaluate malignant IPMNs before surgery.

Pancreatic segmentation: AI using EUS images has also been applied in pancreatic segmentation. A deep learning-based classification system was created to utilize the "station approach" in EUS of pancreas[95]. The system obtained 90.0% accuracy in classification and 0.770 and 0.813 in blood vessel and pancreas segmentation, respectively. The results were similar to that of EUS experts. Thus, this study revealed that AI has the feasibility to detect the station and segmentation of the pancreas.

APPLICATION OF ULTRASOUND-BASED AI IN LOWER GASTROINTESTINAL DISEASE

Colorectal tumors

Colorectal cancer is the third most common cancer worldwide and accounts for the second leading cause of cancer-related deaths. Moreover, a growing number of patients diagnosed with rectal cancer are under 50-years-old[96]. Colorectal cancer is attributed to gene mutations of epithelial cells, such as oncogenes, tumor suppressor genes, and DNA repair genes. The specific molecular mechanisms implicated in this type of cancer may include the instability of chromosomes and microsatellites[97].

Recently, some researchers studied tumor deposits (TDs) of rectal cancer. TDs are described as focal aggregates of adenocarcinoma located in the surrounding fat of the colon or rectum. They are discontinuous with the primary tumor and unrelated to a lymph node[98,99].

It was reported that a patient who is TD-positive has more malignant tumors, with decreased disease-free survival and overall survival[100]. However, TDs are often diagnosed by pathology only after surgery. Hence, the noninvasive preoperative prediction of TDs is important for rectal cancer patients. EUS is currently a common tool to detect rectal masses. Recently, ultrasound-based radiomics have been applied to predict the status of TDs.

Chen *et al*[101] developed an ANN system using ultrasound radiomics and clinical factors to predict TDs. Endorectal ultrasound and SWE examinations were conducted for 127 patients with rectal cancer. The accuracy was 75.0% in the validation group. The model reached 72.7% sensitivity, 75.9% specificity, and 0.743 AUC. The study suggested that ultrasound-based radiomics has the potential for the prediction of TDs before treatment. Table 3 shows the application of ultrasound-based AI in gastrointestinal disease.

CONCLUSION

In recent years, AI models using ultrasound images have developed rapidly. They can offer a more precise and efficient diagnosis and ease the burden of doctors. AI based on ultrasound has been confirmed to be helpful in diffuse liver diseases and FLLs, such as assessing the severity of NAFLD and the grade of liver fibrosis, distinguishing benign and malignant liver lesions, predicting the MVI of HCC, curative TACE effect, and prognoses after thermal ablation. In addition, AI based on EUS has great performance in gastrointestinal diseases, such as distinguishing gastric mesenchymal tumors, differential diagnosis of PC, distinguishing IPMNs, and predicting the status of TDs in rectal cancer.

However, the application of AI based on ultrasound in clinical practice has some limitations. The main reason may be due to the high variability between radiologists in ultrasound image acquisition and interpretation[102]. Hence, it is necessary to unify the ultrasonic image acquisition process as well as the standard of ultrasonic data measurement during the ultrasound examination.

In addition, some studies of AI-powered ultrasound were retrospective and trained on limited data offered by a single hospital with potential data selection bias, and the amount of data in the training set was not enough. Abundant multicenter prospective studies should assure the efficiency and stability of these AI models. Additionally, deep learning needs a large number of images, so it is necessary to establish an abundant database with common collaborative efforts.

In addition, the application of AI based on EUS has some limitations. The number of EUS examinations is overwhelmingly low compared to other examinations such as endoscopy and CT, especially in gastrointestinal diseases.

In the future, AI based on ultrasound may be used to develop highly accurate and more efficient models for more digestive diseases such as peptic ulcers, stomach neoplasms, inflammatory bowel disease, and so on. These models may heavily reduce the workload for doctors by automatic identi-

Table 3 Application of ultrasound-based artificial intelligence in gastrointestinal disease

Ref.	Diseases: number of cases	Type of ultrasound	Algorithm of AI	Performance
Kim *et al*[76]	GISTs: 125	B-mode EUS	CNN	Sensitivity: 83.0%
	Leiomyomas: 33			Specificity: 75.5%
	Schwannomas: 21			Accuracy: 79.2%
Norton *et al*[80]	Chronic pancreatitis: 14	B-mode EUS	Basic neural network	Sensitivity: 89%
	Pancreatic cancer: 21			Accuracy: 80%
Das *et al*[81]	Chronic pancreatitis: 12	B-mode EUS	ANN	Sensitivity: 93%
	Pancreatic cancer: 22			Specificity: 92%
	Normal patient: 22			AUC: 0.93
Zhu *et al*[82]	Chronic pancreatitis: 126	B-mode EUS	SVM	Sensitivity: 96.25%
				Specificity: 93.38%
	Pancreatic cancer: 262			Accuracy: 94.2%
Zhang *et al*[83]	Pancreatic cancer: 153	B-mode EUS	SVM	Sensitivity: 94.32%
	Normal patient: 63			Specificity: 99.45%
				Accuracy: 97.98%
Ozkan *et al*[84]	Pancreatic cancer: 202	B-mode EUS	ANN	Sensitivity: 83.3%
				Specificity: 93.3%
	Normal patient: 130			Accuracy: 87.5%
Tonozuka *et al*[85]	Chronic pancreatitis: 34	B-mode EUS	CNN	Sensitivity: 90.2%
	Pancreatic cancer: 76			
	Normal patient: 29			Specificity: 74.9%
Săftoiu *et al*[88]	Chronic pancreatitis: 47	EUS elastography	ANN	Sensitivity: 87.59%
	Pancreatic cancer: 211			Specificity: 82.94%
Săftoiu *et al*[90]	Chronic pancreatitis: 55	Contrast-enhanced EUS	ANN	Sensitivity: 94.64%
	Pancreatic cancer: 122			Specificity: 94.44%
Kuwahara *et al*[94]	IPMN: 50	B-mode EUS	CNN	Sensitivity: 95.7%
				Specificity: 92.6%
				Accuracy: 94.0%
Zhang *et al*[95]	Training: 291	B-mode EUS	CNN	Accuracy: 90.0%
	Testing: 181			
Chen *et al*[101]	Rectal cancer: 127	Endorectal ultrasound	ANN	Sensitivity: 72.7%
		Shear-wave elastography		Specificity: 75.9%
				AUC: 0.743

AI: Artificial intelligence; ANN: Artificial neural network; CNN: Convolutional neural network; GISTs: Gastrointestinal stromal tumors; IPMN: Intraductal papillary mucinous neoplasm; EUS: Endoscopic ultrasonography; SVM: Support vector machine.

fication of disease on radiologic and histopathologic images. Moreover, the application of AI can enable building individual management for patients as well as predicting disease progression and complications in clinics. Additionally, AI may improve distance teaching by remote monitoring and enhance medical services in undeveloped areas.

FOOTNOTES

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MINIREVIEWS

Oxidative stress bridges the gut microbiota and the occurrence of frailty syndrome

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Abstract

The incidence of frailty gradually increases with age. This condition places a heavy burden on modern society, of which the aging population is increasing. Frailty is one of the most complicated clinical syndromes; thus, it is difficult to uncover its underlying mechanisms. Oxidative stress (OS) is involved in frailty in multiple ways. The association between the gut microbiota (GM) and frailty was recently reported. Herein, we propose that OS is involved in the association between the GM and the occurrence of frailty syndrome. An imbalance between oxidation and antioxidants can eventually lead to frailty, and the GM probably participates in this process through the production of reactive oxygen species. On the other hand, OS can disturb the GM. Such dysbiosis consequently induces or exacerbates tissue damage, leading to the occurrence of frailty syndrome. Finally, we discuss the possibility of improving frailty by intervening in the vicious cycle between the imbalance of OS and dysbiosis.

Key Words: Oxidative stress; Gut microbiota; Frail syndrome; Traditional Chinese medicine

Core Tip: Frailty is defined as a decrease in the reserve and restoring capacity of the body. It is recognized that oxidative stress (OS) is involved in frailty in multiple ways; however, the underlying mechanisms are still unknown. The association between the gut microbiota (GM) and frailty was recently reported. Herein, we propose that OS is involved in the association between the GM and the occurrence of frailty syndrome. The role of the imbalance between OS and dysbiosis is discussed in this review.

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INTRODUCTION

Frailty is one of the most complicated clinical syndromes and is defined as a decrease in the reserve and restoring capacity of the body[1]. For frail people, a slight irritant can result in strong responses, which require a longer period to recover. Thus, frailty can also be regarded as a decline in the ability to maintain homeostasis. Multiple organs and systems, such as the skeletal muscle, immune, endocrine, hematopoiesis, and cardiovascular systems, are involved in the process of frailty[2]. Patients with frailty have a high risk of developing age-related diseases, including neurodegenerative diseases (such as dementia), type II diabetes, atherosclerosis, and chronic heart failure[3]. Frailty places a heavy burden on modern society, which has an extensive elderly population.

Although there are a few hypotheses at present, the mechanisms involved in frailty remain unknown. Researchers have different opinions about the origin of frailty. It is generally accepted that frailty is related to aging. According to Jayanama *et al*'s survey[4], among 9030 volunteers over the age of 20, the average age with a frailty index score of less than 0.1 was 39.7 years old. Moreover, those who had an average age of 65.3 years had a frailty index score of more than 0.3. Miller *et al*[5] reported that 75.4% of 18- to 64-year-old people have an simplified, 5-item frailty index (sFI) = 0, which means they are considered nonfrail. In contrast, 20.1% of the 80 to 89-year-old age group had an sFI ≥ 3, and thus, this age group can be referred to as a frail population. There were 9252 patients included in this analysis.

The incidence of frailty increases with age. However, frailty is not necessarily related to aging. According to an investigation by Xing and Guo[6], 43.2% of 683 older people (≥ 60 years old) from Beijing did not have frailty, while the incidence of prefrailty was 45.7%, and 11.1% of the cohort was frail. These data suggest that healthy aging could be achieved by preventing and improving frailty status.

With the increasing focus on frailty, emerging evidence has increased our understanding of this syndrome. Findings from centenarians suggest that specific gut microbiota (GM) constituents may contribute to healthy aging. For example, *Escherichia*, *Ruminococcus*[7], and *Clostridioides leptum* are increased, whereas *Faecalibacterium prausnitzii* and other species are decreased in centenarians[8]. Biagi *et al*[8] also showed that the proportion of *Clostridioides* cluster XIVa was significantly lower in centenarians than in elderly and younger adults. Furthermore, the diversity and abundance of the GM vary between elderly adults and centenarians. However, the bridge between the GM and the occurrence of frailty remains unclear. In this review, we proposed the possible mechanisms involved in frailty from the perspective of the GM and oxidative stress (OS). The correlations and potential causality among these factors are discussed. The idea of using GM biomarkers to predict frailty is then proposed prospectively. Notably, frailty is not an irreversible status[9]. Timely interventions have the potential to revert the prefrailty or frailty state to a nonfrailty state. According to existing research, dietary interventions are the most commonly used treatment for frailty[10]. Moreover, traditional Chinese medicine (TCM) is a unique application for the treatment of frailty (Figure 1). Achieving healthy aging is becoming a core goal of future research.

THE GM AND FRAILTY

The abundance and diversity of the GM changes with aging. Alterations in the GM partially lead to individual differences in the health status of elderly people by interacting with the host immune system [11]. Evidence has shown that the elderly population has higher abundances of *Clostridioides* cluster XIVa, *Faecalibacterium prauanitzii, Actinomycetes* (mainly *Bifidobacterium*) and *Proteobacteria* than those of young adults[12]. Biagi *et al*[8] showed that the abundance of *Clostridioides* cluster XIVa was significantly higher in the elderly population (49%) than in the centenarians (34%) and the young population (44%). There was no significant difference in the abundance of *Clostridioides* cluster IV among these three age groups, but the subgroups had a different tendency to change. The abundance of *Faecalibacterium*

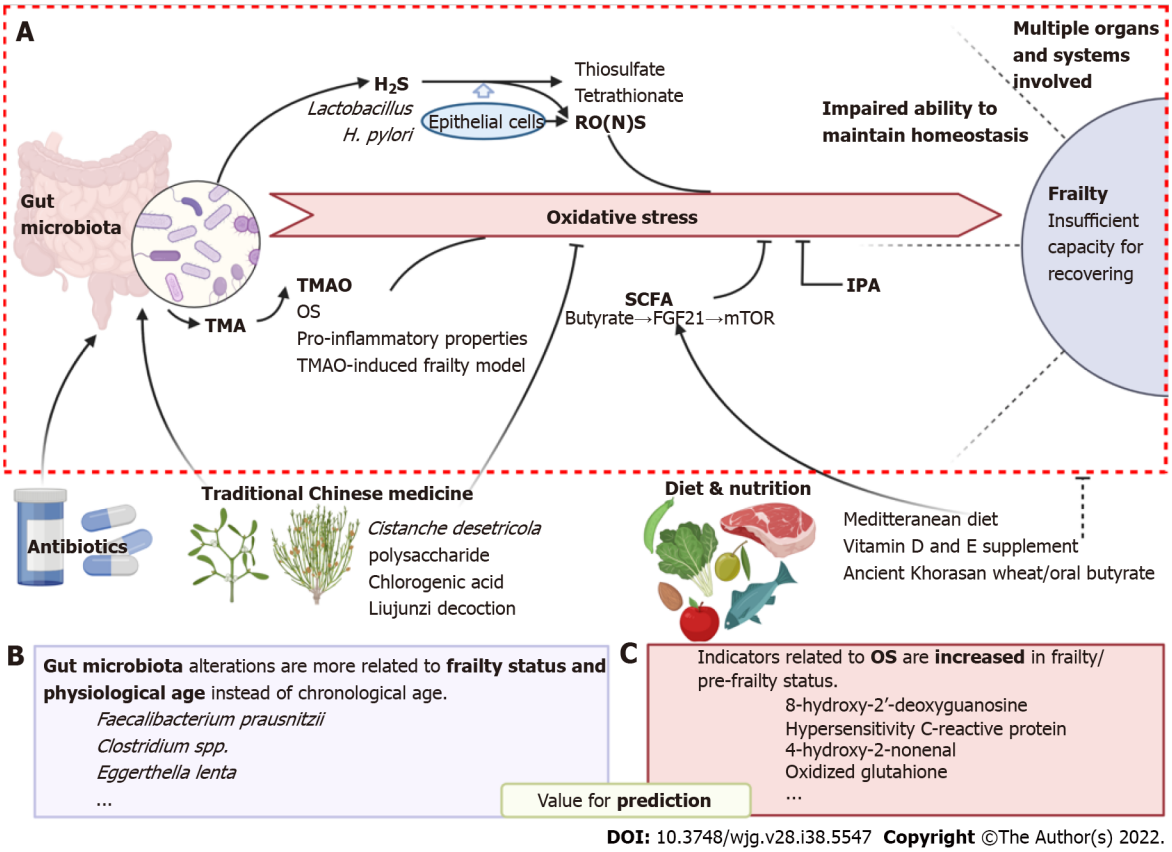

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Figure 1 Oxidative stress is involved in the association between the gut microbiota and frailty syndrome. A: Involving molecular and pathways of how oxidative stress bridges gut microbiota and frailty; B and C: Potential bacterial species and oxidative markers which are valuable for prediction of frailty are listed in B and C, respectively. In addition, possible intervene strategies including traditional Chinese medicine and diet & nutrition are shown at the bottom of the figure. Created with BioRender.com. OS: Oxidative stress; TMA: Trimethylamine; SCFA: Short-chain fatty acid; FGF21: Fibroblast growth factor 21; IPA: Indole-3-propionic acid; RONS: Reactive oxygen and nitrogen species; 8-OdG: 8-hydroxy-2'-deoxyguanosine; 4-HNE: 4-hydroxy-2-nonenal; GSSG: Glutathione.

prausnitzii et rel. decreased in centenarians, and the abundance of *Clostridioides leptum et rel.* was increased. Centenarians had the highest abundance of *Bacilli* (12%) and *Proteobacteria* (2.6%), and the young population and the old population had similar proportions of *Bacilli* (5%) and *Proteobacteria* (1.2%). Jackson *et al*[13] studied the relationship between frailty and the composition of the GM. They found that frailty was negatively correlated with the abundance of *Clostridioides* (in which it accounted for the largest proportion of the GM). Moreover, frailty was positively correlated with the abundance of *Eggerthella lenta*. Centenarians, who are regarded as the "healthy aging" population, have a different GM composition than the aged population. This finding indicates that the GM is not entirely chronological age-correlated but that it is more closely related to the frailty status of the body.

In addition to chronological age, physiological age may better reflect the correlation between frailty and the GM (Figure 1B). In the study by Maffei *et al*[14], 85 volunteers aged 43-79 years were divided into low, medium and upper groups according to either physiological age or chronological age. The chronological age is the number of years from birth to the present. Physiological age was assessed by 34 items of the frailty index (frailty index 34, FI_{34}). According to the FI_{34} value, there were significant differences in the abundance and diversity of the GM among the three age groups. Excluding the effect of sex, body mass index, antibiotic usage, and other confounders, *Eggerthella, Rumen coccus* and *Coprobacillus* were the three main species with the greatest influence. According to the Rockwood FI value, *Faecalibacterium prausnitzii* was negatively correlated with age, while *Eubacterium dolichum* and *Eggerthella lenta* were positively correlated with age[15]. However, there was no significant difference in the GM among the three groups according to chronological age. Although there are contradictory changes in certain strains in the gut, it can be concluded that the change in *Clostridioides* abundance with age is the most remarkable. All the experiments mentioned above suggest that the GM changes with frailty status and that there is a potential link between the GM and frailty syndrome.

In frailty, the most significant change in the GM is a decrease in diversity. In the frail aged population, the microbiota shifts toward a predomination by *Bacteroidetes*, especially in the genus *Prevotella* and its subspecies. Changes in microbiota diversity affect the influence of the metabolites and pathways related to the microbiota. For instance, the GM regulates estrogen levels by secreting β-glucuronidase. β-glucuronidase is an enzyme that can deconjugate estrogen into the active form. Low levels of estrogen downregulate physiological functions that affect neural development, cardiovascular

health, bone density and neoplastic diseases[16]. Scientific evidence suggests that estrogen supplementation could improve physiological functions that prevent or reverse frailty[17]. Said *et al*[18] found that diarylpropionitrile, an estrogen receptor β agonist, had longevity-promoting properties and could reverse frailty.

THE ROLE OF OS IN FRAILTY

OS was regarded as one of the main causes of frailty as early as the 1950s. Harman[19] first proposed the free radical theory of aging; according to this theory, oxygen free radicals damage cell components and intercellular substances, causing the deterioration of body functions and age-related diseases. In the 1980s, OS was discovered. The advent of OS indicates that the balance between oxidation and antioxidation is inclined toward oxidation. This leads to neutrophil inflammatory infiltration, an increase in protease secretion, and the generation of a large number of oxidative intermediates. The study by Viña *et al*[20] suggested that OS was an important negative factor that led to aging and diseases, such as cardiovascular disease, neurodegenerative disease, chronic obstructive pulmonary disease or cancer and other illnesses in aged individuals. Some researchers have thought that oxidative damage may be due to the imbalance between antioxidation defenses and reactive oxygen and nitrogen species (RONS), thus inducing cell apoptosis. This imbalance would lead to alterations in the expression of a number of transcription factors and then cause chemical modifications in macromolecular substances, such as lipids, proteins and DNA[21,22].

Since the free radical theory of aging was proposed, increasing concern has been given to the influence of OS on frailty (Figure 1C). Wu *et al*[23] screened 90 volunteers who were older than 65 years old, dividing them into a frailty group, a prefrailty group and a nonfrailty group according to Fried's phenotype. The serum levels of OS biomarkers such as 8-hydroxy-2′-deoxyguanosine (8-OdG), metabolic markers such as albumin, and inflammatory markers such as hypersensitivity C-reactive protein (hs-CRP) were measured by competitive enzyme-linked immunosorbent assay. The results demonstrated that the serum albumin and hs-CRP levels in the frail group were higher than those in the other two groups. Furthermore, the levels of 8-OdG and hs-CRP increased with the progression of frailty. Serviddio *et al*[24] compared the levels of oxidized glutathione (GSSG), tumor necrosis factor-α, malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and other OS-related indicators in patients with frailty syndrome with those in nonfrail individuals. The data suggested that these indicators of OS were all significantly increased in frail individuals.

In addition to the direct evaluation of the frailty index, OS was also found to be related to sarcopenia. Severe sarcopenia in the elderly was defined as the prophase of frailty by the European Working Group on Sarcopenia. Bernabeu-Wittel *et al*[25] found that in 444 polypathological patients, 97 patients had sarcopenia. The number of patients with a combination of sarcopenia and frailty was 80. The authors also reported that OS markers were significantly increased in patients with sarcopenia, frailty or both. OS was evaluated by the levels of catalase, GSSG reductase, total antioxidant capacity to reactive oxygen species (ROS), and superoxide dismutase (SOD). Coto Montes *et al*[26] found that sarcopenia was closely related to lipid peroxidation. Significant increases in biomarkers such as MDA and 4-HNE were observed in 200 independent 80-year-old persons who had been diagnosed with sarcopenia. Liu *et al*[27] also discovered that the incidence of frailty increased with a rise in the levels of interleukin-6 (IL-6), isoprostaglandin and lipoprotein phosphorylation A2. These studies suggested a correlation between frailty and OS. OS-related biomarkers in serum may be potential biomarkers for predicting frailty. This possibility needs further study.

THE UNDERLYING MECHANISM OF THE INFLUENCE OF THE GM ON FRAILTY SYNDROME WAS ANALYZED BY OS

OS is affected by many factors *in vivo* and *in vitro*. The GM plays a role as "environmental factors" which regulates ROS levels in plasma and maintaining the functions of the digestive, endocrine, immune, skeletal, cardiovascular, and nervous systems. The stability of the GM is fragile and easily affected by external factors, including diet, heavy use of drugs, geographic position, individual lifestyle, and the genetic background of the host[28]. Once the GM is disturbed ("dysbiosis"), it may cause a series of related illnesses, such as inflammatory bowel diseases, obesity, and type II diabetes. These diseases can also affect the oxidative balance, thus forming a vicious cycle.

For example, a variety of colon bacteria, such as *Fusobacterium*, *Clostridioides*, *Escherichia*, *Salmonella*, *Klebsiella*, *Streptococcus*, *Desulfovibrio* and *Enterobacter*, can convert sulfide to hydrogen sulfide (H_2S)[29]. Intestinal epithelial cells protect themselves by converting H_2S into thiosulfate ($S_2O_3^{2-}$). Thiosulfate can be oxidized to tetrathionate ions ($S_4O_6^{2-}$) in the case of intestinal inflammation (Figure 1A). This process is accompanied by the formation of RONS. In both anaerobic and microaerobic environments, tetrathionate can specifically promote the proliferation of *Salmonella enterica Typhimurium* model strains

[30]. It has also been reported that certain GM can cause a sharp increase in ROS. *Lactobacillus* is a strong inducer of ROS. It can stimulate the production of ROS by enhancing the ability to pass through the mucous membrane or increasing the secretion of mucus. Additionally, it can interact with certain cellular receptors, such as formylated peptide receptors or Toll-like receptors[31,32]. In addition, *Lactobacilli* promotes the oxidation of soluble redox proteins, such as GSSG and thioredoxin, and it facilitates the transcription of redox regulatory factors by activating the nuclear factor erythroid 2-related factor 2 pathway[32]. The pathogenic mechanisms of action of *Helicobacter pylori* (*H. pylori*) may also be related to its promotion of ROS production. *H. pylori* can induce severe white blood cell tissue infiltration and release virulence factors that stimulate epithelial cells to produce ROS and secrete myeloperoxidase, chemokines, and proinflammatory cytokines. Consequently, *H. pylori* results in gastrointestinal microbiome dysbiosis and induces nonalcoholic fatty liver disease (NAFLD)[33]. The antioxidant enzyme SOD can be used to treat NAFLD. SOD can convert superoxide anion into hydrogen peroxide and molecular oxygen, thus preventing the accumulation of ROS[34]. In addition to *H. pylori*, the GM has been proposed to be strongly associated with the development and progression of metabolic diseases, including NAFLD[33]. NAFLD patients showed lower fecal microbial diversity than that of healthy controls in one study[35]. At the taxonomic and functional levels, another recent study showed that *Lactobacilli* inhibited the incidence and development of NAFLD by improving hepatic mitochondria and lipid metabolism by promoting the production of the antioxidant GSSG[36].

These studies indicate that the activation of OS by the GM is a possible mechanism of frailty. For example, gut microbes metabolize choline, L-carnitine and betaine in the daily diet to trimethylamine (TMA). These substances are mainly found in red meat, meat products, eggs, and shellfish[37,38]. Nine GM strains can convert choline to TMA: *Anaerococcus hydrogenalis*, *Clostridioides asparagiforme*, *Clostridioides hathewayi*, *Clostridioides sporogenes*, *Escherichia fergusonii*, *Proteus penneri*, *Providencia rettgeri*, *Edwardsiella tarda* and *Providencia rustiganii*. These species belong to *Firmicutes* and *Proteobacteria*[39]. Liver enzymes further convert TMA to TMAO through the flavin-containing monooxygenase (FMO) family members FMO1 and FMO3[37]. Researchers have found that the level of TMAO was increased in the aged population and that TMAO was independently associated with frailty. Nam *et al*[40] reported that the average levels of TMAO were 3.21 μM and 4.04 μM in nonfrail and frail participants, respectively. Moreover, participants who had the highest plasma TMAO levels showed a significant 3.7-fold increase in the incidence of frailty syndrome. A high level of plasma TMAO can increase the risk of stroke, atrial fibrillation, diabetes, congestive heart failure, chronic kidney disease, coronary artery disease, peripheral artery disease and other cardiovascular diseases, which are associated with frailty [40]. Moreover, TMAO significantly increases OS, inflammatory conditions and endothelial dysfunction. TMAO stimulates the TXNIP-NLRP3 inflammasome and activates the release of the inflammatory cytokines IL-1β and IL-18. However, it inhibits the production of endothelial nitric oxide (NO) synthase and NO[41]. It has also been reported that TMAO can promote inflammatory hepatocellular carcinoma by inducing ROS and activating ILK/AKT/mammalian target of rapamycin (mTOR) signaling *via* POSTN[42]. Our previous studies showed that administration of TMAO produced a novel model of frailty in mice[43]. In this TMAO-induced frailty model, the abundance of *Firmicutes* was decreased slightly, while the abundance of *Bacteroidetes* was significantly increased in the gut. The ratio of *Firmicutes* to *Bacteroidetes* (F/B) was significantly decreased. Relevantly, the F/B ratio is reported to be a potential marker of obesity and GM disorder[44], both of which are potential factors of frailty[45,46]. According to an investigation, obese females have higher FI values than those of nonobese females. In addition to TMAO, short-chain fatty acids (SCFAs) are also common metabolites of the GM that are converted from undigested carbohydrates, such as starch and pectin (Figure 1A). Butyrate is a SCFA product. Studies have shown that there is a significant association between SCFAs/butyrate and gut/celiac diseases[47,48]. It is transcriptionally activated by peroxisome proliferator-activated receptor α by replacing histone deacetylase 3 on the fibroblast growth factor 21 (FGF21) promoter. This reaction actives FGF21 and induces the expression and secretion of FGF21[46]. FGF21 regulates the adenosine monophosphate-activated protein kinase-Sirtuin1-mTOR pathway, which is associated with longevity. FGF21 can also alleviate various diseases related to aging[49,50]. In addition, the GM can affect the levels of inflammation by regulating the immune system and can affect insulin resistance by regulating the metabolomic system[51].

In addition to these metabolites, indole-3-propionic acid (IPA), another microbiota-derived metabolite of tryptophan, has been proven to have a positive impact at the cellular level by preventing OS injury and lipoperoxidation and inhibiting the synthesis of proinflammatory cytokines[52], suggesting that IPA may be a promising new target for improving frailty.

The GM and metabolites changes with frailty. Scientific evidence also shows that some antibiotics can reverse frailty. This action may be due to the effect of antibiotics changing the composition of the GM. For example, rapamycin is a broad-spectrum antibiotic. It also acts as an immunosuppressor. Rapamycin regulates protein synthesis and redox reactions. Animal studies have shown that rapamycin prolongs lifespan and improves frailty[50]. These results indicate that the GM plays an important role in the process of frailty *via* OS.

POTENTIAL INTERVENTIONS FOR FRAILTY

By understanding the role of the GM and OS in frailty, several interventions have been proposed to improve this syndrome and to achieve the goal of healthy aging.

Effect of diet on frailty

One possible intervention is to regulate the GM *via* the daily diet. Data from short-lived organisms, such as yeast, worms and fruit flies, showed that diet had an anti-senescence effect and prolonged the lives of these organisms by enhancing stress resistance, reliance on lipid fuel use, and the activation of proteostatic mechanisms[53]. Such effects of diet and nutrition on aging can also be reproduced in humans [54]. For example, the Mediterranean diet can improve frailty. The Mediterranean diet is characterized by an increase in the consumption of vegetables, legumes, fruits, nuts, olive oil and fish[55]. At the same time, the consumption of meat and unsaturated fatty acids is reduced. The Mediterranean diet provides a higher intake of micronutrients, such as antioxidant nutrients, polyphenols and plant bioactive compounds[56]. Ghosh *et al*[57] found that one year of Mediterranean diet intervention can change the composition of the GM and alleviate frailty. Some of the GM that belong to the "diet positive" taxa increased, including *Faecalibacterium prausnitzii*, *Roseburia*, *Eubacterium* (*E. rectale, E. eligens,* and *E. xylanophilum*), *Bacteroides thetaiotaomicron*, *Prevotella copri* and *Anaerostipes hadrus*. An increase in these microbiotas facilitates the production of SCFAs and the enhancement of the anti-inflammatory capacity. On the other hand, the microbiota deemed to be "diet negative" taxa, such as *Ruminococcus torques*, *Collinsella aerofaciens*, *Coprococcus comes*, *Dorea formicigenerans*, *Clostridioides ramosum*, *Veillonella dispar*, *Flavonifractor plautii* and *Actinomyces lingnae*, were reduced. These "diet negative" taxa are associated with type II diabetes, colorectal cancer, atherosclerosis, cirrhosis and inflammatory bowel disease. With the progressive aggravation of frailty symptoms, the "diet-positive" taxa decreased, and the "diet-negative" taxa increased. Proper nutritional supplements can improve frailty. Bo conducted a double-blind experiment and found that mixed supplementation with whey protein, vitamin D and vitamin E can significantly improve muscle mass and strength (handgrip strength)[58]. It was also used to treat elderly individuals with sarcopenia, and the supplementation improved frailty and quality of life. Moreover, the Mediterranean diet was found to tend to increase the plasma concentration of IPA[52]. IPA could reduce the level of OS in the body and help to improve frailty.

In addition to the Mediterranean diet, some other diet regimens can also improve GM and OS. For example, the replacement diet based on ancient Khorasan wheat results in beneficial GM compositional and functional modifications that positively correlate with an improvement in fibromyalgia symptomatology[59]. A habitual diet supplemented with oral butyrate could reduce the production of ROS in leukocytes[60]. Whether these diet regimens could improve frailty remains to be further investigated.

Effect of TCM on frailty

With an increasing number of studies on TCM, many effective monomers and prescription preparations of TCM have been found to improve frailty by regulating the GM and OS. For example, *Cistanche deserticola* and *Eucommia ulmoides* are common TCM with a tonifying effect on the kidneys, which are important organs for aging[61-63]. Modern pharmacological investigations have demonstrated that both TCMs can regulate the GM and OS. *Cistanche deserticola* polysaccharide is one of the main active components of *Cistanche deserticola*. It can produce antiaging effects by scavenging free radicals, reducing telomerase activity, improving mitochondrial antioxidant capacity and improving mitochondrial energy metabolism[64]. Moreover, *Cistanche deserticola* polysaccharide can inhibit the growth of a variety of gut pathogenic bacteria and can promote the growth of probiotics. These effects help to maintain the health of the GM[65]. Chlorogenic acid is one of the main effective components of *Eucommia ulmoides*, and it has strong anti-inflammatory and antioxidant effects. It can reduce OS and improve mitochondrial dysfunction[66]. Furthermore, chlorogenic acid and its hydrolytic form, which is called caffeic acid, have been demonstrated to alleviate inflammation and OS by improving the GM[67]. This effect is manifested by an increase in the content of *Ackermann* bacteria and the restoration of the abundance of GM[68]. It can increase the expression of tight junction proteins in intestinal epithelial cells to repair the intestinal barrier and reduce permeability[69]. These actions may contribute to the antiaging effect of chlorogenic acid[69] and further prolong the lifespan in experiments with *Caenorhabditis elegans*[70]. Liujunzi decoction, a TCM prescription consisting of Dangshen, Tuckahoe, Atracylodes macrocephala, Licorice, Pinellia ternata and Orange peel, was found to significantly improve the frailty state of elderly patients with stable chronic obstructive pulmonary disease. This effect was significantly better than that of tiotropium bromide[71]. In addition, administration of Liujunzi decoction for eight weeks increased the muscle weight and exercise endurance of the frail patients. Supplementation with Chinese medicinal plant extracts from *Lonicera hypoglauca* and *Scutellaria baicalensis*, as well as *Dihydroquercetin* supplementation, could mitigate colonic inflammation by regulating OS and the GM[72,73].

Since TCM is popular in China, Japan and other East Asian regions, most of the available literature to date is limited to the data obtained from Asian populations. However, considering that most modern pharmacological studies are performed with standard experimental animals, the anti-frailty effect of

these TCMs may potentially be extended to Western populations based on these basic research data. However, these medicines still need to be more widely investigated in studies involving a larger population.

CONCLUSION

In summary, the abundance and diversity of the GM were found to change with age. Specific GMs and their metabolites stimulate the production of ROS and affect OS in the body, leading to damage to multiple biological macromolecules. The occurrence of OS may be the intermediate process of the GM that leads to frailty, producing a direct action on the body. This may be one of the precipitating factors of frailty syndrome. Thus, the GM and its metabolites can be used as biomarkers of frailty syndrome. Regulating the GM and OS by diet or TCM could help to improve frailty. Additionally, there is increasing evidence indicating that circadian rhythms may also play important roles in mediating the impact of the GM on chronic diseases[74]. Clarifying the mechanisms involved in frailty from the perspective of the GM may be important to achieving the goal of healthy aging.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Effect of low-dose radiation on thyroid function and the gut microbiota

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Abstract

BACKGROUND

The thyroid-gut axis has a great influence on the maintenance of human health; however, we know very little about the effects of low-dose ionizing radiation (LDR) on thyroid hormone levels and gut microbiota composition.

AIM

To investigate the potential effects of low-dose X-ray radiation to male C57BL/6J mice.

METHODS

Peripheral blood was collected for enzyme-linked immunosorbent assay (ELISA), and stool samples were taken for 16S ribosomal RNA (rRNA) gene sequencing after irradiation.

RESULTS

We found that LDR caused changes in thyroid stimulating hormone (TSH) levels in the irradiated mice, suggesting a dose-dependent response in thyroid function to ionizing radiation. No changes in the diversity and richness of the gut microbiota were observed in the LDR-exposed group in comparison to the controls. The abundance of *Moraxellaceae* and *Enterobacteriaceae* decreased in the LDR-exposed groups compared with the controls, and the *Lachnospiraceae* abundance increased in a dose-dependent manner in the radiated groups. And the abundances of *uncultured_bacterium_g_Acinetobacter*, *uncultured_bacterium_o_Mollicutes_RF39*, *uncultured_bacterium_g_Citrobacter*, and *uncultured_bacterium_g_Lactococcus* decreased in the radiated groups at the genus level, which showed a correlation with radiation exposure and diagnostic efficacy. Analysis of

functional metabolic pathways revealed that biological metabolism was predicted to have an effect on functional activities, such as nucleotide metabolism, carbohydrate metabolism, and glycan biosynthesis and metabolism. Furthermore, Kyoto Encyclopedia of Genes and Genomes pathway annotation also suggested that changes in the gut microbiota were related to processing functions, including translation, replication and repair.

CONCLUSION

LDR can change thyroid function and the gut microbiota, and changes in the abundances of bacteria are correlated with the radiation dose.

Key Words: Low-dose ionizing radiation; Thyroid; Gut microbiota; Thyroid-gut axis

Core Tip: In this study, we administered low-dose X-ray radiation to male C57BL/6J mice to investigate the potential effects. Peripheral blood was collected for enzyme-linked immunosorbent assay, and stool samples were taken for 16S ribosomal RNA gene sequencing after irradiation. We found that LDR caused changes in TSH levels in the irradiated mice and the gut microbiota, and changes in the abundances of bacteria are correlated with the radiation dose. Our research aims to explore the influence of LDR on homeostasis from the "thyroid-gut axis" perspective for the first time.

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INTRODUCTION

According to microecological theory, the ecological balance of the nervous system, endocrine system, metabolic system, microbial system and immune system is the essence of life[1]. The thyroid is an important endocrine gland that regulates multiple metabolic pathways by producing thyroid hormones and has a great impact on the regulation of the internal environment[2]. Several investigations have indicated that the gut microbiota may indirectly participate in disease development and might become a promising biomarker for immune-related adverse events[3]. Studies have found that intestinal flora are related to various thyroid diseases, and the "thyroid-gut axis" theory has been proposed accordingly[4, 5]. The mechanism by which the thyroid-gut axis maintains homeostasis under external stimulation deserves further study.

As one of the environmental stressors affecting people worldwide, low-dose ionizing radiation (LDR) exposure is usually from medical radiotherapy, radiation-contaminated areas and human space exploration[6,7]. The studies previously reported have indicated that the biological responses of LDR exposure on epidemiology, animals, and cells are paradoxical due to discrepancies in the dose rate[8]. The role of LDR in inducing apoptosis, stimulating antioxidant capacity and repairing DNA damage has been well established[9]. Conversely, LDR exposure from radiotherapy for the treatment of autoimmune diseases or carcinoma is associated with an increasing risk of cardiovascular disease or secondary carcinoma[10]. For the thyroid, the levels of thyroid stimulating hormone (TSH), free triiodothyronine (fT3) and free thyroxine (fT4) have been reported to be affected by LDR exposure[11]. In addition, changes between external irradiation and the gut microbiota have been preliminarily confirmed[12]. Thus, we want to know how the thyroid-gut axis responds to radiation exposure and what roles the thyroid and gut microbiota play in an individual's response to radiation injury.

The objective of our research was to investigate changes in thyroid hormone levels and gut microbiota composition in LDR-exposed mice by enzyme-linked immunosorbent assay (ELISA) and 16S rRNA gene sequencing, respectively. Our research revealed the abundances of microbial species in the gut ecosystem following dose-modulated LDR exposure. These investigations predicted possible LDR-induced gut microbiota disorders based on variations in microbial species abundance. Our research aims to explore the influence of LDR on homeostasis from the "thyroid-gut axis" perspective for the first time and may provide a concrete conceptual and analytical basis for the additional study into the long-term impact of LDR on human health.

MATERIALS AND METHODS

Mice and samples

Thirty 6-wk-old female wild-type C57BL/6J mice were divided equally into five groups after one week of adaptive feeding at the Science and Innovation Center of Shanghai Tenth People's Hospital (ID Number: SHDSYY-2022-4381). The low-dose irradiation conditions were as follows: Dose rate, 0.05 Gy/min; 1 min/time; and once every other day. Irradiation was completed by the Irradiation Center of the Institute of Radiation Medicine, Tongji University (160 keV, 1 mA). The two groups were the test group and control group. And the test group was further divided into four groups according to the radiation dose, namely, the 0.05 Gy group, 0.10 Gy group, 0.15 Gy group, and 0.20 Gy group. The general condition of the mice was observed during the experiment.

Assays of thyroid function and acquisition of thyroid tissue

Peripheral blood was collected from the mouse tail vein to measure thyroid hormone levels when the mice completed the planned dose of radiation exposure within 24 h. Serum levels of TSH, fT4, and fT3 were assayed by an ELISA kit (Rayto RT-6100). Histological sections of mouse thyroid tissue were prepared by paraffin embedding and stained with hematoxylin and eosin (HE).

DNA extraction and 16S rRNA gene sequencing

Stool samples were stored in a sterile container and snap-frozen with liquid nitrogen following collection at -80 °C (Sarstedt, 80.734.311, Germany). Using the E.Z.N.A. Soil Kit for DNA extraction (Omega, Bio-Tek, Norcross, GA, United States) and using a Nanodrop 2000 to measure the concentration of bacterial DNA (Thermo Scientific, Wilmington, United States). All the fecal samples were stored at -20 °C until ready for sequencing. Then, the V3-V4 region of the bacterial 16S rRNA was amplified by polymerase chain reaction with barcode index primers using TransStart FastPfu DNA Polymerase (TransGen BioTech, AP221-02, Beijing, China) on an ABI GeneAmp 9700 polymerase chain reaction system (United States). Amplicons were then purified by gel extraction (AxyPrep DNA Gel Extraction Kit, Axygen Biosciences, United States) and quantified using QuantiFluor-ST (Promega, United States). The adapter sequences were added to the purified amplicons using a TruSeq™ DNA Sample Prep Kit (Illumina, San Diego, United States), and paired-end sequencing was performed using an Illumina MiSeq instrument (Illumina, San Diego, United States) (Figure 1).

Microbial analysis

The paired-end readings obtained by MiSeq were first merged by overlapping sequences. Filtering and quality control allow the sequences to be optimized. Operational taxonomic units (OTUs) were clustered using a 97% similarity cutoff with UPARSE (version 10.0). Cohort was randomly generated for a cross-sectional study that included 24 mice exposed to radiation and 6 controls. The alpha diversity was analyzed in terms of the species richness, including CHAO, ACE, SHANNON, and SIMPSON indices. Beta diversity was used to calculate structural variation by principal coordinates analysis (PCoA) and nonmetric multidimensional scaling (NMDS) and was mapped from principal coordinate analysis. Functional pathway analysis was conducted in accordance with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database based on our microbial information.

Statistical analysis

Data that fit the normal distribution are expressed as the mean ± SD, and nonnormally distributed data are expressed as the median (interquartile). The t test or rank-sum test was used for comparing the thyroid hormone levels between the control group (n = 6) and test group (n = 24). Significant differences in the diversity index and microbial abundance between groups was determined by the Mann-Whitney U test. The microbial taxa with differences between the two groups were identified by linear discriminant analysis (LDA) effect size (LEfSe), with an LDA threshold value of > 2.0. The correlation between microbiota abundance and thyroid function was analyzed by Spearman correlation coefficient. The area under the curve (AUC) reflected the diagnostic accuracy of the bacterial indicators based on the receiver operating characteristic (ROC) curve. Unless otherwise stated, all analyses were performed using SPSS Statistics (Version 20.0). Statistical significance was set at $P < 0.05$ for analyses.

RESULTS

LDR caused changes in TSH levels in the mice

To understand changes in thyroid function in the irradiated mice, the levels of thyroid hormones in peripheral blood were determined by ELISA, including TSH, fT3, and fT4. As shown in Figure 2A, the TSH level of mice in the test mice (1.487 ± 0.347 mU/L) was obviously lower than the control mice (1.799 ± 0.289 mU/L), t = 2.188, P = 0.037). The level of TSH was the lowest in the 0.05 Gy group (1.262 ± 0.254 mU/L), which was the lowest among the four test subgroups, and in the 0.20 Gy group, it was the

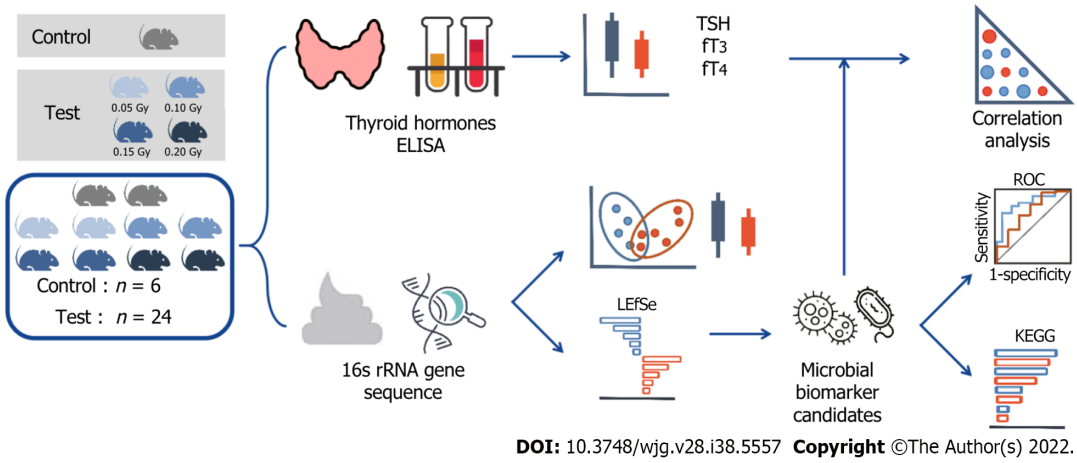

Figure 1 Graphical abstract. Schematic showing sample workflow from sequencing to analysis. TSH: Thyroid stimulating hormone; ELISA: Enzyme-linked immunosorbent assay; ROC: Receiver operating characteristic; KEGG: Kyoto Encyclopedia of Genes and Genomes; LEfSe: Linear discriminant analysis effect size.

highest (1.664 ± 0.264 mU/L) (Figure 2A and B). The change in thyroid hormone levels increased with increasing radiation dose and was consistent with TSH regulation in the test mice. The average levels of fT3 and fT4 in the test mice [386.560 (323.973, 442.594) ng/mL and 6.211 (5.018, 8.536) ng/mL, respectively] were higher than those in the control groups [372.664 (260.701, 384.492) ng/mL and 4.428 (3.175, 6.781) ng/mL, respectively]; nevertheless, the difference was not statistically confirmed between the two groups (Figure 2C and E). Moreover, the changes in fT3 and fT4 Levels from the different dose groups were consistent with the regulation of TSH (Figure 2D and F). Figure 3 shows thyroid tissue from the different groups of mice, and there were no obvious structural abnormalities.

Overview of the 16S rRNA gene sequencing data

An overview of the thyroid hormone levels and taxonomic data of all mice is visualized in Figure 4. High-throughput sequencing results of the 16S rRNA V3-V4 hypervariable regions obtained from stool samples revealed that the tested taxa were abundant, including 11 phyla, 50 families, 110 genera, and 424 OTUs. Rank-abundance curves according to OTU levels indicated that the species were distributed evenly and richly (Figure 5A). Figure 5B indicates that the curves flattened as increasing the sample size, demonstrating that the sequencing data were adequate and appropriately capturing microbial diversity.

LDR does not cause gut microbiota diversity changes in irradiated mice

According to the outcome of alpha diversity analysis, the results showed that the test group had no differences in the diversity and abundances of certain microbes compared with the control group. The Chao and Ace indices, reflecting richness, indicated no considerable difference was found between the two groups ($P > 0.05$) (Figure 5C and D). Moreover, the Shannon and Simpson indices, which represent alpha diversity, indicated no meaningful difference between the two groups ($P > 0.05$, Figure 5E and F). Figure 5G-J shows the Chao, Ace, Shannon and Simpson indices among the different subgroups from the test group.

PCoA and NMDS analyses of the unweighted UniFrac distance were conducted on evaluation the overall microbiota diversity. PCoA indicated that there were no statistically significant variation in diversity of the microbiota between the test and control mice (R = -0.065, $P = 0.715$, Figure 5K). The NMDS results demonstrated no differences between the two groups (R = -0.065, $P = 0.695$, Figure 5L). Figure 5M and N show the results of the PCoA and NMDS analyses in the four test subgroups. These findings suggest that LDR in this study does not lead to altered microbiota diversity.

Changes in the composition of the gut microbiota in irradiated mice

The taxon compositions were further determined in the two groups. Eleven phyla, 50 families, and 110 genera were present in the samples from both groups. Figure 6A shows that *Firmicutes*, *Bacteroidetes*, *Patescibacteria*, and *Deferribacteres* were the four dominant phyla, with total abundances accounting for 97.83% ± 1.23% and 97.68% ± 0.75% of the microbiota of the test and control groups, respectively. The two dominant phyla were Firmicutes and *Bacteroidetes* in both the test and control group. At the phylum level, no meaningful variation in the relative abundances of the microorganisms was observed between the two groups (Figure 6B). We found that the *Firmicutes*-to-*Bacteroidetes* (F/B) ratio in the test group (median = 1.623 ± 0.679) was higher than controls (median = 1.266 ± 0.345), the differences were not confirmed by statistics. In addition, the microbiota was further analyzed according to the distribution at the family and genus levels.

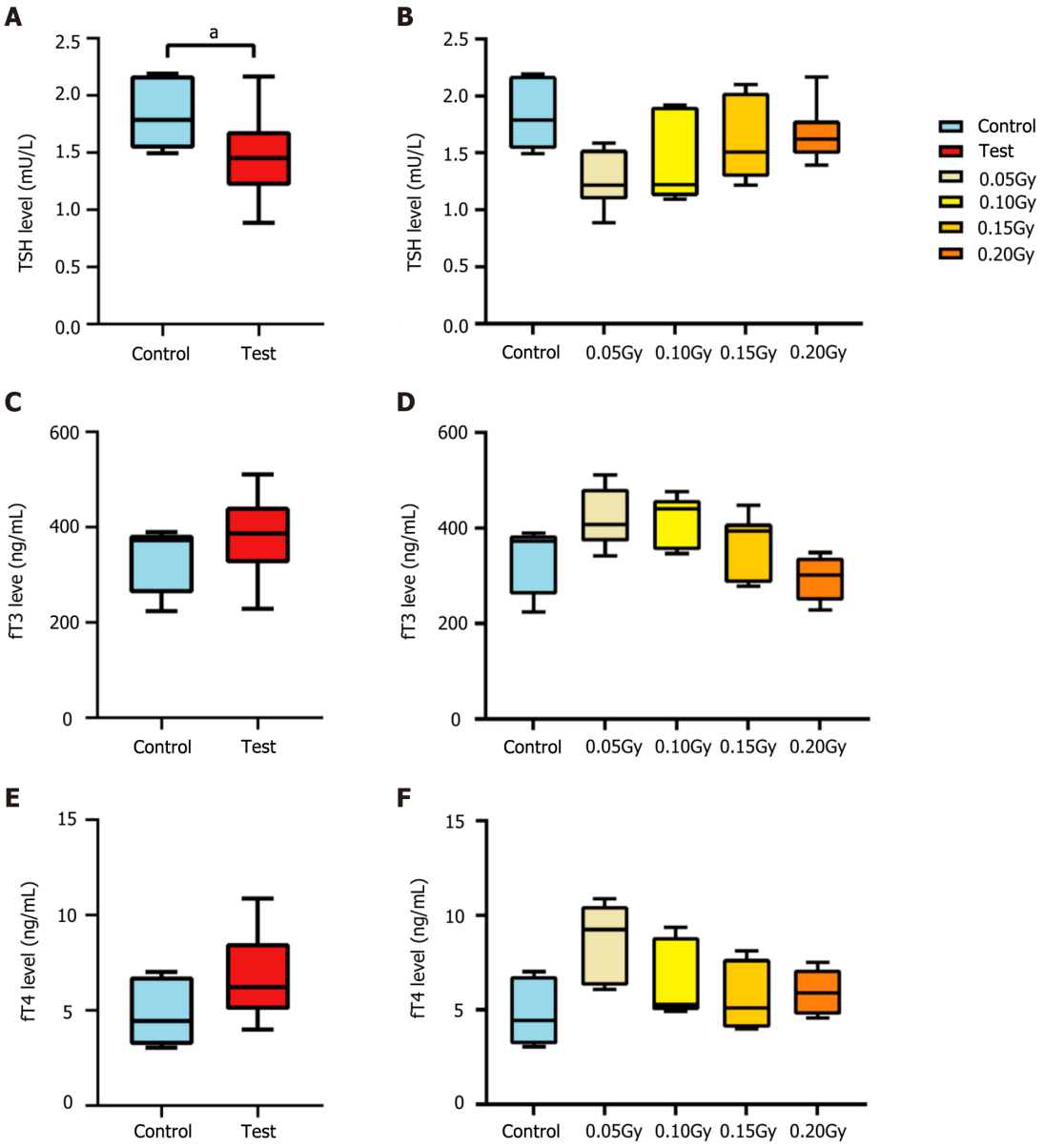

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Figure 2 The level of thyroid hormone in mice exposed to radiation. A: Box and whisker plots of the levels of thyroid stimulating hormone (TSH) in the control (n = 6) and test (n = 24) groups. The TSH level of the mice in the test group was lower than that in the control mice (t = 2.188, P = 0.037); B: Histogram of the levels of TSH from the control and test groups. Statistically significant differences were not confirmed between the two groups; C: Box and whisker plots of the level of fT3 in the control (n = 6) and test groups (n = 24); D: Histogram of the level of fT3 in the control and test subgroups; E: Box and whisker plots of the level of fT4 in the control (n = 6) and test groups (n = 24); F: Histogram of the level of fT4 in the control and test subgroups. TSH: Thyroid stimulating hormone. $^{a}P < 0.05$.

LEfSe analysis results indicated that the control group had relatively high abundance in two families and four genera (LDA > 2, $P < 0.05$, Figure 7A). *Muribaculaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Lactobacillaceae* and *Bacteroidaceae* were the five dominant families, with total abundances accounting for 83.04% ± 4.59% and 80.68% ± 6.93% of the microbiomes of the test group and control group, respectively (Figure 7A). At the family level, the test group had significantly lower levels of *Moraxellaceae* and *Enterobacteriaceae* (all $P < 0.05$, Figure 7B). We also found that the abundance of *Lachnospiraceae* in the test group (20.05% ± 10.79%) was significantly higher than that in the controls (15.08% ± 10.02%), which increased in a dose-dependent manner following LDR exposure (Figure 7B and C).

Uncultured_bacterium_f_Muribaculaceae, *Lactobacillus*, *uncultured_bacterium_f_Lachnospiraceae*, *Bacteroides*, and *uncultured_bacterium_g_Ruminococcaceae_UCG-014* were the five dominant genera, with total abundances accounting for 59.87% ± 11.92% and 67.05% ± 10.38% of the microbiomes of the test group and control group, respectively (Figure 6C and D). At the genus level, the test group had significantly lower levels of *uncultured_bacterium_g_Acinetobacter*, *uncultured_bacterium_o_Mollicutes_RF39*, *uncultured_bacterium_g_Citrobacter*, and *uncultured_bacterium_g_Lactococcus* (all $P < 0.05$, Figure 7D).

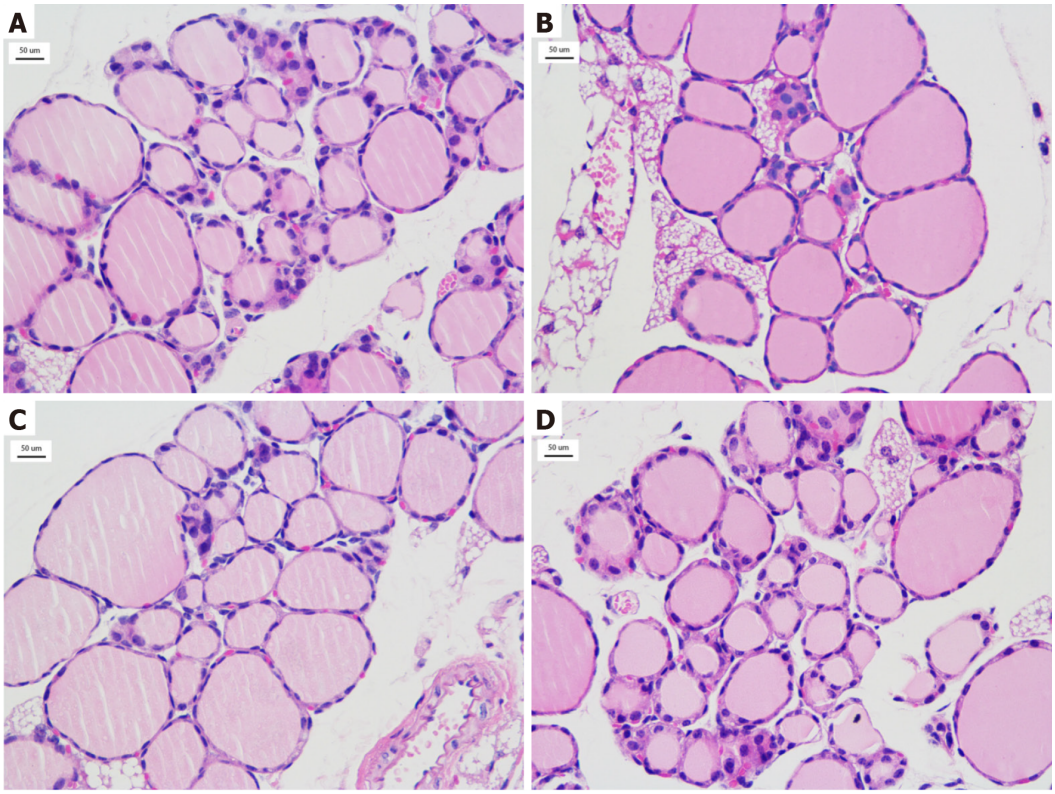

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Figure 3 Thyroid tissue from the mice exposed to radiation (HE, × 400). A-D: Thyroid tissue of radiation-exposed mice from the 0.05 Gy group, 0.10 Gy group, 0.15 Gy group, and 0.20 Gy group. TSH: Thyroid stimulating hormone.

ROC curve analysis reveals the diagnostic value of specific intestinal microbiota

Based on the altered gut microbiota composition between the test and control groups, we found that six kinds of bacteria might serve as diagnostic biomarkers. At the family level, *Moraxellaceae* and *Enterobacteriaceae* are the two different bacteria between the two groups, and we selected them to serve as diagnostic biomarkers. ROC curves were generated to evaluate the diagnostic accuracy of these two families [AUC value of 0.819 (95% confidence interval: 0.640-0.999)] (Figure 8A).

At the genus level, we found four different bacteria and selected them to serve as diagnostic biomarkers, including *uncultured_bacterium_g_Acinetobacter*, *uncultured_bacterium_o_Mollicutes_RF39*, *uncultured_bacterium_g_Citrobacter*, and *uncultured_bacterium_g_Lactococcus*. ROC curves were generated to evaluate the diagnostic accuracy of these four genera [AUC value of 0.882 (confidence interval: 0.728-1.000)]. At the same time, the accuracy of the prognostic variables of two families and four genera was also analyzed by ROC curves [AUC value of 0.854 (confidence interval: 0.673-1.000)] (Figure 8).

The test groups included the 0.05 Gy group, 0.10 Gy group, 0.15 Gy group, and 0.20 Gy group according to the total radiation dose the mice received. On the basis of these results, we investigated the following relationships among thyroid function tests, absorbed dose, and levels of the gut microorganisms: *f_Moraxellaceae*, *f_Enterobacteriaceae*, *uncultured_bacterium_g_Acinetobacter*, *uncultured_bacterium_o_Mollicutes_RF39*, *uncultured_bacterium_g_Citrobacter*, and *uncultured_bacterium_g_Lactococcus*. We made an attempt to demarcate the functional influence of these flora according to the KEGG database to determine which biological pathways they may participate. Spearman correlation coefficient testing showed that *Moraxellaceae* (r = -0.789, P = 7.75E-7), *Enterobacteriaceae* (r=-0.662, P = 2.92E-4), *uncultured_bacterium_g_Acinetobacter* (r = -0.789, P = 7.75E-7), *uncultured_bacterium_o_Mollicutes_RF39* (r = -0.365, P = 0.0106), and *uncultured_bacterium_g_Lactococcus* (r = -0.667, P = 4.77E-3) levels were significantly negatively correlated with the absorbed dose (Figure 9).

The OTU abundance table was standardized to acquire information about the prediction of metabolic function in the KEGG pathway (Figure 10). According to the effect size, the top five pathways included carbohydrate metabolism, nucleotide metabolism, glycan biosynthesis and metabolism, xenobiotics biodegradation and metabolism, and metabolism of other amino acids. The effect size of the genetic information processing function is second only to metabolic function, which includes translation, replication and repair, folding, sorting and degradation, and transcription.

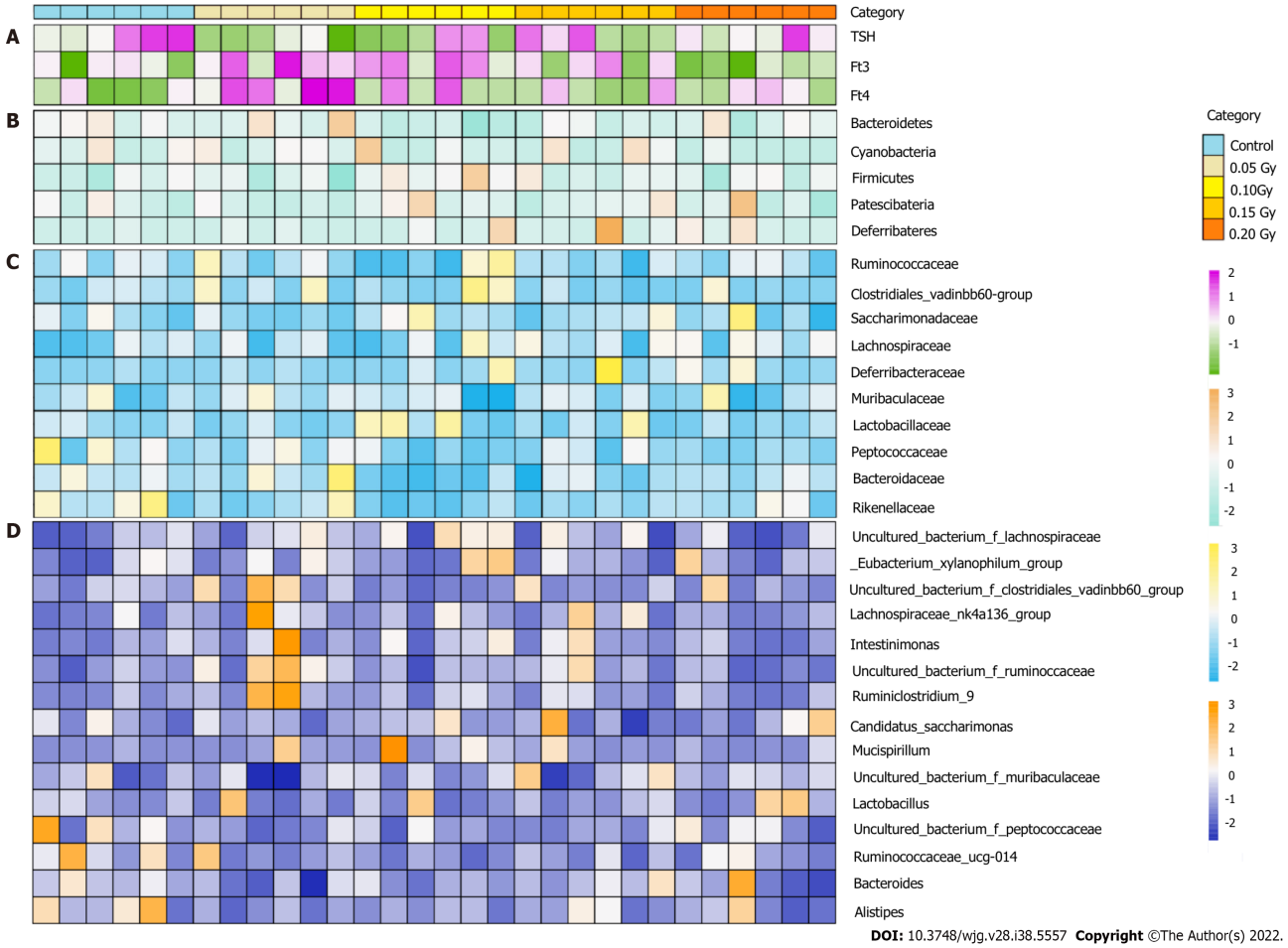

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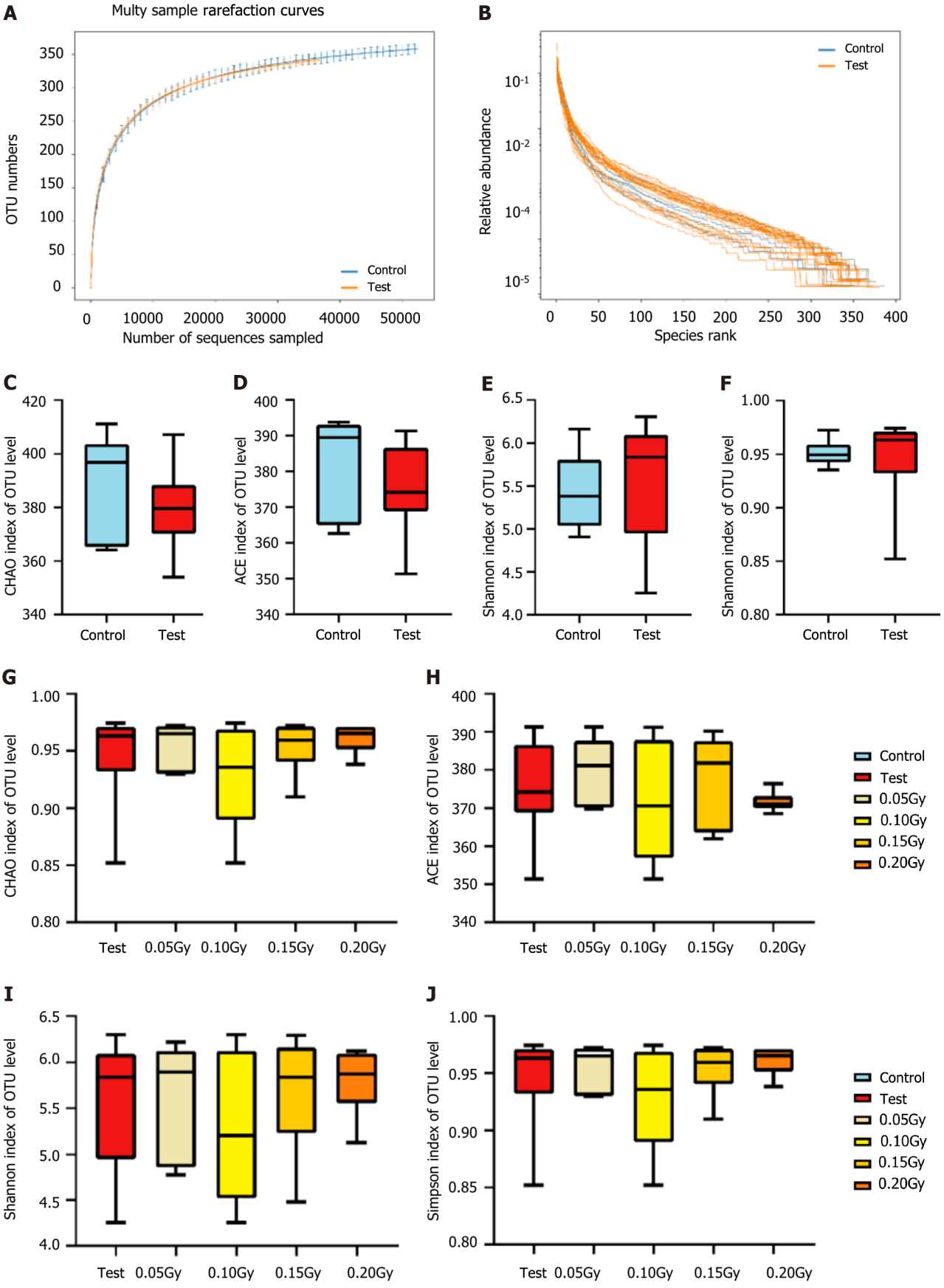
Figure 4 Taxonomic features of the thyroid hormone level and gut microbiota in the control mice (*n* = 6) and radiation-exposed group (*n* = 24). A-D: The thyroid hormone level for all subjects (A), with an overview of the 5 most abundant phyla (B), 10 most abundant families (C) and 15 most abundant genera (D) in fecal samples identified by 16S rRNA gene sequencing. The taxa levels were determined by summing the operational taxonomic units and are presented by relative abundance.

DISCUSSION

As an important organ of the endocrine system, the thyroid participates in homeostatic regulation. Recent studies demonstrated that the gut microbiota, a sophisticated and metabolically active community, is closely related to homeostasis in the host[13]. Although LDR exposure has been demonstrated to induce contradictory effects previously, *i.e.*, beneficial or harmful, the impacts of LDR exposure on the "thyroid-gut axis" have yet to be clarified. Therefore, we analyzed the association among LDR exposure, thyroid function and the gut microbiota in mice in this research. Our group indicated that as the LDR radiation dose increased, subclinical thyroid hyperthyroidism appeared in mice, and the observed species and OTU abundances showed no obvious changes in the radiated groups compared with the controls. It was thought that the low doses of radiation applied in this paper have an influence on the thyroid function of mice but are unable to alter the richness of the intestinal flora.

The thyroid is a radiation-sensitive organ, and internal radionuclide contamination external radiation and exposure (such as therapeutic radiation) may lead to thyroid dysfunction[14,15]. Our research found that the TSH level in the test mice was lower than the controls. Double-strand breaks can lead to cytotoxic, mutagenic and carcinogenic effects if unrepaired or misrepaired, although they only constitute a minority of DNA lesions induced by LDR, which will affect cell function[16,17]. Ishizaki *et al*[18] demonstrated a very small increase in γH2AX-positive foci in a human diploid cell line after exposure to LDR, which indicates the presence of DNA double-strand breaks. Some studies have also suggested that radiation-induced thyroid dysfunction is induced by damage to small thyroid vessels and to the gland capsule, the mechanisms of which include direct thyroid cell injury from radiation damage[19]. Because of the low thyroid dose in patients treated with total body irradiation, the incidence of Graves' disease accompanying this management was also reported in a total body irradiation series[20]. Strikingly, our results indicate pronounced modulation of the TSH level in mice exposed to 0.05 Gy and 0.10 Gy, while those exposed to a much higher dose (0.20 Gy) consistently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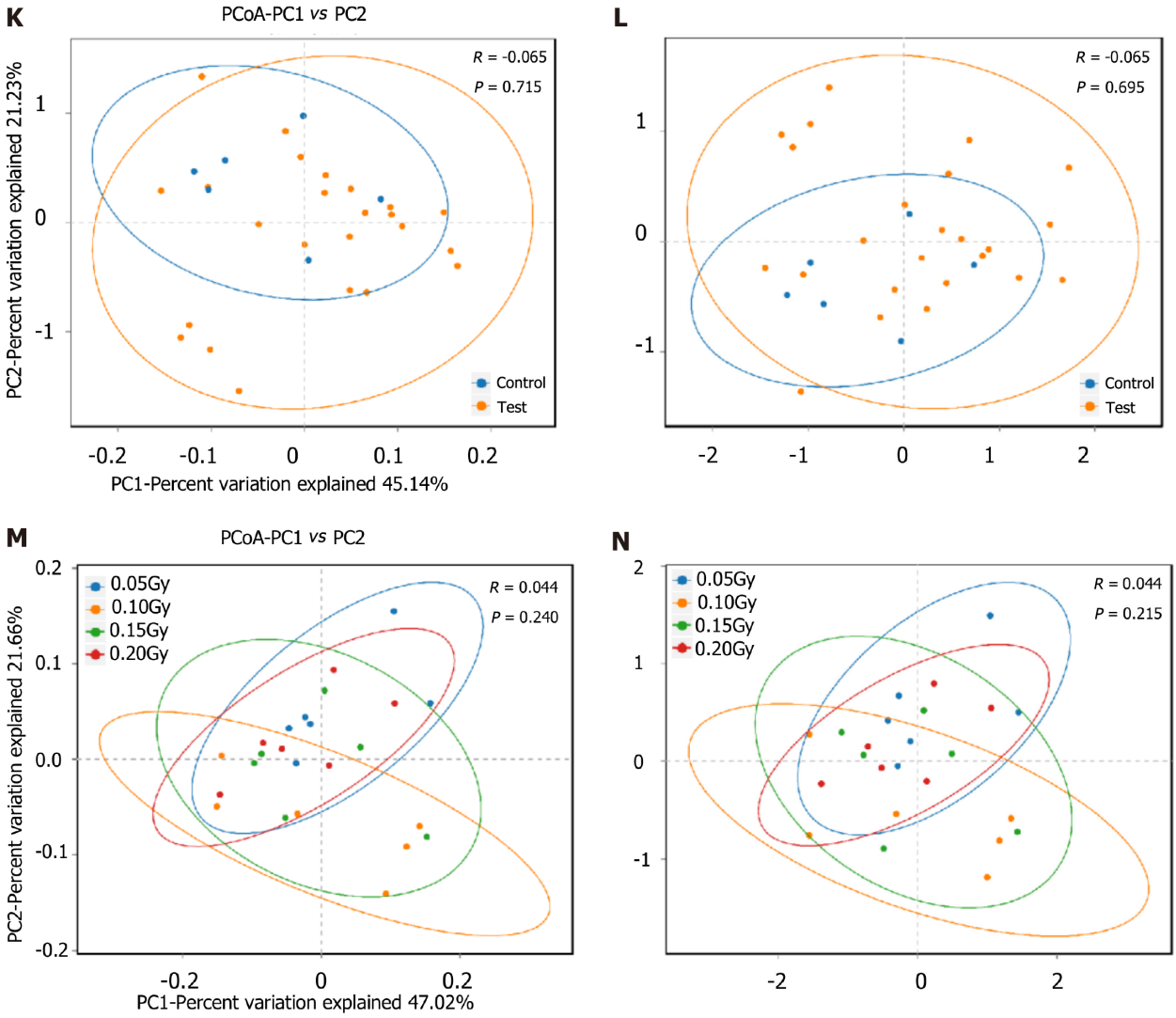

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Figure 5 Bioinformatic analysis of 16S rRNA gene sequencing data in the exploratory cohort at the operational taxonomic units level. A and B: Rank-abundance curves and rarefaction curves (SOB index) of the gut microbiota; C-F: Box and whisker plots of the alpha diversity indices for richness (Chao index and Ace index) and diversity (Shannon index and Simpson index) of the bacterial communities in control mice (n = 6) and the test mice (n = 24); G-J: Box and whisker plots of the alpha diversity indices for richness (Chao index and Ace index) and diversity (Shannon index and Simpson index) of the bacterial communities among the test subgroups (0.05 Gy, 0.10 Gy, 0.15 Gy, and 0.20 Gy; n = 6 in each group); K and L: Principal coordinates analysis (PCoA) and nonmetric multidimensional scaling (NMDS) analyses indicated that the microbial diversity differed nonsignificantly between the control and test groups based on the unweighted UniFrac distance (PCoA, R = -0.065, P = -0.715; NMDS, R = -0.065, P = 0.695); M and N: PCoA and NMDS analyses indicated that the microbial diversity differed nonsignificantly among the test subgroups based on the unweighted UniFrac distance (PCoA, R = 0.004, P = 0.24; NMDS, R = 0.215, P = 0.695).

clustered with controls in terms of thyroid hormone levels. We speculate that there is an intricate dose-dependent response in thyroid function to ionizing radiation, with enhanced sensitivity for the lowest doses employed here. Therefore, thyroid function and microbial communities might be directly affected by LDR exposure.

Firmicutes and *Bacteroidetes* were the two dominant microbiota in these two groups. We found that the *Firmicutes* proportion in the irradiated group was higher than in the controls, while the *Bacteroidetes* proportion was lower in the irradiated group. One study about environmental radionuclides on the gut microbiota from Lavrinienko came to similar conclusions that the F/B ratio was increased almost 2-fold compared with an unirradiated group[21]. Previous investigation have also revealed that patients with metabolic diseases such as obesity, diabetes, and nonalcoholic fatty liver disease have a reduced F/B ratio compared to healthy individuals[22-24]. Recently, our group demonstrated that thyroid diseases, including Graves' disease and thyroid carcinoma, can cause changes in the F/B ratio[25,26]. Therefore, we speculate that the composition and function of microbial communities might be affected by LDR exposure directly or indirectly through changes in thyroid function.

Compared with the controls, the abundances of *Moraxellaceae* and *Enterobacteriaceae* were decreased in the test group at the family level. Obvious differences in the levels of four species were observed between the two groups at the genus level, and *uncultured_bacterium_g_Acinetobacter*, *uncultured_*

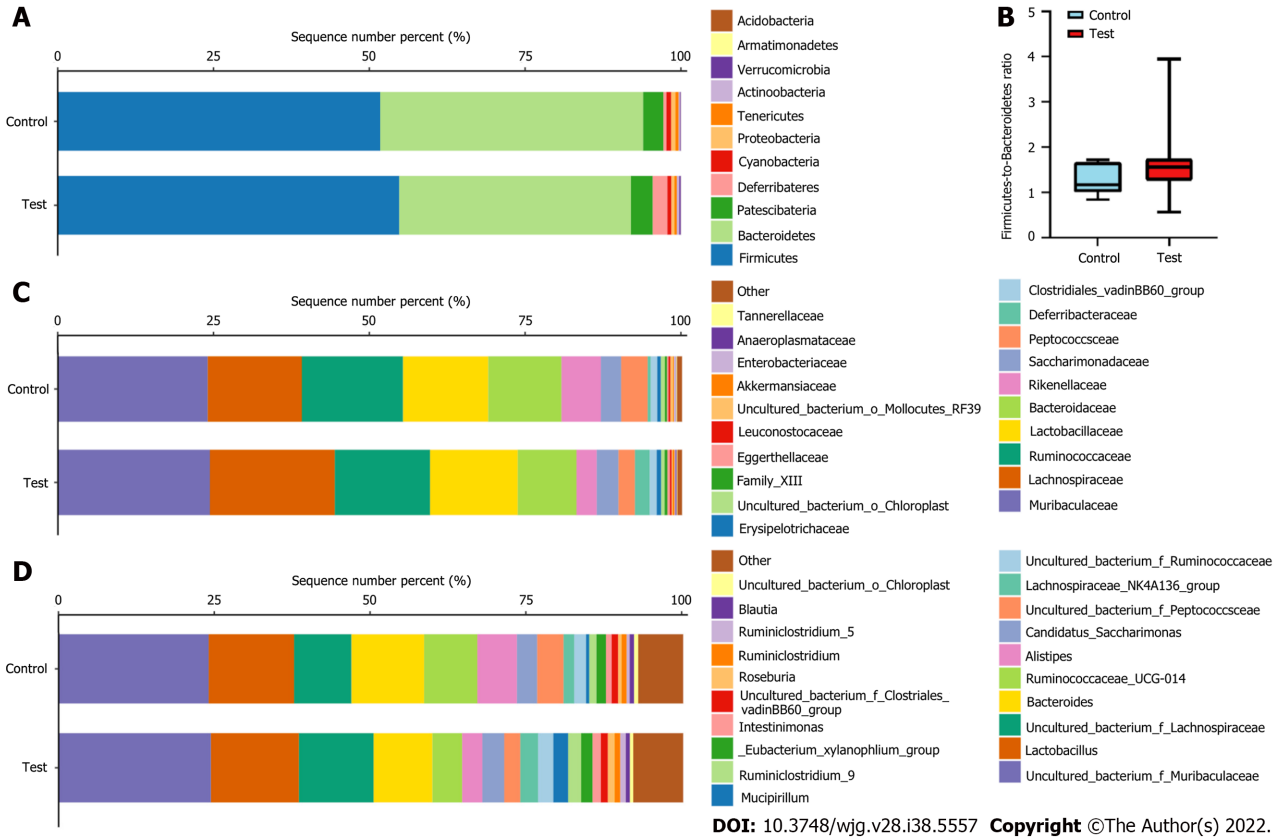

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Figure 6 The gut microbiota composition in the control and test groups. A: Bar plots show the gut microbiota composition at the phylum level; B: Box and whisker plots of the *Firmicutes/Bacteroidetes* ratio in the control (n = 6) and test groups (n = 24), and there was no difference between the groups; C: Bar plots show the gut microbiota composition at the family level; D: Bar plots show the gut microbiota composition at the genus level.

bacterium_o_Mollicutes_RF39, *uncultured_bacterium_g_Citrobacter* and *uncultured_bacterium_g_Lactococcus* were reduced in the test group. On the one hand, the decreased abundance of the bacterium might be directly caused by radiation damage. On the other hand, changes in microbial abundance inhibit the development of gastrointestinal mucositis and promote intestinal repair mechanisms[27]. We hypothesize that metabolites from bacteria impair intestinal barrier function. By regulating bacterial abundance and reducing the release of proinflammatory factors, the intestinal barrier could repair the damage from LDR exposure. Another significant finding in this research was that the abundance of *Lachnospiraceae* increased in a dose-dependent manner following LDR exposure, which was in accordance with the performance outcome of the group of mice that have recovered from high-dose radiation to live a normal life span[28].

The synthesis of short-chain fatty acids (SCFAs) by fermenting polysaccharides in food has made members of the *Lachnospira* family well known[29]. SCFAs are essential substrates for the maintenance of the intestinal epithelium, regulation of the immune system and inflammatory response[30,31]. Broad inhibition of systemic inflammation, through reduction the levels of proinflammatory cytokines or through induction of anti-inflammatory cytokine interleukin-10, mediated by SCFAs may also potentially provide radioprotection. Elevated abundances of *Lachnospiraceae* have been shown to be connected with the repair of gastrointestinal after exposure to radiation[32].

Additionally, we employed KEGG pathway analysis to characterize changes in the gut microbiota associated with the stress from LDR exposure. KEGG pathway analysis based on OTU abundance indicated that biological metabolism was predicted to influence functional activities, including carbohydrate metabolism, glycan biosynthesis and metabolism, and nucleotide metabolism. The gut microbiota composition and metabolic pathways were influenced by LDR exposure. Moreover, processing functions were second only to metabolic functions according to the effect size, including translation, replication and repair. As the most significant influence of radiation, base modifications are a common type of DNA lesion[33]. KEGG pathway analysis may suggest a relationship between radiation damage and functional repair. Nevertheless, our observations provide circumstantial the proof of host-microbiome interactions; more investigations are required to uncover the crosstalk within LDR-responsive alterations and host-derived targets or signals.

Our research did not consider the following issues, and these limitations will also become the focus of follow-up research. First, the effects of exposure dose and recovery time on the thyroid and gut microbiota in the mice were not considered. Second, this was a descriptive study in nature and lacked

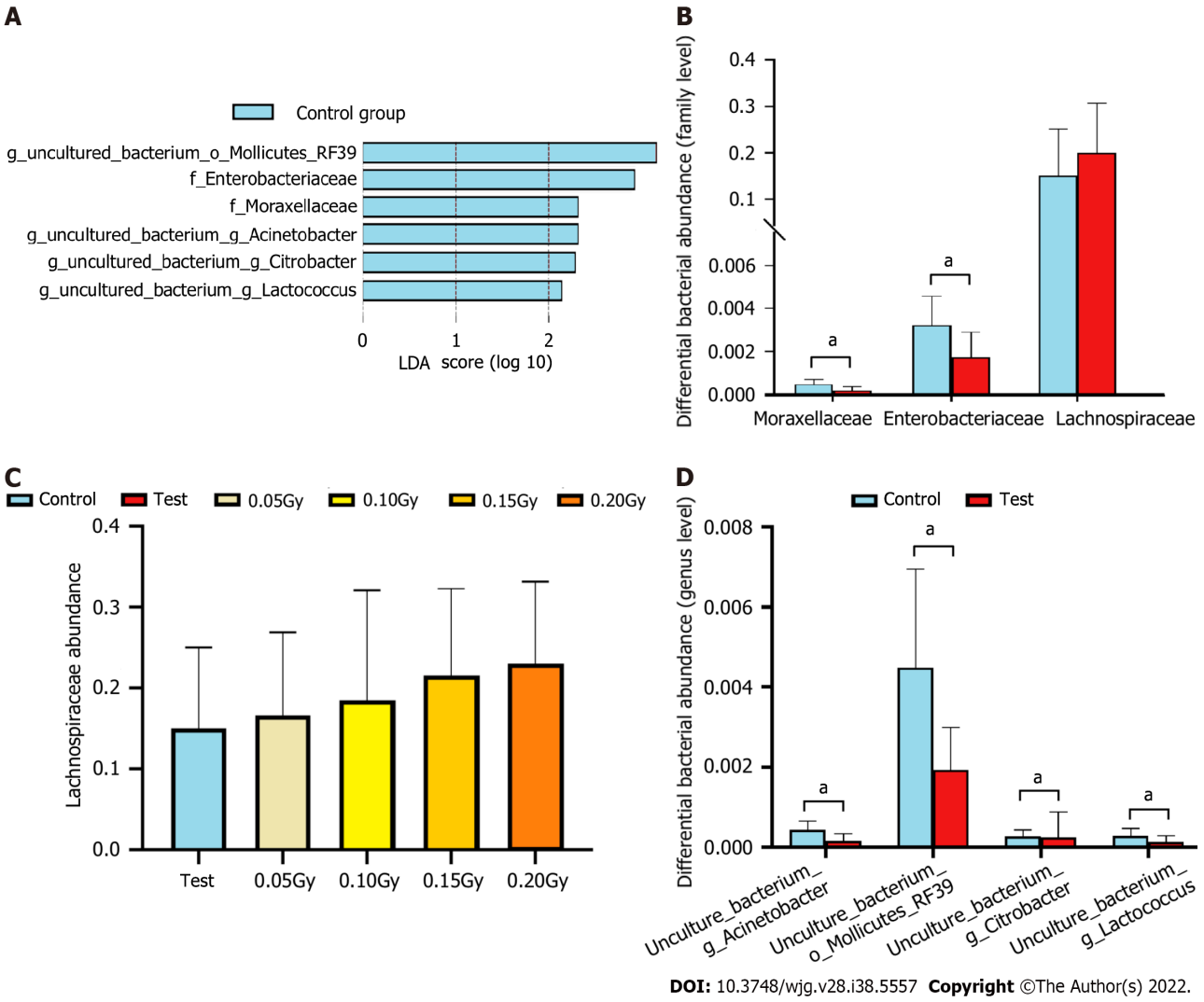

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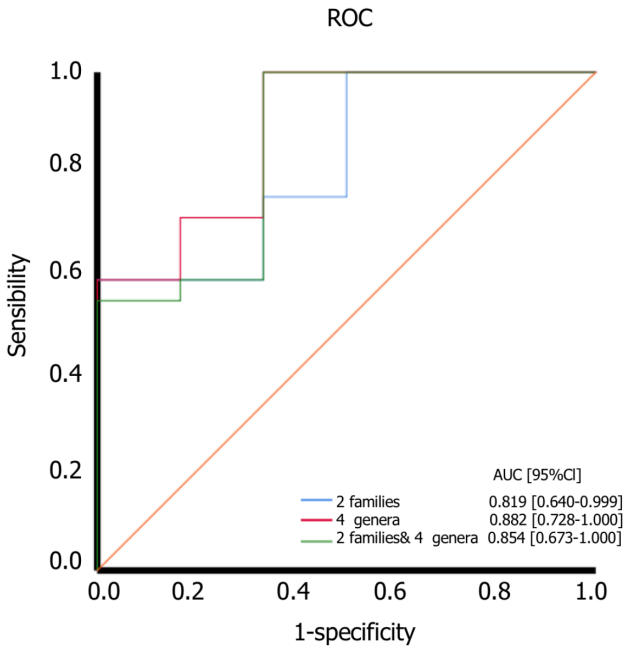
Figure 7 Altered gut microbiota composition in the radiation-exposed group compared with the control group. A: Identification of the significantly different species in the radiation-exposed group and controls at the family and genus levels as determined by linear discriminant analysis (LDA) effect size (LEfSe). Only taxa meeting a significant linear discriminant analysis threshold value of > 2.0 and $P < 0.05$ are shown; B: Comparison of *Moraxellaceae*, *Enterobacteriaceae, and Lachnospiraceae* abundances between the test group and control group at the family level; C: Histogram of the abundance of *Lachnospiraceae* in the control and test subgroups; D: Comparison of the differential bacterial abundances between the test group and controls at the genus level, including *uncultured_bacterium_g_Acinetobacter*, *uncultured_bacterium_o_Mollicutes_RF39*, *uncultured_bacterium_g_Citrobacter*, and *uncultured_bacterium_g_Lactococcus*. $^{a}P < 0.05$.

evidence regarding the underlying mechanisms of the responses to LDR exposure, which could be used to determine the alterations in metabolites from the gut microbiota. Finally, our experiment was a single-center study that lacked external validation. Despite these limitations, our research is one of the few to indicate the effects of LDR on homeostasis from the "thyroid-gut axis" perspective. In summary, our study constitutes a baseline analysis that can not only be used to further study the effects of LDR on human health but also provide potential targets for preventing or predicting radiation damage.

CONCLUSION

LDR can change the thyroid function and gut microbiota of mice, and changes in the abundances of bacteria are correlated with the radiation dose.



DOI: 10.3748/wjg.v28.i38.5557

Figure 8 The diagnostic accuracy of the bacteria based on the linear discriminant analysis effect size results. ROC: Receiver operating characteristic.

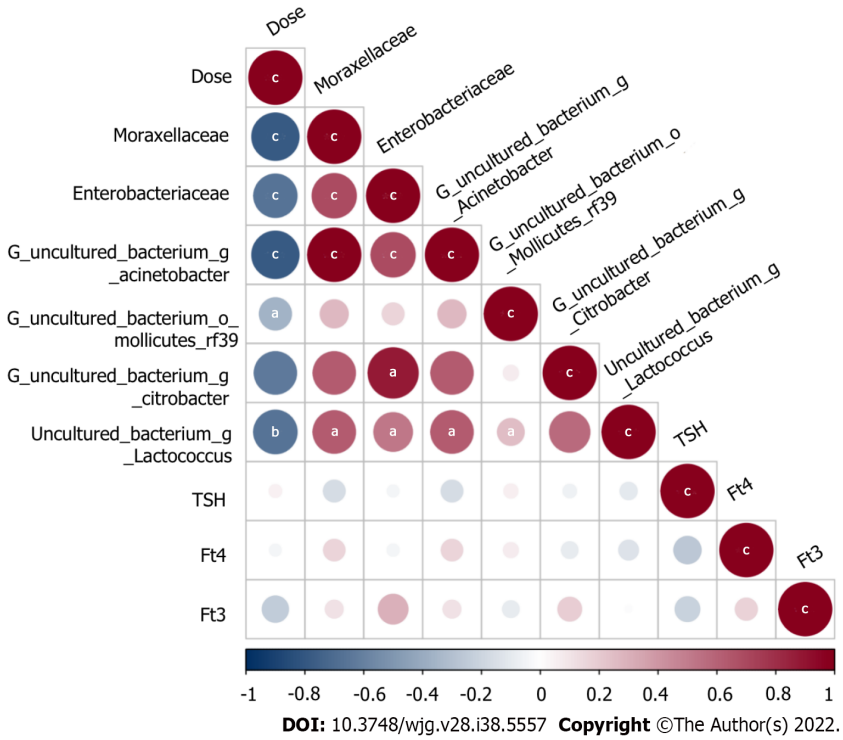


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Figure 9 The relationship between the dose and predictive bacteria as well as thyroid function tests. Positive correlations are represented in red, negative correlations are colored in blue. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

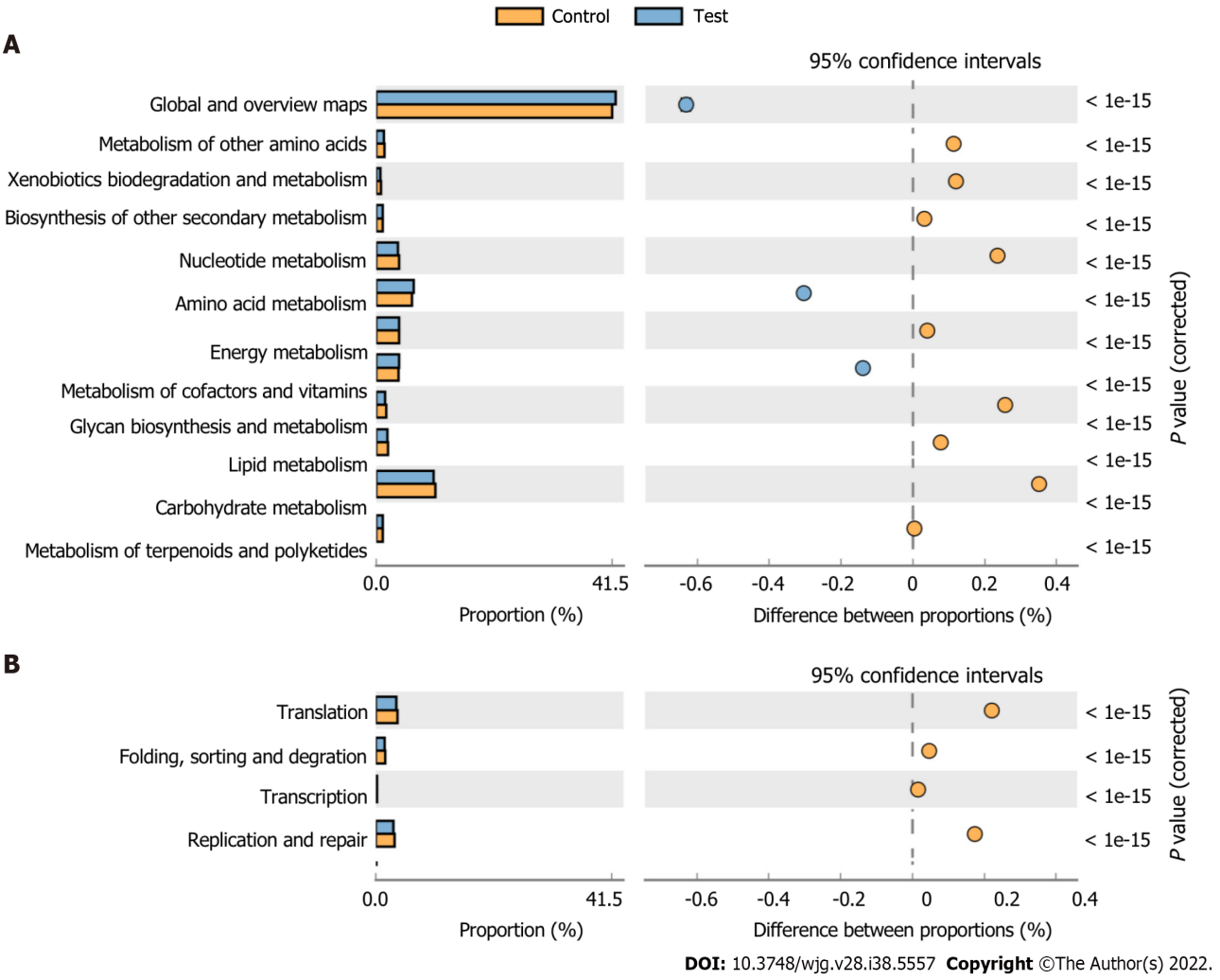

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Figure 10 The histogram shows the Kyoto Encyclopedia of Genes and Genomes categories that differed significantly between the test group and controls. A: Metabolic function; B: Processing function.

ARTICLE HIGHLIGHTS

Research background

The thyroid-gut axis has a great influence on the maintenance of human health.

Research motivation

We know very little about the effects of low-dose ionizing radiation (LDR) on thyroid hormone levels and gut microbiota composition.

Research objectives

To investigate the potential effects by administering low-dose X-ray radiation to male C57BL/6J mice.

Research methods

Peripheral blood was collected for enzyme-linked immunosorbent assay (ELISA), and stool samples were taken for 16S ribosomal RNA (rRNA) gene sequencing after irradiation.

Research results

We found that LDR caused changes in thyroid stimulating hormone (TSH) levels in the irradiated mice, suggesting a dose-dependent response in thyroid function to ionizing radiation. No changes in the diversity and richness of the gut microbiota were observed in the LDR-exposed group in comparison to the controls. The abundance of *Moraxellaceae* and *Enterobacteriaceae* decreased in the LDR-exposed groups compared with the controls, and the *Lachnospiraceae* abundance increased in a dose-dependent manner in the radiated groups. And the abundances of *uncultured_bacterium_g_Acinetobacter*, *uncultured_bacterium_o_Mollicutes_RF39*, *uncultured_bacterium_g_Citrobacter*, and *uncul-tured_*

bacterium_g_Lactococcus decreased in the radiated groups at the genus level, which showed a correlation with radiation exposure and diagnostic efficacy. Analysis of functional metabolic pathways revealed that biological metabolism was predicted to have an effect on functional activities, such as nucleotide metabolism, carbohydrate metabolism, and glycan biosynthesis and metabolism. Furthermore, KEGG pathway annotation also suggested that changes in the gut microbiota were related to processing functions, including translation, replication and repair.

Research conclusions

LDR can change thyroid function and the gut microbiota, and changes in the abundances of bacteria are correlated with the radiation dose.

Research perspectives

We administered low-dose X-ray radiation to male C57BL/6J mice to investigate the potential effects. Peripheral blood was collected for ELISA, and stool samples were taken for 16S rRNA gene sequencing after irradiation.

FOOTNOTES

Author contributions: Tong JY performed the statistical analysis and drafted this manuscript; Jiang W designed and performed the experiments; Yu XQ, Wang R, Lu CH, and Gao DW contributed to data collection; Lv ZW and Li D conceived the project and reviewed the manuscript; all authors approved the final version of the manuscript; Tong JY and Jiang W contributed equally to this work.

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ORIGINAL ARTICLE

Basic Study

Hypoxia inducible factor 1α promotes interleukin-1 receptor antagonist expression during hepatic ischemia-reperfusion injury

Zhao-Yang Wang, Yu Liu, Shi-Peng Li, Jian-Jun Li, Zhen Zhang, Xue-Chun Xiao, Yang Ou, Hang Wang, Jin-Zhen Cai, Shuang Yang

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Abstract

BACKGROUND

Ischemia-reperfusion injury (IRI) is a major risk associated with liver surgery and transplantation, and its pathological mechanism is complex. Interleukin-1 receptor antagonist (IL-1ra) can protect the liver from IRI. However, the regulatory mechanism of IL-1ra expression is still unclear.

AIM

To identify the mechanism that could protect the liver in the early stage of IRI.

METHODS

To screen the key genes in hepatic IRI, we performed RNA sequencing and gene enrichment analysis on liver tissue from mice with hepatic IRI. Subsequently, we verified the expression and effect of IL-1ra in hepatic IRI. We also used promoter mutagenesis and chromatin immunoprecipitation assay to search for the trans-

criptional regulatory sites of hypoxia-inducible factor (HIF)-1α. Finally, to explore the protective mechanism of ischemic preconditioning (IP), we examined the expression of HIF-1α and IL-1ra after IP.

RESULTS

We identified IL-1ra as a key regulator in hepatic IRI. The expression of IL-1ra was significantly upregulated after hepatic IRI both *in vivo* and *in vitro*. Furthermore, we found that HIF-1α regulated *Il-1ra* transcription in response to hypoxia. Increased HIF-1α accumulation promoted IL-1ra expression, whereas inhibition of HIF-1α exhibited the opposite effect. We also confirmed a predominant role for hypoxia response element in the regulation of *Il1ra* transcription by HIF-1α activation. Of note, we demonstrated that IP protects against hepatic IRI by inducing IL-1ra expression, which is mediated through HIF-1α.

CONCLUSION

We demonstrated that ischemia or hypoxia leads to increased expression of IL-1ra through HIF-1α. Importantly, IP protects the liver from IRI *via* the HIF-1α–IL-1ra pathway.

Key Words: Hepatic ischemia-reperfusion injury; Interleukin-1 receptor antagonist; Hypoxia inducible factor 1α; Ischemic preconditioning

Core Tip: Ischemia-reperfusion injury (IRI) is a major risk associated with liver surgery and transplantation, and its pathological mechanism is complex. Interleukin-1 receptor antagonist (IL-1ra) can protect the liver from IRI. The expression of IL-1ra was significantly upregulated after IRI in mice. Hypoxia promoted the expression of IL-1ra. Hypoxia-inducible factor (HIF)-1α accumulation contributed to increased *Il-1ra* transcription. Ischemic preconditioning protected the liver from IRI *via* the HIF-1α-IL-1ra pathway.

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INTRODUCTION

Hepatic ischemia (IS)-reperfusion injury (IRI) is a common complication in liver surgery and transplantation[1]. IRI usually results from restoration of the blood supply after brief IS[2]. The sterile inflammatory response induced by hypoxic stress leads to hepatic IRI[3]. The pathogenesis of hepatic IRI is complex and affected by various factors[4,5], which involve the damage-associated molecular patterns, innate immune response, and inflammation. Among them, the inflammation caused by interleukin (IL)-1β plays an important role[6,7]. During hepatic IRI, injured cells activate the inflammasome pathway, allowing the IL-1β precursor to be cleaved into its mature form by caspase-1 and subsequently released. Mature IL-1β is a broad-acting proinflammatory cytokine that increases the recruitment of endothelial adhesion molecules to innate immune cells and promotes the development of inflammatory phenotypes[8]. However, blocking the action of IL-1 can reduce hepatic damage[9,10]. As a natural anti-inflammatory factor, IL-1 receptor antagonist (IL-1ra) is highly expressed in a variety of inflammatory diseases and is closely related to the occurrence and progression of inflammation[11-14]. Several studies have shown a potential protective effect of IL-1ra in inflammation. For example, IL-1ra competes with IL-1β to bind to IL-1 receptor I, thus playing an anti-inflammatory role[15]. However, the transcriptional regulatory mechanism of IL-1ra during IRI remains unclear.

As a transcription factor, hypoxia-inducible factor (HIF)-1α is an important molecule for cell regulation in the hypoxic environment[16,17]. There have been a few reports confirming that HIF-1α plays an irreplaceable role in hepatic IRI[18]. HIF-1α can regulate the expression of multiple genes in cells after hypoxia[19], and consequently reduce organ IRI by regulating metabolism[20]. Furthermore, the mechanism of HIF-1α protection against hepatic IRI deserves further study.

Ischemic preconditioning (IP) was first reported by Murry *et al*[21] in 1986, who demonstrated that multiple IP reduces cell death after coronary artery occlusion. Although the protective effect of IP on hepatic IRI has been subsequently reported[22], its mechanism of action remains unclear. Therefore, it is

necessary to confirm the mechanism underlying this protective effect, which would be of great clinical significance. In the present study, we found that IL-1ra was expressed in hepatic tissue during IRI and was regulated by IS-induced HIF-1α. We also confirmed that the protective effect of IP was exerted precisely *via* the HIF-1α–IL-1ra pathway, leading to inhibition of IL-1β signaling and subsequent reduction in hepatic IRI.

MATERIALS AND METHODS

Reagents and antibodies

2-MeOE2 (S1233) and DMOG (S7483) were purchased from Selleck (China). Percoll (17089101) was purchased from GE Healthcare (United States). Collagenase IV (C8160) was purchased from Solarbio (China). Mouse monoclonal antibody for β-actin (sc-47778) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, United States). A rabbit monoclonal antibody for IL-1ra (ab124962) was purchased from Abcam (Cambridge, MA, United States). Rabbit antibodies for HIF-1α (36169), BAX (14796), Bcl-2 (3498), and cleaved caspase-3 (9664) were purchased from Cell Signaling Technology (Danvers, MA, United States). ELISA kits for detecting mouse alanine transaminase (ALT) (JL12668), aspartate transaminase (AST) (JL13793), and IL-1ra (JL20255) were purchased from Jonln (China). Recombinant IL-1ra (200-01RA) was purchased from PeproTech (United States).

Animals

Wild-type male C57BL/6 mice, aged 6-8 wk, were purchased from Beijing HFK Bioscience (Beijing, China). The mice were raised in standard conditions[23] and received humanitarian care. The mice were acclimated to the rearing environment for 3-4 d before experimentation.

Animal experiments

Mice were randomly grouped and marked. The mice were weighed and anesthetized with 1% pentobarbital (50 mg/kg). For the hepatic IR experiments, mice were divided into six groups (n = 5 each): Sham group: The portal vein was exposed, and no blood vessels were clamped; ischemia reperfusion (IS) 1.5 h group: We only clamped the left and middle lobe vessels for 1.5 h; IR 3-24 h group: Reperfusion was performed for the corresponding time after the vessels had been clamped for 1.5 h; IP group: We briefly clamped the vessel for 10 min and released it for 10 min before IS; this was named an IP cycle; sh*Il1ra* group: Adenovirus carrying *Il1ra* shRNA was injected *via* the tail vein 48 h before IR. At the end of the experiment, the ischemic hepatic lobes were isolated for subsequent processing or stored at -80 °C. The animal study protocol was approved by the Animal Ethical and Welfare Committee (protocol code: IRM-DWLL-2020173 and date of approval: September 15, 2020).

Nonparenchymal cell and hepatocyte isolation

Nonparenchymal cells (NPCs) and hepatocytes were isolated from mice by collagenase digestion and differential centrifugation using Percoll, as previously described[24].

Histological examination of liver tissue

The left lobe samples of mouse livers were fixed in formaldehyde solution, dehydrated, paraffin-embedded, and cut into 4-μm-thick sections. The IL-1ra levels were determined by immunohistochemistry. The histological analysis was performed using the histochemistry score. A section was selected from the left lobe in every liver, and five views (200 ×) were captured for calculation in each section.

Cell culture

Mouse liver parenchymal cells, AML12 (SCSP-550; American Type Culture Collection, Manassas, VA, United States), were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (11965092; Gibco) supplemented with 10% fetal bovine serum (FBS) (10099133; Gibco) and 1% penicillin/streptomycin at 37 °C in an atmosphere containing 5% CO_2. HEK293T cells were cultured in DMEM (11320033; Gibco) supplemented with 10% FBS, 1% sodium pyruvate, 1% non-essential amino acids, and 2% glutamine.

Cell experiment

AML12 cells were subjected to hypoxia and reoxygenation to simulate the ischemic and reperfusion environment *in vivo*. During hypoxia, cells were cultured in a 37 °C incubator with 95% N_2 and 5% CO_2. After a specific time period, cells were removed and placed back into the 37 °C incubator with 5% CO_2. To interfere with HIF-1α, cells were treated with DMOG or 2-MeOE2 at a concentration of 100 μM for 6 h before subsequent experiments were performed.

Table 1 Primer sequences

Gene	Forward sequence	Reverse sequence
Il1ra	TCTTGGGCATCCACGGG	GAGGCTCACAGGACGGTCAG
β-actin	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC
Il1ra pro-2.1K	CGGAGCTCGCCAGCAAGATTTTAAGTGATTCT	CCAGATCTGGGTGAGCTAAACAGGACACAAGGT
Il1ra pro-1.5K	CGGAGCTCCCCAACAACTTCCAGACTTCCCTC	CCAGATCTGGGTGAGCTAAACAGGACACAAGGT
Il1ra pro-1.1K	CGGAGCTCATCAGTCTAAGGCTGGGCAGGGAG	CCAGATCTGGGTGAGCTAAACAGGACACAAGGT
Il1ra pro-0.6K	CGGAGCTCCCCAGCTCAAATGCCACCATTCTC	CCAGATCTGGGTGAGCTAAACAGGACACAAGGT
Il1ra Mut1	TTTATGCACATTCCCTCTTTCAGC	AGAGGGAATGTGCATAAACTTGT
Il1ra Mut2	GATATGGACTTGCCATTTTGACTC	AAATGGCAAGTCCATATCTTTCTT

Plasmid construction

The mouse *Il1ra* promoter (-2113/+143) sequences were obtained by polymerase chain reaction (PCR) from mouse genomic DNA and cloned into the pGL3-basic vector (Promega, Madison, WI, United States). Mutagenesis of HRE-I and HRE-II in the mouse *Il1ra* promoter was performed using a QuickChange® Lightning Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, United States). The primer sequences are listed in Table 1. The mouse *Il1ra* shRNA plasmid was purchased from Hanbio Biotechnology (Shanghai, China), and packaged into adenoviral particles.

RNA extraction and quantitative qPCR

Total RNA from liver tissue and AML12 cells was extracted using Trizol (Life Technologies, Carlsbad, CA, United States). cDNA was synthesized using reverse transcriptase (Takara, Japan). The specific product of *Il1ra* was amplified by qPCR using the TransStart Green Q-PCR SuperMix Kit (TransGen, China). β-Actin was used as a normalization control. The primer sequences are listed in Table 1.

Immunoblotting assay

Tissues and cell lysates were prepared in RIPA buffer with protease and phosphatase inhibitors. The immunoblotting procedures can be found in the literature[25].

Luciferase assay

Cells were transfected with wild-type or mutant mouse *Il1ra* promoters, followed by treatment with DMOG or 2-MeOE2 under hypoxia in 24-well plates. Lysates were prepared 48 h after transfection, and the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega). The luciferase activity was normalized to the values for Renilla luciferase.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were performed using an EZ-ChIP kit (Millipore, Billerica, MA, United States). The primers and antibodies used in these experiments are shown in Table 1.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.0 (San Diego, CA, United States). All the data are presented as the mean ± SEM and represent three or five independent experiments. One-way analysis of variance was used to compare means among treatment groups. Student's *t*-test for unpaired observations was applied. $P < 0.05$ was considered significant.

RESULTS

Screening of protective genes during hepatic IRI

To screen key genes that potentially play protective roles in the liver in IRI, we constructed a mouse model of hepatic IRI and performed transcriptome sequencing using mRNAs of the liver from the Sham, IS 1.5 h, and IR 3 h groups (Figure 1A). Gene enrichment analysis showed that, after IR 3h, a group of genes enriched in the IL-1 pathway were upregulated (Figure 1B). Expression of 25 genes was significantly increased (> 2-fold) in both IS and IR periods (Figure 1C). These genes were then ranked in descending order of ischemic expression abundance, and *Il1ra*, as a key antagonist of the IL-1 signaling, was shown to be one of the highly differentially expressed genes (Figure 1D). Thus, we chose *Il1ra* for further investigation on its regulatory mechanism in the hepatic IRI.

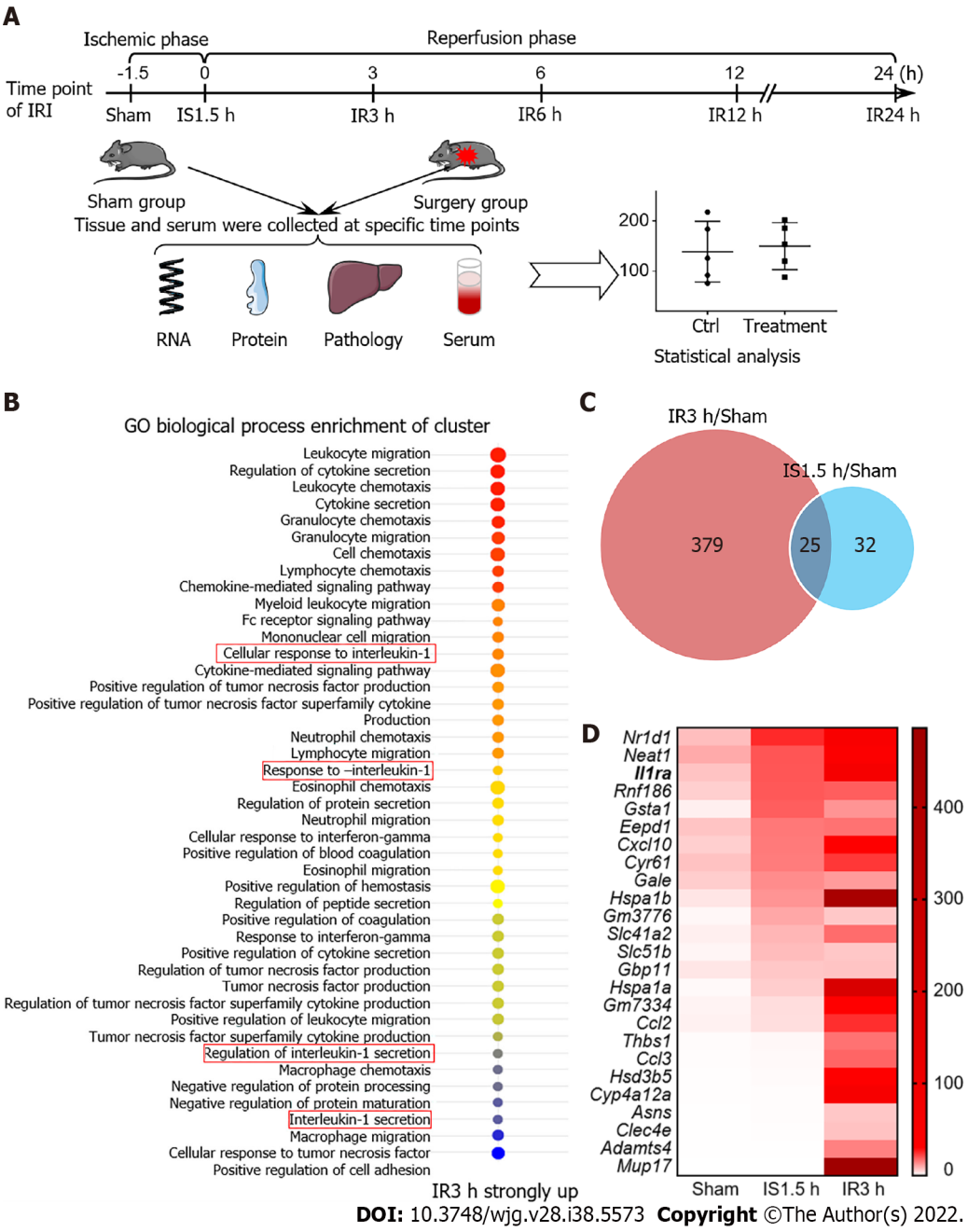

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Figure 1 Construction of a mouse model of hepatic ischemia-reperfusion injury and gene enrichment analysis. A: Model of ischemia-reperfusion injury (IRI) in mice; B: GO enrichment analysis upon IR for 3 h; C: Venn diagram shows that the expression of genes for IR 3 h and ischemia (IS) 1.5 h was more than 2-fold than that in the Sham group; D: Twenty-five genes were elevated in both IR 3 h and IS 1.5 h groups according to IS 1.5 h expression level. IS: Ischemia; IR: Ischemia-reperfusion; IRI: Ischemia-reperfusion injury.

IL-1ra expression is elevated during hepatic IRI

We detected IL-1ra expression in mice with IRI. This confirmed that mRNA expression of *Il1ra* was increased in mice that experienced hepatic IRI as compared with the Sham group (Figure 2A). Upregulation of IL-1ra in liver tissue was confirmed at the protein level by immunoblotting (Figures 2B and C), ELISA (Figure 2D), and immunohistochemistry (Figures 2E and F), demonstrating that expression of IL-1ra is significantly elevated in response to hepatic IRI in mice.

Hepatocytes and hepatic NPCs both express high levels of IL-1ra after IRI

The liver is composed of several different embryonic-derived cell types, including hepatocytes, bile duct epithelial cells, stellate cells, Kupffer cells, and sinusoidal endothelial cells[26]. The liver consists of 80% hepatocytes, and 20% of the other cells are collectively called NPCs. Hepatocytes are the major epithelial cell population of the liver and perform various physiological functions. Therefore, we aimed to identify whether there is a difference in the expression of IL-1ra in hepatocytes and NPCs after IRI. To do so, gradient centrifugation was used to separate hepatocytes and NPCs from mouse liver tissue, and

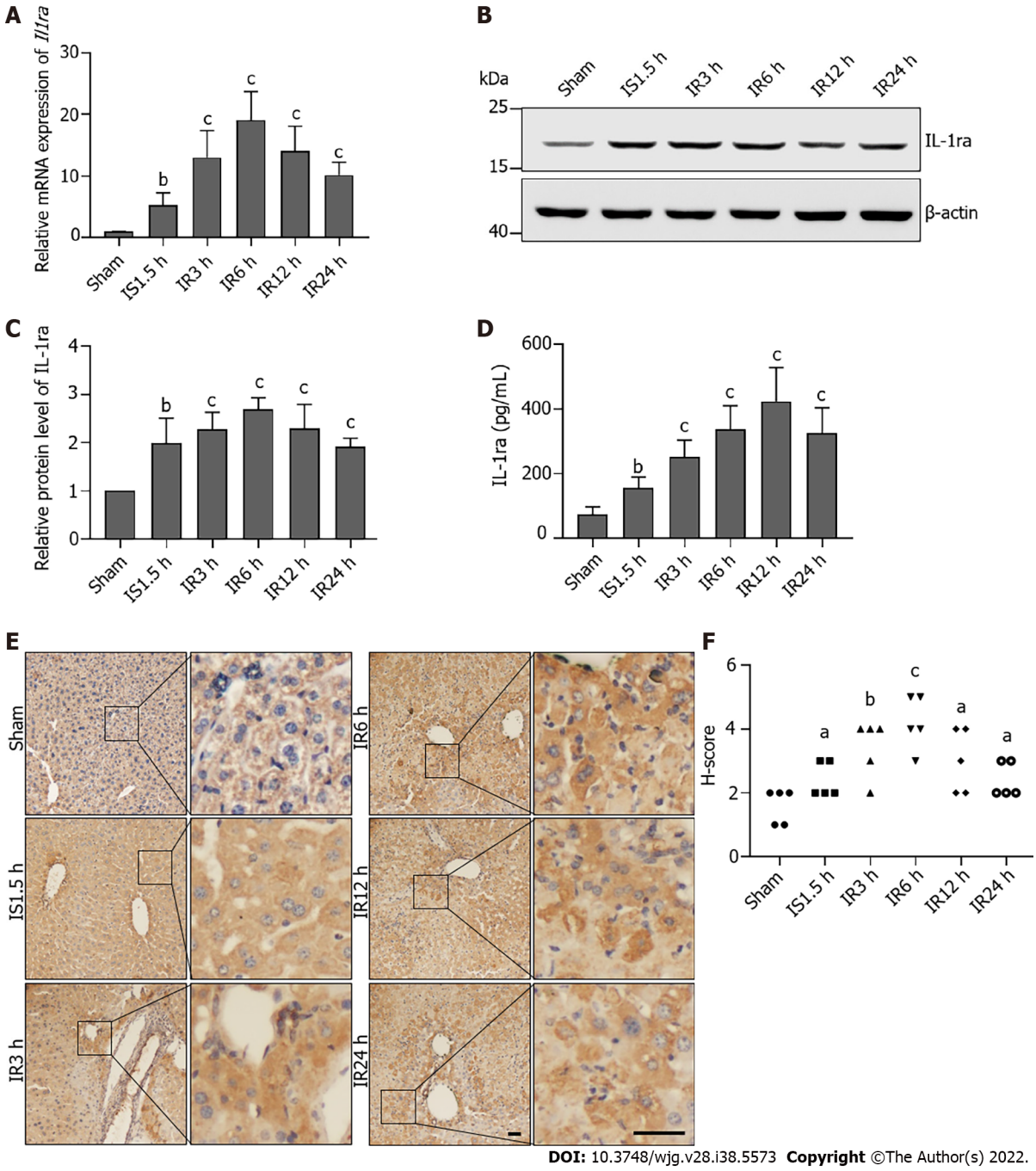

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Figure 2 Interleukin-1 receptor antagonist expression is elevated after hepatic ischemia-reperfusion injury in mice. A: Relative mRNA levels of interleukin-1 receptor antagonist (*Il1ra*) in the liver upon ischemia-reperfusion injury (IRI); B: Protein levels of IL-1ra in the liver upon IRI; C: Statistical analysis of protein levels of IL-1Ra upon IRI; D: Protein content of IL-1ra in serum upon IRI; E. Representative images of immunohistochemical staining for IL-1ra in the liver upon IRI. Scale bars, 100 μm; F: Statistical analysis of immunohistochemical scores. Indicated *P* values were calculated using a two-tailed unpaired Student's *t*-test. Data are presented as the mean ± SEM. Data are representative of five independent experiments. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. IS: Ischemia; IR: Ischemia-reperfusion; IL-1ra: Interleukin-1 receptor antagonist.

expression of IL-1ra was detected. There was no significant difference in the expression of IL-1ra at both the mRNA (Figure 3A) and protein (Figures 3B and C) levels between hepatocytes and NPCs after IR treatment.

To confirm these results *in vitro*, we performed the hypoxia–reoxygenation experiments in the mouse hepatic cell line AML12 to simulate IRI. We found that the mRNA (Figure 3D) and protein (Figures 3E and F) expression of IL-1ra was increased in AML12 cells upon hypoxia and reoxygenation. Similarly, the content of IL-1ra in the culture medium of the hypoxia-reoxygenation group was significantly higher than that in the control group (Figure 3G). Together, these data suggested that IL-1ra is highly expressed in both hepatocytes and NPCs after IRI.

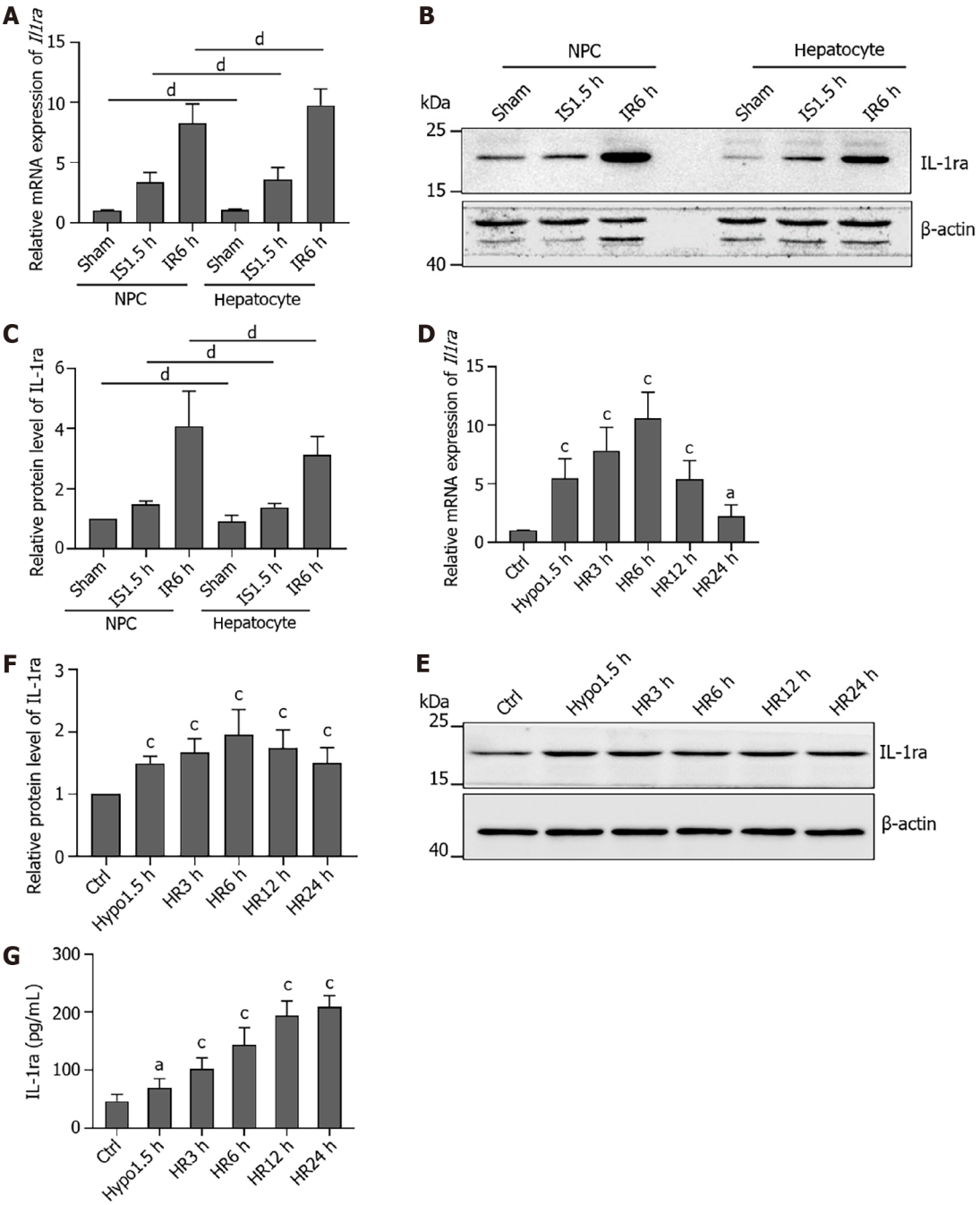

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Figure 3 Interleukin-1 receptor antagonist is expressed in both mouse hepatocytes and nonparenchymal cells. A: Relative mRNA levels of interleukin-1 receptor antagonist (*Il1ra*) in hepatocytes and nonparenchymal cells (NPCs) after ischemia-reperfusion injury (IRI); B: Protein levels of IL-1ra in hepatocytes and NPCs after IRI; C: Statistical analysis of protein levels of IL-1Ra in hepatocytes and NPCs after IRI; D: Relative mRNA levels of *Il1ra* in AML12 cells upon hypoxia-reoxygenation (HR); E: Protein levels of IL-1ra in AML12 cells upon HR; F: Statistical analysis of protein levels of IL-1Ra in AML12 cells upon HR; G: Protein content of IL-1ra in culture medium upon HR. Indicated *P* values were calculated using two-tailed unpaired Student's *t*-tests. Data are presented as the mean ± SEM. Data are representative of five independent experiments. $^{ns}P > 0.05$, $^{a}P < 0.05$, $^{b}P < 0.001$. HR: Hypoxia-reoxygenation; NPCs: Nonparenchymal cells; IL-1ra: Interleukin-1 receptor antagonist.

IL-1ra expression is upregulated upon IS and hypoxia

To verify the regulation of IL-1ra expression in the early stage of hepatic IRI, we constructed hypoxic models at different time points *in vivo* and *in vitro*. Compared with the Sham group, ischemic treatment in mice increased IL-1ra expression in a time-dependent manner at both the mRNA (Figure 4A) and protein (Figures 4B-D) levels. Similar results were also revealed in AML12 cells (Figures 4E-H), demonstrating that IS and hypoxia could promote the expression of IL-1ra.

HIF-1α promotes IL-1ra expression under hypoxic conditions

HIF-1α, as a transcriptional regulator, plays an important role under hypoxic conditions. Therefore, we

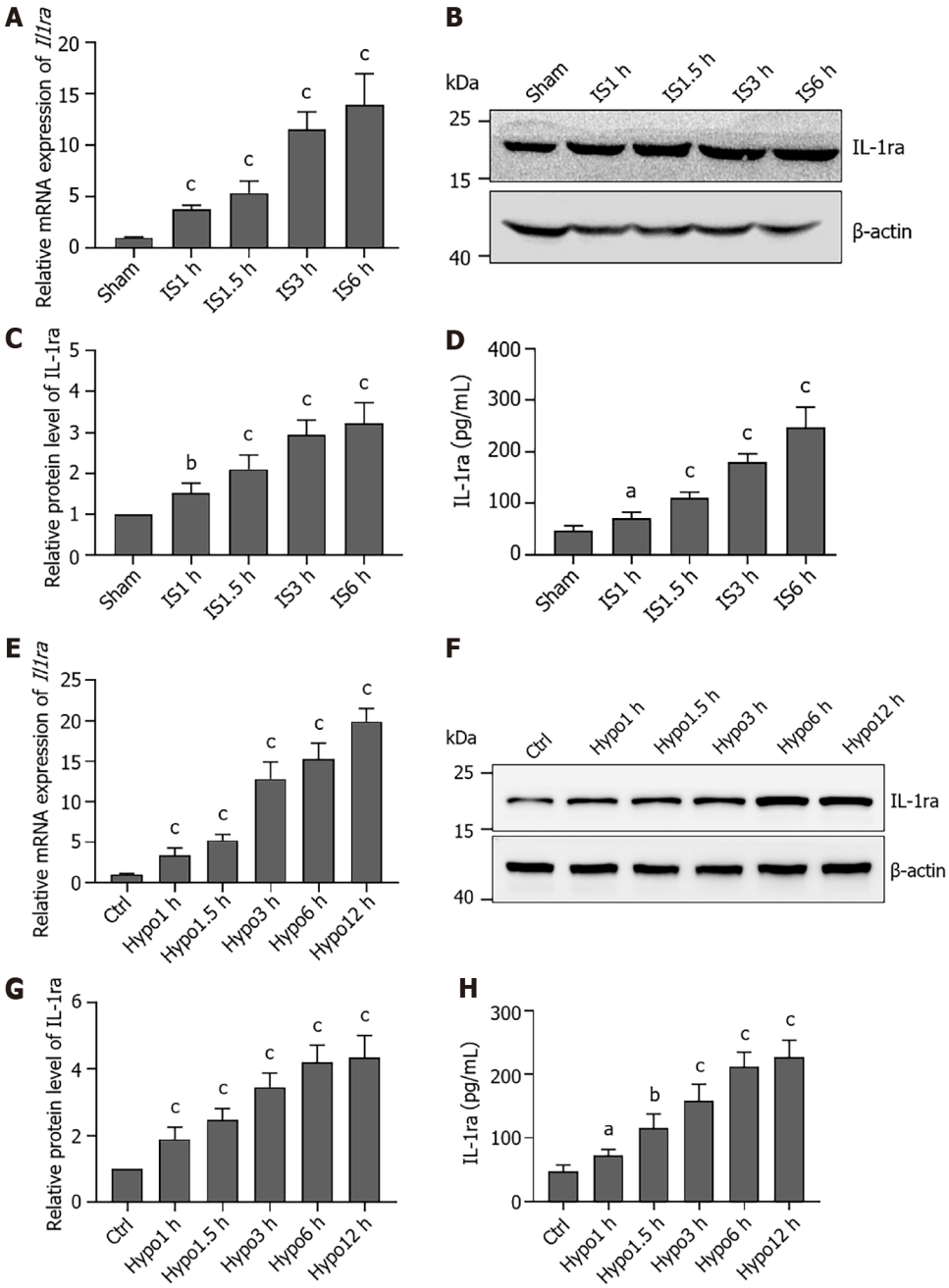

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Figure 4 Interleukin-1 receptor antagonist expression is elevated during ischemia or hypoxia. A: Relative mRNA levels of interleukin-1 receptor antagonist (*Il1ra*) in the liver upon ischemia; B: Protein levels of IL-1ra in the liver upon ischemia; C: Statistical analysis of protein levels of IL-1ra upon ischemia; D: Protein content of IL-1ra in serum upon ischemia; E: Relative mRNA levels of *Il1ra* in AML12 cells upon hypoxia; F: Protein levels of IL-1ra in AML12 cells upon hypoxia; G: Statistical analysis of protein levels of IL-1Ra in AML12 cells upon hypoxia; H: Protein content of IL-1ra in culture medium upon ischemia. Indicated *P* values were calculated using two-tailed unpaired Student's *t*-tests. Data are presented as the mean ± SEM. Data are representative of five independent experiments. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. Hypo: Hypoxia; IS: Ischemia; IL-1ra: Interleukin-1 receptor antagonist.

speculated that HIF-1α would regulate the expression of IL-1ra. Thus, we moved to evaluate the relationship between HIF-1α and IL-1ra expression in hepatic IRI. Increased expression of HIF-1α was observed upon ischemic treatment in mice in a time-dependent manner (Figures 5A and B). Similar results were confirmed in AML12 cells (Figures 5C and D). Immunofluorescence analysis indicated that HIF-1α was localized in the nuclei of AML12 cells after 1.5 h of hypoxia (Figure 5E).

We treated AML12 cells with 2-MeOE2 (a specific HIF-1α inhibitor) or DMOG (a specific HIF-1α agonist). 2-MeOE2 reduced the expression of *Il-1ra* mRNA during hypoxia, while DMOG increased *Il-1ra* expression under normoxia (Figure 5F). Immunoblotting (Figures 5G-I) and ELISA (Figure 5J) confirmed that 2-MeOE2 reduced the protein levels of HIF-1α and IL-1ra in AML12 cells; however, DMOG had the opposite effect. These results indicated that HIF-1α independently promotes the expression of IL-1ra.

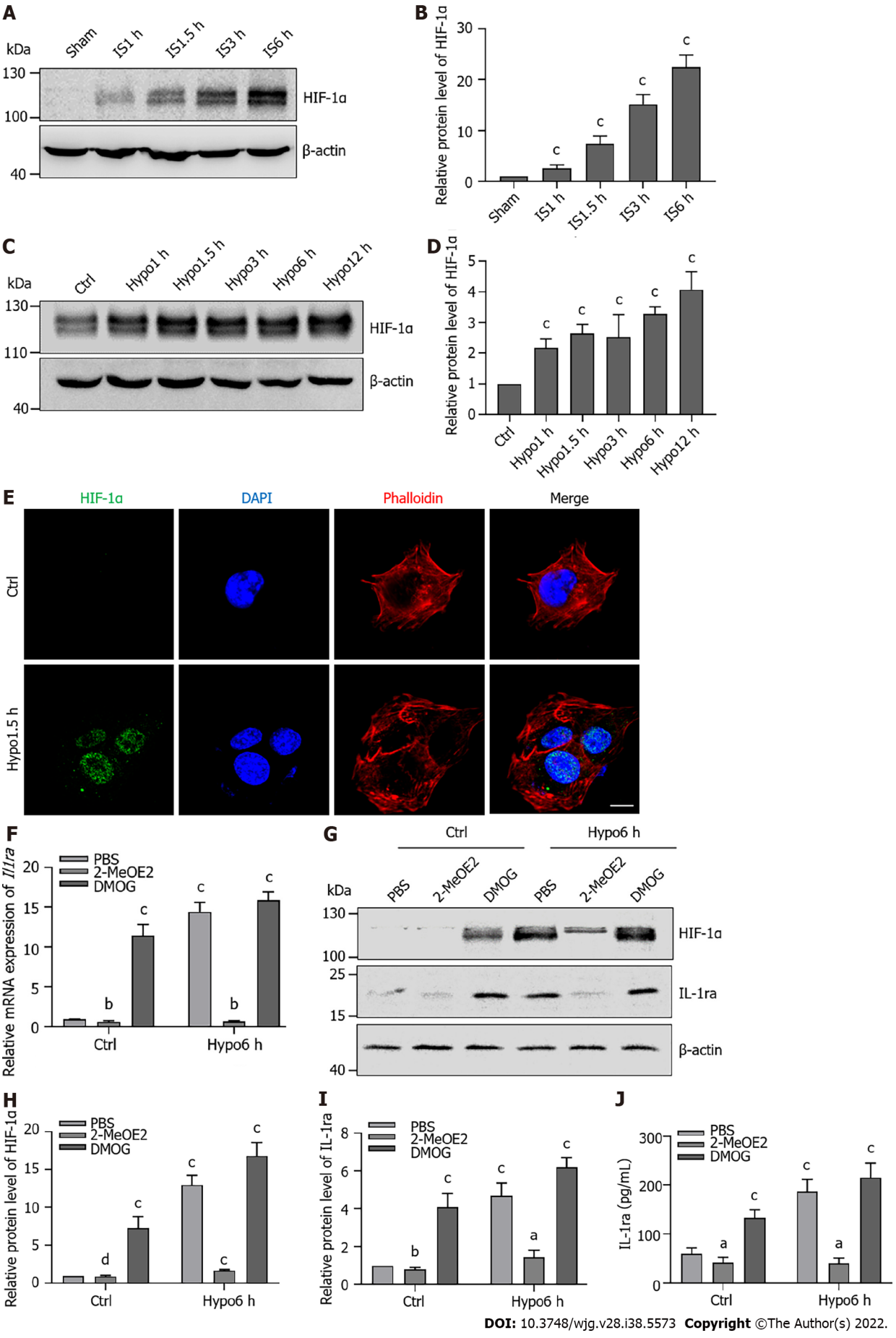

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Figure 5 Hypoxia-inducible factor-1α promotes the expression of interleukin-1 receptor antagonist during ischemia or hypoxia. A: Protein

levels of hypoxia-inducible factor (HIF)-1α in the liver upon ischemia; B: Statistical analysis of protein levels of HIF-1α in the liver upon ischemia; C: Protein levels of HIF-1α in AML12 cells upon hypoxia; D: Statistical analysis of protein levels of HIF-1α in AML12 cells upon hypoxia; E: Immunofluorescence staining for HIF-1α in AML12 cells upon hypoxia for 1.5 h. Scale bars, 10 μm; F: Relative mRNA levels of interleukin-1 receptor antagonist (*Il1ra*) in AML12 cells treated with 2-MeOE2 (100 μM) and DMOG (100 μM) upon hypoxia; G: Protein levels of HIF-1α and IL-1ra in AML12 cells treated with 2-MeOE2 and DMOG upon hypoxia; H: Statistical analysis of protein levels of HIF-1α in AML12 cells treated with 2-MeOE2 and DMOG upon hypoxia; I: Statistical analysis of protein levels of IL-1ra in AML12 cells treated with 2-MeOE2 and DMOG upon hypoxia; J: Protein content of IL-1ra in culture medium of in AML12 cells treated with 2-MeOE2 and DMOG upon hypoxia. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-tests. Data are presented as the mean ± SEM. Dots depict individual samples. $^{ns}P > 0.05$, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. Hypo: Hypoxia; IS: Ischemia; HIF: Hypoxia-inducible factor; IL-1ra: Interleukin-1 receptor antagonist; IgG: Immunoglobulin G.

HIF-1α transcriptionally upregulates IL-1ra expression

To investigate the regulatory mechanism of IL-1ra by HIF-1α, we transfected a mouse *Il1ra* promoter-reporter construct into AML12 cells (Figure 6A). Upon treatment with hypoxia, we found that *Il1ra* promoter activity was significantly increased (Figure 6B). To explore the transcriptional regulatory elements of the *Il1ra* promoter in response to hypoxia, we generated a series of truncated *Il1ra* promoter–reporter constructs. *Il1ra*-p-2.1k promoter activity was significantly increased upon hypoxic treatment, while the truncated promoter activity of *Il1ra*-p-1.5k, *Il1ra*-p-1.1k, and *Il1ra*-p-0.6k was not altered by hypoxia (Figure 6C).

We used the JASPAR database to predict the binding site of HIF-1α on the *Il1ra*-p-2.1k promoter and found two possible binding elements, HRE I (-2018/-2011) and HRE II (-1895/-1888) (Figure 6D). Site-directed mutagenesis showed that mutation of HRE II did not affect hypoxia-induced activation of the *Il1ra* promoter, while mutation of either HRE I or HRE I + II eliminated this effect (Figure 6D). Importantly, we used the ChIP assay to verify that HIF-1α was able to be recruited to the HRE I element in the *Il1ra* promoter (Figures 6E and F).

IP protects the liver from IRI via the HIF-1α-IL-1ra pathway

It has been reported that IP can alleviate hepatic IRI[27]. However, the protective mechanism of IP is not yet clear. Here, we speculated that IL-1ra might play a potential role in the regulation of IP. Thus, the mice were subjected to IP with the indicated cycles. Compared with the control group, the IP group had significantly lower ALT and AST levels in their serum, which were further reduced by increased IP cycles (Figures 7A and B). Similar results of IL-1ra upregulation were observed by immunoblotting (Figures 7C and D) and ELISA (Figure 7E).

To verify whether IL-1ra plays a protective role in IP, we injected adenovirus carrying *Il1ra* shRNA *via* the tail vein to knock down the expression of IL-1ra in mice. Expression of IL-1ra in the liver and serum of mice injected with adenovirus was significantly decreased (Figures 7F-H). The ALT and AST levels were increased by knockdown of IL-1ra in the liver (Figures 7I and J).

It has been reported that HIF-1α plays an important role in IP[28], and we thus speculated that the expression of IL-1ra in IP is regulated by HIF-1α. We found that IP induced accumulation of HIF-1α in the liver (Figures 7K-M); however, 2-MeOE2 significantly attenuated this effect (Figures 7M-P). These results collectively suggested that IP might exert a protective effect on the liver from IRI through regulating the HIF-1α–IL-1ra signaling.

DISCUSSION

Hepatic IRI is a common complication of liver surgery. Previous studies have demonstrated that the IL-1 signaling pathway plays a pivotal role in the regulation of hepatic IRI. Here, we extended the study, showing that the expression of IL-1ra, an inhibitor of the IL-1 signaling pathway, is increased in response to hepatic IRI, especially in the ischemic stage. Mechanistically, hypoxia induces the transcriptional expression of IL-1ra through HIF-1α accumulation. Of note, we proved that IP could protect the liver from IRI by promoting IL-1ra expression in a HIF-1α-dependent manner. Thus, our study has provided additional evidence for the regulatory mechanism of IL-1ra during IS, indicating a practical strategy for alleviating hepatic IRI.

Previous studies have shown that hepatic IRI not only causes damage to the liver itself, but also leads to the abnormal functioning of various organs, and induces systemic inflammatory response syndrome [29]. Therefore, the question of how to reduce or even eliminate hepatic IRI has received constant attention among clinicians and researchers. Hepatic IRI is a complex pathophysiological process with numerous and relational influencing factors. Among them, the early inflammatory response plays a crucial role in the occurrence and development of hepatic IRI[30], because it is located in the upstream region of the injury response chain. Upon activation, downstream factors often promote each other and thus aggravate the injury[31]. If the activation of early inflammation can be reduced or even blocked, it may be possible to prevent the occurrence and development of hepatic IRI at source.

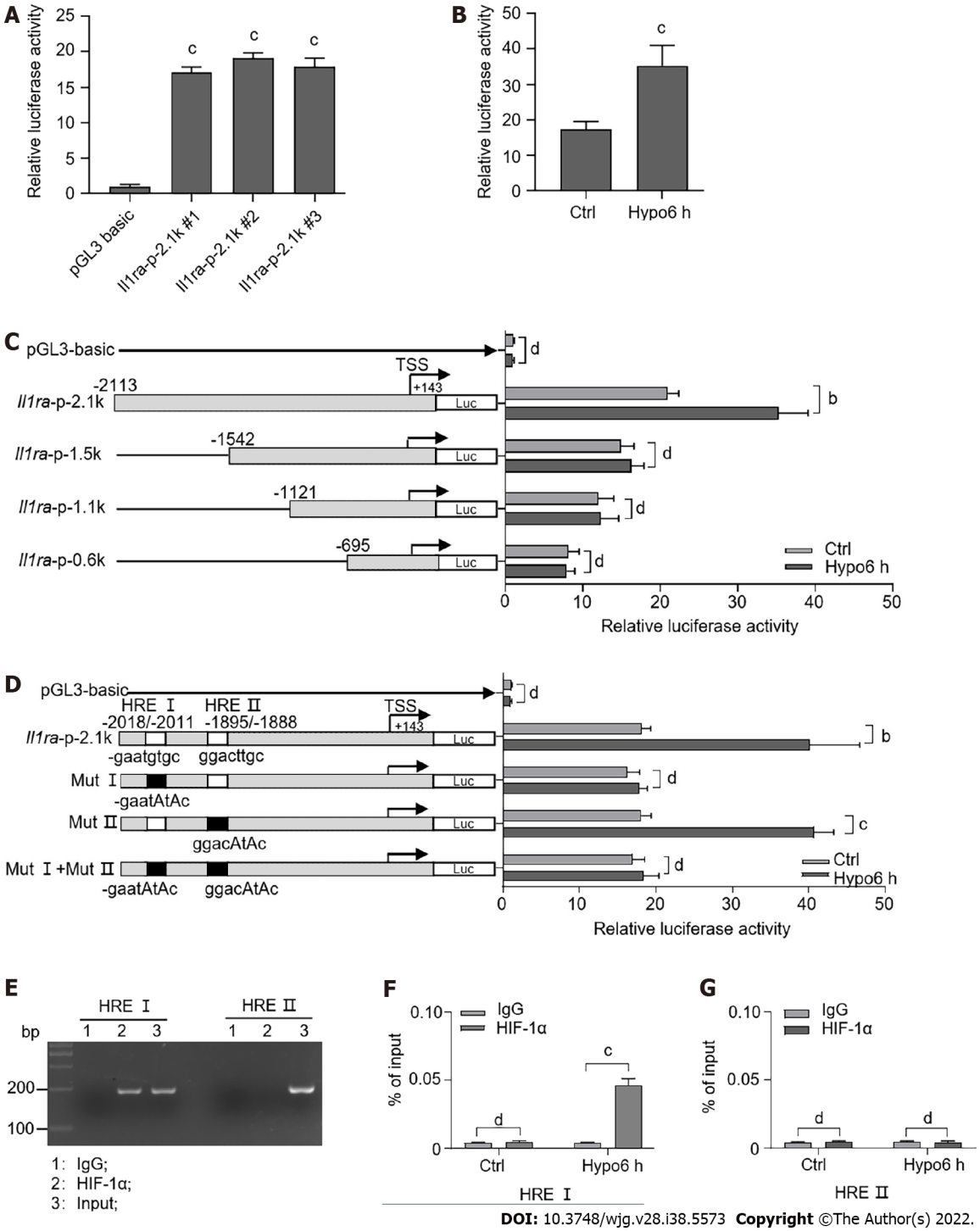

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Figure 6 Hypoxia-inducible factor transcriptionally upregulates interleukin-1 receptor antagonist expression. A: Luciferase assay for the wild-type promoter (-2113/+143) of interleukin-1 receptor antagonist (*Il1ra*) in AML12 cells; B: Luciferase assay for the wild-type promoter of *Il1ra* in AML12 cells upon hypoxia; C: Luciferase assay for wild-type and truncated promoters of *Il1ra* in AML12 cells upon hypoxia; D: Luciferase assay for wild-type (-2113/+143) and HRE-mutated promoters of *Il1ra* upon hypoxia; E: Chromatin immunoprecipitation assay for hypoxia-inducible factor (HIF)-1α binding to HRE sites upon hypoxia; F and G: Quantitative polymerase chain reaction analysis for the recruitment of HIF-1α to the endogenous *Il1ra* promoter in AML12 cells upon hypoxia. Indicated *P*-values were calculated using two-tailed unpaired Student's *t*-tests. Data are presented as the mean ± SEM. Data are representative of independent experiments. $^{ns}P > 0.05$, $^{a}P < 0.01$, $^{b}P < 0.001$. Hypo: Hypoxia; IS: Ischemia; HIF: Hypoxia-inducible factor; IL-1ra: Interleukin-1 receptor antagonist; IgG: Immunoglobulin G.

Current studies have shown that many proinflammatory cytokines are released in the early stage of hepatic IRI[4]. For example, blocking the activation of proinflammatory IL-1 signaling significantly prevents IRI[32]. IL-1 is a major inflammatory response mediator[33]. Experimental results have shown that large amounts of IL-1β and tumor necrosis factor-α are produced in the tissue after hepatic IS and reperfusion[34]. At the same time, this can also stimulate the secretion of inflammatory cells, leading to the inflammatory destruction of local tissues. IL-1β and the nucleotide-binding and leucine-rich repeat protein 3 (NLRP3) inflammasome play a role through high mobility group box 1 protein[35], nuclear

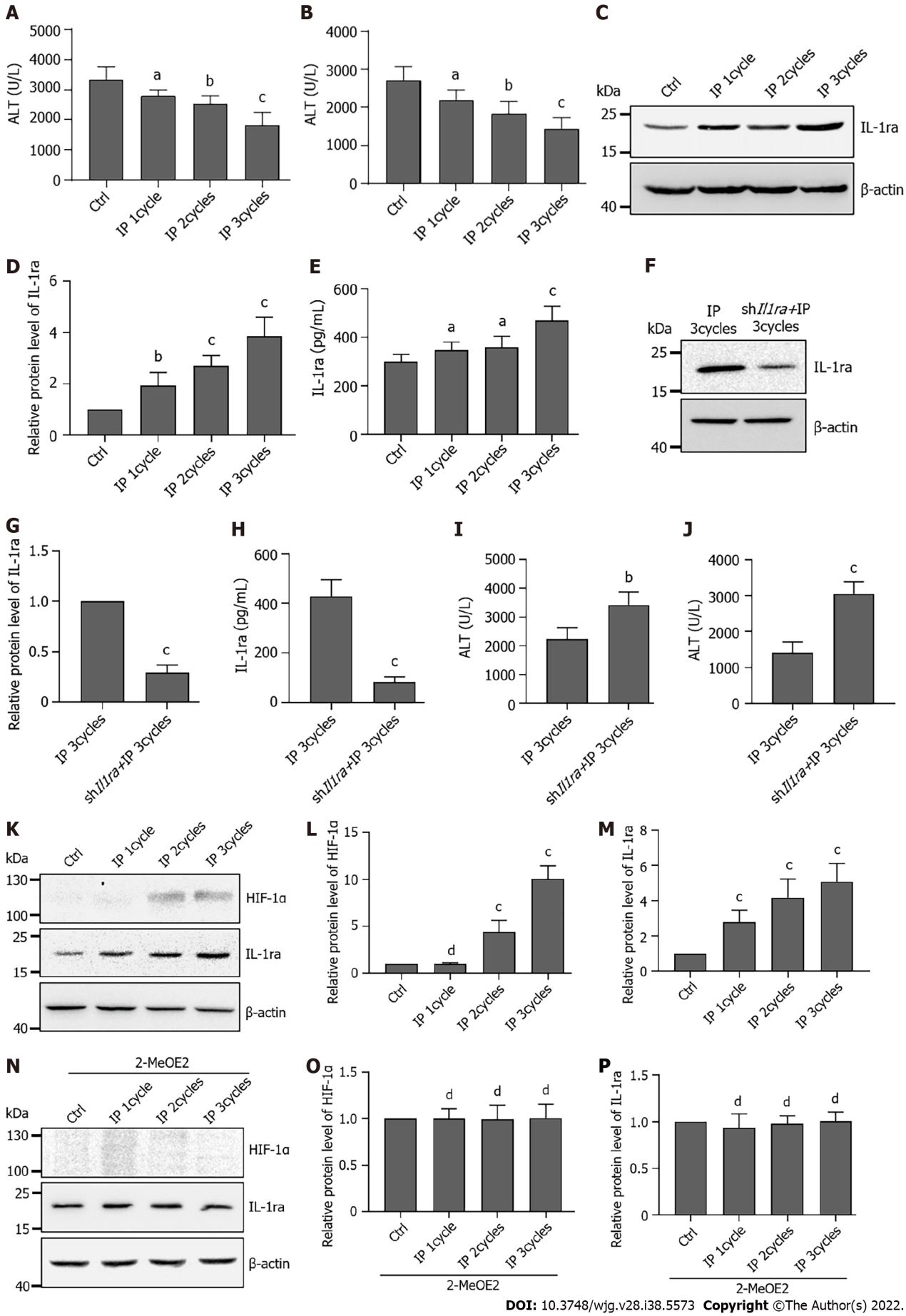

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Figure 7 Ischemic preconditioning protects the liver by promoting interleukin-1 receptor antagonist expression *via* hypoxia-inducible

factor-1α. A: Serum alanine transaminase (ALT) levels in mice undergoing ischemia-reperfusion injury (IRI) after different ischemic preconditioning (IP) cycles; B: Serum aspartate transaminase (AST) levels in mice undergoing IRI after different IP cycles; C: Protein levels of interleukin-1 receptor antagonist (IL-1ra) in the liver after different IP cycles; D: Statistical analysis of protein levels of IL-1ra after different IP cycles; E: Protein content of IL-1ra in serum after different IP cycles; F: Protein levels of IL-1ra in the liver with sh*Il1ra* injection after three IP cycles; G: Statistical analysis of protein levels of IL-1ra with sh*Il1ra* injection after three IP cycles; H: Protein content of IL-1ra in serum with sh*Il1ra* injection after three IP cycles; I: Serum ALT levels in mice with sh*Il1ra* injection after three IP cycles; J: Serum AST levels in mice with sh*Il1ra* injection after three IP cycles; K: Protein levels of hypoxia-inducible factor (HIF)-1α and IL-1ra in the liver after different IP cycles; L: Statistical analysis of protein levels of HIF-1α after different IP cycles; M: Statistical analysis of protein levels of IL-1ra after different IP cycles; N: Protein levels of HIF-1α and IL-1ra in the liver with 2-MeOE2 treatment after different IP cycles; O: Statistical analysis of protein levels of HIF-1α with 2-MeOE2 treatment after different IP cycles; P: Statistical analysis of protein levels of IL-1ra with 2-MeOE2 treatment after different IP cycles; Indicated *P* values were calculated using two-tailed unpaired Student's *t*-tests. Data are presented as the mean ± SEM. Data are representative of five independent experiments. $^{ns}P > 0.05$, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. IP: Ischemic preconditioning; AST: Aspartate transaminase; ALT: Alanine transaminase; IL-1ra: Interleukin-1 receptor antagonist; HIF: Hypoxia-inducible factor.

factor-κB and Toll-like receptor 4 in lesions caused by warm IRI[7]. Of note, NLRP3 may be involved in IRI independently of the inflammasome pathway by recruiting neutrophils. Macrophages secrete pro-IL-1β, which is subsequently cleaved and activated by neutrophil-derived proteases in a mouse model. Mature IL-1β is required to induce inflammation during hepatic IR, and the interaction between macrophages and neutrophils is essential in this process[6]. Therefore, the antagonism of proinflammatory IL-1 cytokines has important therapeutic potential for reducing IRI.

IL-1ra is a naturally occurring polypeptide. Recent research has focused on the role of IL-1ra in various pathophysiological states or the effects of recombinant IL-1ra administration in animals and humans[36]. It has been reported that the delivery of the *Il-1ra* gene to the rat liver *via* the adenovirus vector or lipofection can significantly reduce IR-induced proinflammatory cytokine production and hepatocyte injury[15]. This is consistent with our results showing that the knockdown of IL-1ra significantly increased the damage during hepatic IRI, suggesting a potential inhibitory effect of IL-1ra on inflammation in response to hepatic IR.

In the process of IRI, IS is a prerequisite. It is precisely regulated because the depletion of oxygen and energy in the ischemic period cause a series of injuries in the subsequent reperfusion period. In the state of hepatic IS, the metabolic mode switches from aerobic to anaerobic, the redox process in hepatocytes is blocked, the ATP-dependent cellular metabolic activity gradually stops, and the intracellular ATP is rapidly depleted. At the same time, the absence of oxygen causes HIF-1α to gradually accumulate. The increased transport of HIF-1α into the nucleus triggers the expression of genes involved in oxygen transport, oxygen utilization, glycolytic metabolism, cell death, cell survival, and other processes that affect cell survival during IS. Recent studies have demonstrated that HIF-1α can alleviate IRI by regulating the expression of inducible NO synthase[37]. HIF-1α may also enhance tissue anti-inflammatory effects by increasing heme oxygenase-1 expression, thereby reducing damage[38]. In this study, we further showed that HIF-1α, as an important protective factor in IRI, could protect the liver from injury by promoting the expression of IL-1ra and subsequently inhibiting activation of the IL-1 signaling pathway. In terms of the mechanism, we found that HIF-1α recognizes and binds to the basic helix-loop-helix protein sequence in the *Il1ra* promoter region, resulting in the increased transcription of *Il1ra*.

Recent studies have shown that IP has broad protective effects against IRI[39]. However, due to the complexity of IRI, the underlying cellular and molecular mechanisms of IP remain largely unknown. In the present study, we provided evidence that IP promotes the expression and release of IL-1ra into the hepatic microenvironment, thus blocking the downstream activation of IL-1β and protecting the liver from the cascading effects of inflammatory factors. We demonstrated that IP can induce the accumulation of HIF-1α, which increases with the number of IP cycles. These results would explain the protective effect of IP at a mechanistic level and lay a theoretical foundation for the better application of IP.

CONCLUSION

Our study demonstrated a protective effect of IL-1ra on hepatic IRI through the transcriptional regulation of IL-1ra by HIF-1α. Importantly, we found that IP can regulate the expression of IL-1ra through HIF-1α at early stage of hepatic IRI, thereby blocking the inflammatory IL-1 signaling pathway to protect the liver from IRI (Figure 8).

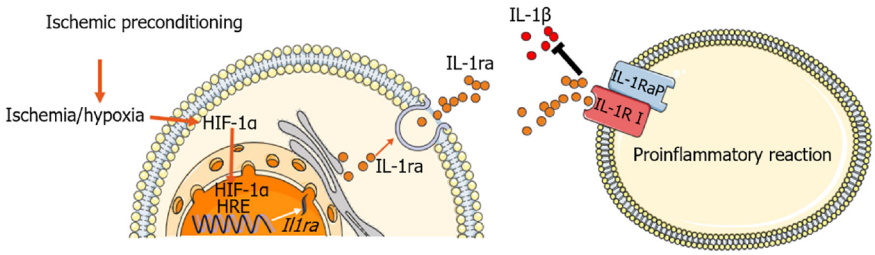

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Figure 8 Model of ischemic preconditioning-induced hypoxia-inducible factor-1α-interleukin-1 receptor antagonist signaling to alleviate hepatic ischemia-reperfusion injury. Ischemic preconditioning promotes the accumulation of hypoxia-inducible factor (HIF)-1α, which subsequently increases HIF-1α entry into the nucleus and promotes the transcriptional expression of interleukin-1 receptor antagonist (IL-1ra). In turn, this inhibits the pro-inflammatory effects of IL-1β and eventually protects against hepatic ischemia-reperfusion injury. HIF: Hypoxia-inducible factor; IL-1ra: Interleukin-1 receptor antagonist.

ARTICLE HIGHLIGHTS

Research background

Ischemia-reperfusion injury (IRI) is associated with transplant failures, graft dysfunction, and a relatively poor prognosis. Interleukin-1 receptor antagonists (IL-1ra) play an important role in protecting the liver from IRI. However, the mechanism of its regulatory expression remains unclear.

Research motivation

Inhibition of hepatic inflammation by promoting the expression of IL-1ra is one of the key targets for the treatment of hepatic IRI.

Research objectives

To investigate the regulatory mechanism of hepatocyte-derived IL-1ra expression in the process of hepatic IRI to provide a therapeutic target for hepatic IRI.

Research methods

A 70% hepatic IRI model was established in mice, and AML12 cells were subjected to hypoxia/reoxygenation for the simulation of IRI *in vitro*. The *Il-1ra* promoter-reporter system was constructed to detect the regulatory effect of hypoxia. The ischemic preconditioning (IP) model was established to investigate its regulatory effect on IL-1ra.

Research results

IL-1ra is a key regulator of hepatic IRI. Il-1ra expression was significantly up-regulated after hepatic IRI *in vivo* and *in vitro*. Hypoxia inducible factor (HIF)-1α regulates *Il-1ra* transcription during hypoxia. IP prevents hepatic IRI by inducing the expression of IL-1ra, which is mediated by HIF-1α.

Research conclusions

Ischemia or hypoxia leads to increased IL-1ra expression through HIF-1α. In addition, IP protects the hepatic tissue from IRI through the HIF-1α-IL-1ra pathway.

Research perspectives

HIF-1α-IL-1ra pathway is a potential mechanism of hepatic protection during hepatic IRI, and also a key target for the treatment of hepatic IRI.

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FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Cohort Study

No long-term survival benefit with sustained-release 5-fluorouracil implants in patients with stages II and III gastric cancer

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Abstract

BACKGROUND

The prognosis of gastric cancer in an advanced stage remains poor. The exact efficacy of the use of intraoperative sustained-release chemotherapy with 5-fluorouracil (5-FU) in advanced-stage gastric cancer is still unelucidated.

AIM

To explore the long-term survival benefit of using sustained-release 5-FU implants

in stage II and stage III gastric cancer patients.

METHODS

Patients with gastric cancer in a locally advanced stage and who underwent an R0 radical resection between Jan 2014, to Dec 2016, in this single institution were included. Patients with pathological diagnoses other than adenocarcinoma were excluded. All included patients were grouped according to whether intraoperative sustained-release (SR) chemotherapy with 5-FU was used or not (NSR). The primary end-point was 5-year overall survival. Kaplan–Meier method with log-rank test was used to analyze the overall survival of patients and Cox analysis was used to analyze prognosis factors of these patients.

RESULTS

In total, there were 563 patients with gastric cancer with locally advanced stage, who underwent an R0 radical resection. 309 patients were included in the final analysis. 219 (70.9%) were men, with an average age of 58.25 years. Furthermore, 56 (18.1%) received neoadjuvant chemotherapy, and 191 (61.8%) were in TNM stage III. In addition, 158 patients received intraoperative sustained-release chemotherapy with 5-FU and were included in the SR group, while the other 161 patients were included in the NSR group. The overall complication rate was 12.94% in the whole group and 10.81%, 16.46% in SR and NSR groups, respectively. There were no significant differences between the two groups in overall survival and complication rate ($P > 0.05$). The multivariate cox analysis indicated that only N Stage and neoadjuvant therapy were independent influencing factors of survival.

CONCLUSION

Intraoperative sustained-release chemotherapy usage with 5-FU, did not improve the survival of patients who underwent an R0 radical resection in locally advanced stage of gastric cancer.

Key Words: Sustained-release 5-fluorouracil implants; Gastric cancer; 5-year survival rate; Safety; Prognostic factor; R0 radical resection

Core Tip: Intraoperative drug administration shows promise in preventing the recurrence of gastric cancer. Sustained-release 5-fluorouracil (5-FU) implant is a new convenient and continuous drug release method that ensures locally high drug concentration for approximately 1 mo. 5-FU implants are widely used to treat various gastric tumors. However, presently, only retrospective research and a small-scale clinical study conducted in China have reported on its use in patients with gastric cancer. In our real-world study, we collected complete datasets, making this the largest study in China. Unfortunately, 5-FU implants did not improve the long-term survival of gastric cancer patients.

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INTRODUCTION

Gastric cancer is the fifth most common newly diagnosed cancer and the third most common cause of cancer mortality worldwide[1-3]. In China, there are approximately 400000 new patients with gastric cancer, and nearly 300000 people die of gastric cancer every year[4]. At present, locally advanced gastric cancer with the American Joint Committee on Cancer TNM stages II and III accounts for the majority of these cases[5]. For locally-advanced gastric cancer patients, the current standard treatment is D2 gastrectomy, followed by adjuvant chemotherapy[6]. However, even in those who received standard treatment, 30–60% of them may relapse either locally or distantly[7].

Patients with stages II and III gastric cancer have been found to have a tendency to relapse after treatment[8]. According to the findings of a study by Yago *et al*[9], the 5-year recurrence-free survival rate was 88.3% for stage IIA, 73.8% for stage IIB, 67.4% for stage IIIA, 55.7% for stage IIIB, and 29.9% for stage IIIC. Of the three major recurrence patterns namely hematogenous, peritoneal, and lymph nodal

recurrences after curative gastrectomy, the latter two account for the majority of the cases[10-13]. In order to decrease local recurrence after resection, methods, such as extended peritoneal lavage[14], hyperthermic intraperitoneal perfusion chemotherapy[15], peritoneal lavage with 5-FU, and continuous intraperitoneal chemotherapy using a retained tube or pump, have been developed[16]. Furthermore, it has been reported that intraoperative intraperitoneal chemotherapy can reduce the mortality risk[17]. In addition, increasing the temperature of chemotherapeutic drugs has a synergistic effect with the increase of intraperitoneal chemotherapy. The CYTO-CHIP study group reported that compared with the routine method of cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy, it can improve median overall survival time and increase the 3-and 5-year overall survival rates [18]. In another study, the use of catheter-based intraperitoneal chemotherapy showed similar results of increased the 5-year overall survival rate and reduced the peritoneal recurrence rate[19]. The use of sustained-release 5-FU implants intraoperatively is a relatively newly developed method and has been used widely in almost all types of digestive tract cancer in China[20]. When this drug was placed intraoperatively into the tumor bed, it was released continuously, and it maintained a locally high drug concentration for approximately 1 mo[21]. The potential remnant tumor tends to grow quickly in the first year after resection owing to the decreased immunity and absence of adjuvant chemotherapy[9]. This is the theoretical basis for the use of sustained-release 5-FU implants.

Moreover, optimistic results concerning the use of sustained-release 5-FU implants in the treatment of gastric cancer have been shown. Recently, a multi-center, randomized, open-label, controlled clinical study showed that for cTNM stage III gastric cancer patients, the use of intraoperative 5-FU implants, combined with postoperative adjuvant chemotherapy, may reduce the risk of peritoneal recurrence and prolong progression-free survival significantly[22]. Another retrospective study with 5-FU implants in advanced gastric cancer patients showed that the use of 5-FU implants may improve 5-years overall survival (OS) and progression-free survival rates after surgery in gastric cancer patients[21]. However, these studies had limitations. The sample size of these studies was limited, the follow-up times were insufficient, and the rate of loss of follow-up was extremely high. In order to explore the long-term survival benefit of the use of sustained-release 5-FU implants, we conducted a real-world study using our own clinical data from a single institution.

MATERIALS AND METHODS

Data Collection and Primary Outcomes

In total, we treated 563 stages II and III gastric cancer patients between January 2014 and December 2016. The inclusion criteria were the presence of clinical TNM stage II and stage III, pathologically diagnosed adenocarcinoma of the stomach, a complete record of history, and age between 18–80 years. The exclusion criteria were the presence of gastric remnant cancer, distant metastases, positive peritoneal cytology, palliative resection, and any unsuitable condition for 5-FU chemotherapy. The primary clinical outcome was 5-year OS. Information on the patients' age, sex, body mass index, tumor (TNM Stage, Borrmann classification, Lauren classification, pathological classification, differentiation, number of resected lymph nodes, number of positive lymph nodes, as well as the extent of nerve invasion, and presence of a vascular tumor thrombus), treatment (resections, neoadjuvant therapy, and use of 5-FU implants), postoperative complications (pulmonary infections, anastomotic fistulas, bleeding, abdominal infections, intestinal obstructions, and admissions to the intensive care unit), and their follow-up results were extracted from our hospital's electronic records.

Treatment methods

All our patients underwent a standard D2 gastric resection with or without neoadjuvant therapy. In addition, all the patients received 6–8 cycles of adjuvant chemotherapy with an S-1 + oxaliplatin regimen. In the sustained-release (SR) group, sustained-release 5-FU implants in a fixed dosage of 1000 mg were placed intraoperatively near the tumor bed after resection. Other patients were divided into non-sustained-release (NSR) group. The placement of 5-FU implants near vessels and anastomotic stoma should be avoided.

Statistical analyses

The continuous variables were presented as central tendencies (means or medians) and dispersions (standard deviations or interquartile ranges). For the group comparisons of the numeric variables, the Student's *t*-test was used when the data were normally distributed, and the Mann–Whitney test for the variables in which distribution was not normal. When the categorical predictors were compared between the groups, we used Pearson's χ^2 test or Fisher's exact test. The survival analysis included the use of the Kaplan–Meier estimator for OS. A Cox regression analysis was performed to obtain the crude and adjusted hazard ratios for OS. Significance level for all tests was reached when the two-tailed P value was < 0.05. Statistical analyzes were performed using the IBM SPSS Statistics (version 26.0, IBM Inc., Armonk, NY, United States), and Prism software (version 9, GraphPad Software Inc., San Diego, CA, United States).

RESULTS

Clinicopathological characteristics

In total, 241 patients with incomplete data and 13 patients with a pathological diagnosis of gastric neuroendocrine tumor were excluded. Finally, 309 patients were included in this study, and they were divided into two groups according to whether sustained-release 5-FU was used or not. Therefore, there were 158 patients in the SR group and 164 patients in the NSR group (Figure 1).

The average age of the whole group was 58.25 ± 11.2 years, with male patients being the majority (70.9%). All the patients were diagnosed pathologically as having a stomach adenocarcinoma of TNM stages II and III. Furthermore, 32.4% of patients had signet ring cell carcinoma. All of the patients underwent an R0 resection surgery. As shown in Table 1, we compared the differences in the clinicopathological characteristics between the two patient cohorts. There were no significant differences between the two groups with respect to age, sex, body mass index, and TNM stage. Similarly, there were no differences between the two groups with respect to their pathological characteristics such as the Borrmann classification, Lauren classification, and differentiation. Although the number of resected lymph nodes in the SR group was higher than that in the NSR (P = 0.0145), the number of positive lymph nodes number was similar (P = 0.2319). Lymph node dissection was satisfactory in both groups, even though there was a gap between the two groups (Table 1).

Postoperative complications in patients

Table 2 shows the major postoperative complications, such as pulmonary inflections, anastomotic fistulas, postoperative bleeding, abdominal infections, and intestinal obstructions. There were no significant differences between the two groups with respect to the incidence of postoperative complications (P > 0.05).

Prognostic factors in gastric cancer patients

Figure 2 shows the results of the univariate and multivariate Cox analyses. From the univariate analysis, N stage (P < 0.001), T stage (P = 0.001), TNM stage (P < 0.001) neoadjuvant therapy (P < 0.001), Borrmann classification (P = 0.001), and resection type (P = 0.002) were found to be influencing prognostic factors in this group of patients. As per the multivariate Cox analysis, only N stage (P = 0.030) and neoadjuvant therapy were found to be independent influencing prognostic factors (Figure 2 and Table 3). The use of sustained-release 5-FU implants was not an independent influencing prognostic factor in this group (P = 0.786).

Survival outcomes

The 5-years OS rate of the SR and NSR groups were 68.2% and 67.9%, respectively (P = 0.9850). The median survival time for both groups was not obtained, as patient mortality numbers were too low to calculate. Furthermore, we performed subgroup comparisons according to the patient's age, sex, and TNM stage. There were no significant differences in any of the subgroups (P > 0.05) (Figures 3 and 4, Table 3).

DISCUSSION

Gastric cancer remains one of the most lethal cancers in China[23,24]. Almost 70% of the relapses occur within 2 years and more than 90% within 5 years[7,25]. In clinical practice, the recurrence patterns are classified as locoregional, peritoneal, and hematogenous. As reported by Wu, approximately all of the recurrences (99.5%) occurred within 7 years after the surgery, with 53.5% having had peritoneal dissemination, 43.3% hematogenous metastases, and 28.6% distant lymphatic spread[26]. The locoregional and peritoneal recurrences, which usually occur in gastric cancer patients, were found to be a critical problem. Peritoneal recurrence has been reported as the most common recurrence pattern in locally advanced gastric cancer patients, which accounts for 50% of the cases and occurs within 2 years after surgery[27]. In another study, almost 60% of the gastric cancer patients experienced a recurrence, which included 32.4% locoregional recurrences, 13.7% peritoneal metastases, and 44.3% distant metastases[28].

To reduce locoregional recurrences, intraperitoneal chemotherapy has been used widely worldwide. Different from other methods, the use of intraoperative sustained-release 5-FU is promising because of the convenient usage and a long effective time of approximately 1 mo. The use of sustained-release 5-FU implants has shown survival benefits in different types of digestive system tumors such as colon cancer and primary hepatic cellular cancer[29,30]. Furthermore, a few studies on the treatment of gastric cancer, which showed similar results, were also reported by Chinese researchers[21,22]. However, P values of survival benefits obtained in previous studies on gastric cancer were lower, but close to 0.05. Therefore, we conducted this real-world study to confirm the findings.

In the present study, the use of sustained-release 5-FU implants did not improve the long-term survival of gastric cancer patients with either TNM stage II or III. The estimated 5-year survival of patients with gastric cancer with TNM stage III in SR and NSR group was 50% and 49%, respectively (P

Table 1 Clinicopathological characteristics of different gastric cancer group

	SR group (*n* = 148)	NSR group (*n* =161)	*P* value
Age (yr)	57.15 ± 10.71	59.27 ± 11.52	0.2917
Gender, *n* (%)			0.781
Male	106 (71.6)	113 (70.2)	
Female	42 (28.4)	48 (29.8)	
BMI (kg/m^2)	23.97 ± 3.591	23.68 ± 3.488	0.9716
Neoadjuvant therapy, *n* (%)			0.52
Yes	29 (19.6)	27 (16.8)	
No	119 (80.4)	134 (83.2)	
Cardiovascular disease, *n* (%)			0.503
Yes	32 (21.6)	40 (24.8)	
No	116 (78.4)	121 (75.2)	
Diabetes, *n* (%)			0.093
Yes	10 (6.8)	20 (12.4)	
No	138 (93.2)	141 (87.6)	
T stage, *n* (%)			0.699
T1 and T2	23 (15.5)	21 (13.0)	
T3 and T4	96 (64.9)	113 (70.2)	
yp T1 and T2	5 (3.4)	3 (1.9)	
yp T3 and T4	24 (16.2)	24 (14.9)	
N stage, *n* (%)			0.533
N0 and N1	44 (29.7)	51 (31.7)	
N2 and N3	75 (50.7)	83 (51.6)	
yp N0 and yp N1	11 (7.4)	15 (9.3)	
yp N2 and yp N3	18 (12.2)	12 (7.5)	
M stage, *n* (%)			> 0.999
M0	148 (100.00)	161 (100)	
TNM stage, *n* (%)			0.91
Stage II	57 (38.5)	61 (37.9)	
Stage III	91 (61.5)	100 (62.1)	
Borrmann classification, *n* (%)			0.207
Superficial type	3 (2.0)	1 (0.6)	
Type I	10 (6.8)	9 (5.6)	
Type II	41 (27.7)	36 (22.4)	
Type III	73 (49.3)	78 (48.4)	
Type IV	2 (1.4)	9 (5.6)	
NA	19 (12.8)	28 (17.4)	
Lauren classification, *n* (%)			0.782
Intestinal type	42 (28.4)	48 (29.8)	
Diffuse type	64 (43.2)	69 (42.9)	
Mixed type	34 (23.0)	39 (24.2)	
NA	8 (5.4)	5 (3.1)	

Differentiation, *n* (%)			0.666
Low	88 (59.5)	91 (56.5)	
Low-medium	36 (24.3)	39 (24.2)	
Medium	19 (12.8)	26 (16.1)	
Medium-high and high	2 (1.4)	4 (2.5)	
NA	3 (2.0)	1 (0.6)	
Nerve invasion, *n* (%)			0.26
Yes	73 (49.3)	92 (57.1)	
No	24 (16.2)	27 (16.8)	
NA	51 (34.5)	42 (26.1)	
Vascular tumor thrombus, *n* (%)			0.23
Yes	57 (38.5)	57 (38.5)	
No	40 (27.0)	54 (33.5)	
NA	51 (34.5)	42 (26.1)	
Pathological classification, *n* (%)			0.481
Signet-ring cell carcinoma	45 (30.4)	55 (34.2)	
Another adenocarcinoma	103 (69.6)	106 (65.8)	
Resection type, *n* (%)			0.057
Total gastrectomy	53 (35.8)	54 (33.5)	
Proximal gastrectomy	7 (4.7)	20 (12.4)	
Distal gastrectomy	88 (59.5)	87 (54.0)	
R0 resection	148 (100.00)	161 (100)	> 0.999
Resected lymph nodes number	39.21 ± 14.13	35.73 ± 13.53	0.0145
Positive lymph nodes number	7.393 ± 8.864	6.856 ± 9.743	0.2319

SR: Sustained-release; NSR: Not sustained-release; BMI: Body mass index; NA: Not available.

Table 2 Postoperative complications in patients

	SR group (*n* = 148), *n* (%)	NSR group (*n* = 161), *n* (%)	*P* value
Pulmonary infection	3 (2.0)	6 (3.8)	0.583
Anastomotic fistula	6 (4.0)	6 (3.8)	0.882
Postoperative bleeding	1 (0.7)	3 (1.9)	0.675
Abdominal infection	3 (2.0)	9 (5.6)	0.105
Intestinal obstruction	3 (2.0)	1 (0.6)	0.556

SR: Sustained-release; NSR: Not sustained-release.

= 0.775), slightly higher than that of previous studies[31,32]. This may have been due to the R0 radical resection decreasing the risk of local-regional recurrence and compromising the effects of the sustained-release drug. Therefore, we may hypothesize that in cases of incomplete resections, such as an R1 resection, or if the lymph node dissection did not reach the D2 resection standard, the use of a sustained-release drug may improve survival. Previous results of drug use in the treatment of unresectable tumors, such as pancreatic cancer and colorectal cancer, provided evidence for this hypothesis[33].

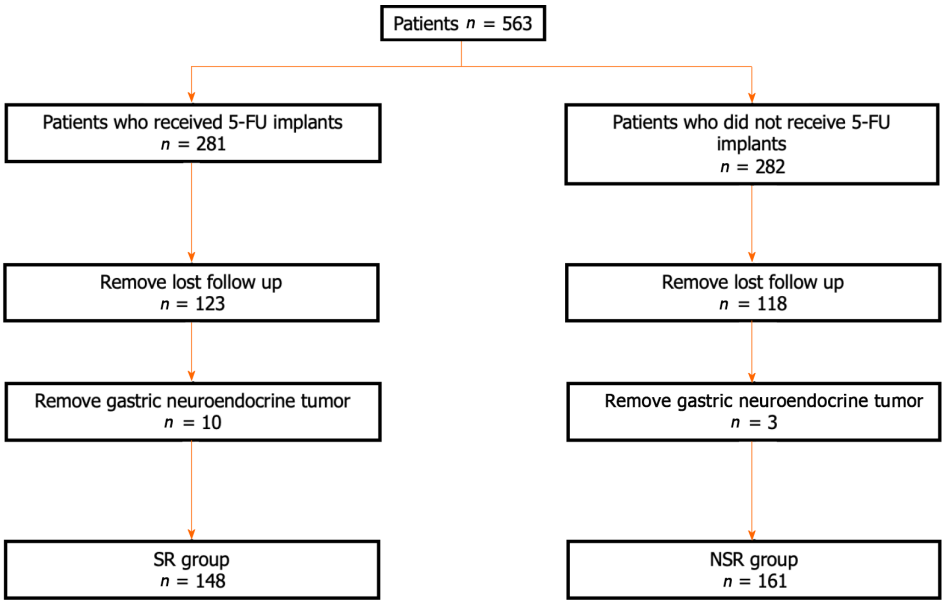
Similar to the findings of previous studies, the use of the sustained-release 5-FU implants did not increase postoperative complications, and systemic toxicity was rare in the present study. The use of 5-FU implants is usually recommended in gastric cancer patients, particularly at TNM stage T4 or N1–3

Table 3 Univariate and multivariate Cox analysis of patients

	Univariate analysis			Multivariate analysis		
Characteristics	**HR**	**95%CI**	***P* value**	**HR**	**95%CI**	***P* value**
Gender						
Male	Reference					
Female	1.058	0.712-1.572	0.781			
Age, yr						
< 60	Reference					
≥ 60	1.03	0.717-1.481	0.872			
Neoadjuvant therapy						
No	Reference					
Yes	2.188	1.448-3.306	< 0.001	2.118	1.194-3.759	0.01
BMI						
Normal < 24	Reference					
Abnormal ≥ 24	0.752	0.516-1.098	0.14			
Cardiovascular disease						
No	Reference					
Yes	0.943	0.606-1.467	0.794			
Diabetes						
No	Reference					
Yes	0.891	0.479-1.658	0.715			
Drug administration						
No	Reference					
Yes	1.052	0.731-1.513	0.786			
Resection type						
Total gastrectomy	Reference					
Partial gastrectomy	0.558	0.387-0.804	0.002	0.663	0.428-1.027	0.066
Borrmann classification						
Superficial type, Type I and II	Reference					
Type III and IV	2.203	1.378-3.522	0.001	1.562	0.951-2.566	0.078
Lauren classification						
Intestinal type	Reference					
Others	1.158	0.762-1.762	0.492			
Differentiation type						
Low	Reference					
Others	0.874	0.535-1.430	0.593			
TNM stage						
Stage II	Reference					
Stage III	3.12	1.977-4.926	< 0.001	1.315	0.561-3.084	0.528
T stage						
T1 and T2	Reference					
T3 and T4	3.382	1.649-6.937	0.001	1.9	0.777-4.645	0.159
N stage						

N0 and N1	Reference					
N2 and N3	2.516	1.636-3.869	< 0.001	2.224	1.082-4.572	0.03
Signet-ring cell carcinoma						
Yes	Reference					
No	1.188	0.736-1.916	0.481			
Nerve invasion						
No	Reference					
Yes	1.703	0.938-3.093	0.08			
Vascular tumor thrombus						
No	Reference					
Yes	1.099	0.701-1.722	0.681			

HR: Hazard ratio; CI: Confidence interval; BMI: Body mass index.



DOI: 10.3748/wjg.v28.i38.5589

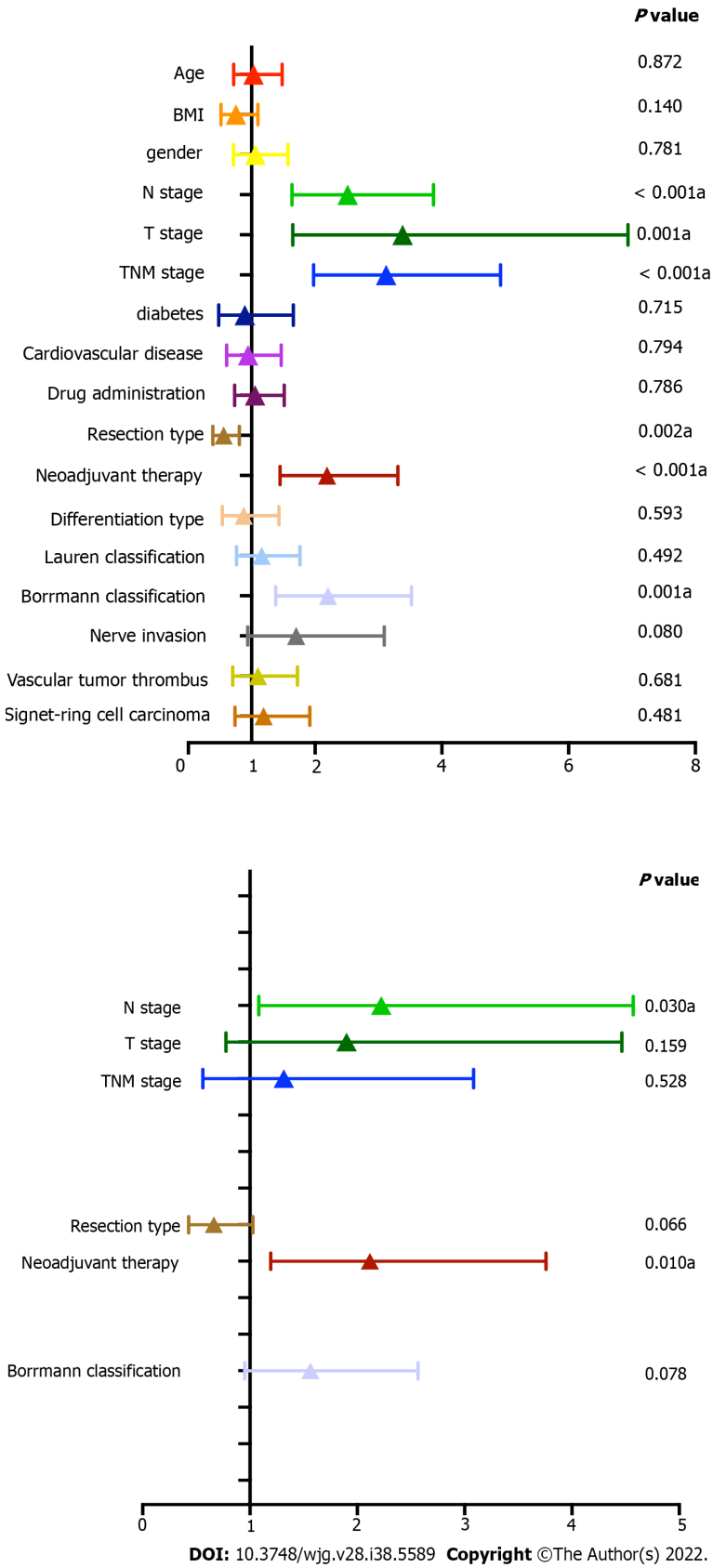
Figure 1 Flowchart of the selection of gastric cancer patients. 5-FU: 5-fluorouracil; NSR: Non-sustained-release.

[34]. In our study, the selection criteria were based on preoperative image examinations. Patients treated with 5-FU implants were in stage II and III, which is in accordance with the recommendations from the Chinese expert consensus[34].

Our study had some limitations. First, the sample size was insufficient. However, to date it remains the largest group studied. Second, selection biases existed. Many patients had incomplete history records, which may have contributed to a contrary result. Finally, the exact relapse time and the first site of recurrence were recorded incompletely. Owing to a lack of analysis of the relapses, the results were compromised. In order to understand the survival benefit of the sustained-release 5-FU plants on gastric cancer, a randomized controlled large-scale study is needed.

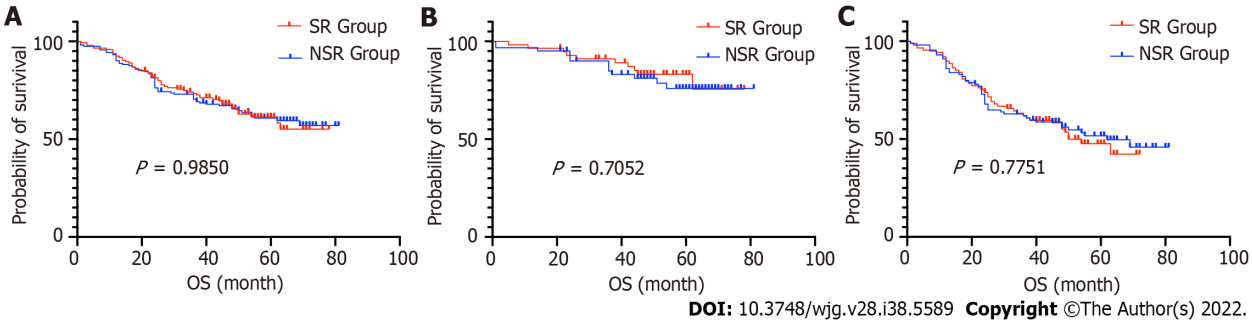
CONCLUSION

While the use of intraoperative sustained-release chemotherapy with 5-FU did not improve the survival of patients in an advanced stage of gastric cancer who underwent an R0 radical resection, it was a safe method, and it did not increase the complication rate.



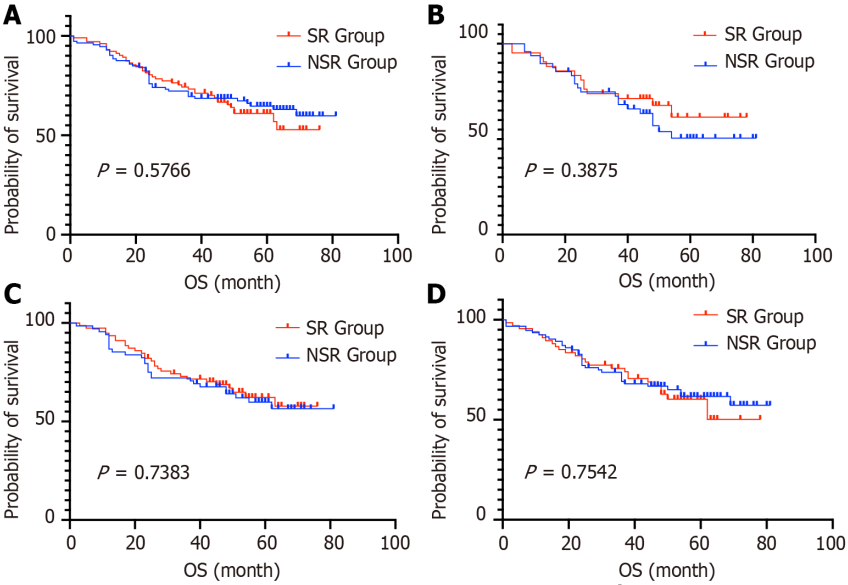
DOI: 10.3748/wjg.v28.i38.5589

Figure 2 Univariate and Multivariate Cox analyses of prognostic factors of 5-year survival in this group. N stage (N0 and N1 *vs* N2 and N3), T stage (T1 and T2 *vs* T3 and T4), TNM stage (stage II and stage III), surgery type (Total gastrectomy *vs* Partial gastrectomy), Neoadjuvant therapy (No *vs* Yes) and Borrmann classification (Superficial Type, Type I and Type II *vs* Type III and Type IV) are the prognosis factor of gastric cancer patients.



DOI: 10.3748/wjg.v28.i38.5589

Figure 3 Survival Outcomes in gastric cancer patients. A: Overall survival analysis in all gastric cancer patients; B: Overall survival analysis in stage II gastric cancer patients; C: Overall survival analysis in stage III gastric cancer patients. NSR: Non-sustained-release.



DOI: 10.3748/wjg.v28.i38.5589

Figure 4 Survival analysis in the age and gender subgroups. A: Survival analysis in male gastric cancer patients; B: Survival analysis in female gastric cancer patients; C: Survival analysis in gastric cancer patients less than 60 years old; D: Survival analysis in gastric cancer patients is mor than 60 years old. NSR: Non-sustained-release.

ARTICLE HIGHLIGHTS

Research background

The prognosis of gastric cancer in an advanced stage remains poor. The exact efficacy of the use of intraoperative sustained-release chemotherapy with 5-fluorouracil (5-FU) in advanced-stage gastric cancer is still unelucidated.

Research motivation

To explore the long-term survival benefit of using sustained-release 5-FU implants in stage II and stage III gastric cancer patients.

Research objectives

To explore the long-term survival benefit of using sustained-release 5-FU implants in stage II and stage III gastric cancer patients.

Research methods

All included patients were grouped according to whether intraoperative sustained-release (SR) chemotherapy with 5-FU was used or not (NSR). The primary end-point was 5-year overall survival. Kaplan–Meier method with log-rank test was used to analyze the overall survival of patients and Cox analysis was used to analyze prognosis factors of these patients.

Research results

309 patients were included in the final analysis. In addition, 158 patients received intraoperative sustained-release chemotherapy with 5-FU and were included in the SR group, while the other 161 patients were included in the NSR group. The overall complication rate was 12.94% in the whole group and 10.81%, 16.46% in SR and NSR groups, respectively. There were no significant differences between the two groups in overall survival and complication rate ($P > 0.05$).

Research conclusions

Intraoperative sustained-release chemotherapy usage with 5-FU, did not improve the survival of patients who underwent an R0 radical resection in locally advanced stage of gastric cancer.

Research perspectives

5-FU implants did not improve survival.

FOOTNOTES

Author contributions: Wu YZ and Xie YB contributed to the manuscript writing; Wu YZ, Zheng XH, Ming Wu, and Ding SK contributed to the data collection; Wu YZ and Ren JS contributed to the data analysis; Wang BZ and Xue LY contributed to the pathological diagnosis; Xie YB, Tian YT, and Yang L contributed to the clinical treatment and manuscript modification; all the authors contributed to the manuscript and approved the submitted version.

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Informed consent statement: Informed consent from patients was waived by the ethics committee of the National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College.

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Data sharing statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Retrospective Cohort Study

Timing of endoscopic retrograde cholangiopancreatography in the treatment of acute cholangitis of different severity

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Abstract

BACKGROUND

The optimal timing of endoscopic retrograde cholangiopancreatography (ERCP) in acute cholangitis (AC) is uncertain, especially in patients with AC of varying severity.

AIM

To report whether the timing of ERCP is associated with outcomes in AC patients with different severities.

METHODS

According to the 2018 Tokyo guidelines, 683 patients who met the definite diagnostic criteria for AC were retrospectively identified. The results were first compared between patients receiving ERCP ≤ 24 h and > 24 h and then between patients receiving ERCP ≤ 48 h and > 48 h. Subgroup analyses were performed in patients with grade I, II or III AC. The primary outcome was 30-d mortality. Secondary outcomes were intensive care unit (ICU) admission rate, length of hospital stay (LOHS) and 30-d readmission rate.

RESULTS

Taking 24 h as the critical value, compared with ERCP > 24 h, malignant biliary obstruction as a cause of AC was significantly less common in the ERCP ≤ 24 h group (5.2% *vs* 11.5%). The proportion of cardiovascular dysfunction (11.2% *vs* 2.6%), respiratory dysfunction (14.2% *vs* 5.3%), and ICU admission (11.2% *vs* 4%) in the ERCP ≤ 24 h group was significantly higher, while the LOHS was significantly shorter (median, 6 d *vs* 7 d). Stratified by the severity of AC, higher ICU

admission was only observed in grade III AC and shorter LOHS was only observed in grade I and II AC. There were no significant differences in 30-d mortality between groups, either in the overall population or in patients with grade I, II or III AC. With 48 h as the critical value, compared with ERCP > 48 h, the proportion of choledocholithiasis as the cause of AC was significantly higher in the ERCP ≤ 48 h group (81.5% *vs* 68.3%). The ERCP ≤ 48 h group had significantly lower 30-d mortality (0 *vs* 1.9%) and shorter LOHS (6 d *vs* 8 d). Stratified by AC severity, lower 30-d mortality (0 *vs* 6.1%) and higher ICU admission rates (22.2% *vs* 10.2%) were only observed in grade III AC, and shorter LOHS was only observed in grade I and II AC. In the multivariate analysis, cardiovascular dysfunction and time to ERCP were two independent factors associated with 30-d mortality.

CONCLUSION

ERCP ≤ 48 h conferred a survival benefit in patients with grade III AC. Early ERCP shortened the LOHS in patients with grade I and II AC.

Key Words: Acute cholangitis; Endoscopic retrograde cholangiopancreatography; severity; Timing; Thirty-day mortality; Length of hospital stay

Core Tip: Compared with endoscopic retrograde cholangiopancreatography (ERCP) > 24 h, ERCP ≤ 24 h group had a significantly higher intensive care unit (ICU) admission rate and shorter length of hospital stay (LOHS). Subgroup analysis showed higher ICU admission rate was only in grade III acute cholangitis (AC); shorter LOHS was only in grade II and I AC. Compared with ERCP > 48 h, ERCP ≤ 48 h group had significantly lower 30-d mortality and shorter LOHS. Subgroup analysis revealed lower 30-d mortality was only in grade III AC; shorter LOHS was only in grade II and I AC. We concluded that ERCP ≤ 48 h conferred a survival benefit in grade III AC; early ERCP shortened LOHS in grade II and I AC.

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INTRODUCTION

Without treatment, patients with acute cholangitis (AC) may progress to septicemia and organ failure resulting in mortality[1,2]. Over the past two decades, endoscopic retrograde cholangiopancreatography (ERCP) has been generally accepted as a first-line treatment for AC[3,4]. Although delayed biliary drainage may not affect the risk of complications in patients who respond well to antibiotics, some patients with AC require early ERCP to avoid (persistent) organ failure or mortality[2,5,6]. Despite consensus on the need for biliary drainage, the optimal timing for early ERCP remains unclear due to mixed results in the literature[7]. Different definitions of early ERCP have been used in the literature, ranging from 12 h to 72 h[2,5,8-11]. The varied definitions among studies have led to inconsistent conclusions. In addition, the definitions of AC are not uniform across studies. More importantly, most studies did not define the timing of ERCP by stratifying the severity of AC[7]. In this context, the recently published Tokyo Guidelines 2018 (TG18) provide not only a diagnosis of AC but also a severity grading, which is important for predicting prognosis and determining treatment strategies[3]. However, although TG18 recommends early or urgent biliary drainage for moderate or severe cholangitis, there is no specific timing for early or urgent ERCP. Therefore, this study aims to investigate whether the timing of ERCP is associated with improved outcomes in AC patients with different severities.

MATERIALS AND METHODS

This was a retrospective study conducted at Chang Gung Memorial Hospital Linkou Center. At our center, ERCP has been the first-line of treatment for patients with AC for the past two decades. This study was reviewed and approved by the Ethics Committee of the Chang Gung Memorial Hospital (IRB No. 202200881B0). Since this was a retrospective study using routine clinical treatment or diagnostic

medical records, the Chang Gung Medical Foundation Institutional Review Board approved the waiver of the participant's consent. All methods were carried out under relevant guidelines and regulations.

Definition of acute cholangitis

The diagnostic criteria for AC were based on the 2018 Tokyo Guidelines, including systemic inflammation, cholestasis and imaging findings[3]. Systemic inflammation included fever (body temperature > 38 °C) or evidence of an inflammatory response [white blood cell (WBC) count < 4000 or > 10000/μL or C-reactive protein ≥ 1 mg/dL]. Cholestasis included jaundice (serum total bilirubin ≥ 2 mg/dL) or abnormal liver function tests (serum alkaline phosphatase, r-glutamyl transferase, aspartate aminotransferase, or alanine aminotransferase > 1.5 times the upper limit of the normal value). Imaging findings included bile duct dilatation or imaging evidence of etiology such as strictures, stones or stents. A definite diagnosis of AC was defined as one item in systemic inflammation, one item in cholestasis and one item in imaging findings.

Definition of the severity of acute cholangitis

AC severity was divided into three grades based on the 2018 Tokyo Guidelines[3]. Grade III (severe) AC was AC associated with the onset of dysfunction in at least one of the following organs/systems: cardiovascular dysfunction (defined as hypotension requiring dopamine ≥ 5 μg/kg per min, or any dose of norepinephrine), neurological dysfunction (presence of conscious disturbance), respiratory dysfunction (defined as PaO_2/FiO_2 ratio < 300), renal dysfunction (oliguria, serum creatinine > 2.0 mg/dL), hepatic dysfunction [defined as prothrombin time-international normalized ratio (PT-INR) > 1.5] or hematological dysfunction (defined as platelet count < 100 × 10^3/μL). Grade II (moderate) AC was AC associated with any two of the following conditions: abnormal WBC count (> 12000/μL or < 4000/μL), high fever (≥ 39 °C), old age (≥ 75 years), hyperbilirubinemia (serum total bilirubin ≥ 5 mg/dL), or hypoalbuminemia (< lower limit of normal value × 0.7). Grade I (mild) AC was AC that did not meet the criteria of "Grade III" or "Grade II" AC at initial diagnosis.

Definition of time to ERCP

Time to ERCP was defined as the time from the emergency department visit to the commencement of ERCP.

Patient selection and clinical variables

The study flow chart is shown in Figure 1. Between 2016 and 2017, 2121 patients who underwent ERCP in our center were retrospectively collected from the computer database of the Therapeutic Endoscopy Center. The inclusion criteria were patients who met the TG18/TG13 criteria for a definite diagnosis of AC. The exclusion criteria were: (1) Patients who did not meet the criteria for a definite diagnosis of AC; (2) inpatients who developed AC after hospitalization; and (3) patients who received ERCP 7 or more days after an emergency department visit. For patients readmitted for AC during the study period, we included only the first admission and the ERCP procedure.

Medical records were reviewed, and the following data were obtained: sex; age; clinical manifestations, including body temperature, systolic blood pressure, heart rate, saturation, respiratory rate and urine output; laboratory values including WBC count, platelet count, PT-INR, C-reactive protein, creatinine, bilirubin, alkaline phosphatase, r-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase and albumin; diagnosis and treatment of ERCP, including causes of obstruction (such as stones, malignant strictures or stent dysfunction); and the timing of ERCP.

Outcome assessments

The primary outcome was 30-d mortality. Secondary outcomes were ICU admission rate, length of hospital stay (LOHS) and 30-d readmission rate. The results were first compared for patients receiving ERCP ≤ 24 h *vs* > 24 h and then for patients receiving ERCP ≤ 48 h *vs* > 48 h. Subgroup analyses were also performed in patients with grade I, II, and III AC.

Statistical analysis

Continuous variable data are represented by the median and interquartile range (IQR); categorical variables are presented as a number (%). For comparisons, the Kruskal-Wallis test was used for continuous variable data and the chi-square test or Fisher's exact test was used for suitable categorical variables. Logistic regression analysis was performed to identify factors associated with 30-d mortality. Only variables with a P value < 0.05 in the univariate analysis were included in the multivariate analysis. Two-tailed P values < 0.05 were considered statistically significant and P values = 0.05 were considered marginally significant. Statistical analysis was performed using SPSS software (version 22.0; SPSS, Inc., Chicago, IL, United States).

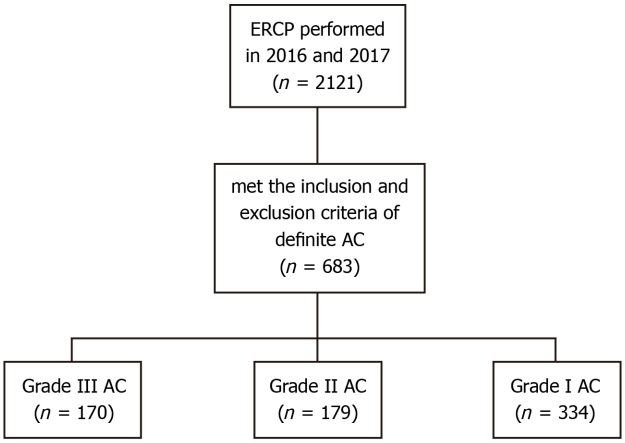

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Figure 1 Study flow chart. AC: Acute cholangitis; ERCP: Endoscopic retrograde cholangiopancreatography.

RESULTS

A total of 683 patients who met the eligibility criteria were included in the study. Among them, there were 170 (24.9%) grade III AC patients, 179 grade II AC patients (26.4%) and 334 grade I AC patients (48.9%).

Patient characteristics

The baseline characteristics of the patients are presented in Table 1. The median (IQR) age of the patients was 66 (53-78) years; 57.2% were male. The median body temperature was 37.5 (36.8-38.4) °C and 58.4% of patients had abnormal WBC counts. The median platelet count was 198 × 10^3 (148 × 10^3-251 × 10^3)/μL. The median serum levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were 150 U/L, 166 U/L and 265 U/L, respectively. Serum amylase and lipase data were available for 307 (44.9%) and 487 (71.3%) patients with median levels of 65 U/L and 37 U/L, respectively. The median serum bilirubin level was 3.7 (2.3-6.3) mg/dL and the median creatine level was 0.93 (0.73-1.24) mg/dL. The median PT/INR was 1.1 (1.1-1.2). Only 133 (19.5%) patients had data on serum albumin and the median level was 3.56 (3.05-3.98) g/dL. Twenty-nine (4.2%) patients had cardiovascular dysfunction, 35 (5.1%) patients had neurological dysfunction and 48 (7%) patients had respiratory dysfunction.

Comparisons between ERCP ≤ 24 h and ERCP > 24 h: Compared with ERCP > 24 h, patients with ERCP ≤ 24 h had significantly lower body temperature (median, 37.2 °C *vs* 37.5 °C. P = 0.003), significantly higher serum alanine aminotransferase (median, 194 U/L *vs* 156 U/L, P = 0.02) and serum creatinine levels (median, 0.96 *vs* 0.93, P = 0.004), and significantly higher proportions of abnormal WBC counts (70.9% *vs* 55.4%, P = 0.001), cardiovascular dysfunction (11.2% *vs* 2.6%, P < 0.001) and respiratory dysfunction (14.2% *vs* 5.3%, P < 0.001).

Comparisons between ERCP ≤ 48 h and ERCP > 48 h: Compared with ERCP > 48 h, patients with ERCP ≤ 48 h had significantly lower body temperature (median, 37.4 °C *vs* 37.6 °C, P = 0.009), significantly higher serum alanine aminotransferase levels (median, 188 U/L *vs* 142 U/L, P = 0.004) and significantly lower PT/INR (median, 1.1 *vs* 1.2, P = 0.001).

ERCP characteristics and causes of AC

The characteristics of ERCP are listed in Table 2. Causes of AC included common bile duct stones (CBDS, 74.4%), malignant biliary obstruction (MBO, 10.2%), biliary stent dysfunction (8.9%), benign biliary stricture (4.5%) and others (1.9%). ERCP failed in 1% of patients. For patients with successful ERCP, endoscopic treatments during ERCP included endoscopic sphincterotomy (81.7%), endoscopic papillary balloon dilatation (0.7%), bile duct stone retrieval (73.5%), stone-free bile duct clearance (5.4%), removal of old biliary stents (8.8%), insertion of new biliary stents (27.4%), dilation of biliary strictures (1%) and others (0.4%).

Comparisons between ERCP ≤ 24 h and ERCP > 24 h: The median time to ERCP was 17.7 (9.0-20.4) h in the ERCP ≤ 24 h group and 67.6 (43.6-98.9) h in the ERCP > 24 h group. Only malignant biliary obstruction as a cause of AC was significantly less common in the ERCP ≤ 24 h group (5.2% *vs* 11.5%, P = 0.032). In therapeutic ERCP, bile duct stone retrieval was higher in the ERCP ≤ 24 h group (80.6% *vs* 71.8%, P = 0.038) whereas the old biliary stent removal rate was lower (4.5% *vs* 9.8%, P = 0.049).

Table 1 Characteristics of the patients

Patient characteristics	Total, *n* = 683	Time to ERCP divided by 24 h			Time to ERCP divided by 48 h		
		ERCP ≤ 24 h, *n* = 134	ERCP > 24 h, *n* = 549	*P* value	ERCP ≤ 48 h, *n* = 314	ERCP > 48 h, *n* = 369	*P* value
Age in yr[1]	66 (53-78)	64 (56-75.5)	66 (52-78)	0.803	65 (54-79)	66 (51-79)	0.518
Male sex, *n* (%)	391 (57.2)	76 (56.7)	315 (57.4)	0.890	171 (54.5)	220 (59.6)	0.174
Body temperature in °C[1]	37.5 (36.8-38.4)	37.2 (36.7-38.1)	37.5 (36.9-38.5)	0.003	37.4 (36.8-38.28)	37.6 (36.9-38.5)	0.009
Abnormal WBC count[2], *n* (%)	399 (58.4)	95 (70.9)	304 (55.4)	0.001	195 (62.1)	204 (55.3)	0.072
Platelet count as /μL[1]	198 (148-251)	205 (142-256)	196.5 (149-250)	0.806	195 (148-249.8)	200 (148-251.5)	0.844
AST in U/L[1]	150 (83-305)	175 (97-406)	145 (80-289)	0.063	164 (95-311)	139 (76-302)	0.08
ALT in U/L[1]	166 (82-300)	194 (118-337)	156 (79-292)	0.02	188 (101-311)	142 (69-277)	0.004
ALK-P in U/L[1]	205 (133-327)	198 (123-321)	209 (133-327)	0.486	190 (123-329)	211 (140-324)	0.208
Amylase in U/L[1]	65 (41-170) (*n* = 307)	68 (42-634) (*n* = 66)	65 (40-145) (*n* = 241)	0.126	68 (41-367) (*n* = 138)	62 (40-139) (*n* = 169)	0.118
Lipase in U/L[1]	37 (26-144) (*n* = 487)	46 (25-1297) (*n* = 97)	36 (26-99) (*n* = 390)	0.104	41 (26-339) (*n* = 232)	35 (26-76) (*n* = 255)	0.094
Bilirubin in mg/dL[1]	3.7 (2.3-6.3)	4.1 (2.6-5.8)	3.7 (2.3-6.3)	0.395	3.8 (2.4-6.2)	3.7 (2.3-6.5)	0.878
PT/INR[1]	1.1 (1.1-1.2)	1.1 (1.1-1.3)	1.1 (1.1-1.2)	0.685	1.1 (1.1-1.2)	1.2 (1.1-1.3)	0.001
Creatinine in mg/dL[1]	0.93 (0.73-1.24)	0.96 (0.78-1.45)	0.93 (0.72-1.20)	0.040	0.95 (0.75-1.28)	0.93 (0.72-1.21)	0.389
Albumin in g/dL[1]	3.56 (3.05-3.98) (*n* = 133)	3.83 (3.24-4.0) (*n* = 23)	3.48 (3.05-3.98) (*n* = 110)	0.218	3.69 (3.21-3.96) (*n* = 43)	3.46 (2.98-3.99) (*n* = 90)	0.334
Cardiovascular dysfunction, *n* (%)	29 (4.2)	15 (11.2)	14 (2.6)	< 0.001	18 (5.7)	11 (3.0)	0.076
Neurological disturbance, *n* (%)	35 (5.1)	9 (6.7)	26 (4.7)	0.351	16 (5.1)	19 (5.1)	0.975
Respiratory dysfunction, *n* (%)	48 (7)	19 (14.2)	29 (5.3)	< 0.001	24 (7.6)	24 (6.5)	0.562

[1]Expressed as median (interquartile range).
[2]Abnormal WBC count is defined as WBC < 4000/μL or > 12000/μL.
ALK-P: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BT: Body temperature; ERCP: Endoscopic retrograde cholangiopancreatography; PT/INR: Prothrombin time/international normalized ratio; WBC: White blood cell count.

Comparisons between ERCP ≤ 48 h and ERCP > 48 h: The median time to ERCP was 26.0 (18.8-40.1) h in the ERCP ≤ 48 h group and 88.5 (67.2-114.6) h in the ERCP > 48 h group. Regarding indications for ERCP, CBDS was more common in the ERCP ≤ 48 h group (81.5% *vs* 68.3%, $P < 0.001$) whereas malignant biliary obstruction (6.1% *vs* 13.8%, $P < 0.001$) and stent dysfunction were less common (5.4% *vs* 11.9%, $P = 0.003$). In therapeutic ERCP, endoscopic sphincterotomy (85.7% *vs* 78.3%, $P = 0.013$) and bile duct stone retrieval (79.6% *vs* 68.3%, $P = 0.001$) were more frequent in the ERCP ≤ 48 h group whereas the removal of old biliary stents (5.4% *vs* 11.7%, $P = 0.004$) and the insertion of new biliary stents (22.3% *vs* 31.7%, $P = 0.006$) were less frequent.

Primary and secondary outcomes

The primary and secondary outcomes are summarized in Table 3.

Overall patients: Overall, the 30-d mortality rate was 1.02% (or 7/683). The ICU admission rate was 5.4%; the median LOHS was 7 (5-10) d; and the 30-d readmission rate was 12.7%.

(1) Comparisons between ERCP ≤ 24 h and ERCP > 24 h: The overall 30-d mortality rate was 0 in the ERCP ≤ 24 h group and 1.3% in the ERCP > 24 h group. However, the difference did not reach statistical significance ($P = 0.356$). Regarding secondary outcomes, the ERCP ≤ 24 h group had significantly higher ICU admission rates (11.2% *vs* 4.0%, $P = 0.001$) and shorter LOHS (median, 6 d *vs* 7 d, $P = 0.018$).

(2) Comparisons between ERCP ≤ 48 h and ERCP > 48 h: Overall, the 30-d mortality rate was significantly lower in the ERCP ≤ 48 h group than in the ERCP > 48 h group (0 *vs* 1.9%, $P = 0.017$). For secondary outcomes, the ERCP ≤ 48 h group had a significantly shorter LOHS (median, 6 d *vs* 8 d, $P <$

Table 2 Characteristics of endoscopic retrograde cholangiopancreatography

Patient characteristics	Total, *n* = 683	Time to ERCP divided by 24 h			Time to ERCP divided by 48 h		
		ERCP ≤ 24 h, *n* = 134	ERCP > 24 h, *n* = 549	*P* value	ERCP ≤ 48 h, *n* = 314	ERCP > 48 h, *n* = 369	*P* value
Time to ERCP in h[1]	53.8 (28.4-90.7)	17.7 (9.0-20.4)	67.6 (43.6-98.9)	-	26.0 (18.8-40.1)	88.5 (67.2-114.6)	
Indications of ERCP, *n* (%)							
Common bile duct stones	508 (74.4)	107 (79.9)	401 (73)	0.106	256 (81.5)	252 (68.3)	< 0.001
Malignant obstruction	70 (10.2)	7 (5.2)	63 (11.5)	0.032	19 (6.1)	51 (13.8)	< 0.001
Stent dysfunction	61 (8.9)	8 (6.0)	53 (9.7)	0.180	17 (5.4)	44 (11.9)	0.003
Benign stricture	31 (4.5)	8 (6.0)	23 (4.2)	0.375	15 (4.8)	16 (4.3)	0.783
Others	13 (1.9)	4 (3.0)	9 (1.6)	0.296	7 (2.2)	6 (1.6)	0.565
Treatment during ERCP, *n* (%)							
ES	558 (81.7)	112 (83.6)	446 (81.2)	0.529	269 (85.7)	289 (78.3)	0.013
EPBD	5 (0.7)	0	5 (0.9)	0.589	2 (0.6)	3 (0.8)	1
Stone retrieval	502 (73.5)	108 (80.6)	394 (71.8)	0.038	250 (79.6)	252 (68.3)	0.001
Clearance without stone	37 (5.4)	5 (3.7)	32 (5.8)	0.336	16 (5.1)	21 (5.7)	0.732
Removal of old stents	60 (8.8)	6 (4.5)	54 (9.8)	0.049	17 (5.4)	43 (11.7)	0.004
Stent insertion	187 (27.4)	35 (26.1)	152 (27.7)	0.715	70 (22.3)	117 (31.7)	0.006
Biliary stricture dilatation	7 (1.0)	1 (0.7)	6 (1.1)	1	1 (0.3)	6 (1.6)	0.132
Others	3 (0.4)	0	3 (0.5)	1	0	3 (0.8)	0.254
ERCP failure, *n* (%)	7 (1.0)	0	7 (1.3)	0.356	1 (0.3)	6 (1.6)	0.132

[1]Expressed as median (interquartile range). EPBD: Endoscopic papillary balloon dilation; ERCP: Endoscopic retrograde cholangiopancreatography; ES: Endoscopic sphincterotomy.

0.001).

Patients with Grade III AC: The 30-d mortality rate was 3.5% (or 6/170) for patients with grade III AC. The ICU admission rate was 15.3%; the median LOHS was 7 (7-14) d; and the 30-d readmission rate was 13.5% in this patient group.

(1) Comparisons between ERCP ≤ 24 h and ERCP > 24 h: The 30-d mortality rate for grade III AC patients was 0 in the ERCP ≤ 24 h group and 4.6% in the ERCP > 24 h group. However, the difference did not reach statistical significance ($P = 0.338$). Regarding secondary outcomes, the ERCP ≤ 24 h group had significantly higher ICU admission rates (9.0% *vs* 2.6%, $P = 0.002$).

(2) Comparisons between ERCP ≤ 48 h and ERCP > 48 h: Among grade III AC patients, the 30-d mortality rate was significantly lower in the ERCP ≤ 48 h group than in the ERCP > 48 h group (0 *vs* 6.1%, $P = 0.039$). Regarding secondary outcomes, the ERCP ≤ 48 h group had significantly higher ICU admission rates (22.2% *vs* 10.2%, $P = 0.031$).

Patients with Grade II AC: The 30-d mortality rate was 0 for patients with grade II AC. The ICU admission rate was 2.8%; the median LOHS was 7 (5-10) d; and the 30-d readmission rate was 13.4% in this patient group.

(1) Comparisons between ERCP ≤ 24 h and ERCP > 24 h: The only significant finding in grade II AC patients was that the LOHS was shorter (median, 6 d *vs* 7 d, $P = 0.047$) in the ERCP ≤ 24 h group.

(2) Comparisons between ERCP ≤ 48 h and ERCP > 48 h: Among grade II AC patients, the only significant finding was that the LOHS was shorter (median, 6 d *vs* 8 d, $P = 0.001$) in the ERCP ≤ 48 h group.

Patients with Grade I AC: The 30-d mortality rate was 0.3% (or 1/334) for patients with grade I AC. The ICU admission rate was 1.8%; the median LOHS was 6 (5-9) d; and the 30-d readmission rate was 12% in this patient group.

(1) Comparisons between ERCP ≤ 24 h and ERCP > 24 h: The only significant finding in grade I AC patients was that the LOHS was shorter (median, 6 d *vs* 7 d, $P = 0.005$) in the ERCP ≤ 24 h group.

Table 3 Primary and secondary outcomes

Outcomes	Total	Time to ERCP divided by 24 h			Time to ERCP divided by 48 h		
		ERCP ≤ 24 h	ERCP > 24 h	*P* value	ERCP ≤ 48 h	ERCP > 48 h	*P* value
Overall	*n* = 683	*n* = 134	*n* = 549		*n* = 314	*n* = 369	
30-d mortality, *n* (%)	7 (1.02)	0	7 (1.3)	0.356	0	7 (1.9)	0.017
ICU admission, *n* (%)	37 (5.4)	15 (11.2)	22 (4)	0.001	22 (7.0)	15 (4.07)	0.091
LOHS in d[1]	7 (5-10)	6 (4-10)	7 (5-10)	0.018	6 (4-9)	8 (6-11)	< 0.001
30-d readmission, *n* (%)	87 (12.7)	16 (11.9)	71 (12.9)	0.757	40 (12.7)	47 (12.7)	0.999
Grade III AC	*n* = 170	*n* = 39	*n* = 131		*n* = 72	*n* = 98	
30-d mortality, *n* (%)	6 (3.5)	0	6 (4.6)	0.338	0	6 (6.1)	0.039
ICU admission, *n* (%)	26 (15.3)	12 (9.0)	14 (2.6)	0.002	16 (22.2)	10 (10.2)	0.031
LOHS in d[1]	7 (7-14)	10 (6-16.5)	9 (7-12)	0.637	9 (6-15)	9 (7-12)	0.448
30-d readmission, *n* (%)	23 (13.5)	4 (3)	19 (14.5)	0.602	10 (13.9)	13 (13.3)	0.906
Grade II AC	*n* = 179	*n* = 39	*n* = 140		*n* = 88	*n* = 91	
30-d mortality, *n* (%)	0	0	0	-	0	0	-
ICU admission, *n* (%)	5 (2.8)	2 (5.1)	3 (2.1)	0.299	3 (3.4)	2 (2.2)	0.679
LOHS in d[1]	7 (5-10)	6 (4-9.5)	7 (5.8-10)	0.047	6 (4.8-9)	8 (6-11)	0.001
30-d readmission, *n* (%)	24 (13.4)	4 (10.3)	20 (14.3)	0.514	8 (9.1)	16 (17.6)	0.096
Grade I AC	*n* = 334	*n* = 56	*n* = 278		*n* = 154	*n* = 180	
30-d mortality, *n* (%)	1 (0.3)	0	1 (0.36)	1	0	1 (0.56)	1
ICU admission, *n* (%)	6 (1.8)	1 (1.8)	5 (1.8)	1	3 (1.9)	3 (1.7)	1
LOHS in d[1]	6 (5-9)	6 (4-8.3)	7 (5-9.8)	0.005	6 (4-7)	7 (6-11)	< 0.001
30-d readmission, *n* (%)	40 (12)	8 (14.3)	32 (11.5)	0.56	22 (14.3)	18	0.229

[1]Expressed as median (interquartile range). AC: Acute cholangitis; ERCP: Endoscopic retrograde cholangiopancreatography; ICU: Intensive care unit; LOHS: Length of hospital stay.

(2) Comparisons between ERCP ≤ 48 h and ERCP > 48 h: Among grade I AC patients, the only significant finding was that the LOHS was shorter (median, 6 d *vs* 7 d, $P < 0.001$) in the ERCP ≤ 48 h group.

Factors associated with 30-d mortality

The results of the univariate and multivariate analyses are listed in Table 4. The univariate analysis revealed that malignant biliary obstruction (OR: 6.817, 95%CI: 1.494-31.109, $P = 0.013$), hepatic dysfunction (OR: 8.896, 95%CI: 1.645-48.119, $P = 0.011$), respiratory dysfunction (OR: 10.517, 95%CI: 2.284-48.431, $P = 0.003$), neurological dysfunction (OR: 15.094, 95%CI: 3.241-70.298, $P = 0.001$), cardiovascular dysfunction (OR: 18.750, 95%CI: 3.990-88.112, $P < 0.001$), severity of AC (severe *vs* moderate + mild, OR: 18.732, 95%CI: 2.239-156.728, $P = 0.007$), ICU admission (OR: 7.326, 95%CI: 1.373-39.101, $P = 0.02$), and time to ERCP (every 1-d delay, OR: 1.950, 95%CI: 1.252-3.038, $P = 0.003$) were associated with 30-d mortality. The multivariate analysis revealed that time to ERCP (every 1-d delay, OR: 2.081, 95%CI: 1.154-3.753, $P = 0.015$) was the only independent factor associated with 30-d mortality. However, cardiovascular dysfunction (OR: 17.756, 95%CI: 0.994-317.241, $P = 0.050$) was of marginal significance.

DISCUSSION

Kiriyama *et al*[3] reported that early or urgent ERCP significantly reduced 30-d mortality only in patients with grade II AC compared with patients who did not receive early or urgent ERCP. This result may be due to the lack of well-defined timing for early or urgent ERCP. In a meta-analysis published in 2020, Du *et al*[7] reported that early ERCP reduced in-hospital mortality regardless of whether it was defined as < 24 h, < 48 h or < 72 h. In the present study, we found that ERCP ≤ 48 h but not ERCP ≤ 24 h

Table 4 Univariate and multivariate analyses of the factors associated with 30-d mortality

Variables		Univariate analysis		Multivariate analysis	
		OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age	≥ 75 yr	1.772 (0.393-7.991)	0.456		
	< 75 yr	Reference			
Common bile duct stones	Yes	0.254 (0.056-1.146)	0.075		
	No	Reference			
Malignant biliary obstruction	Yes	6.817 (1.494-31.109)	0.013	7.718 (0.664-89.660)	0.102
	No	Reference			
Fever, BT ≥ 39 °C	Yes	1.045 (0.124-8.777)	0.968		
	No	Reference			
Abnormal WBC count	Yes	1.789 (0.345-9.289)	0.489		
	No	Reference			
Hyperbilirubinemia, ≥ 5 mg/dL	Yes	1.382 (0.307-6.228)	0.673		
	No	Reference			
Hepatic dysfunction, PT-INR > 1.5	Yes	8.896 (1.645-48.119)	0.011	2.257 (0.275-18.553)	0.449
	No	Reference			
Hematological dysfunction, PLT < 100 × 10^3/μL	Yes	4.885 (0.919-25.649)	0.063		
	No	Reference			
Renal dysfunction, Cr > 2.0 mg/dL	Yes	4.548 (0.862-23.998)	0.074		
	No	Reference			
Respiratory dysfunction, PaO_2/FiO_2 ratio > 300	Yes	10.517 (2.284-48.431)	0.003	1.644 (0.172-15.676)	0.666
	No	Reference			
Neurological dysfunction, conscious disturbance	Yes	15.094 (3.241-70.298)	0.001	2.773 (0.380-20.238)	0.315
	No	Reference			
Cardiovascular dysfunction[1]	Yes	18.750 (3.990-88.112)	< 0.001	17.756 (0.994-317.241)	0.050
	No	Reference			
Severity of AC	Grade III	18.732 (2.239-156.728)	0.007	3.603 (0.274-47.356)	0.329
	Grade II + I	Reference			
ICU admission	Yes	7.326 (1.373-39.101)	0.02	2.463 (0.204-29.703)	0.478
	No	Reference			
Time to ERCP	Every 1-d delay	1.950 (1.252-3.038)	0.003	2.081 (1.154-3.753)	0.015

[1]Defined as hypotension requiring dopamine ≥ 5 μg/kg per min, or any dose of norepinephrine. AC: Acute cholangitis; BT: Body temperature; ERCP: Endoscopic retrograde cholangiopancreatography; ICU: Intensive care unit; PT/INR: Prothrombin time/international normalized ratio; WBC: White blood cell count.

significantly reduced 30-d mortality. Our results were consistent with the 2021 American Society for Gastrointestinal Endoscopy guidelines recommending ERCP ≤ 48 h in AC patients[12]. In that study, however, the data were insufficient to stratify by disease severity. In a subgroup analysis, we found that the same survival benefit was observed only in patients with grade III AC but not in patients with grade II or I AC. These results were because patients with grade III AC had significantly higher 30-d mortality than those with grade II or I AC (3.5% *vs* 0 *vs* 0.3%, $P = 0.001$). Hakuta *et al*[10] reported that time to ERCP was not associated with clinical outcomes (including in-hospital mortality) in patients with non-grade III AC. Therefore, we recommend emergent ERCP (≤ 48 h) for patients with grade III AC in terms of survival benefit.

However, 30-d mortality in AC has been reported to range from 1% to 16% between studies, which may be one of the reasons leading to inconsistent conclusions about the optimal timing of ERCP[11,13-

15]. Differences in mortality may be due to different patient populations in different studies, *e.g.*, patients with AC due to CBDS and MBO may have different clinical courses and prognoses. Kiriyama *et al*[14] reported that patients with AC associated with MBO had a higher 30-d mortality rate than those with AC associated with CBDS. In our univariate analysis, MBO was a factor associated with 30-d mortality. Therefore, one of the reasons for the low 30-d mortality in our study was the low proportion of patients with MBO (10.2%). In contrast, Tan *et al*[11] included 43% of MBO patients in their study and reported a 30-d mortality rate of 16%. However, Park *et al*[15] included only patients with AC associated with distal MBO and reported an overall 30-d mortality rate of 4.8%. Therefore, there may be some other factors associated with 30-d mortality between studies.

Of the five organ failure criteria used to diagnose grade III AC, cardiovascular dysfunction was the only independent factor associated with 30-d mortality in the current study. Therefore, among grade III AC patients, those with cardiovascular dysfunction may need to be treated differently[16,17]. Karvellas *et al*[17] reported an overall mortality rate of 37% in 260 patients with AC-related septic shock. They found that delayed biliary decompression > 12 h from the onset of shock was one of three independent factors associated with mortality. The 2019 European Society of Gastrointestinal Endoscopy guidelines recommend biliary drainage (preferably endoscopic) within 12 h of shock onset for AC patients with CBDS-related septic shock[16]. Therefore, cardiovascular dysfunction should be weighed when developing new guidelines in the future.

Some studies found no survival benefit but did find early ERCP to reduce the LOHS[18-21]. Hou *et al*[20] reported that in a multivariate analysis, the LOHS increased by 1.44 d for every 1-d delay in ERCP ($P < 0.001$). Similar results were seen in the study by Zhu *et al*[18]: LOHS increased by 1.49 d for every 1-d delay in biliary drainage ($P < 0.0001$). However, these findings were not stratified by disease severity. Although we did not perform a multivariate analysis of the LOHS, our results suggested that the LOHS could be significantly reduced regardless of ERCP ≤ 24 h or ≤ 48 h. In subgroup analyses stratified by disease severity, this benefit was only observed in patients with grade I or II AC. The benefit of early ERCP in shortening the LOHS might be offset by higher ICU admission rates in grade III AC patients. Similar findings were found by Jang *et al*[19], who recommended urgent ERCP (≤ 24 h) for patients with grade I or II AC because it can shorten the LOHS.

This study has several limitations. First, this retrospective, single-center study might have inherent selection bias. Patients with cardiovascular dysfunction and respiratory dysfunction tended to receive ERCP ≤ 24 h. Therefore, caution is required when interpreting the results of this subgroup analysis. Second, we identified patients from the endoscopy database. AC patients who died before receiving ERCP might not have been included in this study, resulting in an underestimation of 30-d mortality. Third, data on albumin, one of the criteria for class II AC, were available in only 19.5% of patients. Therefore, some patients with grade II AC may be misclassified as grade I AC and vice versa.

CONCLUSION

ERCP ≤ 48 h but not ≤ 24 h has a survival benefit in AC patients; this benefit is only observed in patients with grade III AC. Early ERCP is also recommended for patients with grade I or II AC because it shortens the LOHS.

ARTICLE HIGHLIGHTS

Research background

The optimal timing of endoscopic retrograde cholangiopancreatography (ERCP) for acute cholangitis has been inconsistently reported and there are few studies on the timing of ERCP in acute cholangitis of varying severity.

Research motivation

On the one hand, unnecessary emergent ERCP increases medical costs and the burden on physicians and technicians; on the other hand, delayed ERCP may increase morbidity and mortality in patients with acute cholangitis. The findings of this study may guide the avoidance of unnecessary urgent and delayed ERCP for acute cholangitis.

Research objectives

This study aims to answer the optimal timing of ERCP for acute cholangitis of different severity according to 30-d mortality after ERCP. Answering this question can serve as important evidence for future guideline development.

Research methods

The retrospective cohort study included 683 patients who met the diagnostic criteria for acute cholangitis defined by the 2018 Tokyo Guidelines. Among them, there were 170 (24.9%) grade III acute cholangitis patients, 179 grade II acute cholangitis patients (26.4%) and 334 grade I acute cholangitis patients (48.9%). Results are first compared between patients receiving ERCP ≤ 24 h and > 24 h, and then between patients receiving ERCP ≤ 48 h and > 48 h. Subgroup analyses are performed on patients with grade III, II or I acute cholangitis.

Research results

When 24 h was considered a critical value for ERCP timing, we found that patients with malignant biliary obstruction received ERCP ≤ 24 h less frequently when compared with ERCP > 24 h (5.2% *vs* 11.5%). Patients with organ dysfunction such as cardiovascular dysfunction (11.2% *vs* 2.6%) and respiratory dysfunction (14.2% *vs* 5.3%) or those admitted to the ICU (11.2% *vs* 4%) tended to receive ERCP ≤ 24 h. Patients with ERCP ≤ 24 h had significantly shorter hospital stays (median, 6 d *vs* 7 d). Stratified by the severity of acute cholangitis, higher ICU admission was only observed in grade III acute cholangitis and a shorter length of hospital stay was only observed in grade I and II acute cholangitis. Regarding 30-d mortality, the results of ERCP ≤ 24 h and > 24 h were not significantly different, either in the overall population or in patients with grade I, II or III acute cholangitis. When 48 h was considered a critical value for ERCP timing, patients with choledocholithiasis received ERCP ≤ 48 h more frequently (81.5% *vs* 68.3%). Patients who received ERCP ≤ 48 h had significantly lower 30-d mortality (0 *vs* 1.9%) and shorter hospital stays (6 d *vs* 8 d). Stratified by the severity of acute cholangitis, lower 30-d mortality (0 *vs* 6.1%) and higher ICU admission rates (22.2% *vs* 10.2%) were only observed in grade III acute cholangitis and a shorter length of hospital stay was only observed in grade I and II acute cholangitis. In the multivariate analysis, cardiovascular dysfunction and time to ERCP were two independent factors associated with 30-d mortality.

Research conclusions

ERCP ≤ 48 h but not ≤ 24 h has a survival benefit in acute cholangitis patients; this benefit is only observed in patients with grade III acute cholangitis. Early ERCP is also recommended for patients with grade I and II acute cholangitis because it shortens the length of hospital stay.

Research perspectives

Of the five organ failure criteria used to diagnose grade III AC, cardiovascular dysfunction was the only independent factor associated with 30-d mortality in the current study. Therefore, cardiovascular dysfunction should be weighed more heavily in the development of new guidelines in the future.

FOOTNOTES

Author contributions: Huang YC contributed to the conceptualization of the study and original manuscript; Wu CH, Lee MH and Wang SF contributed to data planning, interpretation and formal analysis; Lin CH and Sung KF contributed to data collection; Tsou YK is committed to the conceptualization of the study, manuscript writing, review and editing; Liu NJ contributed to revising the final version of the manuscript for submission.

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ORIGINAL ARTICLE

Retrospective Study

Clearance of the liver remnant predicts short-term outcome in patients undergoing resection of hepatocellular carcinoma

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Abstract

BACKGROUND

Estimation of the functional reserve of the remnant liver is important to reduce morbidity and mortality.

AIM

To estimate the functional reserve of the remnant liver in patients with hepatocellular carcinoma (HCC).

METHODS

We reviewed the medical records of 199 patients who underwent resection of HCC. Hepatic clearance of the remnant liver was calculated using fusion images of ^{99m}Tc-labelled galactosyl-human serum albumin liver scintigraphy and computed tomography. Posthepatectomy liver failure (PHLF) was classified according to the International Study Group of Liver Surgery. Complications was classified according to Clavien–Dindo classification. We analyzed by the risk factors for PHLF, morbidity and mortality with multivariate analysis.

RESULTS

Twenty-seven (30%) patients had major complications and 23 (12%) developed PHLF. The incidence of major complications increased with increasing albumin–bilirubin (ALBI) grade. The area under the curve values for hepatic clearance of the remnant liver, liver to heart-plus-liver radioactivity at 15 min (LHL15), and ALBI score predicting PHLF were 0.868, 0.629, and 0.655, respectively. The area under the curve for hepatic clearance of the remnant liver, LHL15, and ALBI score predicting major complications were 0.758, 0.594, and

0.647, respectively. The risk factors for PHLF and major complications were hepatic clearance of the remnant liver and intraoperative bleeding.

CONCLUSION
The measurement of hepatic clearance may predict PHLF and major complications for patients undergoing resection of HCC.

Key Words: Liver function; Hepatectomy; Cirrhosis; Fusion image; Complication; Mortality

Core tip: Little is known about the association of remnant hepatic clearance with morbidity and mortality. The aim of present study was to evaluate the effectiveness of measuring hepatic clearance of the remnant liver and to determine its association with morbidity and mortality in patients undergoing hepatectomy for hepatocellular carcinoma. Risk factors significantly associated with morbidity and mortality were remnant liver clearance and intraoperative blood loss. Hepatic clearance was associated with posthepatectomy liver failure and the development of major complications. The estimation of hepatic clearance of the remnant liver may provide guidance for determining the extent of resection in a patient-specific manner.

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INTRODUCTION

Advances in surgical technique and postoperative care have improved the outcomes of patients undergoing hepatectomy. However, posthepatectomy liver failure (PHLF) can lead to increased rates of morbidity and mortality in patients with hepatocellular carcinoma (HCC) especially in patients with chronic liver damage[1]. Major hepatectomy must be performed to preserve the maximal remnant liver function. However, adequate hepatectomy must be performed to ensure adequate surgical margins around the tumor[2]. Therefore, preoperative assessment of remnant liver function reserve is important to determine the appropriate surgical procedure.

An algorithm including the presence of ascites, serum bilirubin, serum albumin concentration, prothrombin time and encephalopathy is commonly used to determine the indications for resection of HCC. The indocyanine green (ICG) test is the most commonly used test and is considered relatively reliable for assessment of liver functional reserve[3]. However, the Child–Pugh score and ICG test do not accurately predict the development of PHLF[4]. A simple method using conventional data has been reported. The albumin–bilirubin (ALBI) score is an effective predictor of PHLF in patients with HCC compared to that of ICG test[5].

Computed tomography (CT) volumetry can accurately determine the regional liver volume, and has been used to estimate remnant liver function[6]. However, CT volumetry can never reflect the function of the remnant liver. The liver function of each lobe varies with progression of chronic liver disease or steatosis, which indicate that liver function is not distributed homogeneously[7]. Liver function is unevenly modified, resulting from impaired blood circulation[8], biliary stenosis, or induction by the tumor[9]. Changes in portal hemodynamics and a regional reduction in liver function must be considered to determine the optimal surgical procedure[7,10]. A novel method is needed to preoperatively plan for hepatic resection.

Taniguchi *et al*[11] described that ^{99m}Tc-labelled galactosyl-human serum albumin (GSA) hepatic clearance strongly correlates with the degree of liver fibrosis and conventional liver function tests. GSA scintigraphy is widely used to evaluate liver function[10,12-16]. Asialoglycoprotein receptors exist predominantly in the liver on the surface of hepatocytes and are responsible for the metabolism of serum glycoproteins[17]. The receptor density in the liver is closely related to serum asialoglycoprotein level and hepatocyte function[18]. However, little is known about the clinical utility of hepatic clearance for the prediction of PHLF, morbidity and mortality. The aim of present study was to evaluate the effectiveness of measuring hepatic clearance of the remnant liver and to verify risk factors based on the standardized PHLF criteria and complications in patients undergoing hepatectomy.

MATERIALS AND METHODS

Patients

We included patients who underwent hepatectomy between July 2011 and March 2021 at Jichi Medical University (Shimotsuke, Tochigi, Japan). The protocol for this research project was approved by a suitably constituted Ethics Committee of the institution and it conformed to the provision of the Declaration of Helsinki (Committees of Jichi Medical University, Approval No. A21-029). Blood samples obtained preoperatively were analyzed for conventional liver tests.

The procedures for hepatectomy were categorized according to the Brisbane Nomenclature from the International Hepato-Pancreato-Biliary Association[19]. The anatomical resection was defined as resection of the tumor together with the related portal vein branches and the corresponding hepatic territory. The procedure was classified as a hemihepatectomy, an extended hemihepatectomy (hepatectomy plus removal of additional contiguous segments), a sectionectomy (resection of two Couinaud subsegments), or segmentectomy (resection of one Couinaud subsegment). All other nonanatomical procedures were classified as limited resections.

Contrast-enhanced CT

A three-phase enhanced helical CT scan of the liver was used to confirm tumor location and margins before surgery. A 16-row multi-detector CT scan was performed at 3 mm intervals with 100 mL iohexol (Omnipaque 300; Daiichi Sankyo, Tokyo, Japan) (3 mL/s) injected intravenously.

GSA single photon emission CT image

Patients underwent preoperative GSA scintigraphy using a dual-head rotating gamma camera system and a dedicated data processing unit (Prism Axis; Picker Prism International, Cleveland, OH, USA). A single bolus of 3 mg GSA (185 MBq; Nihon Medi-Physics, Tokyo, Japan) was injected intravenously. After confirmation that the detector covered the area in the liver and heart, acquisition of planar images was begun with an acquisition time of 15 s each for 16 min immediately after injection. After acquisition of planar images, dynamic single photon emission CT (SPECT) acquisition was started with an acquisition time of 20 s every 5 min. To generate a set of images equivalent to static SPECT images, projection data from dynamic SPECT were merged. Total liver function was calculated as the total liver GSA clearance, expressed as mL/min by the Patlak plot method.

Region of interest (ROI) was also generated over the entire liver on the tomographic images using isocount methods (25% cutoff of minimal count) to estimate the liver functional volume (mL). Functional liver volume does not include function parameters.

Estimation of function of the remnant liver

Hepatic clearance and functional volume of the remnant liver were estimated from the fusion with CT scan images (Figure 1). Images from the CT scan were aligned with the slice of the liver SPECT image with reference to the hepatic vein on every 3-mm liver cross-slice as a landmark on contrast-enhanced helical CT (Figure 1). After the transection line was set on the SPECT images based on the surgical procedure, the remnant liver with the resection line was determined manually. Remnant liver function was calculated from the proportional allocation of voxel count in static SPECT by the Patlak plot method and expressed by GSA clearance (mL/min). Regional functional liver volume (mL) was also calculated from the SPECT data by the outline extraction method[7].

Definition of major complication and PHLF

Postoperative complications were defined according to the Clavien–Dindo classification[20]. A major complication was defined as grade IIIa or higher. Postoperative mortality was defined as death within 30 d after surgery. PHLF was defined following the definition of the International Study Group of Liver Surgery[21]. Patients with increased prothrombin time–international normalized ratio (PT-INR) and hyperbilirubinemia (according to the normal cut-off levels defined by the local laboratory) on or after postoperative day 5 were considered to have PHLF. PHLF Grade A resulted in abnormal laboratory parameters and required no change in clinical management. Grade B was a deviation from the regular, postoperative clinical pathway, but patients could be managed without invasive treatment. Grade C resulted in deviation from the regular clinical management and required invasive treatment.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation. All categorical data were analyzed by Pearson's χ^2 test. Normally distributed values were analyzed by Student's t test. Non-normally distributed values were analyzed using the Mann–Whitney U test. We analyzed the power for the prediction of PHLF, morbidity and mortality with the parameters of GSA scintigraphy with the area under the receiver-operating characteristic (ROC) curves, and the area under the ROC curve was calculated. In multivariate analysis, risk factors for PHLF were determined by logistic regression multivariate analysis with JMP statistical software (version 13; SAS, Inc. Cary, NC, USA). The level of statistical significance was set at $P < 0.05$.

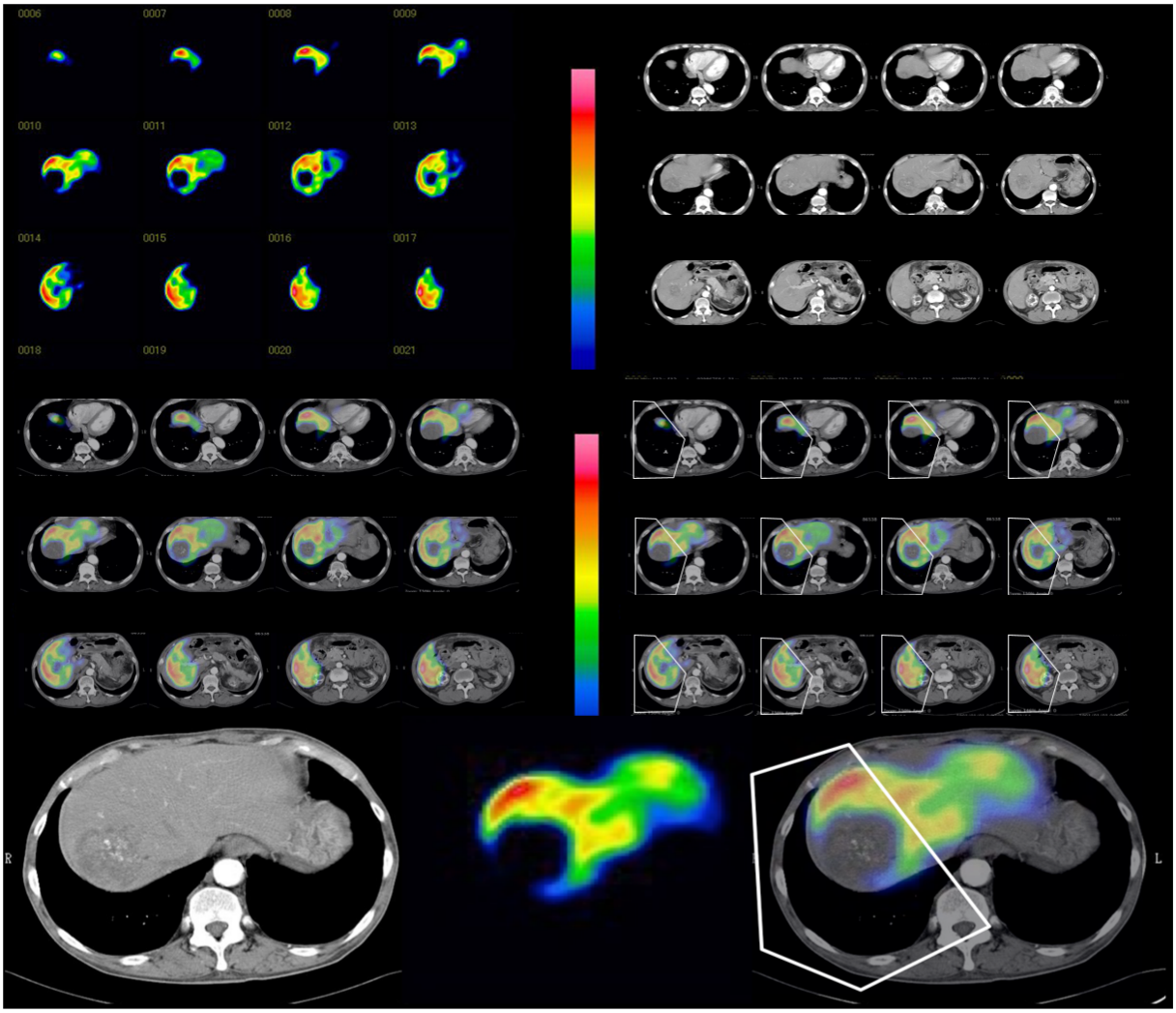

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Figure 1 Schematic model for analysis of regional hepatic clearance with computed tomography fusion images. The images of ^{99m}Tc-galactosyl serum albumin scintigraphy single photon emission computed tomography and computed tomography scans were merged using software. The cutting line was set based on tumor location and size on each fusion image. The liver function was calculated automatically as ^{99m}Tc-galactosyl serum albumin scintigraphy parameters. CT: Computed tomography; SPECT: Single photon emission computed tomography.

RESULTS

Clinicopathological characteristics

A total of consecutive 199 patients with HCC were included, including 156 men and 43 women, with a median age of 70 (range, 24–87 years) (Table 1). Among the 199 patients, 94 (47%) had hepatitis C virus infection and 22 (11%) had hepatitis B virus infection. Most patients were Child–Pugh class A (197/199, 99%) and the remaining patients were class B (2/199, 1%). According to ALBI grade, 68% (135/199) of patients were stratified into Grade 1, 32% (64/199) Grade 2, and 1% (2/199) Grade 3. There were 6% of ALBI Grade 1 patients who developed major complications and 18% ALBI Grade 2 patients had major complications (P = 0.04).

Postoperative morbidity, PHLF and mortality

Among the 199 patients, 41 (21%) developed postoperative complications (Table 2). The most common complication was PHLF (12%, 23/199), followed by wound infection (5.0%, 10/199). Thirty-three (17%) patients developed minor complications, including Grade I complications in 25 (13%) patients and Grade II complications in eight (4.0%) patients. Major complications occurred in 27 (14%) patients, including Grade IIIa (11%, 21/199), Grade IIIb (1.5%, 3/199), Grade IVa (0.5%, 1/199) and Grade V (1%, 2/199). Eleven patients (5%) had PHLF Grade A, eight (4%) had PHLF Grade B, and four (2%) had PHLF Grade C. Two patients died of PHLF within 30 d after surgery, for a postoperative mortality rate of 1% (Table 2).

Table 1 Patient characteristics

Variables	All patients (*n* = 199)	No PHLF < Grade B (*n* = 187)	PHLF ≥ Grade B (*n* = 12)	*P* value
General				
Age (yr)	69.1 ± 9.0	69.2 ± 9.12	66.9 ± 7.05	0.53
Gender (male : female)	154 : 45	144 : 43	10 : 2	0.61
Child–Pugh class (A : B : C)	196 : 3 :0	184 : 3 : 0	12 : 0 : 0	0.31
Preoperative laboratory tests				
Total bilirubin (mg/dL)	0.85 ± 0.38	0.82 ± 0.38	1.06 ± 0.32	0.01
PT-INR	1.12 ± 0.20	1.12 ± 0.21	1.13 ± 0.08	0.70
Albumin (mg/dL)	4.1 ± 0.5	4.1 ± 0.5	3.9 ± 0.4	0.16
AST (IU/L)	43 ± 41	41 ± 40	56 ± 47	0.11
ALT (IU/L)	40 ± 34	39 ± 33	47 ± 40	0.31
Choline esterase(U/L)	262 ± 77	267 ± 76	226 ± 73	0.02
PNI score	48.6 ± 6.0	48.9 ± 6.0	46.4 ± 5.6	0.08
ALBI score	-2.76 ± 0.4	-2.78 ± 0.4	-2.57 ± 0.35	0.14
NLR	2.48 ± 2.03	2.50 ± 2.12	2.31 ± 1.07	0.82
PLR	0.12 ± 0.08	0.13 ± 0.09	0.10 ± 0.05	0.29
Procedure-related factors				
Limited resection	80 (39.8%)	77 (38.7%)	3 (1.5%)	0.43
Segmentectomy	57 (28.4%)	51 (25.6%)	6 (3.0%)	
Secteionectomy	36 (17.9%)	33 (16.6%)	1 (0.5%)	
Hemihepatectomy	24 (11.9%)	22 (11.1%)	2 (1.0%)	
Trisectionectomy	4 (2.0%)	4 (2.1%)	0 (0%)	
Operative time (min)	304 ± 109	299 ± 104	345 ± 135	0.06
Intraoperative blood loss (mL)	848 ±1006	743 ± 889	1604 ± 1456	< 0.001
Liver function tests				
Total Liver hepatic clearance (mL/min)	285 ± 98	315 ± 106	258 ± 91	0.02
Total Liver Functional volume (mL)	1321 ± 280	1340 ± 278	1202 ± 270	0.04
Hepatic clearance of the remnant liver (mL/min)	248 ± 95	261 ± 91	149 ± 47	< 0.001
Functional volume of the remnant liver (mL)	1057 ± 334	1104 ± 317	710 ± 239	< 0.001
LHL15	0.92 ± 0.03	0.925 ± 0.03	0.906 ± 0.05	0.02
HH15	0.60 ± 0.07	0.602 ± 0.07	0.651 ± 0.07	0.01
Surgical outcome				
PHLF grade (0 : A : B : C)	176 : 11 : 8 : 4	176 : 11 : 0 : 0	0 : 0 : 8 : 4	-
Hospital length of stay (d)	15 (7-119)	14 (7-119)	25 (12-74)	< 0.001

PNI: Prognostic nutrition index; ALBI: Albumin–bilirubin; NLR: Neutrophil–lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; LHL15: liver to heart-plus-liver radioactivity at 15 min; PHLF: Posthepatectomy liver failure; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PT-INR: Prothrombin time international normalized ratio.

Correlations between hepatic clearance of the remnant liver and PHLF

ROC curve analysis of hepatic clearance of the remnant liver, liver to heart-plus-liver radioactivity at 15 min (LHL15), and ALBI score were used to predict the risk of developing PHLF (Figure 2A). The area under the ROC curve for hepatic clearance of the remnant liver, LHL15, and ALBI score for predicting

Table 2 Postoperative morbidity in patients with hepatocellular carcinoma

Complication	Clavien–Dindo classification							Total (%)
	I	II	IIIa	IIIb	IVa	IVb	V	
Pleural effusion or ascites	6		1					7 (3.5)
Pneumonia		1	1					2 (1.0)
Biliary leakage		1	5					6 (3.0)
Arrhythmia	1							1 (1.0)
Wound infection	10							10 (5.0)
Intra-abdominal abscess		1	8					9 (4.5)
Intra-abdominal hemorrhage			1	1				2 (1.0)
PHLF	8	5	5	2	1		2	23 (11.6)
Total (%)	25 (12.5)	8 (4.0)	21 (10.6)	3 (1.5)	1 (0.5)	0	2 (1.0)	60 (30.0)

PHLF: Posthepatectomy liver failure.

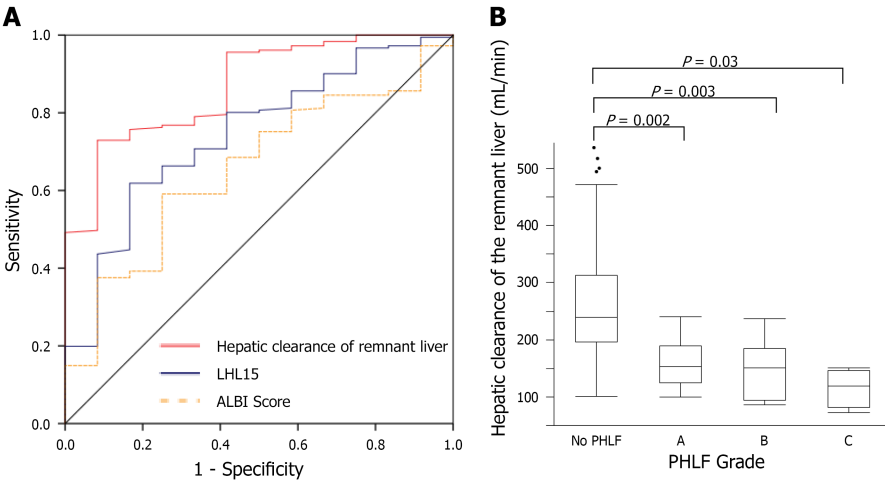

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Figure 2 Analysis of hepatic clearance for post hepatectomy liver failure. A: Receiver-operating characteristic curve analysis of hepatic clearance of the remnant liver, LHL15, and albumin–bilirubin (ALBI) score in predicting PHLF. The area under the receiver-operating characteristic curve values for analysis of hepatic clearance of the remnant liver, LHL15, and ALBI score in predicting PHLF were 0.868, 0.629, and 0.655, respectively; B: Hepatic clearance of the remnant liver for each PHLF. The median hepatic clearances of the remnant liver were 239, 153, 150.5, and 119.5 mL/min for normal, Grades A, B, and C, respectively. LHL15: liver to heart-plus-liver radioactivity at 15 min; ALBI score: Albumin–bilirubin score; PHLF: Post hepatectomy liver failure.

the development of PHLF were 0.868, 0.629, and 0.655, respectively. Hepatic clearance of the remnant liver had the highest area under the curve for predicting the development of PHLF. The cutoff values for predicting PHLF with highest sensitivity and specificity were 192 mL/min (sensitivity, 87.0%; specificity, 76.1%) for hepatic clearance of the remnant liver, 0.91 (sensitivity, 47.8%; specificity, 73.3%) for LHL15, 2.96 (sensitivity, 34.9%; specificity, 95.7%) for ALBI score. The relationship between hepatic clearance of the remnant liver and PHLF grade was evaluated (Figure 2B). The median hepatic clearances of the remnant liver were 239, 153, 150.5 and 119.5 mL/min for no PHLF, Grades A, B and C, respectively. The differences were significant for no PHLF and Grade A (P = 0.002), no PHLF and Grade B (P = 0.003), and no PHLF and Grade C (P = 0.02).

Correlation between hepatic clearance of remnant liver and morbidity and mortality

ROC curve analysis of hepatic clearance of the remnant liver, LHL15, and ALBI score were used to predict the risk of developing major complications (Figure 3A). The area under ROC curves for hepatic clearance of the remnant liver, LHL15, and ALBI score for predicting major complications were 0.758, 0.594, and 0.647, respectively. Hepatic clearance of the remnant liver had the highest area under the curve for predicting the development of major complications. The cutoff values for predicting PHLF with highest sensitivity and specificity were 237 mL/min (sensitivity, 100%; specificity, 51.9%) for

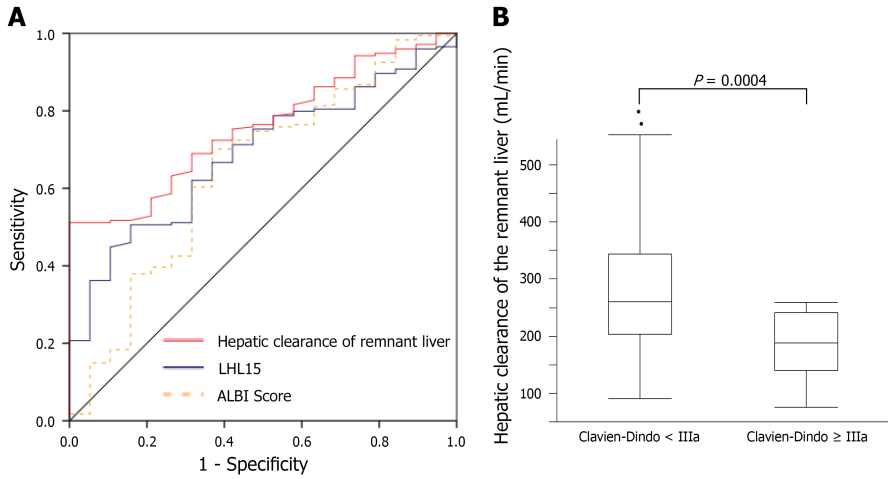

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Figure 3 Analysis of hepatic clearance for morbidity and mortality. A: Receiver operating characteristic curve analysis of hepatic clearance of the remnant liver, LHL15, and albumin–bilirubin (ALBI) score in predicting major morbidity. The area under the receiver operating characteristic curve analysis of hepatic clearance of the remnant liver, LHL15, and ALBI score in predicting major morbidity were 0.758, 0.594, and 0.647, respectively; B: Hepatic clearance of the remnant liver for Clavien–Dindo classification. The median hepatic clearances of the remnant liver were 238 and 179 mL/min for Clavien–Dindo < III and Clavien–Dindo ≥ III. LHL15: liver to heart-plus-liver radioactivity at 15 min; ALBI score: Albumin–bilirubin score.

hepatic clearance of the remnant liver, 0.94 (sensitivity, 84.2%; specificity, 36.2%) for LHL15, 2.63 (sensitivity, 69.3%; specificity, 63.2%) for ALBI score. The relationship between hepatic clearance of the remnant liver and Clavien–Dindo classification was evaluated (Figure 3B). The median hepatic clearances of the remnant liver were 238 and 179 mL/min for Clavien–Dindo < IIIa and Clavien–Dindo ≥ IIIa. The differences were significant for Clavien–Dindo < IIIa and Clavien–Dindo ≥ IIIa (P = 0.0004).

Multivariate analysis for PHLF Grade B or C

Multivariate regression analysis was performed between variables, with statistically significant differences following the univariate analysis regarding PHLF Grade B or C (Table 3). Hepatic clearance of the remnant liver [P = 0.001, odds ratio (OR): 0.973, 95% confidence interval (CI): 0.952–0.995] and intraoperative blood loss (P = 0.006, OR: 1.001, 95%CI: 1.0002–1.002) were independent risk factors for PHLF Grade B or C.

Multivariate analysis for major complication and mortality

Multivariate regression analysis was performed between variables, with significant differences following the univariate analysis regarding major complications (Table 4). Hepatic clearance of the remnant liver (P = 0.004, OR: 0.988, 95%CI: 0.979–0.999) and intraoperative blood loss (P = 0.005, OR: 1.0005, 95%CI: 1.0002–1.0014) were independent risk factors for developing major complications.

DISCUSSION

Hepatic clearance was associated with PHLF and major complications. The independent risk factors for developing PHLF and major complications were the hepatic clearance of the remnant liver, and intraoperative blood loss. The results of this study show that the measurement of hepatic clearance of the remnant liver is reliable for predicting the development of PHLF and major complications.

The results of this study support the use of hepatic clearance of the remnant liver, LHL15, and ALBI score for predicting the development of PHLF and postoperative major complications in patient with HCC. LHL15 and HH15, which are hepatic uptake and blood clearance ratios in GSA scintigraphy, are the most popular and widely used in many institutions. However, they may be insufficient for accurately estimating the degree of liver function because these indices are calculated from planar scintigraphic images, which do not correctly reflect hepatocyte volume[11]. In contrast, hepatic clearance measured by SPECT analysis contains volumetric information and may correctly estimate the hepatocyte volume[11]. LHL15 reflects the function of the whole liver, but the hepatic clearance of remnant liver shows the liver function of remnant liver, therefore, hepatic clearance may reflect functional reserve and short term outcome.

Many studies have investigated the relationship between GSA scintigraphy and PHLF. However, this is the first report comparing residual liver function and major complications using GSA scintigraphy. Patients with lower remnant liver function are at higher risk for PHLF, morbidity and mortality. The

Table 3 Predictive factors for post-hepatectomy liver failure in grade B, or C

	Univariate analysis			Multivariate analysis		
	P	OR	95%CI	*P*	OR	95%CI
Age (yr)	0.53	1.02	0.96-1.08			
Gender (male : female)	0.59	0.67	0.10-2.66			
Child–Pugh class	0.47	1.63	0.06-1.63			
PT-INR	0.86	0.79	0.05-11.0			
AST	0.34	0.99	0.98-1.00			
ALT	0.14	0.99	0.98-1.00			
Choline Esterase	0.24	1.00	0.99-1.01			
ALBI score	0.15	0.37	0.09-1.41	0.375	3.31	0.37-29.3
Operation time	0.02	1.01	0.99-1.00	0.110	1.010	0.99-1.01
Intraoperative blood loss	0.001	1.0007	1.0003-1.0012	0.033	1.001	1.0002-1.002
Hepatic clearance of the remnant liver	< 0.001	0.972	0.958-0.987	0.002	0.973	0.952-0.995
Functional volume of the remnant liver	0.002	0.997	0.994-0.999	0.351	0.998	0.995-1.00
LHL15	0.02	52	2.03-1382	0.362	9.597	0.08-1211

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PT-INR: Prothrombin time international normalized ratio; ALBI: Albumin-bilirubin; LHL15: liver to heart-plus-liver radioactivity at 15 min; OR: Odds ratio; CI: Confidence interval.

quality and volume of the postoperative remnant liver have been shown to be associated with postoperative outcomes[22]. Surgeons should emphasize the remnant liver functional reserve rather than the resected liver volume[12,23]. Elevation of serum bilirubin and PT-INR are associated with morbidity and mortality, regardless of the extent of resection[24]. Liver failure after limited resection can develop. Patients with reduced reserve of the remnant liver are at higher risk for the development of PHLF and major complications[22,25,26]. The extent of surgery should be considered to preserve as much liver function as possible. Moreover PHLF grade C is most severe types of liver failure that may lead to in-hospital death[24]. In ROC curve analysis, the cutoff line of PHLF grade C was 151 mL/min (sensitivity 87.5%, specificity 100%). Patients below the cutoff line should be given special consideration by surgeons before surgery and may not be ideal candidates for hepatic resection. Hepatic clearance of the remnant liver below 100 mL/min is associated with a high mortality rate. Therefore, PTPE should be performed when hepatic clearance of the remnant liver is below 100 mL/min, and surgery should be considered when the clearance is above 100 mL/min. In addition, if hepatic clearance of the remnant liver is greater than 100 mL/min preoperatively, unnecessary PTPE can be avoided.

The risk for developing PHLF and major complications is determined by patient and surgical factors. Intraoperative blood loss is a well-known risk factor for morbidity and mortality after hepatic resection [25,27-30]. Hemorrhage can lead to the development of metabolic acidosis as a consequence of intracellular derangements in oxygen and substrate utilization[29]. Reduced levels of cytokines and humoral factors, such as interleukin-6, hepatocyte growth factors, and growth hormone after extensive blood loss may result in decreased liver regeneration because of loss of growth factors needed for regeneration[28].

The present study had some limitations, including a retrospective design, and being a single center study. Preoperative GSA scintigraphy was routinely performed to estimate total liver function in this hospital. Total liver function, remnant liver function, laboratory data, and liver failure were objectively assessed in advance, which limited the risk of observation bias. Prospective multicenter trials are needed to validate the results of this study.

CONCLUSION

Lower functional reserve of the remnant liver results in a higher risk of developing PHLF and major complications in patients undergoing resection of HCC. The estimation of hepatic clearance of the remnant liver may provide guidance for determining the extent of resection in a patient-specific manner.

Table 4 Predictive factors for morbidity (Clavien–Dindo classification ≥ IIIa)

	Univariate analysis			Multivariate analysis		
	P	OR	95%CI	*P*	OR	95%CI
Age (yr)	0.68	1.01	0.96-1.06			
Gender (male : female)	0.69	0.80	0.27-2.36			
Child–Pugh class	0.89	1.09	0.29-2.91			
PT-INR	0.97	1.03	0.09-11.6			
AST	0.27	0.99	0.98-1.00			
ALT	0.11	0.99	0.98-1.00			
Cholinesterase	0.58	1.00	0.99-1.01			
ALBI score	0.02	3.57	1.19-10.7	0.790	1.22	0.286-5.19
Operation time	0.01	1.01	1.00-1.01	0.166	1.004	0.991-1.015
Intraoperative blood loss	0.001	1.0006	1.0002-1.001	0.005	1.0005	1.0002-1.0014
Hepatic clearance of the remnant liver	< 0.001	0.986	0.978-0.994	0.004	0.988	0.979-0.999
Functional volume of the remnant liver	0.003	0.998	0.996-0.999	0.329	0.99	0.997-1.00
LHL15	0.13	9.9	0.53-183	0.914	1.27	0.02-99.2

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PT-INR: Prothrombin time international normalized ratio; ALBI: Albumin-bilirubin; LHL15: liver to heart-plus-liver radioactivity at 15 min; OR: Odds ratio; CI: Confidence interval.

ARTICLE HIGHLIGHTS

Research background

Preoperative assessment of liver function is important to reduce the rate of complications.

Research motivation

Few studies evaluate the relationship of residual liver function to complications and prognosis using hepatic clearance.

Research objectives

To measure hepatic clearance of the remnant liver using ^{99m}Tc-galactosyl serum albumin (GSA) scintigraphy, single photon emission computed tomography, to elucidate the association between residual liver function and morbidity and mortality, and to identify risk factors for those factors.

Research methods

We collected data from 199 patients who underwent resection of hepatocellular carcinoma. Hepatic clearance of the remnant liver was measured using fusion images of ^{99m}Tc-labelled GSA liver scintigraphy and computed tomography scans. Risk factors were determined using logistic regression multivariate analysis.

Research results

Risk factors for posthepatectomy liver failure, morbidity, and mortality were low hepatic clearance of the remnant liver and intra-operative bleeding.

Research conclusions

Lower residual liver function is associated with a poor short-term prognosis.

Research perspectives

Preoperative estimation of remnant liver function is useful to determine the surgical approach.

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FOOTNOTES

Author contributions: Miki A, Sakuma Y, Shimizu A and Yasuda Y designated the overall concept and outline the manuscript; Ohzawa H, Saito A, Meguro Y, Watanabe J, Morishima K, Endo K, Sasanuma H, and Sata N contributed to the discussion and design of the manuscript; Miki A and Lefor AK contributed to the writing, editing the manuscript, illustrations, and review of literature.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of Jichi Medical University, Approval No. A21-029.

Informed consent statement: Written informed consent from any patient for data collection in a prospectively collected data base is available. However, the need for written informed consent for this study was waived by the Institutional Review Board of Jichi Medical University in view of the retrospective design of the study, based on national and local guidelines such as the fact that all clinical/ laboratory measurements and procedures were part of routine care.

Conflict-of-interest statement: The authors declare no conflicts of interest for this study.

Data sharing statement: The database contains highly confidential data which may provide insight in clinical and personnel information about patients and lead to their identification. Therefore, according to organizational restrictions and regulations these data cannot be made publicly available. However, the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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ORIGINAL ARTICLE

Retrospective Study

A new scoring system to evaluate adjuvant chemotherapy for patients with T2N0M0 gastric cancer after D2 gastrectomy

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Abstract

BACKGROUND

At present, there is insufficient medical evidence to determine whether adjuvant chemotherapy is necessary for T2N0M0 gastric cancer.

AIM

To obtain a risk score to assess the need for adjuvant chemotherapy in patients with T2N0M0 gastric cancer.

METHODS

We identified 325 patients with pathological T2N0M0 stage primary gastric cancer at the National Cancer Center between 2011 and 2018. Univariate and multivariate Cox regression analyses were performed to predict factors affecting prognosis. Vascular invasion, tumor site, and body mass index were assessed, and a scoring system was established. We compared the survival outcomes and benefits of adjuvant chemotherapy between the different subgroups.

RESULTS

Five-year survival rates of the score 0, 1, 2, and 3 groups were 92%, 95%, 80%, and 50%, respectively ($P < 0.001$). In the score 2-3 group, five-year survival rates for patients in the adjuvant chemotherapy group and postoperative observation group were 95% and 61%, respectively ($P = 0.021$).

CONCLUSION

For patients with T2N0M0 stage gastric cancer and two or more risk factors, adjuvant chemotherapy after D2 gastrectomy may have a survival benefit.

Key Words: Gastric cancer; Risk score; T2N0M0; Adjuvant chemotherapy; D2

gastrectomy; Survival

Core Tip: It is controversial whether adjuvant chemotherapy is necessary for stage T2N0M0 gastric cancer. In our study, we assessed the risk score of patients with pathologic T2N0M0 gastric cancer after D2 gastrectomy, based on clinicopathological factors, and identified a high-risk subgroup that could benefit from adjuvant chemotherapy. For patients with T2N0M0 stage gastric cancer with two or more risk factors, adjuvant chemotherapy after D2 gastrectomy may have a survival benefit.

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INTRODUCTION

Gastric cancer is one of the most common malignancies worldwide[1-3]. D2 gastrectomy combined with postoperative adjuvant chemotherapy is the main treatment modality for advanced gastric cancer[4-8]. According to the 8th edition of the American Joint Committee on Cancer (AJCC) guidelines for gastric cancer, T2 was defined as tumor invasion of the muscularis propria[9]. It is controversial whether adjuvant chemotherapy is necessary for stage T2N0M0 gastric cancer[10-16]. Previous studies have suggested that patients with stage I gastric cancer cannot benefit from adjuvant chemotherapy[17]. However, there are some risk factors for recurrence of T2N0M0 gastric cancer, such as lymphatic and/or blood vessel invasion, tumor diameter, perineural invasion, proximal tumor location, and poor differentiation[14,18]. Postoperative adjuvant chemotherapy may inhibit the recurrence in these patients. To further clarify the indications for the use of postoperative adjuvant chemotherapy in T2N0M0 gastric cancer, we reviewed 325 patients with T2N0M0 gastric cancer admitted to the National Cancer Center between 2011 and 2018. In this study, we assessed the risk score of patients with pathologic T2N0M0 gastric cancer after D2 gastrectomy based on clinicopathological factors and identified a high-risk subgroup that could benefit from postoperative adjuvant chemotherapy.

MATERIALS AND METHODS

Patient selection

We identified 402 patients with pathological T2N0M0 stage primary gastric carcinoma and gastroesophageal junction carcinoma (as defined by the AJCC guidelines, 8th edition) who were admitted to the Department of Pancreatic and Gastric Surgery, National Cancer Center, from 2011 to 2018. Three hundred and twenty-five patients were included in our study, all of whom underwent D2 gastrectomy. A total of 63 patients received post-operative adjuvant chemotherapy. The major chemotherapy regimens included platinum + 5-FU; paclitaxel + platinum + 5-FU; and others. Adjuvant chemotherapy is usually performed for 4-6 cycles. Exclusion criteria included loss to follow-up, lack of adenocarcinoma, neoadjuvant chemotherapy, adjuvant radiotherapy, Siewert I type/Siewert II type gastroesophageal junction carcinoma invading the dentate line, and postoperative survival time < 1 mo. Patients were followed-up by telephone. The follow-up was completed on April 30, 2020. The median follow-up time was 65.4 mo.

Statistical analyses

Univariate and multivariate Cox regression analyses were performed to screen for prognostic variables. Variables with a P value < 0.05 and < 0.25 in the univariate and multivariable Cox regression analyses were included in the study. Three variables were included in total: Vascular invasion, tumor site, and body mass index (BMI). The tumor site was classified as cardiac or non-cardiac. Cardiac cancer refers to Siewert type II gastroesophageal junction carcinoma that does not invade the dentate line and Siewert type III gastroesophageal junction carcinoma. BMI of < 18.5 or > 23.9, positive result of vascular invasion, and cardiac cancer were defined as risk factors. Each risk factor was assigned one point, and a total of four groups were obtained, which were defined as scores 0, 1, 2, and 3, respectively. We found that patients with a score ≥ 2 had a poor prognosis, and chemotherapy significantly improved

Table 1 Clinicopathologic variables of 325 T2N0M0 gastric cancer patients

Variable	Overall	Adjuvant chemotherapy group	Postoperative observation group	*P* value
	325	63	262	
Age (yr) *n* (%)				0.609
< 40	20 (6.2)	3 (4.8)	17 (6.5)	
≥ 40	305 (93.8)	60 (95.2)	245 (93.5)	
Sex, *n* (%)				0.878
Male	250 (76.9)	48 (76.2)	202 (77.1)	
Female	75 (23.1)	15 (23.8)	60 (22.9)	
Smoking history, *n* (%)				0.363
Yes	169 (52.0)	36 (57.1)	133 (50.8)	
No	156 (48.0)	27 (42.9)	129 (49.2)	
Family history of gastric cancer, *n* (%)				0.852
Yes	24 (7.4)	5 (7.9)	19 (7.3)	
No	301 (92.6)	58 (92.1)	243 (92.7)	
BMI, *n* (%)				0.150
< 18.5 or > 23.9	176 (54.2)	29 (46.0)	147 (56.1)	
18.5-23.9	149 (45.8)	34 (54.0)	115 (43.9)	
Postoperative hospital stay, *n* (%)				0.747
≤ 14 d	285 (87.7)	56 (88.9)	229 (87.4)	
> 14 d	40 (12.3)	7 (11.1)	33 (12.6)	
Tumor site, *n* (%)				0.004
Cardia cancer	105 (32.3)	30 (47.6)	75 (28.6)	
Non-cardia gastric cancer	220 (67.7)	33 (52.4)	187 (71.4)	
The degree of differentiation, *n* (%)				0.571
Poorly differentiated	121 (37.2)	24 (38.1)	97 (37.0)	
Moderately differentiated	178 (54.8)	36 (57.1)	142 (54.2)	
Highly differentiated	26 (8.0)	3 (4.8)	23 (8.8)	
Vascular invasion, *n* (%)				0.014
Yes	54 (16.6)	17 (27.0)	37 (14.1)	
No	271 (83.4)	46 (73.0)	225 (85.9)	

BMI: Body mass index.

prognosis. According to the study results, scores of 2-3 were defined as the high-risk group. The Kaplan-Meier method was used to calculate the 5-year survival rate and compare the overall survival (OS) between the different score groups.

Statistical analysis was performed using the R software 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) and the SPSS 22.0 software (SPSS Inc., Chicago, IL, United States). Each test was bilateral, and statistical significance was set at $P < 0.05$.

RESULTS

Clinicopathological characteristics, univariate and multivariable cox regression analyses

A total of 325 patients were recruited for this study. Table 1 summarizes the clinicopathological characteristics of the patients enrolled in this study. Univariate Cox regression analysis demonstrated that the tumor site ($P = 0.022$, Table 2), vascular invasion ($P < 0.001$, Table 2), and BMI ($P = 0.036$, Table 2) were

Table 2 Univariate Cox Proportional Hazards Modeling for overall survival

Clinicopathological features	HR (95%CI)	*P* value
Sex		
Male	Reference	
Female	0.278 (0.066-1.172)	$P = 0.081$
Smoking history		
Yes	Reference	
No	0.605 (0.276-1.323)	$P = 0.208$
Family history of gastric cancer		
Yes	Reference	
No	0.550 (0.075-4.057)	$P = 0.558$
BMI		
18.5-23.9	Reference	
> 23.9 or < 18.5	2.509 (1.060-5.937)	$P = 0.036$
Postoperative hospital stay		
≤ 14 d	Reference	
> 14 d	0.990 (0.298-3.292)	$P = 0.987$
Tumor site		
Cardia cancer	Reference	
Non-cardia gastric cancer	0.411 (0.192-0.878)	$P = 0.022$
The degree of differentiation		
Poorly differentiated	Reference	
Moderately differentiated	1.330 (0.574-3.082)	$P = 0.507$
Highly differentiated	0.857 (0.182-4.043)	$P = 0.846$
Vascular invasion		
Yes	Reference	
No	0.097 (0.044-0.212)	$P < 0.001$

BMI: Body mass index; HR: Hazard ratio; 95%CI: 95% confidence interval.

significant risk factors for OS. Multivariate Cox regression analysis demonstrated that vascular invasion was an independent risk factor for OS ($P < 0.001$, Table 3).

Vascular invasion, tumor site, and BMI were assessed in the study, and a scoring system was established.

Survival results of different groups

Figure 1A summarizes the survival curves of patients with scores of 0, 1, 2, and 3. There were significant differences among all groups except for the score 0 and 1 groups (score 0 group *vs* score 1 group, $P = 0.537$; score 0 group *vs* score 2 group, $P = 0.049$; score 0 group *vs* score 3 group, $P < 0.001$; score 1 group *vs* score 2 group, $P = 0.003$; score 1 group *vs* score 3 group, $P < 0.001$; score 2 group *vs* score 3 group, $P = 0.008$). For all patients, 5-year survival rates of the adjuvant chemotherapy and postoperative observation groups were 96% and 90%, respectively ($P = 0.676$, Table 4). Five-year survival rates of the score 0, 1, 2, and 3 groups were 92%, 95%, 80%, and 50%, respectively ($P < 0.001$, Table 4). In the score 0 and score 1 groups, there were no differences in the 5-year survival rates between the postoperative observation and adjuvant chemotherapy groups. In the score 2-3 group, 5-year survival rates for patients in the adjuvant chemotherapy group and postoperative observation group were 95% and 61%, respectively ($P = 0.021$, Table 4).

Figure 1B-D summarizes the survival curves of patients with scores of 0, 1, and score 2-3 T2N0M0 gastric cancer in the adjuvant chemotherapy and postoperative observation groups. Table 5 summarizes the distribution of the different risk factors in each risk score group.

Table 3 Multivariable Cox Proportional Hazards Modeling for overall survival

Clinicopathological features	HR (95%CI)	*P* value
Sex		
Male	Reference	
Female	0.390 (0.076-1.988)	*P* = 0.257
Smoking history		
Yes	Reference	
No	0.725 (0.308-1.710)	*P* = 0.463
Family history of gastric cancer		
Yes	Reference	
No	0.495 (0.058-4.224)	*P* = 0.521
BMI		
18.5-23.9	Reference	
> 23.9 or < 18.5	1.848 (0.760-4.490)	*P* = 0.175
Postoperative hospital stay		
≤ 14 d	Reference	
> 14 d	1.198 (0.350-4.100)	*P* = 0.960
Tumor site		
Cardia cancer	Reference	
Non-cardia gastric cancer	0.620 (0.277-1.390)	*P* = 0.246
The degree of differentiation		
Poorly differentiated	Reference	
Moderately differentiated	0.517 (0.206-1.300)	*P* = 0.161
Highly differentiated	0.390 (0.077-1.960)	*P* = 0.305
Vascular invasion		
Yes	Reference	
No	0.106 (0.045-0.246)	*P* < 0.001

HR: Hazard ratio; 95%CI: 95% confidence interval.

DISCUSSION

Our study found that adjuvant chemotherapy is necessary for the treatment of T2N0M0 gastric cancer patients with two or more risk factors. The risk factors included vascular invasion, BMI, and tumor site. Based on these results, we obtained a simple risk score to assess the need for adjuvant chemotherapy in patients with T2N0M0 gastric cancer. Patients with a score 2-3 were assigned to the high-risk group.Previous studies have shown that adjuvant chemotherapy can prolong OS in advanced gastric cancer and reduce recurrence[19]. However, evidence of the survival benefits of adjuvant chemotherapy for early gastric cancer is lacking. Although there is no lymph node metastasis in T2N0M0 gastric cancer, some patients still experience recurrence[10-16]. Therefore, it is important to identify patients with stage T2N0M0 gastric cancer who are at high risk of recurrence and may require adjuvant chemotherapy. Univariate Cox regression analysis demonstrated that tumor site (P = 0.022, Table 2), vascular invasion (P < 0.001, Table 2), and BMI (P = 0.036, Table 2) were significant risk factors for OS in patients with T2N0M0 disease. Multivariate Cox regression analysis showed that vascular invasion was an independent prognostic indicator in patients with T2N0M0 disease[14]. Tumor site has been reported to be a prognostic risk factor for stage IB gastric cancer[11]. The 5-year OS rate of patients with stage IB gastric cancer whose tumors are located in the upper third of the stomach is only 81.8%, which is lower than that of patients with stage II disease receiving S-1 adjuvant chemotherapy[5]. Another study that followed 532 patients reported poorer long-term survival in patients with proximal gastric cancer than in those with distal gastric cancer[20]. Proximal gastric cancer has a higher proportion of undifferentiated tumors, and tumors located in this region can metastasize to almost all lymph nodes, except in the

Table 4 Five-year survival rates of different groups

	Group	*n*	5-year survival rate	Log rank test
All patients				$P = 0.676$
	Adjuvant chemotherapy	63	96%	
	Postoperative observation	262	90%	
Risk score				$P < 0.001$
	Score 0 group	98	92%	
	Score 1 group	152	95%	
	Score 2 group	69	80%	
	Score 3 group	6	50%	
Score 0 group				$P = 0.825$
	Adjuvant chemotherapy	13	92%	
	Postoperative observation	85	92%	
Score 1 group				$P = 0.308$
	Adjuvant chemotherapy	22	92%	
	Postoperative observation	130	96%	
Score 2-3 group				$P = 0.021$
	Adjuvant chemotherapy	28	95%	
	Postoperative observation	47	61%	

Table 5 Distribution of three different risk factors in each score group

	BMI < 18.5 or > 23.9	Vascular invasion	Cardia cancer
All	149	54	105
Score 0 group	0	0	0
Score 1 group	92	15	45
Score 2 group	51	33	54
Score 3 group	6	6	6

BMI: Body mass index.

five groups[11]. These factors may account for the lower survival rates of patients with proximal gastric cancer. Several studies have shown that BMI affects the prognosis of patients with gastric cancer[21-26]. Low BMI was associated with malnutrition, whereas high BMI was associated with a higher risk of surgery and a higher rate of postoperative complications. A high BMI also increases the risk of stomach cancer[27]. The degree of tumor differentiation was not included in the study, possibly because poorly differentiated tumors do not show significant aggressiveness in the early stages of tumor development.

Based on these findings, we developed a scoring system to assess the need for the use of adjuvant chemotherapy in patients with T2N0M0 gastric cancer. Patients with no or only one risk factor had good prognosis after D2 gastrectomy and did not require adjuvant chemotherapy. Patients in the score 2-3 group had a significantly worse prognosis and could benefit from adjuvant chemotherapy. Our study may help to provide targeted treatment for patients with stage T2N0M0 gastric cancer.This study had some limitations. This was a single-center retrospective study with a lower level of evidence than that of a prospective study. We did not classify the patients into subgroups based on the number of lymph nodes removed. The number of lymph node dissections has a significant effect on OS. For patients with stage T1-2 node-negative gastric cancer, the 5-year survival rate increased by 7.6% for every 10 Lymph nodes examined[28]. No recurrence-free survival or recurrence pattern was observed. We did not discuss the genetic characteristics of patients with gastric cancer included in the study. Genetic characteristics of patients with gastric cancer may influence the efficacy of adjuvant chemotherapy.

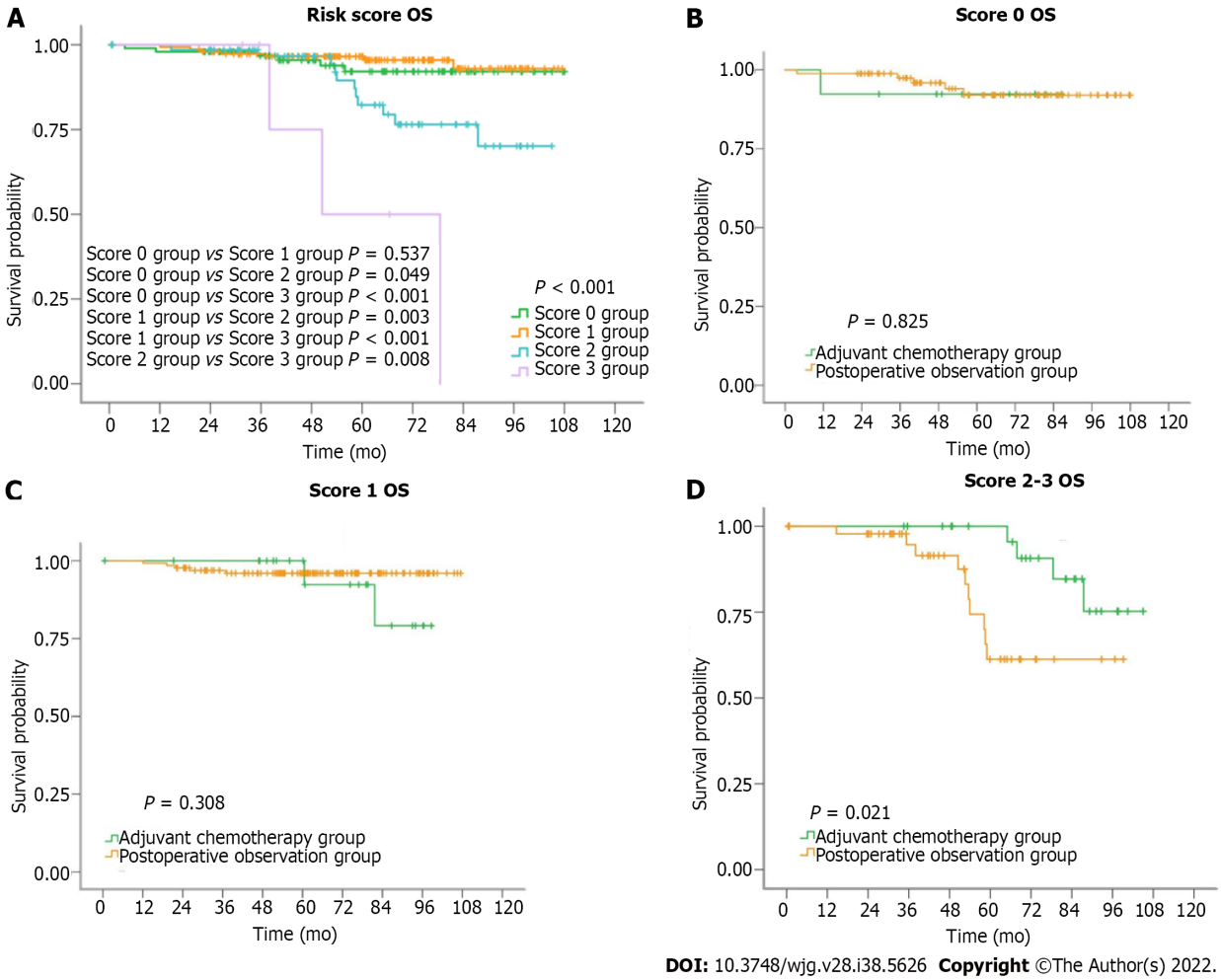

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Figure 1 Survival curves. A: The survival curves of score 0 group, score 1 group, score 2 group and score 3 group patients; B: The survival curves of score 0 T2N0M0 gastric cancer patients in adjuvant chemotherapy group and postoperative observation group; C: The survival curves of score 1 T2N0M0 gastric cancer patients in adjuvant chemotherapy group and postoperative observation group; D: The survival curves of score 2-3 T2N0M0 gastric cancer patients in adjuvant chemotherapy group and postoperative observation group. OS: Overall survival.

CONCLUSION

For patients with T2N0M0 stage gastric cancer and two or more risk factors, adjuvant chemotherapy after D2 gastrectomy may have a survival benefit. Individualized treatment should be adopted according to examination and pathological results in patients with T2N0M0 gastric cancer.

ARTICLE HIGHLIGHTS

Research background

It is controversial whether adjuvant chemotherapy is necessary for stage T2N0M0 gastric cancer.

Research motivation

To further clarify the indications for the use of postoperative adjuvant chemotherapy in patients with T2N0M0 gastric cancer.

Research objectives

To obtain a risk score to assess the need for adjuvant chemotherapy in patients with T2N0M0 gastric cancer.

Research methods

Univariate and multivariate Cox regression analyses were performed to predict factors affecting prognosis. Vascular invasion, tumor site, and BMI were assessed, and a scoring system was established.

We compared the survival outcomes and benefits of adjuvant chemotherapy between the different subgroups.

Research results

Five-year survival rates of the score 0, 1, 2, and 3 groups were 92%, 95%, 80%, and 50%, respectively ($P < 0.001$). In the score 2-3 group, five-year survival rates for patients in the adjuvant chemotherapy group and postoperative observation group were 95% and 61%, respectively ($P = 0.021$).

Research conclusions

For patients with T2N0M0 stage gastric cancer and two or more risk factors, adjuvant chemotherapy after D2 gastrectomy may have a survival benefit.

Research perspectives

Individualized treatment should be adopted according to examination and pathological results in patients with T2N0M0 gastric cancer.

FOOTNOTES

Author contributions: Xu Q and Kang WZ contributed equally to this work; Tian YT and Xu Q designed the research; Kang WZ, Xiong JP, and Shao XX analyzed the data and wrote the paper; Li WK and Hu HT collected the patient's clinical data.

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ORIGINAL ARTICLE

Observational Study

Red blood cell distribution width derivatives in alcohol-related liver cirrhosis and metabolic-associated fatty liver disease

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Abstract

BACKGROUND

Looking for undiscovered blood markers of liver fibrosis and steatosis still remains an issue worth exploring. There are still plenty of unresolved issues related to the actual role of hematological indices as potential markers of liver function.

AIM

To study red blood cell distribution width (RDW), RDW-to-platelet ratio (RPR) and RDW-to-lymphocyte ratio (RLR) in alcohol-related liver cirrhosis (ALC) and metabolic-associated fatty liver disease (MAFLD).

METHODS

The study group was composed of 302 people: 142 patients with ALC and 92 with MAFLD; 68 persons were included as controls. RDW, RPR and RLR were measured in each person. Indirect and direct parameters of liver fibrosis were also assessed [aspartate transaminase to alkaline transaminase ratio, aspartate transaminase to platelet ratio index (APRI), fibrosis-4 (FIB-4), gamma-glutamyl transpeptidase to platelet ratio (GPR), procollagen I carboxyterminal propeptide, procollagen III aminoterminal propeptide, transforming growth factor-α, platelet-derived growth factor AB, laminin]. MELD score in ALC patients and non-alcoholic fatty liver disease (NAFLD) fibrosis score together with BARD score were obtained in the MAFLD group. The achieved results were compared to controls. Then a correlation between assessed markers was done. Diagnostic value of each investigated parameter and its suggested cut-off in the research group

were evaluated with area under the curve (AUC).

RESULTS

RDW, RPR and RLR values turned out to be significantly higher in ALC and MAFLD groups compared to controls (ALC: $P < 0.0001$; NAFLD: $P < 0.05$, $P < 0.0001$ and $P < 0.0001$, respectively). RPR correlated positively with MELD score ($P < 0.01$) and indirect indices of liver fibrosis (FIB-4 and GPR; $P < 0.0001$) in ALC patients; negative correlations were found between PDGF-AB and both: RDW and RPR ($P < 0.01$ and $P < 0.0001$, respectively). RPR correlated positively with NAFLD fibrosis score and APRI ($P < 0.0001$) in the MAFLD group; a positive relationship was observed between RDW and FIB-4, too ($P < 0.05$). AUC values and suggested cut-offs for RDW, RPR and RLR in ALC patients were: 0.912 (> 14.2%), 0.965 (> 0.075) and 0.914 (> 8.684), respectively. AUC values and suggested cut-offs for RDW, RPR and RLR in MAFLD patients were: 0.606 (> 12.8%), 0.724 (> 0.047) and 0.691 (> 6.25), respectively.

CONCLUSION

RDW with its derivatives appear to be valuable diagnostic markers in patients with ALC. They can also be associated with a deterioration of liver function in this group.

Key Words: Hematological indices; Alcohol-related liver cirrhosis; Metabolic-associated liver disease; Red blood cell distribution width; Red blood cell distribution width-to-platelet ratio; Red blood cell distribution width-to-lymphocyte ratio

Core Tip: Red blood cell distribution width (RDW), RDW-to-platelet ratio (RPR) and RDW-to-lymphocyte ratio (RLR) remain uninvestigated in Polish patients suffering from alcohol-related liver cirrhosis (ALC) and metabolic-associated liver disease. Based on the available literature, association between RDW, RPR, RLR and serological (indirect and direct) indices of liver fibrosis have never been explored in a single study to date. We consider RPR as a potential reliable diagnostic tool in the assessment of ALC patients, corresponding with MELD score and serous markers of liver cirrhosis. Hematological parameters could be perceived as possible indices in the noninvasive evaluation of people with liver disorders.

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INTRODUCTION

Alcohol-related liver cirrhosis (ALC) and metabolic-associated fatty liver disease (MAFLD) belong to pathologies with an undeniable global impact. Their monitoring and prediction of a possible outcome are of crucial importance. Due to the progress in hepatology and increased access to new imaging techniques, the evaluation of advanced liver fibrosis (even without a biopsy) turned out to be available. Nevertheless, liver elastography in an ultrasound or magnetic resonance mode are not commonly accessible tools in all medical centers. Direct and indirect markers of liver fibrosis obtained from the blood still remain quite trustworthy indices, but they are not perfect, either. Thus, looking for potentially novel parameters of liver disease progression is a key goal in the field of hepatology[1-4]. The most meaningful and valuable future diagnostic tools are new noninvasive blood markers of liver fibrosis; red blood cell distribution width (RDW) together with RDW-to-platelet ratio (RPR) were suggested to be such probable indices. Nevertheless, their probable role in the course of liver steatosis has been poorly explored. RDW reflects the diversity of red blood cells in volume and size. It is obtained from automated hematology analyzers in everyday clinical practice. In the past, RDW was clinically applied only to diagnose anemia or related maladies. Nowadays the spectrum of its possible clinical performance has been significantly broadened. RDW and RPR have already been proven to achieve higher levels in the course of the neoplastic process, being connected with an overall poor survival (*e.g.*, in the course of cervical, colorectal and prostate cancers), as well[5-7]. Cardiovascular disorders and stroke constitute other pathologies that were explored in the context of RDW. Its high level was even

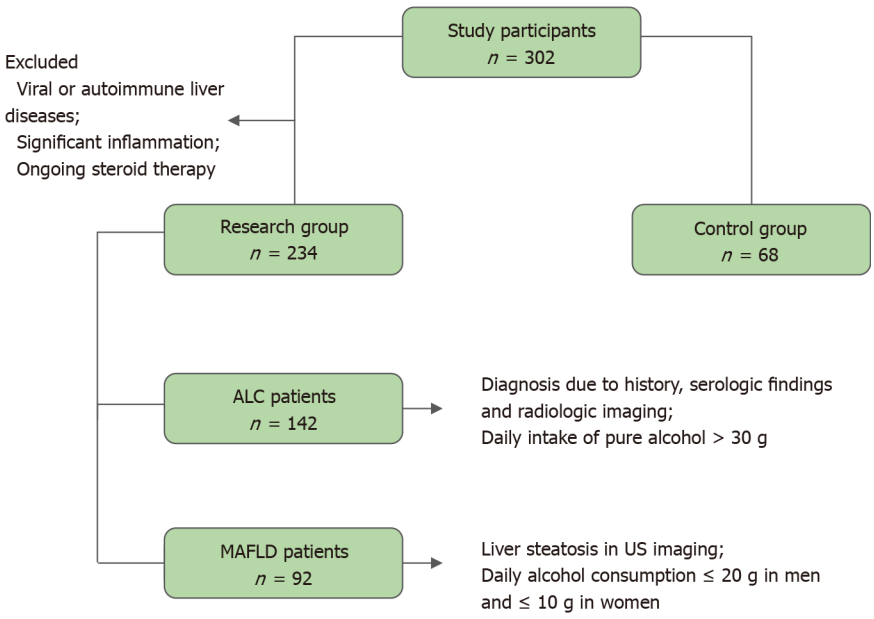

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Figure 1 Flow chart demonstrating the selection of study participants. ALC: Alcohol-related liver cirrhosis; MAFLD: Metabolic-associated fatty liver disease; US: Ultrasound.

proved to be associated with a 3-mo poor functional outcome in the course of thrombolytic therapy due to acute ischemic stroke. RDW was additionally described as a marker of acutely decompensated chronic heart failure and an indicator of increased mortality among critically ill patients with myocardial infarction[8-11]. Of note, except for RDW derivatives, hematocrit was found to be inseparably connected with the development of atherosclerosis (evaluated as internal carotid intima-media thickness) in MAFLD patients, suggesting a common potential role of hematological markers - not only in MAFLD, but also due to MAFLD-followed cardiovascular disorders[12]. Thus, RDW derivatives can be perceived as systemic markers of various pathologies. Moreover, according to previously conducted studies, RDW and RPR can even correlate with MELD score and are suggested to behave as markers of poor survival in patients with chronic liver pathologies. RDW-to-lymphocyte ratio (RLR) appears to be a relatively uninvestigated parameter in patients with liver disorders[13-16]. We suspect that no survey to date has explored the diagnostic role of RDW derivatives and their relationships with serological indices of liver fibrosis in the course of ALC and MAFLD. Furthermore, our investigation appears to be the first one performed on Polish patients to evaluate existing dependences between hematological indices and serological markers of liver fibrosis.

MATERIALS AND METHODS

The survey was accepted by the local ethics committee of the Medical University of Lublin (No. KE-0254/86/2016) and all participants signed an informed written consent on the basis of the Helsinki Declaration for the procedures they followed.

Materials

The investigation was conducted retrospectively on 302 persons. The study group was formed by 142 patients with ALC and 92 with MAFLD. The control group was represented by 68 healthy volunteers. Figure 1 displays the selection of the participants included in the survey. Table 1 shows clinical features of the study population. The diagnosis of liver cirrhosis (LC) was established in accordance with commonly used criteria. The doppler mode abdominal ultrasound examination was applied to confirm the presence of portal hypertension (diameter of portal vein ≥ 13 mm). Different underlying factors leading to portal hypertension were ruled out. Panendoscopy was performed in each of the 142 persons belonging to the ALC group - in 126 of them varices in the esophagus or stomach of the different stages were visualized. Ascites was found in 92 of the ALC patients, leading consequently to the paracentesis in 84 of them. None from the study group suffered from hepatic encephalopathy or spontaneous bacterial peritonitis. All persons enrolled to the survey were given 0/9 points on the CHESS scale. Alcohol-related etiology of LC was figured out due to the proven daily intake of pure ethanol more than 30 g. A detailed history of alcohol addiction was obtained directly from the patients or their trustworthy family members. In addition, all included in the study of ALC patients got positive results on the

Table 1 Clinical characteristics of study participants

Parameter	ALC, *n* = 142	MAFLD, *n* = 92	Controls, *n* = 68	Together, *n* = 302
Sex (F/M)	36/106	33/59	36/32	105/197
Age (yr), (mean ± SD; median; min-max)	54 ± 12; 55; 31-84	60 ± 15; 61; 22-90	46 ± 16; 45; 20-85	54 ± 15; 55; 20-90
BMI (kg/m^2) (mean ± SD; median; min-max)	25.89 ± 9.31; 25.91; 16.7-36.71	29.49 ± 4.9; 28.7; 16.26-43.01	21.95 ± 2.62; 22.45; 16.18-24.86	-
DM type 2	0/142	22/92	-	-
AH	32/142	46/92	-	-

F: Female; M: Male; min: Minimum; max: Maximum; BMI: Body mass index; DM: Diabetes mellitus; AH: Arterial hypertension.

conducted CAGE test. On the other hand, MAFLD was diagnosed according to the previous history, physical examination, laboratory findings and ultrasound imaging. A daily alcohol consumption amount was not greater than 20 g in men and 10 g in women. Disorders potentially responsible for the development of liver steatosis (celiac disease, Wilson's disease, hepatobiliary infections and alpha-1-antitrypsin deficiency) had been ruled out. Diabetes mellitus type 2 was identified among 22 persons. The presence of diabetes mellitus type 1 caused the exclusion from the survey. None of the MAFLD patients was diagnosed with impaired fasting glucose. Forty six of them were found to suffer from arterial hypertension; 84 persons presented features of metabolic syndrome. Viral/autoimmune liver diseases, clinically relevant inflammatory process and anemia constituted other excluding criteria. None of the survey participants was on steroid therapy.

Procedures

Venous blood samples obtained from peripheral blood were collected from all study participants. EDTA was applied to evaluate hematological indices and citrate to evaluate clotting. Biochemical indices were obtained from the blood samples without an anticoagulant. We collected the blood after at least 12 h of fasting. Hematological and biochemical procedures were performed 4 h after blood sample collection. All of the measurements were conducted in the laboratory of Clinical Hospital Number 4, Lublin, Poland. The remaining part of the blood samples, without an anticoagulant, were centrifuged at the speed of 2000 *g* for 10 min within 15 min after blood collection. The obtained serum was then stored in 1 mL Eppendorf test tubes at the temperature of -80 °C until the evaluation of direct markers of liver fibrosis with ELISA. Among morphotic parameters of the blood, the RDW, RPR and RLR were obtained. The evaluation of indirect indices of liver cirrhosis concerned: Aspartate transaminase to alkaline transaminase ratio (AAR), aspartate transaminase to platelet ratio index (APRI), FIB-4 (fibrosis-4) and gamma-glutamyl transpeptidase to platelet ratio (GPR) (GGT to PLT Ratio). MELD score was assessed in the ALC patients and non-alcoholic fatty liver disease (NAFLD) fibrosis score and BARD score were applied to the MAFLD group. Procollagen I carboxyterminal propeptide (PICP), procollagen III aminoterminal propeptide (PIIINP), platelet-derived growth factor AB (PDGF-AB), transforming growth factor-α (TGF-α) and laminin belonged to direct indices of liver fibrosis that were measured. Laboratory procedures were performed in the Department of Biochemistry and Molecular Biology, Medical University of Lublin according to the recommended indications. The assessment of PICP and PIIINP was conducted with quantitative ELISA tests. PDGF-AB and TGF-α were evaluated with the use of R&D Systems Quantikine ELISA Kits. Finally, the measurement of the concentration of laminin was possible because of the Takara Laminin EIA Kit without Sulphuric Acid.

Statistical analysis

Statistical analysis of the results was carried out with Statistica 13.0 (StatSoft Polska Sp. z o.o., Kraków, Poland) for Windows system. The demographic characteristics of study participants and findings of laboratory investigations were shown as the mean ± SD and Student's *t* test was applied to compare these data. Deviation from normality was assessed with the use of Kolmogorov-Smirnov test. Data collected in the study were shown as the median and range (minimum–maximum). The Mann-Whitney *U* test was applied for between-group comparisons due to non-normal distribution. The correlations were verified with Spearman correlation analyses. All probability values were two-tailed, and a value of *P* less than 0.05 was perceived as statistically significant. Receiver operating characteristic (ROC) curves and area under the curve (AUC) values were used to evaluate the sensitivity and specificity of investigated indices and to verify suggested cut-offs of tested markers in ALC and MAFLD patients.

Table 2 Results of used scores in research group

Score	ALC				MAFLD			
	mean ± SD	Median	Min	Max	mean ± SD	Median	Min	Max
MELD	17 ± 8	16	6	45	-	-	-	-
BARD	-	-	-	-	2 ± 1	2	0	4
NAFLD fibrosis score	-	-	-	-	-1.36 ± 1.5	-1.16	-5.83	1.74

Min: Minimum; Max: Maximum; MELD: Model for end-stage liver disease; NAFLD: Non-alcoholic fatty liver disease.

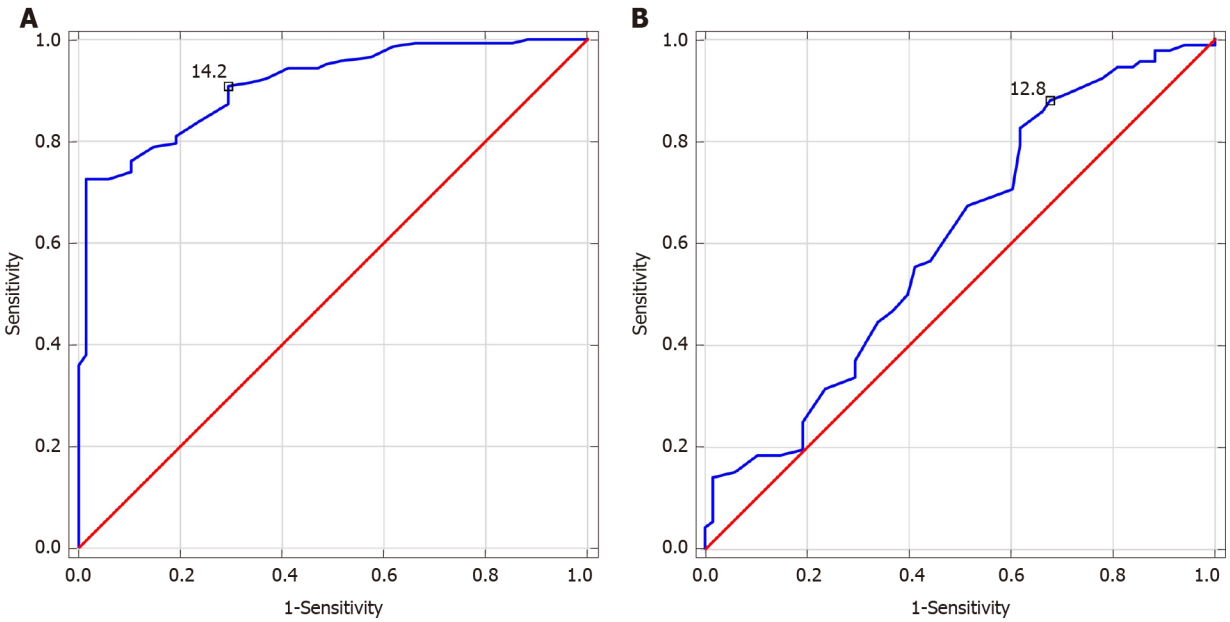

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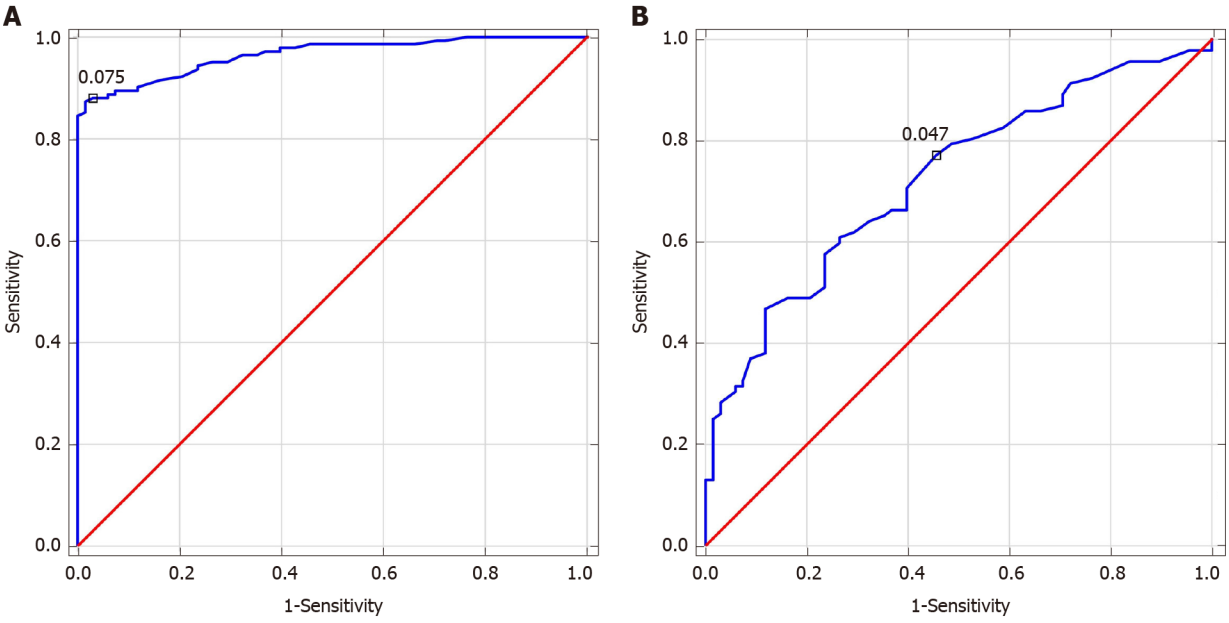
Figure 2 Receiver operating characteristics for red blood cell distribution width. A: Alcohol-related liver cirrhosis (ALC), area under the curve (AUC) = 0.912 (cut-off > 14.2%); B: Metabolic-associated fatty liver disease (MAFLD), AUC = 0.606 (cut-off > 12.8%). Youden index cut-off for red blood cell distribution width in ALC and MAFLD groups = 15.1% and 13%, respectively.

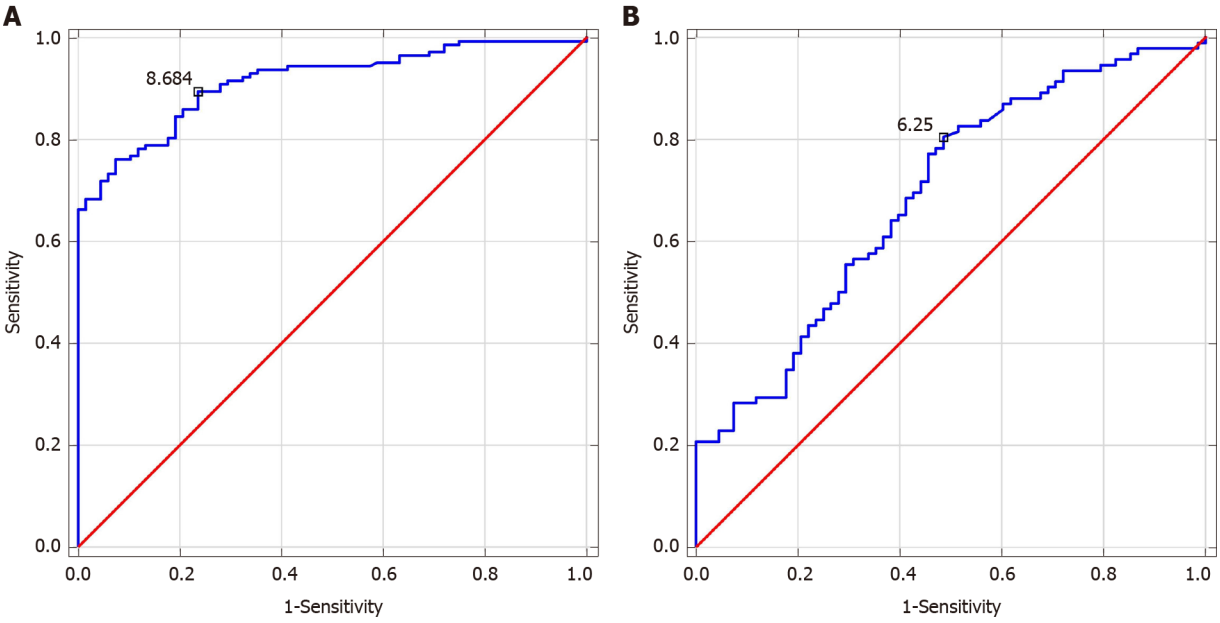
RESULTS

Table 2 presents results of applied scores in the research group. Results of hematological parameters are presented in Table 3. The RDW level was above the reference range in both the ALC and MAFLD groups; its level was significantly higher in comparison to the controls ($P < 0.0001$ and $P < 0.05$, respectively). RPR and RLR values turned out to be significantly higher among ALC and MAFLD patients compared to the control group ($P < 0.0001$). Table 3 presents results of indirect and direct indices of liver fibrosis, as well. The medians of AAR, APRI, FIB-4 and GPR were shown to be significantly higher within the ALC group in comparison to the controls ($P < 0.0001$). Excluding AAR, patients with MAFLD were observed to present notably higher values of all the enumerated above indirect markers of liver fibrosis compared to the control group ($P < 0.0001$). According to the direct parameters of liver fibrosis, laminin value in the course of ALC was importantly higher than in the control group ($P < 0.05$). Except for PICP, medians of PIIINP, PDGF-AB and TGF-α had notably lower levels ($P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively). Concentrations of TGF-α and laminin in MAFLD patients in comparison to controls were found to be remarkably lower ($P < 0.0001$). PICP, PIIINP and PDGF-AB medians did not differ in a significant pattern. Table 4 presents noted correlations between evaluated indices in ALC and MAFLD groups. We observed meaningful positive dependences between RPR and indirect parameters of liver cirrhosis - FIB-4 and GPR ($P < 0.0001$) in the ALC patients. RDW and RPR correlated in a negative pattern with PDGF-AB ($P < 0.01$ and $P < 0.0001$, respectively). A positive correlation was found between RPR and MELD scores ($P < 0.01$). Other positive dependences were observed between: RDW and FIB-4 ($P < 0.05$), and between RPR and APRI ($P < 0.0001$), in the MAFLD group. RPR correlated positively with the NAFLD fibrosis scores ($P < 0.0001$), as well. Table 5 shows diagnostic accuracy of evaluated hematological markers. ROCs describing investigated parameters in the ALC and MAFLD groups are shown below in Figures 2-4. AUC values with



DOI: 10.3748/wjg.v28.i38.5636

Figure 3 Receiver operating characteristics for red blood cell distribution width-to-platelet ratio. A: Alcohol-related liver cirrhosis (ALC), area under the curve (AUC) = 0.965 (cut off > 0.075); B: Metabolic-associated fatty liver disease (MAFLD), AUC = 0.724 (cut-off > 0.047). Youden index cut-off for red blood cell distribution width-to-platelet ratio in ALC and MAFLD groups = 0.08 and 0.06, respectively.



DOI: 10.3748/wjg.v28.i38.5636

Figure 4 Receiver operating characteristics for red blood cell distribution width-to-lymphocyte ratio. A: Alcohol-related liver cirrhosis (ALC), area under the curve (AUC) = 0.914 (cut-off > 8.684); B: Metabolic-associated fatty liver disease (MAFLD), AUC = 0.691 (cut-off > 6.25). Youden index cut-off for red blood cell distribution width-to-lymphocyte ratio in ALC and MAFLD groups = 11.16 and 6.25, respectively.

suggested cut-offs for RDW, RPR and RLR in ALC patients were: 0.912 (> 14.2%), 0.965 (> 0.075) and 0.914 (> 8.684), respectively. AUC values and proposed cut-offs for RDW, RPR and RLR in the MAFLD groups were: 0.606 (> 12.8%), 0.724 (> 0.047) and 0.691 (> 6.25), respectively.

DISCUSSION

New reliable blood markers required in the evaluation of liver fibrosis and clinical prognosis of cirrhosis are permanently in the interest of scientists. Several years ago, basic hematological indices, used in

Table 3 Results of indirect and direct markers of liver fibrosis together with hematological markers in examined patients

Parameter (reference range)	ALC				MAFLD				Controls			
	mean ± SD	Median	Min	Max	mean ± SD	Median	Min	Max	mean ± SD	Median	Min	Max
RDW (11%-15%)	17.27 ± 3.26	**16.7**[d]	12.2	27.9	14.25 ± 2.52	**13.85**[a]	4.2	25	13.45 ± 1.1	**13.4**	11.1	15
RPR	0.26 ± 0.37	**0.18**[d]	0.04	3.55	0.06 ± 0.02	**0.06**[d]	0.02	0.18	0.05 ± 0.01	**0.05**	0.03	0.08
RLR	19.14 ± 12.27	**15.73**[d]	1.6	79.23	10.89 ± 8.55	**8.13**[d]	0.66	60	7.09 ± 2.48	**6.24**	3.38	12.5
FIB-4	11.67 ± 25.46	**6.34**[d]	0.69	287.59	1.92 ± 1.63	**1.57**[d]	0.23	11.58	0.85 ± 0.54	**0.71**	0.28	3.27
GPR	15.73 ± 28.54	**6.65**[d]	0.18	188.71	2.76 ± 5.57	**0.54**[d]	0.13	35.41	0.25 ± 0.1	**0.24**	0.06	0.63
PICP (ng/mL)	63.32 ± 31.53	**60.53**	6.15	161.12	52.14 ± 27.56	**46.08**	10.10	147.27	58.26 ± 37.39	**44.18**	0	202.89
PIIINP (ng/mL)	9.28 ± 4.33	**8.4**[b]	2.43	28.65	11.41 ± 3.99	**11.00**	2.18	25.35	11.07 ± 5.61	**10.25**	4.35	43.63
PDGF-AB (pg/mL)	18280.47 ± 8061.06	**17343.71**[c]	1925.68	42823.84	26858.68 ± 7335.09	**26682.83**	10821.02	49808.07	23579.28 ± 10068.8	**25623.2**	1638.2	47758.7
TGF-α (pg/mL)	24 ± 45.33	**13.77**[d]	0.872	507.09	17.89 ± 19.18	**12.09**[d]	1.39	142.63	28.44 ± 17.21	**24.59**	1.31	93.55
Laminin (ng/mL)	976.34 ± 705.29	**832.06**[a]	101.933	3301.00	48 ± 230.24	**375.23**[d]	72.87	1335.92	718.24 ± 386.1	**663.27**	140.88	1813.88

[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.
[d]$P < 0.0001$.

ALC: Alcoholic liver cirrhosis; MAFLD: Metabolic associated fatty liver disease; Min: Minimum; Max: Maximum; AAR: Aspartate transaminase to alkaline transaminase ratio; APRI: Aspartate transaminase to platelet ratio index; FIB-4: Fibrosis-4; GPR: Gamma-glutamyl transpeptidase to platelet ratio; PICP: Procollagen I carboxyterminal propeptide; PIIINP: Procollagen III aminoterminal propeptide; PDGF-AB: Platelet-derived growth factor AB; TGF-α: Transforming growth factor-α; RDW: Red blood cell distribution width; RPR: Red blood cell distribution width-to-platelet ratio; RLR: Red blood cell distribution width-to-lymphocyte ratio.

everyday life, were proposed as potential candidates in this area, *e.g.*, mean platelet volume, platelet crit and RDW. It is assumed that their linkage with liver disorders is due to the inflammation and the release of interleukin-6, interfering hemolytic anemia, hypersplenism and bone marrow activation. The greatest number of surveys in this area of hepatology seems to concern hepatitis B virus- and hepatitis C virus-related cirrhosis; the ALC and MAFLD patients were not explored as much[17-20]. Moreover, a quick diagnosis and further staging of liver steatosis is also a crucial goal nowadays, due to the global prevalence of MAFLD together with its possible severe consequences. RDW derivatives require further investigations in the context of liver disorders. Being the marker of red blood cells heterogeneity, RDW was commonly used as an indicator of anemia. However, it turned out that its potential role can be even more complex. Former surveys proved that the progression of liver cirrhosis is accompanied by an increase in RDW and its elevation might even be a marker of poor prognosis. Similar observations were made with reference to its derivative - RPR. Recent meta-analyses by Cai *et al*[21] and Milas *et al*[22] confirmed these data. RDW and RPR were even found to be markers of increased mortality in ALC

Table 4 Correlations between examined parameters in examined alcohol-related liver cirrhosis and metabolic-associated fatty liver disease patients

Pair	R Spearman	*P* value
ALC		
RPR *vs* FIB-4	0.733	$P < 0.0001$
RPR *vs* GPR	0.398	$P < 0.0001$
RPR *vs* MELD	0.267	$P < 0.01$
RDW *vs* PDGF-AB	-0.257	$P < 0.01$
RPR *vs* PDGF-AB	-0.380	$P < 0.0001$
MAFLD		
RDW *vs* FIB-4	0.215	$P < 0.05$
RPR *vs* APRI	0.469	$P < 0.0001$
RPR *vs* NFS	0.688	$P < 0.0001$

ALC: Alcoholic liver cirrhosis; MAFLD: Metabolic-associated fatty liver disease; RDW: Red blood cell distribution width; RPR: Red blood cell distribution width-to-platelet ratio; MELD: Model of end stage liver disease; APRI: Aspartate transaminase to platelet ratio index; FIB-4: Fibrosis-4; GPR: Gamma-glutamyl transpeptidase to platelet ratio; PDGF-AB: Platelet-derived growth factor AB; NFS: NAFLD fibrosis score.

patients - independently of MELD score. Lately, RDW elevation was found to accompany severe inflammation and liver fibrosis in three independent studies on autoimmune hepatitis patients[23-25]. RDW was even proposed as a prognostic marker in the course of hepatocellular carcinoma[26,27]. What is more, recently a higher level of RLR has been explored as a potential marker of its recurrence[28]. In our ALC group, RPR correlated positively with MELD score and the diagnostic accuracy of both: RDW and RPR turned out to be high (AUC = 0.912 and AUC = 0.965, respectively). A close relationship between RDW derivatives and indirect indices of liver fibrosis in the course of cirrhosis was already established and our results support this association. However, it seems that previous surveys did not explore relationships between RDW derivatives and indirect parameters of liver fibrosis. According to the available literature, we are the first to note a negative correlation between PDGF-AB concentration and both: RDW and RPR levels in the course of ALC. RLR appears to be explored so far, among liver pathologies, only in the course of primary biliary cholangitis (PBC) and in people affected with acute hepatitis E virus (HEV) infection. Its diagnostic accuracy in the assessment of liver fibrosis in PBC patients and in the diagnosis of HEV infection in symptomatic patients was better compared to APRI, FIB-4 and RPR[29-31]. Our study appears to be the first one concentrating on the role of RLR in ALC and MAFLD. Interestingly, its diagnostic accuracy in the ALC patients turned out to be very high (AUC = 0.914). The data on RDW derivatives in MAFLD patients are very limited. However, previous research observed that elevation in RDW can be assumed as a sign of the progression of simple steatosis to steatohepatitis and the progression of liver fibrosis in the course of MAFLD[32-36]. In our examined MAFLD patients, RPR correlated positively with indirect parameters of liver fibrosis and NAFLD fibrosis score, however, its diagnostic accuracy was quite disappointing (AUC = 0.606). In our survey, we did not aim to perform the comparison of selected hematological markers between the ALC and MAFLD patients. The purpose of our study was to find out whether deviations in certain hematological parameters might accompany an isolated liver steatosis. Our investigation focused on the population of people with MAFLD without the evaluation of possible features of hepatitis in liver biopsy. In the future, we would like to differentiate patients with a simple steatosis and already developed steatohepatitis confirmed by liver biopsy in the context of potential deviations among hematological markers.

CONCLUSION

In conclusion, our study gives a new insight into the tight relationship between serological indices of liver fibrosis and RDW derivatives in patients with liver pathologies. Especially, RLR seems to be a valuable unexplored parameter in the course of ALC. Its high diagnostic accuracy in our ALC patients is a promising message for subsequent studies. Undoubtedly, this direction of research should be continued to define a potential role of commonly accessible RDW derivatives as indicators of liver disorders in everyday clinical life.

Table 5 Diagnostic accuracy of hematological indices in examined alcohol-related liver cirrhosis and metabolic-associated fatty liver disease patients

Parameter	ALC								MAFLD							
	Diagnostic accuracy								Diagnostic accuracy							
	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	*P* value	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	*P* value
RDW	**0.912**	91	71	87	79	3.14	0.13	$P < 0.0001$	**0.606**	88	32	64	67	1.29	0.38	$P < 0.05$
RPR	**0.965**	88	97	98	80	29.33	0.12	$P < 0.0001$	**0.724**	77	54	70	64	1.67	0.43	$P < 0.0001$
RLR	**0.914**	89	77	89	78	3,87	0,14	$P < 0.0001$	**0.691**	80	52	69	66	1.67	0.38	$P < 0.0001$

ALC: Alcoholic liver cirrhosis; MAFLD: Metabolic-associated fatty liver disease; AUC: Area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; PLR: Positive likelihood; NLR: Negative likelihood ratio.

ARTICLE HIGHLIGHTS

Research background

The most meaningful and valuable future diagnostic tools are new noninvasive blood markers of liver fibrosis, and red blood cell distribution width (RDW) together with RDW-to-platelet-(PLT)-ratio (RPR) were suggested to be such probable indices. Their potential role in the course of liver disorders still requires further elucidation.

Research motivation

In light of the above, we decided to study the diagnostic role of RDW derivatives and their relationships with direct and indirect indices of liver fibrosis in patients with alcohol-related liver cirrhosis (ALC) and metabolic-associated fatty liver disease (MAFLD). It seems to be the first investigation performed among Polish patients to evaluate the potential dependences between hematological parameters and serological markers of liver fibrosis.

Research objectives

The aim of the study was the evaluation of RDW, RPR and RDW-to-lymphocyte ratio (RLR) in ALC and MAFLD patients.

Research methods

The study group was comprised of 302 persons: 142 patients with ALC, 92 with MAFLD and 68 volunteers as controls. RDW, RPR and RLR were assessed in each participant. Indirect and direct indices of liver fibrosis were also measured [aspartate transaminase to alkaline transaminase ratio, aspartate transaminase to platelet ratio index (APRI), fibrosis-4 (FIB-4), gamma-glutamyl transpeptidase to platelet ratio (GPR), procollagen I carboxyterminal propeptide, procollagen III aminoterminal propeptide, transforming growth factor-α, platelet-derived growth factor AB, laminin]. MELD score in the ALC patients and NAFLD fibrosis score together with BARD score were obtained in the MAFLD

group. The achieved results were compared to controls. Then a correlation between investigated parameters was done. Diagnostic value of each evaluated marker together with suggested cut-off in the research group were assessed with area under the curve (AUC).

Research results

RDW, RPR and RLR values were significantly higher in ALC and MAFLD patients in comparison to controls. RPR correlated positively with MELD score and indirect parameters of liver fibrosis in the ALC group. RPR correlated positively with the NAFLD fibrosis score and APRI in the MAFLD patients; a positive dependency was noted between the RDW and FIB-4. The AUC values and suggested cut-offs for RDW, RPR and RLR in ALC patients were: 0.912 (> 14.2%), 0.965 (> 0.075) and 0.914 (> 8.684), respectively. AUC values and proposed cut-offs for RDW, RPR and RLR in MAFLD patients were: 0.606 (> 12.8%), 0.724 (> 0.047) and 0.691 (> 6.25), respectively.

Research conclusions

Our study gives a new insight into the tight relationship between serological indices of liver fibrosis and RDW derivatives in ALC and MAFLD patients. RLR seems to be a valuable unexplored parameter in the course of ALC patients. Its high diagnostic accuracy in ALC is a promising message for subsequent studies. Undoubtedly, this direction of research should be continued to define a potential role of commonly accessible RDW derivatives as indicators of liver disorders in everyday clinical life.

Research perspectives

It seems that our results have important clinical implications and could be applied in everyday diagnostics of patients with liver disorders.

FOOTNOTES

Author contributions: Michalak A, Lach T and Cichoż-Lach H prepared data; Michalak A and Cichoż-Lach H invented and conducted the study; Guz M, Kozicka J and Jeleniewicz W applied the experiments, gained and interpreted data; Cybulski M statistically analyzed the data; Michalak A and Cichoż-Lach H wrote the article; All authors accepted the current version of the manuscript.

Institutional review board statement: The survey was accepted by the local ethics committee of the Medical University of Lublin (No. KE-0254/86/2016).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

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SCIENTOMETRICS

Comparison of evaluation indexes for Gastroenterology and Hepatology journals in different databases

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Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

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Grade B (Very good): B
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Grade D (Fair): 0
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Abstract

BACKGROUND

Accurate assessment of the quality of academic journals is of great significance. While Journal Impact Factor (JIF), calculated by Clarivate and based upon the Web of Science literature database, and CiteScore (CS), developed by Elseiver and based upon the Scopus database, have enjoyed high uptake worldwide, efforts continue towards creation of other scientometric indexes that will provide ever-greater qualitative insights into journal impact. Such efforts have yielded the newly-launched *Journal Article Influence Index* (*JAII*), which is based on the *Reference Citation Analysis* (*RCA*) database, an open multidisciplinary citation analysis database based on artificial intelligence technology.

AIM

To evaluate and summarize the similarities and differences between *JAII* and JIF/CS as journal evaluation indicators, and provide an intuitive method for visual representation of the related data.

METHODS

We searched the Journal Citation Reports to obtain the 2021 JIF list, downloaded the CS list updated in July on the Scopus website, and collected the comprehensive list of 2022 *JAII*s from the *RCA* database (www.referencecitationanalysis.com).

RESULTS

Our research results revealed that by breaking through the time limit of mainstream journal evaluation methods, the *JAII* is able to perform well in data reliability, establishing its benefit as a complementary scientometric index to JIF and CS.

CONCLUSION

JAII provides comprehensive assessment of the quality and performance of journals.

Key Words: *Journal Article Influence Index*; Journal Impact Factor; CiteScore; Gastroenterology and Hepatology; Scientometric index

Core Tip: Compared with Journal Impact Factor (JIF) and CiteScore (CS), the newly-launched *Journal Article Influence Index* (*JAII*) breaks through the time limit feature of the former indexes. A key benefit of the *JAII* is that it does not require the temporal path (wait-time) of JIF and CS to accurately evaluate a journal's impact. As such, *JAII* is immediately useful for assessing the performance of journals and the drawbacks of time randomness are overcome. Here, we describe the features of *JAII* as a comprehensive assessment of the quality and performance of journals, in its functionality based upon the *Reference Citation Analysis* (*RCA*) database that covers some more specific journals than other literature databases.

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INTRODUCTION

The quality assessment of peer-reviewed published research is important for the reputation, substance and growth of various professional associations, individual scientists, and academic institutions, as well as the funding organizations that evaluate and support them[1]. The quality of scientific contributions is primarily assessed on a temporal basis, with quantitative evaluation of the long-term impact in a field or discipline. The impact of an individual scientific article can be inferred from the citations that it receives. A similar principle is applied to evaluation of the journals that publish these scientific articles[2]. These long-standing efforts have led to researchers proposing various methods that improve the assessment of the quality of scientific journals[3,4]. What most of these methods have in common, though, is the use of complex mathematical algorithms to analyze networks of scientific papers to estimate citation quality.

First proposed by Eugene Garfield in 1955, the Scientific Citation Index, Journal Citation Reports [(JCR); published by the Institute for Scientific Information (ISI)] aims to rank, evaluate, classify, and compare journals[5]. The involved metrics are calculated based on the number of articles published by a journal and the number of times that a journal is cited. Moreover, they have been widely adopted as tools to evaluate researchers and research work in a wide range of scientific settings. One of the most prominent among such indicators is the Journal Impact Factor (JIF).

In addition to the JIF, other metrics provided by the ISI include total citation frequency, immediacy index, number of source entries published in the current year, frequency of citations in the previous 2 years, cited half-life, and the ratio of different citations for each article. The ISI introduced a simplified system in 1974, along with a list of topic categories and an accompanying catalog of the total 176 JCR journals. In recent decades, the different journal categories have been subjected to many holistic analyses. The resultant definitions of the common characteristics that underpin particular types of journals and relate to the JIF have served as a useful tool for researchers, both in the scientometric field and in general as contributing authors, to better evaluate journal impact[6,7]. To this day, journals are ranked by JIF within their assigned category. The journals listed in the JCR are further subgrouped by the ranking of JIF-related indicators (*i.e.* JIF variation coefficient, *etc*); this greater detailed categorization has allowed scholars to perlustrate the impact factor values more intuitively from a holistic and comprehensive perspective.

JIF has been the most widely used indicator of quality of scientific journals over the past decades[8]. However, in accordance with the 1999 announcement by the ISI/JCR that the accuracy of JIF is not fully guaranteed[9], it is important to note that the methodological considerations in the JIF calculation still include a lack of assessment of the quality of citations, the inclusion of self-citations, poor comparability between different scientific fields, and an analysis of publications mainly in English[10]. This is in addition to the fact that JIFs of journals representing different disciplines are not comparable to each

other.

On December 8, 2016, Scopus launched the CiteScore (CS) quality metric, in direct competition of JIF but which was developed specifically for journals indexed by Scopus. Over the past few years, the number of journals assigned a CS has increased dramatically, especially for journals that are not included in the JIF annual assignments. Scientometric studies evaluating the relationship between CS and JIF have revealed that although there is a strong correlation between the two metrics, there are also obvious complex differences[11,12]. While CS may be more balanced and most certainly is more transparent[13], it also shares some key limitations with the JIF[14,15].

Reference Citation Analysis (*RCA*) is a very recently launched open multidisciplinary citation analysis database based on artificial intelligence technology. This database covers a wide array of seemingly disparate disciplines such as business, economics and management, chemistry and materials science, engineering and computer science, health and medical sciences, humanities, literature and arts, life sciences and earth sciences, physics and mathematics, and social sciences. Users can search the collective literature based on fields such as author, category, DOI, ISSN, keyword, ORCID number, publication name, PubMed ID, and title to track original innovative research results and cutting-edge progress; they can also sort results by an article impact index metric. Importantly, the results analysis functionality culminates in a comprehensive and customizable report of the retrieved results.

Based on the *RCA* database, the *Journal Article Influence Index* (*JAII*) metric is officially available as a new indicator of journal quality that is calculated *via* the normal approach of quantifiable citations. Systematically comparing this new metric to traditional journal evaluation metrics will help ensure the accuracy of *JAII*. With acknowledgement of the continuous deepening of research in the field of Gastroenterology and Hepatology of recent years[16], we performed such a comparative analysis to determine the similarities and differences between *JAII* and JIF/CS as journal evaluation indicators, with the ultimate aim of providing an intuitive method for visual representation of the related data.

MATERIALS AND METHODS

Data sources

The raw data for this study was obtained in July 2022 from the official websites of the institutions that released each metric under consideration. We searched the JCR to obtain the 2021 JIF list, downloaded the CS list updated in July from the Scopus website, and collected the 2022 *JAII* list from the *RCA* database (www.referencecitationanalysis.com). In addition, we also searched for information related to the characteristics of these scientific journal quality indexes for reference.

Besides, based on the results of *RCA* search by the Gastroenterology and Hepatology category, we compared *JAII* to JIF and CS respectively. The resultant data from the *RCA* database were used as the matching benchmark, and the matching method was based on ISSN, EISSN, and journal name.

RESULTS

Statistical analysis and visualization

The Gastroenterology and Hepatology-categorized journals identified in each database are presented in Table 1 (grouped by the evaluation indicator and in descending order according to the respective quality metric value). In total, 102 journals carried a *JAII*, 81 carried a JIF, and 76 carried a CS (all assigned in 2021).

Next, in order to make an intuitive comparison between the three evaluation indicators, we drew a scatter distribution plot for JIF-*JAII* (Figure 1A) and CS-*JAII* (Figure 1B), and plotted a single-timepoint uniform curve using the least squares method[17]. In this case, we took an intersection, considering that some journals with *JAII* have no JIF or CS. It can be seen from the figure that in the evaluation of lower-quality journals, the linearity of *JAII* and JIF/CS has greater overlap, but in the evaluation of higher-quality journals, the randomness of the data is greater. Journals with a large deviation between JIF and *JAII* include *Nature Reviews Gastroenterology & Hepatology*, *Lancet Gastroenterology & Hepatology*, *Seminars in Liver Disease*, and so on. Journals with a large deviation between CS and *JAII* include *Gut*, *Journal of Hepatology*, *Gastroenterology*, and so on.

The results of the combined analysis of the three journal evaluation indicators are visualized in Figure 2A-C[18]. Figure 2A gives a comparison of the values between the three evaluation indicators of the same journal (73 in total, taking the intersection). Figure 2B gives the JIF-*JAII* ratio and CS-*JAII* ratio for each journal. Figure 2C gives the values of JIF and CS in descending *JAII* order.

Finally, we combined the three journal evaluation indicators together, and through a histogram (Figure 2D), we can more clearly see the impact of the joint evaluation of the three on the ranking of journals without weight. This can also be used as a reference evaluation method.

Table 1 Comparison of *Journal Article Influence Index*, Journal Impact Factor, and CiteScore in decreasing order of *Journal Article Influence Index* values

Journal name	2022 *JAII*	2021 JIF	2021 CS
Seminars in Liver Disease	48.011	6.512	9.4
Hepatology	43.087	17.298	25.8
Gastroenterology	37.347	33.883	33
Gut	36.77	31.793	40.1
Nature Reviews Gastroenterology & Hepatology	35.564	73.082	-
Gut Microbes	31.922	9.434	9.4
Alimentary Pharmacology & Therapeutics	28.815	9.524	-
Journal of Hepatology	28.63	30.083	39.2
Best Practice & Research Clinical Gastroenterology	28.443	2.695	-
Diseases of the Colon & Rectum	26.986	4.412	-
Liver Transplantation	26.916	6.112	8
Gastric Cancer	24.132	7.701	12.5
Lancet Gastroenterology & Hepatology	23.661	45.042	-
The American Journal of Gastroenterology	23.599	12.045	-
Journal of Gastroenterology	22.863	6.772	13.7
Clinical Gastroenterology and Hepatology	22.413	13.576	12.2
Neurogastroenterology and Motility	22.381	3.96	6.5
World Journal of Gastroenterology	21.897	5.374	8.1
American Journal of Physiology-Gastrointestinal and Liver Physiology	21.407	4.871	-
Journal of Gastrointestinal Surgery: Official Journal of the Society for Surgery of the Alimentary Tract	20.787	3.267	-
Liver International	19.971	8.754	11.2
Clinics in Liver Disease	19.939	6.265	8
Journal of Viral Hepatitis	19.545	3.517	6.1
Digestive Diseases and Sciences	19.37	3.487	5.5
World Journal of Gastrointestinal Pathophysiology	18.735	-	-
Scandinavian Journal of Gastroenterology	18.364	3.027	3.6
Gastrointestinal Endoscopy	18.175	10.396	9.8
Helicobacter	18.162	5.182	8.6
Inflammatory Bowel Diseases	17.936	7.29	9.8
Gastroenterology Clinics of North America	17.833	3.867	6.1
Journal of Pediatric Gastroenterology and Nutrition	17.742	3.288	4.8
Hepatology International	17.664	9.029	8.9
Journal of Clinical Gastroenterology	16.888	3.174	5.5
Journal of Gastroenterology and Hepatology	16.793	4.369	6
World Journal of Hepatology	16.007	-	3.6
International Journal of Colorectal Disease	15.433	2.796	3.9
Gut Pathogens	15.39	5.324	6.5
World Journal of Gastrointestinal Pharmacology and Therapeutics	14.797	-	-
Pancreas	14.71	3.243	4.4
HPB: The Official Journal of the International Hepato Pancreato Biliary Association	14.453	3.842	-

International Journal of Hepatology	14.249	-	6.1
European Journal of Gastroenterology & Hepatology	14.227	2.586	-
Therapeutic Advances in Gastroenterology	13.823	4.802	5.8
Journal of Neurogastroenterology and Motility	13.594	4.725	7.4
Pancreatology	13.497	3.977	5.8
Hepatology Research	13.332	4.942	7.8
Gut and Liver	13.193	4.321	6.6
Digestive Diseases	13.081	3.421	4.2
BMC Gastroenterology	12.991	2.847	3.3
Endoscopy	12.541	9.776	11
Journal of Crohn's & Colitis	12.432	10.02	
Colorectal Disease	12.341	3.917	4.4
Liver Cancer	12.174	12.43	12.6
Digestive and Liver Disease: Official Journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver	12.096	5.165	-
Diseases of the Esophagus: Official Journal of the International Society for Diseases of the Esophagus	11.969	2.822	-
Current Opinion in Gastroenterology	11.929	2.741	4.9
World Journal of Gastrointestinal Oncology	11.552	3.404	3.6
United European Gastroenterology Journal	11.453	6.866	7.9
Clinical and Molecular Hepatology	11.251	8.337	8.9
Digestive Surgery	11.226	2.459	4.2
Expert Review of Gastroenterology & Hepatology	10.885	4.095	-
World Journal of Gastrointestinal Endoscopy	10.598	-	-
World Journal of Gastrointestinal Surgery	10.579	2.505	-
Clinical and Translational Gastroenterology	10.45	4.396	5.2
Clinical and Experimental Gastroenterology	10.149	-	5
Gastroenterology Research and Practice	9.902	1.919	3.7
Journal of Digestive Diseases	9.302	3.366	4.2
Cellular and Molecular Gastroenterology and Hepatology	9.277	8.797	-
Digestion	9.189	3.672	5.1
Clinics in Colon and Rectal Surgery	9.059	2.403	3.5
Techniques in Coloproctology	9.056	3.699	4.6
Journal of Gastric Cancer	9.031	3.197	4.4
Hepatic Medicine: Evidence and Research	8.847	-	-
Annals of Hepatology	8.782	3.388	4.7
JHEP Reports	8.693	9.917	8.1
BMJ Open Gastroenterology	7.884	-	3.5
Clinical Endoscopy	7.72	-	3.5
Intestinal Research	7.651	-	6
Canadian Journal of Gastroenterology & Hepatology	7.615	2.605	-
Digestive Endoscopy	7.111	6.337	7.5
Hepatobiliary & Pancreatic Diseases International	7.052	3.355	-
Esophagus: Official Journal of the Japan Esophageal Society	6.775	3.671	-

Endoscopy International Open	6.725	-	-
Gastroenterology Report	6.685	4.063	4.9
Clinics and Research in Hepatology and Gastroenterology	6.59	3.189	3.1
Journal of Clinical and Experimental Hepatology	6.236	-	5.3
Saudi Journal of Gastroenterology	6.205	3.214	4.3
Hepatitis Monthly	6.037	1.214	1.1
Hepatology Communications	6.006	5.701	7.7
Liver Research	5.941	-	6.3
Endoscopic Ultrasound	5.932	5.275	5.9
Gastrointestinal Tumors	5.556	-	-
Indian Journal of Gastroenterology: Official Journal of the Indian Society of Gastroenterology	5.311	-	-
Frontline Gastroenterology	4.933	-	3.8
Journal of Clinical and Translational Hepatology	4.562	5.065	6.4
Inflammatory Intestinal Diseases	4.474	-	0.2
Annals of Gastroenterological Surgery	4.427	3.583	5.5
Case Reports in Gastroenterology	4.117	-	1
Annals of Coloproctology	3.946	-	2.4
Translational Gastroenterology and Hepatology	3.945	-	5.5
Clinical Liver Disease	3.934	-	2.4
Journal of Gastrointestinal Oncology	3.029	2.587	3.3

CS: CiteScore; *JAII*: *Journal Article Influence Index*; JIF: Journal Impact Factor. "-" denotes lack of score assigned by the corresponding institution/database.

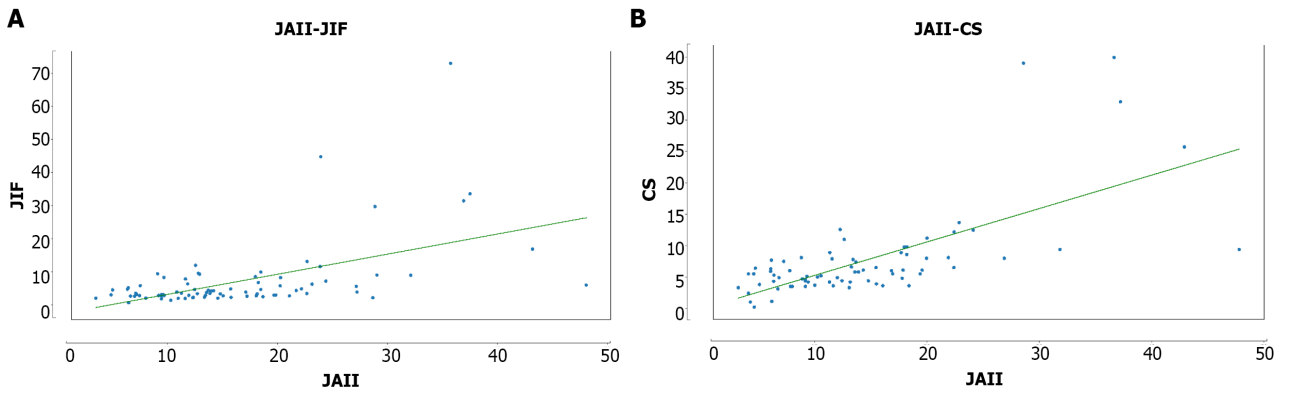

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Figure 1 Scatter distribution plots for Journal Impact Factor-*Journal Article Influence Index* and CiteScore-*Journal Article Influence Index*.
A: Journal Impact Factor-*Journal article influence index* (*JAII*); B: CiteScore-*JAII*. JIF: Journal Impact Factor; CS: CiteScore; *JAII*: *Journal Article Influence Index*.

DISCUSSION

Comparison of databases and calculation principles

JIF: JIFs are obtained through the Web of Knowledge database using the Science Edition of JCR which collects citation data from more than 7300 science and technology journals worldwide. The IF of a T-year journal is defined as the number of times that the journal has been cited in years T-1 and T-2 divided by the number of documents that can be cited in the journal in years T-1 and T-2[19].

CS: CSs are calculated using data from the Scopus database. CS has a publication window of 3 years before the 1-year reference window and counts the references from one document type to another[20]. In other words, CS calculates the average number of citations of papers published in a journal for 3

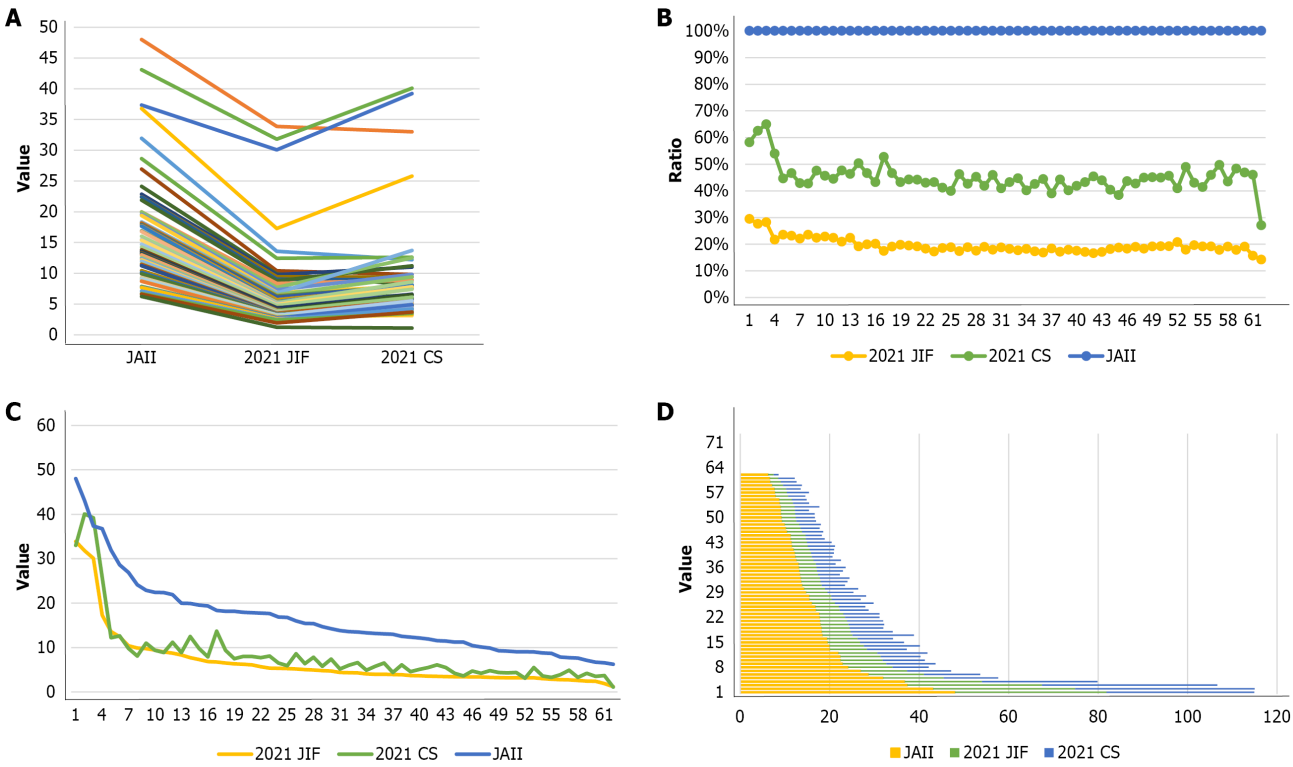

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Figure 2 Visualization of the three journal evaluation indicators. A: Comparison of the values obtained upon application of the three evaluation indicators; B: Journal Impact Factor (JIF)-*Journal Article Influence Index* (*JAII*) and CiteScore (CS)-*JAII* ratios for each journal; C: Values of JIF and CS in descending *JAII* order; D: Histogram combining the three journal evaluation indicators together. JIF: Journal Impact Factor; CS: CiteScore; *JAII*: *Journal Article Influence Index*.

consecutive years in the 4th year. In a given year, CS is calculated as the amount of times that documents published in the previous 3 years were cited in that year divided by the number of documents published in those 3 years that were included in the Scopus database.

JAII: *JAII*s, calculated as total citations divided by total articles, are based on journals and their citations included in the *RCA* database.

Advantages and disadvantages of JAII

It is undeniable that the *JAII* metric has its merits as a journal evaluation indicator. (1) Compared with JIF and CS, *JAII* is able to break through the time limit disadvantage of the first two. Journals do not need to meet the waiting-time thresholds of JIF and CS to be accurately evaluated. As such, *JAII* is able to evaluate more journals accurately in a near-real time manner, which explains why there are more journals with a *JAII* than those with a JIF/CS. (2) Compared with JIF and CS, *JAII* is useful for assessing the performance of journals immediately upon its creation. Moreover, since a small number of articles in journals will result in a high JIF and CS at a given time, *JAII* relieves the chance of biased evaluation of journals. *JAII* is also more conducive to a comprehensive assessment of the quality and performance of journals. And (3), compared with JIF and CS, *JAII* is more conducive to high-quality journal evaluation. In addition to these advantages, *JAII* has a high degree of compliance with JIF and CS in the evaluation of journals with a lower impact.

Another important feature related to the *JAII* is that the *RCA* database, upon which it is based, can enable queries to journals by category, such as focused query of Gastroenterology and Hepatology, representing a ready convenience to researchers.

There exist disadvantages in the *JAII*. These include the lack of statistical timeliness, a feature by which *JAII* is slightly inferior to JIF and CS, and the lack of consideration to different developments of the same journal in different periods. *JAII* also shares some of the drawbacks of both JIF and CS, such as the lack of evaluation of citation quality and the inclusion of self-citations.

Non-linearity interpretation

As we have shown in Figure 1 and described textually in the "Results" section above, the linearity of *JAII*-JIF-CS was clear for lower-quality journals but failed to match each other perfectly for higher-quality journals.

Our explanation is that JIF and CS are subject to changes in citation frequency and number of published articles in different years, and their correlation with time exacerbates the influence of human manipulability[21]. *JAII* reduces this time randomness. In addition, the JIF and CS of high-quality journals may be more susceptible to this effect, and their fluctuations can be effectively explained.

Threats to validity

In addition to the lack of evaluation of citation quality and self-citation, other factors may threaten the effectiveness of the evaluation parameters in use. Research on JIF, CS and other statistical standards for journal quality has shown that there are still many statistical violations at play, including those related to and arising from reliability, incomplete reporting of validity, insignificant results, insignificant effect sizes, and hypothesis checking, as well as uncorrected inferences and multiple comparisons from descriptive statistics[22]. *JAII* is also inevitably affected by the same, to at least some extent, and this limitation cannot be ignored.

CONCLUSION

The main differences between *JAII* and JIF/CS come from the differences in the scientific databases used as the cited sources, as well as the differences in the evaluation methods underpinning each of these indicators. Due to the JIF/CS time factor limitation, the *JAII* method based on the *RCA* database is able to evaluate more journals. Besides, *JAII* provides more focused quantitative insight by considering categories of journal papers. In terms of practicality, the novelty introduced by the *JAII* indicator is its open-accessibility to users (as opposed to a subscription service to select users). To summarize, *JAII* is a reliable index to evaluate the quality of journals in near-real time.

In the future, scientometric researchers can focus on the differences of the different journal evaluation indexes to aid in their studies on the origin of nonlinear characteristics in order to put forward a more perfect journal evaluation standard. Meanwhile, researchers in general can exploit the distinct advantages of each as they currently stand to better understand journal quality and promote the impact of their own scientific communications.

ARTICLE HIGHLIGHTS

Research background

The evaluation of journal quality is very important for researchers. Journal Impact Factor (JIF) and CiteScore (CS) are two of the most popular and authoritative journal evaluation indicators. With the ongoing scientometric research into their advantages and disadvantages, there is a consequent emergence of new journal evaluation indicators. The logical next-step is comparative judgement of the reliability and innovative novelty of such new journal evaluation indexes.

Research motivation

The recently-launched *Reference Citation Analysis* database of Baishideng Publishing Group is an open multidisciplinary citation analysis database founded in artificial intelligence technology. Based on this database, *Journal Article Influence Index* (*JAII*) has been proposed as a new journal evaluation indicator.

Research objectives

To compare the advantages and disadvantages of *JAII* with those of JIF and CS.

Research methods

For comparisons between *JAII* and 2021 JIF/2021 CS, we conducted statistical analyses and provided an intuitive method for visual representation of the related data.

Research results

For lower-quality journals, *JAII*, 2021 JIF, and 2021 CS had a good linear correlation. However, their results of assessments of higher-quality journals varied widely. These three evaluation indexes have their own advantages and disadvantages, including the avoidance of time randomness and ability for near-real time evaluation of the *JAII*.

Research conclusions

JAII is a comprehensive assessment tool to assess the quality and performance of journals.

Research perspectives

In the future, we hope to better explain the current existent nonlinear relationship among the three evaluation indexes, and combine a variety of journal evaluation indicators to allow for more comprehensive evaluation of journal quality by scientometric-focused and general researchers.

FOOTNOTES

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