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Contents

Weekly Volume 29 Number 26 July 14, 2023

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Study

Clinical Trials Study

CASE REPORT

ABOUT COVER

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REVIEW

Impact of COVID-19 in individuals with and without pre-existent digestive disorders with a particular focus on elderly patients

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Abstract

Coronavirus disease 2019 (COVID-19) has several extrapulmonary symptoms. Gastrointestinal (GI) symptoms are among the most frequent clinical manifestations of COVID-19, with severe consequences reported in elderly patients. Furthermore, the impact of COVID-19 on patients with pre-existing digestive diseases still needs to be fully elucidated, particularly in the older population. This review aimed to investigate the impact of COVID-19 on the GI tract, liver, and pancreas in individuals with and without previous digestive diseases, with a particular focus on the elderly, highlighting the distinctive characteristics observed in this population. Finally, the effectiveness and adverse events of the anti-COVID-19 vaccination in patients with digestive disorders and the peculiarities found in the elderly are discussed.

Key Words: COVID-19; Elderly; Inflammatory bowel disease; Liver disease; Cirrhosis; Pancreatic disease

Core Tip: Gastrointestinal symptoms are frequent in coronavirus disease 2019 (COVID-19), with more severe consequences reported in elderly patients. Patients with pre-existing liver disease are at an increased risk for worse outcomes, while no definitive conclusions can be drawn regarding patients with inflammatory bowel disease or pancreatic diseases. Elderly patients with digestive disorders, although the available data are limited, have no worse COVID-19 outcomes than those without these diseases.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the ongoing pandemic of coronavirus disease 2019 (COVID-19). Since its first report in December 2019 in Wuhan, China[1], COVID-19 has quickly spread worldwide with 635229101 confirmed cases, including 6602552 deaths according to the World Health Organization (WHO) at the moment of writing[2]. Although symptomatic COVID-19 patients exhibit various signs and symptoms, the typical clinical presentation has been predominantly respiratory[3]. The most common symptoms include cough (60%-86%), shortness of breath (53%-80%), and taste and smell alteration (64%-80%)[4-6]. However, as caseloads rise, many extrapulmonary effects have been observed, affecting the cardiovascular, neurologic, gastrointestinal (GI), and dermatologic systems. Liver and pancreatic impairment may also occur, especially in critically ill patients[7]. Although their exact prevalence is still the subject of debate, GI symptoms are more and more frequently reported[8]. However, it is impossible to exclude a bias thriving from the increased awareness of GI manifestations[9]. Some authors reported a prevalence of GI involvement of around 31.9%[10,11], while others up to 61%[12]. Regarding the impact of SARS-CoV-2 infection on older age, more severe outcomes are described [13,14]. The reason is the various age-related changes and the higher number of comorbidities typical of these patients. However, increasing evidence suggests that age is the most significant risk factor for worse outcomes[15]. Immunosenescence, the age-associated decline of immune system function, is the primary reason for the increased susceptibility to viral infection[16,17].

Methods

Considering a broad topic such as SARS-CoV-2 infection, this narrative review aimed to provide an overview of current knowledge of its impact on the GI tract, liver, and pancreas, with a particular interest in the elderly population. First, we created a list of keywords related to our research question. Articles were searched in the PubMed, Scopus, and EMBASE databases using the following search terms: "COVID-19", "SARS-CoV2 infection", "Elderly people", "Elderly population", "Old people", "Gerontal population", "Extrapulmonary manifestations", "Gastrointestinal symptoms", "Gastrointestinal bleeding (GIB)", "Inflammatory bowel disease (IBD) ", "Liver injury", "Liver dysfunction", "Chronic liver disease", "Drug liver injury", "Pancreatic involvement", "Acute pancreatitis" and "COVID-19 vaccination". We then synthesized, analyzed, and critically evaluated the related data to identify trends and patterns, in theory, debates, conflicts, and, most of all, persistent gaps in the existing knowledge.

Mechanisms of GI involvement in COVID-19

It is well known that SARS-CoV-2 enters cells using angiotensin-converting enzyme 2 (ACE2)[8,18]. In addition to the respiratory tract, ACE2 has also been found in the GI tract, especially in the tongue, esophageal, gastric, and rectal mucosa[19]. Mechanisms underlying the probable pathological effect of SARS-CoV-2 are varied. First, the virus's entry into cells may lead to a direct cytopathic effect[20]. SARS-CoV-2 infection emphasizes inflammatory pathways determining the "cytokine storm", which is characterized by the overproduction of mediators such as interleukin-2 (IL-2), IL-7, tumor necrosis factor (TNF) and granulocyte monocyte colony-stimulating factors[21]. This condition could generate diarrhea due to the alteration of gut motility and GI flora[22]. Notably, gut microbiota alterations have been found in these patients, probably due to factors such as exposition to antimicrobial agents or expression of viral proinflammatory mediators. Moreover, ACE2 seems to be involved in the antimicrobial peptide secretion through target of rapamycin activity; an aberrant functioning of this pathway has been described during COVID-19[23]. In addition to this mechanism, disorders of the gut-lung axis may be involved in the pathophysiology of GI symptoms. For example, it has been reported that lung flora alteration can correlate with intestinal microbiota modifications, probably due to increased recruitment of lung-derived CCR9+CD4+T cells into the bowel[24,25]. Regarding nausea and vomiting, these symptoms are likely

related to SARS-CoV-2 infection of the vagus nerve and a cytokine-mediated stimulation of the central and autonomic nervous systems[26]. Moreover, anorexia might be related to nausea and vomiting or associated with acute viral prodrome and cytokine storm[27]. Some studies have identified a lower prevalence of GI symptoms in the elderly population. This finding could be related to differences in gene expression of the ACE2, which seems to be directly correlated with age[9,28,29]. Older patients showed higher intestinal ACE2 mRNA expression, which could modify susceptibility to GI symptoms by influencing intestinal immunity and microbiota composition[29,30]. However, studies on this relation are few and with uncertain results.

GI symptoms in patients with COVID-19

In one-third of patients with COVID, GI symptoms occur as the first presentation. Nausea and vomiting may be present in up to two-thirds, diarrhea in up to 50%, loss of appetite in approximately 40%, and less than 10% present abdominal pain[7]. Moreover, extreme cases, such as the autoptic finding of segmental dilatation and stenosis of the small intestine in an 85-year-old man with COVID-19, have been reported in the literature[31]. However, the causal role of SARS-CoV-2 has yet to be established.

GI manifestations in elderly patients with COVID-19

Anorexia and diarrhea were the most frequent symptoms reported in the elderly, with variable frequencies depending on the study. In a large Brazilian cohort of 9807 patients, low frequencies of GI symptoms were reported, with diarrhea being registered only in 2% of patients[32]. Marziliano *et al*[33] reported a prevalence of GI symptoms of near 9% in a population of older adults (65 years and older). Ramos-Rincon *et al*[34] reported that very old patients (age > 80 years old) had higher frequencies of GI symptoms, with diarrhea presenting in 14% and vomiting in 5%, and anorexia in nearly 21% of patients. In the studies mentioned above, GI symptomatology did not impact mortality. Other studies pointed out an inverse correlation between age and the prevalence of GI symptoms[9,28]. Aroniadis *et al*[9] reported that older COVID-19 patients were less likely to exhibit digestive symptoms. A lower prevalence of GI symptoms was reported in 70 and older patients compared with those < 70 years (32% *vs* 41%). This potential age-related protective effect is found in a dysregulation of the immune system activation and a different expression of the ACE2 receptor in the digestive system [28]. Surprisingly, some studies highlighted a possible protective role of GI symptoms in COVID-19. Belgian research observed a prevalence of 30% of GI symptoms in frail older patients (aged over 80 years) and a positive correlation between their presence and patients' survival[35]. These results were also aligned with those of Vrillon *et al*[36], who described a positive outcome in older people presenting with GI symptoms, whereas younger adults with digestive symptoms had a higher prevalence of complications. On the contrary, Atalla *et al*[37] in their retrospective study, revealed a higher incidence of loss of appetite (83.3% *vs* 44.4%) and diarrhea (50% *vs* 28.6%) in the deceased patients than in those who survived. However, the onset of diarrhea during hospitalization was not necessarily related to SARS-CoV-2 infection, and the etiological analyses are not presented in the article[37]. Patients with SARS-CoV-2 infection are also at risk of other potential causes of diarrhea, such as Clostridium difficile infection and antibiotic-associated diarrhea due to frequent antimicrobic therapy and hospitalization. These could be confounding factors that alter the analysis of GI symptoms during SARS-Cov-2 infection, especially when they appear during infection and not at onset[18,24]. GI bleeding (GIB) in COVID-19 patients can represent a fatal complication. In the literature, a rate of GIB between 1.1% and 13% during SARS-CoV-2 infection has been reported[38]. However, the actual incidence and prevalence of GIB in COVID-19 patients are extremely difficult to assess. The underlying mechanisms for GI hemorrhage in COVID-19 patients could involve conditions that increase the risk of thromboembolism, such as dehydration, which may follow fever or diarrhea[39]. Anticoagulation drugs prescribed for preventing thrombotic events should be considered additional risk factors[40]. Although Ion *et al*[41] sustained that the use of anticoagulation or antiplatelet agents was not a risk factor for GIB, at least in hospitalized COVID-19 patients. Stress ulcers may be another major cause of bleeding unrelated to SARS-COV-2[41]. Furthermore, assisted ventilation techniques could cause stress ulceration and generate GIB[42]. However, rates of thrombosis and bleeding related to COVID-19 were congruent to those reported in hospitalized patients with comparable grades of critical illness. Except for case reports, no elective studies on the elderly population are available in the literature[43]. An overview of the analyzed studies is shown in Table 1.

COVID-19 in inflammatory bowel disease patients

Due to the immunosuppressive therapies, malnutrition, and chronic inflammation, patients affected by inflammatory bowel disease (IBD) are potentially at augmented risk for SARS-CoV-2 infections and complications due to the immunosuppressive therapies, malnutrition, and chronic inflammation[44,45]. However, various studies have investigated the incidence of COVID-19 in IBD patients, concluding that it is not increased compared to the general population [46-48]. Interestingly, COVID-19 in IBD patients has a clinical presentation similar to the general population. A recent meta-analysis found that the most common symptoms in these patients are extraintestinal, such as fever and cough[49]. However, GI manifestations appear more common than in the general population. Diarrhea can occur in 27.2% of patients, followed by abdominal pain, nausea, and vomiting, with a pooled prevalence rate of 13%, 10%, and 8.8%, respectively, and sporadically GI symptoms can be the only clinical presentation, which could be challenging to differentiate from IBD reactivation[49]. The prognosis of COVID-19 patients affected by IBD is not different from that of the general population. A recent meta-analysis observed that IBD was not correlated with augmented risk of death, intensive care unit (ICU) admission, or hospitalization related to COVID-19, and the pooled odds ratios were 0.67 [95% confidence interval (CI): 0.32-1.42], 1.09 (95%CI: 0.27-4.47), and 0.58 (95%CI: 0.28-1.18), respectively[50]. On the other hand, another meta-analysis found that in IBD patients affected by COVID-19, the risk ratio (RR) of adverse outcomes was increased (RR = 1.32; 95%CI: 1.06-1.66) compared to patients without IBD[51]. The risk factors for worse outcomes of COVID-19,

Table 1 Overview of studies evaluating the course of gastrointestinal symptoms during severe acute respiratory syndrome coronavirus 2 infection in the elderly population

Ref.	*n*	Age (yrs)	GI symptoms	Diarrhoea	Nausea/vomiting	Anorexia	Abdominal pain	Outcomes
de Souza *et al* [32]	9807	70.21 ± 8	-	2%	-	-	-	No association
Ramos-Rincon *et al* [34]	2772	86.3 ± 3	-	14%	5%	22%	-	No association
Marziliano *et al*[33]	4961	77 ± 8	9%	-	-	-	-	No association
Atalla *et al* [37]	111	87.0 median (IQR: 77.0-92.0)		7% (38% all ages)	2%	17% (61% all ages)	-	Mortality was associated with a disease course beginning with a loss of appetite, and the incidence of diarrhea was more frequent in the deceased
Lanthier *et al* [35]	50	88 median (IQR: 83-92)	30%	24%	6%	10%	6%	Digestive symptoms were associated with a favorable outcome
Aroniadis *et al*[9]	434	Age > 70	31%	19%	-	-	-	Older patients were less likely to exhibit gastrointestinal symptoms
Zhan *et al*[23]	39	Age > 75	36%	-	-	-	-	No association
Vrillon *et al* [36]	76	90 median (IQR: 86-92)	22%	-	-	-	-	Digestive symptoms were associated with a favorable outcome

The sample's age was expressed either by the mean or the median in the studies analyzed. For some non-specific studies performed on older people, only the characteristics of the population mentioned above have been shown in the table. However, some of these have yet to express a category-specific significant trend measure. For example, the relationship between the course of the infection and the outcome was reported in the last column. SD: Standard deviation; IQR: Interquartile range; GI: Gastrointestinal.

such as age, male sex, and comorbidities, are the same as observed in the general population. In IBD patients with COVID-19, an active disease was considered a risk factor for poor outcome[52,53], and in a cohort of 79 patients with IBD and COVID-19, active IBD, especially in elderly patients, was correlated with worse outcomes such as pneumonia, hospitalization, respiratory support, and death[52]. Another study reported that the correlation between IBD activity and the risk of severe COVID-19 appears to vary with age and is more relevant in younger patients[53]. The impact of IBD medications on the course of COVID-19 is still under investigation. A meta-analysis by Tripathi *et al*[54] has shown that the therapy with TNF-α antagonists is associated with favorable hospitalization and mortality outcomes, while the use of mesalamine was correlated with worse outcomes in terms of hospitalization, ICU admissions, and death. Another recent study, including 6144 patients, found that systemic corticosteroids were associated with severe COVID-19, while mesalamine and sulfasalazine were not associated with adverse outcomes[55]. In addition, combination therapy with TNF-α antagonists plus thiopurines was correlated with an augmented risk of hospitalization or death, but not the combination with methotrexate. Moreover, biologics were not associated with worse COVID-19 outcomes and could have a protective effect without differences when comparing biologic classes such as TNF-α, IL-12/23, or integrin antagonists [56]. Therefore, the correct management of IBD therapy is relevant because SARS-CoV-2 is not correlated with the risk of IBD relapse. Conversely, the discontinuation or delay of the IBD therapy, regardless of SARS-CoV-2 infection, is significantly associated with the disease activity[55,56] (Table 2).

COVID-19 in elderly patients with IBD

Elderly-onset IBD is defined as onset at 60 years or older[57], and up to 30% of the IBD population is older than 60 years, while 15% of IBD patients have been diagnosed after age 65. It has been observed that in elderly-onset IBD, there is a different natural history and disease phenotype. The disease outcomes are less influenced by genetics, while frailty, immunosenescence, and dysbiosis have a more significant role[58]. In IBD patients, as in the general population, age and comorbidities increase the risk of severe COVID-19 and disease-related mortality[45,59]. A prospective observational study that included 482 patients confirmed that age over 60 years was correlated with severe COVID-19 [odds ratio (OR) = 4.59, 95%CI: 1.3-15.9] and was an independent risk factor related to death (OR = 7.1, 95%CI: 1.8-27.4), as to have two or more comorbidities (OR = 3.9, 95%CI: 1.3-11.6)[60]. Wetwittayakhlang *et al*[61] examined a cohort of 3516 IBD patients, of whom 82 were diagnosed with COVID-19 infection, and they observed that age over 55 years was an independent risk factor for developing severe COVID-19. The study by Brenner *et al*[59], through an extensive international registry, corroborates these observations, finding that advanced age [adjusted OR (aOR) = 1.04; 95%CI: 1.01-1.06] and having at

Table 2 Risk of severe coronavirus disease 2019 in elderly inflammatory bowel disease patients

Ref.	Total number of IBD patients	Number of COVID-19-positive IBD patients	Age threshold considered (yrs)	Risk of severe COVID-19
Ludvigsson *et al*[45]	67292	179 (hospitalized patients)	60	HR = 1.42; 95%CI: 0.94-2.13
Brenner *et al*[59]	SECURE-IBD database	525	Increasing age on multivariable analysis	OR = 1.04; 95%CI: 1.01-1.06
Zabana *et al*[60]	53682	482	60	OR = 4.59, 95%CI: 1.3-15.9, *P* = 0.02
Wetwittayakhlang *et al*[61]	3516	82	55	OR = 11.09, 95%CI: 1.81-68.09, *P* = 0.02

OR: Odds ratio; CI: Confidence interval; IBD: Inflammatory bowel disease; COVID-19: Coronavirus disease 2019; HR: Hazard ratio.

least two comorbidities (categorized into lung disease, cardiovascular disease, hypertension, history of stroke, cancer, liver disease, kidney disease, and diabetes) (aOR = 2.9; 95%CI: 1.1-7.8) were positively associated with severe COVID-19.

Regarding the risk of SARS-CoV-2 infection, a study by Gubatan *et al*[62] observed that IBD patients older than 66 years have an augmented risk of acquiring SARS-CoV-2 infection compared to younger patients. On the other hand, a retrospective cohort study by Calafat *et al*[63] that included 418 IBD patients over 65 years of age, of whom 32 were diagnosed with COVID-19, found that the incidence of COVID-19 in elderly IBD patients is similar to that reported in the age-adjusted general population. Furthermore, the incidence of COVID-19 was not influenced by the use of immunosuppressants, but the authors observed a worse prognosis among the patients who did not use immunosuppressants. Medication use in older patients with IBD differs from younger ones since they are treated less often with biological agents and immunosuppressants. However, corticosteroid use is similar[63,64], and it is possible to hypothesize that these differences could have a role in the course of SARS-CoV-2 infection. Overall, in the elderly population, IBD does not seem to increase the risk of SARS-CoV-2 infection or severe COVID-19[65] (Table 2).

LIVER MANIFESTATIONS ASSOCIATED WITH COVID-19

Introduction

Among the extrapulmonary manifestations of SARS-CoV-2, abnormal liver function reflecting hepatocellular and cholangiocellular injury is often reported[12,66]. COVID-19-associated liver injury includes any liver abnormality due to the disease course or the treatment. Indeed, it is not always possible to determine whether the liver injury is due to the infection or other concomitant conditions, such as the co-administration of hepatotoxic agents or ischemic hepatitis from severe and prolonged hypotension. Moreover, the cytokines storm observed in the severe forms of COVID-19 and caused by systemic hyper-inflammation may result in multiple severe organs injury and, in turn, it represents another cause of liver damage[67]. However, the frequency of liver dysfunction in COVID-19 infection has not yet been well understood [68].

Liver test abnormalities

Among abnormal liver function, liver test abnormalities have often been described. The incidence of elevated liver transaminases, alanine transaminase (ALT), and aspartate aminotransferase (AST) in COVID-19 patients range from 2.5% to 76.3%[69]. A systemic review pointed out that elevated liver chemistries occurred in 23.1% of adult patients with COVID-19 at initial presentation[66]. In comparison, 24.4% develop elevated liver chemistries during the illness, and up to 10.7% have severe liver injury[66]. In detail, the pooled incidence of AST and ALT elevation at initial presentation of COVID-19 was 22.5% and 17.9%, respectively[66]. The incidence of hyperbilirubinemia at the onset of symptoms was 13.4%, while the incidence of alkaline phosphatase (ALP) and gamma-glutamyltransferase (gamma-gt) was 6.1% and 21.1%, respectively[66]. In addition, hypoalbuminemia ranged from 1.1% to 45.8% in non-severely infected patients, reaching 72.9% in those severely infected[66].

The cause of the elevated liver enzymes in COVID-19 patients without pre-existent liver diseases still needs to be well elucidated. In the study of Kulkarni *et al*[66], only 3.6% of the analyzed patients had underlying chronic liver disease (CLD), suggesting that liver damage might be directly caused by the viral infection of liver cells. It is believed that SARS-CoV-2 could penetrate the liver cells thanks to the ACE2 receptor, which is expressed in the liver and bile duct cells[70]. Recent data show that ACE2 is expressed in 2.6% of hepatocytes and 59.7% of cholangiocytes. The level of ACE2 expression in cholangiocytes was similar to type 2 alveolar cells of the lungs. Therefore, liver dysfunction may result from SARS-CoV-2 attachment to ACE2 on cholangiocytes[71]. Of note, studies on both mice and humans reveal an increased ACE2 expression in hepatocytes when liver fibrotic/cirrhotic conditions are present[69]. This finding leads us to believe that preexisting liver injury could exacerbate SARS-CoV-2 hepatic tropism. Although COVID-19 may contribute to liver dysfunction directly through an inflammatory response, postmortem pathological findings of the liver suggest that COVID-19-related liver dysfunction may be mainly caused by secondary liver damage by respiratory distress syndrome-induced hypoxia, multiple organ failure, and the use of potentially hepatotoxic drugs[72]. Microscopically, the most

significant findings in postmortem hepatic tissue of patients with COVID-19 were microvesicular steatosis and mild lobular inflammation[73]. Zhao *et al*[72] presented unique findings, such as platelet-fibrin microthrombi in hepatic sinusoids, central vein or portal vein, histolytic hyperplasia in portal tracts, and megakaryocytic in sinusoids.

Liver test abnormalities and COVID-19 outcomes

Liver test abnormalities can predict the severity of COVID-19 disease[74]. Patients with abnormal liver tests at admission or during hospitalization, classified as hepatocyte type or mixed type, had significantly higher risks of progressing to severe COVID-19 and mortality when compared to patients with normal liver tests[66,75]. Elevated liver enzyme levels are linked to adverse manifestations such as shock, admission to an ICU, and mechanical ventilation. Although, Săbiescu *et al*[76] proved that only elevation over five times the upper limit is strongly correlated with high mortality risk. Also, hypoalbuminemia is a strong predictor of severe COVID-19 course and, in combination with AST or total bilirubin (TBIL), has a remarkable association with mortality[77], even in patients without chronic illness[78]. Furthermore, Da *et al*[79] reported a correlation between ALT levels and levels of inflammatory markers such as C-reactive protein, D-dimers, ferritin, and IL-6.

It appears evident that liver injury in COVID-19 patients is linked to the severity of the hyperinflammatory response, thus, reinforcing the hypothesis that the entity of liver damage is related to the severe forms of SARS-CoV-2 infection. Pazgan-Simon *et al*[80] reported that liver injury in patients with COVID-19 with no underlying liver disease did not correlate with higher mortality. On the other hand, patients with preexisting liver disease, particularly those with cirrhosis, have a higher risk of death than those without any known liver pathology[81].

Impact of SARS-CoV-2 infection on patients with CLD

Wang *et al*[82] showed an increased risk of COVID-19 infection in patients with a recent diagnosis of CLD. A multicentric retrospective study revealed that nearly one-fifth of hospitalized COVID-19 patients had CLD[82]. However, the elevated aminotransferases on admission were higher in patients with CLD than those without CLD. On the contrary, during hospitalization, the aminotransferase level did not differ between patients with or without CLD. Iavarone *et al*[83] described the impact of SARS-CoV-2 infection on ALT levels in cirrhotic patients, revealing that acute liver injury was observed in almost 50% of patients with previously average ALT values. However, more data are necessary to clarify the impact of an ALT increase on the natural history of cirrhosis and COVID-19. It has been described that CLD can negatively influence the clinical outcomes of patients with COVID-19[84,85]. The overall mortality rate of COVID-19 is estimated at 0%-2% in these patients[86]. However, currently, there is no convincing evidence that patients with stable CLD without advanced fibrosis/cirrhosis, primary biliary cholangitis, or primary sclerosing cholangitis have increased susceptibility to severe COVID-19 infection[87]. Contradictory data exist on the risk of developing severe illnesses of non-alcoholic fatty liver disease (NAFLD)[88]. Patients with NAFLD often suffer from metabolic comorbidities such as diabetes, hypertension, and obesity and, for this reason, present an increased risk of a severe course of COVID-19[89]. However, Sachdeva *et al*[90] affirmed that NAFLD might represent a predictor of severe COVID-19, even after adjusting to the presence of confounding factors. The risk of a worse prognosis of COVID-19 is directly related to the severity of the liver disease, and cirrhosis may appear independently associated with an increased risk of death in patients hospitalized with COVID-19[91]. A large multinational cohort study determined that baseline liver disease severity is the primary determinant of SARS-CoV-2 infection outcome[92]. This study resulted in a mortality of 32% in patients with cirrhosis compared to only 8% of those with CLD without cirrhosis. In addition, patients with CLD without cirrhosis appear to have a similar risk of SARS-CoV-2 infection-related mortality compared to patients without liver disease. Furthermore, since only 19% of cirrhosis patients' mortality was related to liver complications, the leading cause of death remained COVID-19-related lung injury. Patients with cirrhosis may also have underlying complications such as hepato-pulmonary syndrome, porto-pulmonary hypertension, or hepatic hydrothorax, which can increase the risk of respiratory failure[93]. On the contrary, a Korean cohort study showed no significant association between developing severe complications from COVID-19, including mortality, or the presence of liver cirrhosis[94]. A possible explanation could be attributed to the different etiologies of cirrhosis in the patients analyzed. In particular, chronic hepatitis B is responsible for more than 70% of cirrhosis cases in Korea[95], and NAFLD and alcoholic liver disease are the most common etiologies of liver cirrhosis in Europe and North America. Consequently, the different etiologies of cirrhosis may play a critical role in developing severe complications from COVID-19. For example, a United States multicentric study pointed out that, among patients with CLD, those with decompensated cirrhosis, alcohol-related liver disease, and hepatocellular carcinoma (HCC) were more vulnerable to adverse outcomes from COVID-19. These patients also had a higher risk for all-cause mortality from COVID-19[96] (Table 3).

Drug-induced liver injury caused by COVID-19 treatment

Liver injury can be caused by several medications used in the treatment of COVID-19. Drug metabolites can cause cellular stress that can lead to apoptosis or necrosis of liver cells[97]. Since most of these drugs cause an elevation of liver enzymes alone, it is important to correctly define acute liver injury to avoid withdrawing this medication improperly[98]. Drug-induced liver injury (DILI) is defined as an increased level of ALT ≥ 5-times upper limit of normal (ULN), or increased level of ALP ≥ 2-times ULN (in the absence of bone pathology), or a simultaneous increase of ALT ≥ 3-times ULN and TBIL concentration > 2-times ULN. While analyzing the different medications used for COVID-19 infection, liver-related adverse effects were more common in patients who used hydroxychloroquine and azithromycin and those who did not receive any targeted therapy[99]. In contrast, drugs, including lopinavir/ritonavir (LPV/v), were associated with 4 × higher odds of liver injury[75]. According to another study, no apparent side effects were found in the LPV/r group, except for transient ALT elevation (< 125 U/L)[96]. A meta-analysis revealed that the incidence of DILI in patients treated

Table 3 Summary of studies on the relationship between liver disease and coronavirus disease 2019 in elderly patients

Ref.	Outcome considered	Results
Khateri *et al* [108]	Incidence of acute liver injury in patients affected by COVID-19	Acute liver injury has no relationship with age
Metawea *et al* [110]	Mortality of patients with hepatocellular carcinoma infected with COVID-19	Age is associated with poorer outcomes and higher mortality
Ji *et al*[111]	Severe COVID-19 in patients affected by NAFLD	Associated in patients older than 60 yr
Zhou *et al* [112]	Severe COVID-19 in patients affected by NAFLD	Associated in patients younger than 60 yr
Hartl *et al* [113]	Liver-related death due to COVID-19 between different age groups	More frequent in the 40-69 years old group than in the over 70 years old group (6.5% *vs* 2.2%)
Ioannou *et al* [115]	Predictors of mortality among patients with cirrhosis and SARS-CoV-2 infection	Advanced age was one of the main risk factors for mortality among patients with cirrhosis and SARS-CoV-2 infection
Brozat *et al* [116]	The case fatality rate in patients with cirrhosis and SARS-CoV-2 infection	The case fatality rate in cirrhotic patients and SARS-CoV-2 infection aged 65 yr and older was nearly three times that in patients younger than 65 yr (43.6% *vs* 16.1%)

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; NAFLD: Non-alcoholic fatty liver disease.

with remdesivir was 15.2%, while the incidence of DILI in patients treated with LPV/v was 37.2%[66]. Although some extensive reviews concluded that remdesivir does not affect liver function[100], AST and ALT elevations have been described in a cohort of patients treated with remdesivir. However, in most cases, elevated levels of AST and ALT do not progress to severe liver injury[101]. Hepatotoxicity is documented among the possible Tocilizumab-related side effects. In registration trials, serum aminotransferase elevations occurred in up to 40% of patients receiving tocilizumab. After its licensure, it has been linked to several instances of clinically apparent liver injury with jaundice. Also, liver failure and transplantation may occur in patients treated with tocilizumab[102]. Interestingly, the median age of COVID-19 patients with DILI ranged from 54.3 to 56 years; therefore, age does not appear to significantly influence the risk of developing DILI[103]. Although animal studies have demonstrated changes in hepatic physiology that affect drug metabolism in the aging liver, there is no evidence that this leads to any appreciable deterioration of liver function in healthy elder patients. Moreover, several large international DILI registries do not support elder age as an independent risk factor for developing hepatic injury[104]. On the contrary, it has been described that age does affect the incidence rates of liver injury in COVID-19 patients. Older patients have a higher incidence of liver injury. In addition, impaired liver function in the elderly increases the drug concentrations in their livers. The decline in liver function also explains the higher incidence of DILI in the elderly[105]. Therefore, intensive liver function monitoring should be considered for patients treated with drugs such as remdesivir, LPV/v, and tocilizumab.

COVID-19 liver manifestations in elderly patients

It has been debated if age may represent a risk factor in developing severe complications of SARS-CoV-2 infection in patients with CLD. Several studies demonstrated that older patients are more susceptible to developing severe COVID-19 [106] but are also more likely to develop liver function abnormalities[68]. However, advanced age as a risk factor for more severe forms of COVID-19 has not yet been well assessed. Older patients with COVID-19 have a higher risk of liver injury [107]. Indeed, Khateri *et al*[108] have revealed that the prevalence of acute liver injury has no relationship with age. Spearman *et al*[109] have pointed out that age represents one of the main risk factors for adverse outcomes in individuals with CLD and COVID-19. In patients with HCC infected with COVID-19, age is considered one of the factors responsible for poorer outcomes and higher mortality[109,110]. In a Chinese retrospective study of patients with COVID-19, NAFLD and age over 60 years were associated with a more severe course of COVID-19[111]. On the contrary, Zhou *et al*[112] demonstrated that the association between metabolic-associated fatty liver disease and the development of severe COVID-19 was significant in patients aged less than 60 years. The higher prevalence of severe COVID-19 in patients aged under 60 years with NAFLD compared to those without NAFLD may be attributed to hepatic and systemic immune responses caused by NAFLD, which may increase the severity of the cytokine storm in younger patients with COVID-19. Hartl *et al*[113] analyzed the frequency and the predictive role of abnormal liver chemistries in different age groups. Interestingly, the study revealed that patients aged 40-69 years had a significant risk for COVID-19-associated liver injury. The median levels of hepatocellular injury were highest in patients aged between 40 and 69 years, while cholestatic liver injury was similar within both groups (40-69 years and > 70 years). However, the patients aged over 70 had the highest risk of COVID-19-related mortality; liver-related death due to COVID-19 occurred significantly more often in 40-69-year-old patients than those aged over 70 years (6.5% *vs* 2.2%)[113]. Moreover, this study revealed that increased AST levels were linked to a shorter survival time in patients older than 70, while elevated AST seems to predict a severe course of COVID-19 in all age strata[113]. Of all patients with liver-related death, only 1.7% had no preexisting liver disease. Another study pointed out that among patients with CLD, the highest risk of death was found in their eighth decade of life[92]. Age was associated with higher 30-d mortality in patients with cirrhosis and SARS-CoV-2 infection compared with patients without SARS-CoV-2 infection[114]. Ioannou *et al*[115] also highlighted that higher age, decompensation,

and high model for end-stage liver disease scores were mortality predictors. Also, a more recent study pointed out that older age > 65 and Child-Pugh class C were associated with a high mortality rate[116]. In contrast, Marjot *et al*[92] revealed that mortality in patients with cirrhosis was more evenly distributed across age categories, including a high mortality rate under age 40. A multicentric retrospective Italian study confirmed that the outcomes of cirrhosis patients with COVID-19 were poor. According to a previous study, cirrhotic patients had a higher mortality rate and lower age at death[83].

In summary, COVID-19 is frequently associated with liver function abnormality. However, liver dysfunction may predict a severe form of COVID-19. Therefore, special attention should be paid to older patients, especially those with preexisting CLD and after using hepatotoxic agents. Lastly, cirrhotic patients deserve special attention because they have a high risk of liver function deterioration and mortality with COVID-19 infection, regardless of age (Table 3).

Pancreatic manifestations associated with COVID-19 in elderly patients

The pancreatic involvement of COVID-19, both in terms of clinical implications and underlying mechanisms, is highly manifold and individual-specific[97]. Clinical presentations may range from asymptomatic increases in pancreatic enzyme levels to episodes of acute pancreatitis (AP) and its related complications included pseudocyst formation, peripancreatic fluid collection, pancreatic necrosis, and walled-off necrosis[97,117-119]. Several studies also reported the impact of COVID-19 on metabolic and endocrinologic pancreatic function; manifestations include the development of glucose intolerance and the exacerbation of hyperglycemia, both leading to the development of new-onset diabetes[120, 121].

COVID-19 and AP: Examining the causality

The mechanisms underlying COVID-induced pancreatic damage can be direct, due to the cytopathic effect of local SARS-CoV-2 replication, or indirect, caused by the infection's systemic inflammatory and immune response. Moreover, drug-induced pancreatic injury resulting from antipyretics, anti-inflammatories, and corticosteroids, should also be considered as an additional risk factor[122,123]. However, despite several proposed explanations, no comprehensive theory of COVID-induced pancreatic impairment is universally accepted. The most accredited theory views the engagement of several complexes and interrelated processes. For example, whether the pancreatic injury is caused by SARS-CoV-2 or is just an epiphenomenon is often unclear.

Incidence of COVID-induced pancreatic impairment

From an analysis of the present literature, it can be inferred that the range of incidence of COVID-19-caused pancreatic damage is susceptible to the definition of pancreatic impairment itself. Studies accounting for amylase or lipase serum level increases as an index of pancreatic involvement report an incidence level of 8.5%-17.3%[122,124]. When the more stringent Atlanta criteria are considered, lower incidence values of 1.7%-1.8% are reported[125-127]. An example of this can be found in McNabb-Baltar *et al*[128]'s work; who pointed out that despite mild hyperlipasemia being observed in 9 out of 71 patients (12.1%), only 2 of those (2.8%) had levels more than three times higher than the ULN and that none of them showed any characteristic imaging findings of AP. Similar conclusions are supported by the works of Bansal *et al* [129], Rasch *et al*[130], Barlass *et al*[131], and Bacaksiz *et al*[132], all of which call out for caution when addressing the interrelation between the alteration of pancreatic enzymes and COVID-induced pancreatic impairment. Despite the increase in pancreatic biomarkers typical of COVID-19 patients, no direct correlation to pancreatic impairment is established, as these imbalances could result from concurrent clinical conditions[86,133].

On the other hand, many authors have recognized and described a significant prognostic role of amylase and lipase levels in poor outcomes in COVID-19. Liu *et al*[125] reported a pancreatic enzyme alteration incidence about nine times higher in patients with severe COVID conditions compared to those with non-severe disease (17.4% *vs* 1.85%). Barlass *et al*[131] showed an association between lipase elevation and worse disease outcomes, especially in terms of the need for intensive care (92.9% in patients with elevated lipase levels *vs* 32.8% in those with lower levels) and rate of intubation (78.6% *vs* 23.5%). Ultimately, the multicenter retrospective cohort study by Singh *et al*[134] played a vital role in strengthening this theory, and if its findings are confirmed, serum lipase can be utilized as a marker of disease severity in patients with COVID-19.

Prevalence and outcomes of acute and chronic pancreatitis in COVID-19

After carefully examining the existing literature, a bidirectional relationship between COVID-19 disease and AP can be inferred, at least in terms of outcomes. As part of their retrospective observational cohort study, Inamdar *et al*[135] reported the point prevalence, risk factors, and outcomes among hospitalized patients with pancreatitis with or without COVID-19. This work illustrated a point prevalence of pancreatitis of 0.27%, a higher need for intensive care (mechanical ventilation), and a longer length of hospital stay (OR = 5.65 and OR = 3.22, respectively) among COVID-19 patients. Comparable data were obtained in the works of Karaali and Topal[136], Dirweesh *et al*[137], and from the COVID PAN collaborative study[138], the last of which reported longer length of hospital-stay, persistent organ failure, and higher 30-d mortality in patients with SARS-CoV-2 co-infection. As clearly described by Ye *et al*[139], COVID-19 patients with comorbidities had worse clinical outcomes and greater risk of adverse events proportionally to the number of comorbidities. Focusing on AP, only two studies have compared outcomes in COVID-19 patients with and without pancreatic impairment. Mirò *et al*[140] found comparable results for these two groups, except for the former, being more frequently in need of hospitalization. Similarly, Akarsu *et al*[141] reported that COVID-19 patients who suffered from pancreatitis were more likely to have higher hospitalization and mortality rates. Gubatan *et al*[62] were the first to evaluate the prevalence and outcomes of COVID-19 among patients with a history of pancreatitis. As thoroughly

described by Huang *et al*[142], preexisting pancreas condition was associated with an increased risk of COVID-19 hospitalization and mortality compared to pancreatitis-free patients. Specifically, the highest hospitalization and ICU admission rates were registered in those with a history of chronic pancreatitis. Multicenter research by Hadi *et al*[143] have confirmed that COVID-19 patients with convalescent plasma (CP) bear higher hospitalization rates despite showing no difference in mortality and critical care need. A plausible explanation looks at the higher pancreatic fibrosis grade and the lower inflammatory state, typical of these patients, as predisposing agents to the burden of comorbidities and worse COVID-19 outcomes (Tables 4 and 5).

Distinctive features of COVID-19-associated AP in elderly patients

Focusing on the elderly population, it seems that AP has some particular clinical features that lead to a clinically severe evolution, systemic complications, and, therefore, higher mortality rates[145]. AP has been increasing globally because of an aging population. However, it is worth noting that even if the WHO's definition of elderly is over > 65 years, the age cut-off used for "elderly" in AP prevalence studies differs. In detail, the highest prevalence was found in subjects between 55 and 65 years of age[145-147]. The non-specific presentation and the last occurrence of the symptoms typical of elderly patients make the clinical assessment more difficult for physicians. For example, the typical abdominal pain radiating to the back is absent or mild in 53.8% of cases[148]. For these reasons and the overlap of coexisting comorbidities, AP is often clinically indistinguishable from other clinical conditions in this population. Márta *et al*[149] reported that aging greatly influenced AP's outcome. Their work demonstrated a direct relationship between age and the mortality rate with mortality increase of 0.08% per year between the ages of 20 and 59 and up to 0.76% per year between 59 and 70[149]. These findings, suggesting the involvement of additional deteriorating factors in the elderly population, led the way to other/further studies. COVID-19-induced AP in the elderly population is increasingly reported. Unfortunately, no studies account for only COVID-19 elderly patients with pancreatic impairment, and the few data available are insufficient to draw general conclusions. In Wang *et al*[122]'s work, which describes the incidence of comorbidities in a COVID-19-affected population, the nine patients with pancreatic damage had an average age of 55 years, ranging from 25 to 71 years. Similar data are reported in Inamdar *et al*[135]'s (average age of 54 years), Bruno *et al*[124]'s (average age of 56), and Bulthuis *et al*[150]'s work (60 years). Moreover, a systematic review of case reports and case series pointed out that COVID-19-associated AP affected primarily females with a median age of 53.5 years[144]. The average age of all these study populations aligns with the definition mentioned above of elderly in AP, but the range does not[145]. Conversely, from what was stated before about geriatric patients solely affected by AP[142], 11 case reports of AP in COVID-19-affected elderly patients showed the typical clinical presentation with all the patients experiencing abdominal pain radiating to the back[151-160]. However, these findings are insufficient to suggest a direct or, most likely, indirect effect of SARS-CoV-2 infection on AP's clinical presentation. Therefore, further studies are necessary to establish a causative role. As previously reported, aging is considered a risk factor for a worse outcome not only in COVID-19 disease[161] but also in pancreatitis[149]. Advancing age is one of the 8 modified Glasgow Imrie severity Criteria for AP [162]. Three or more positive criteria, generally assessed within 48 h from admission, are indicative of severe pancreatitis and may require transfer to an intensive care unit[163]. Three of the 11 case reports had to be excluded from the analysis for insufficient data availability. Half of the remaining reports showed a result equal to or higher than three, suggesting severe pancreatitis. However, it is essential to highlight that due to the intrinsic limitation of retrospective analysis, information about the specific timings of the blood test is missing. To the authors' knowledge, no studies evaluating the isolated impact of age as a risk factor for severe prognosis exist for specific subgroups of patients like COVID-19 and AP. Moreover, in the aforementioned prognostic studies[136-138], the demographic composition of the age of the analyzed groups was so similar that no meaningful information could be inferred on the topic.

Impact of SARS-CoV-2 vaccines on patients with IBD

Initially, SARS-CoV-2 vaccines caused hesitancy in the IBD, mainly due to fear of poor response and safety concerns. IBD represents impaired immune function due to the disease and the therapies used to control this illness[60]. Jena *et al*[164] in a systematic review and meta-analysis, analyzed the response to complete vaccination in IBD patients. They reported positive seroconversion rates (95%), although these rates were slightly lower than non-IBD controls (98%). However, if considering only mRNA vaccines, the seroconversion data overlapped between the two populations. Similar results are reported by Bhurwal *et al*[165], which confirmed the adequate response to vaccination in the IBD population (96%), again showing improved outcomes with mRNA vaccines. Moreover, these two meta-analyses found no significant difference in seroconversion concerning the therapy administered (anti-TNF alone, vedolizumab, ustekinumab, or JAK inhibitors), in contrast to other studies which found lower responses to vaccination during anti-TNF alpha and JAK inhibitors therapy [166,167]. In addition, the analysis of breakthrough infections suggests an overall frequency similar to the general population. Nevertheless, a more rapid decline in antibody titers is described in IBD patients, particularly those treated with anti-TNF, immunomodulators, or a combination of these drugs[164]. Another parameter used to assess the efficacy of vaccination is the T cellular response, which plays an essential role in preventing disease progression[168]. Patients with IBD maintain this response even when receiving immune-targeted therapies, confirming that the immunocompromised state does not necessarily prevent a response to vaccination[169,170]. Interestingly, the T cellular response appears to be increased in patients taking anti-TNF-α due to unclear mechanisms[170]. Regarding the safety of vaccinations, several studies have observed similar side effect frequencies between the IBD population and the general population. However, no worsening or flare-up of the disease following vaccination has been proved. Thus, vaccines are considered a safe and well-tolerated strategy for IBD patients[171,172].

Focusing on the elderly population, it is well known that the response to vaccines may be lower than in young people [173], which may also occur with SARS-CoV-2 vaccines. This is probably the result of immune-senescence phenomena, which leads to quantitative and qualitative alterations in the immune system, including a reduction in naive T

Table 4 Evidence regarding pancreatic involvement in coronavirus disease 2019 patients

Ref.	Study design	No. of patients with pancreatic injury/total no. of patients	Remarks
Wang *et al*[122]	CHS	9/52 (4.68%)	Potential mild pancreatic involvement in patients with COVID-19 pneumonia
Bruno *et al*[124]	CHS	6/70 (8.5%)	Pancreatic involvement in hospitalized patients with documented COVID-19
Liu *et al*[125]	RS	13/121 (10.74%)	Pancreatic enzyme alteration incidence was higher in patients with severe COVID-19-related conditions than those with the non-severe disease. However, only a minority of patients with pancreatic enzyme alteration had a confirmed diagnosis of AP as defined by the AC
Stephens *et al*[126]	RS	158/234 (67.5%)	Raised serum amylase in patients with COVID-19 may not be associated with pancreatitis
Akkus *et al*[127]	RS	127/309 (15.7%)	Pancreatic injuries or AP are frequent during COVID-19 infection, especially in those with pre-existing DM
McNabb-Baltar *et al*[128]	RS	9/71 (12.1%)	Although a mild elevation in serum lipase was observed in some patients with COVID-19, acute clinical pancreatitis was not seen, according to the AC
Bansal *et al*[129]	RS	14/42 (33%), 7/29 patients (24.1%)	Pancreatic injury showed no statistically significant relation to the severity or outcome of COVID-19
Rasch *et al*[130]	CHS	22/38 (57.8%)	Patients with lipasemia needed more extended periods of mechanical ventilation than patients with COVID-19-associated ARDS
Barlass *et al*[131]	CCS	14/83 (16.8%)	Elevated lipase is associated with worse disease outcomes and increased ICU admission and intubation
Bacaksiz *et al*[132]	RS	316/1378 (23%)	Hyperamilasemia was significantly associated with COVID-19 severity
Magro *et al*[86]	Review	NA	Increased amylase or lipase levels might not be associated with AP in COVID-19 and may be a consequence of concurrent clinical conditions
Hunt *et al*[133]	Review	NA	No direct correlation between COVID-19 and pancreatic impairment could be established
Singh *et al*[134]	MS	1406/435731 (0.32%)	Worse clinical outcomes
Inamdar *et al*[135]	MS	189/11.883 (0.01%)	COVID-19 patients with pancreatitis were more likely to require mechanical ventilation and had a more extended hospital stay than patients without COVID-19
Karaali and Topal[136]	RS	189/562 (33.6%)	COVID-19 patients with AP had a higher rate of severe AP and a higher need for ICU admission
Dirweesh *et al*[137]	RS	75/339 (22.1%)	Higher mortality, MOF, and POF rates were registered in patients with AP and coexisting COVID-19
Pandanaboyana *et al*[138]	CHS	149/1777 (8.3%)	SARS-CoV-2 infection in acute pancreatitis increases 30-d mortality and disease severity
Mirò *et al*[140]	MS	45/63.822 (0.0007%)	Higher need for hospitalisation in COVID-19 patients with pancreatitis
Akarsu *et al*[141]	CCS	40/316 (12.6%)	Higher mortality rate and increased need for hospitalisation in COVID-19 patients with pancreatitis
Gubatan *et al*[62]	RS	100% total population 14235	Patients with a history of pancreatitis may be more susceptible to COVID-19
Huang *et al*[142]	RCS	4706/326993 (1.4%)	Pre-existing pancreatitis was associated with an increased risk of COVID-19–related hospitalisation and mortality
Hadi *et al*[143]	CS	2/3 (66.6%)	COVID-19 patients with CP bear higher hospitalisation rates
Georgakopoulou *et al*[144]	RS	100%	COVID-19-associated acute pancreatitis affected primarily females with a median age of 53.5 yr

CS: Case series; CR: Case reports; RS: Retrospective studies; RCS: Retrospective cohort study; MS: Multicentric study; CCS: Case-control study; CHS: Cohort study; CSS: Cross-sectional study; NA: Not available; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; AC: Atlanta criteria; CP: Convalescent plasma; ICU: Intensive care unit; MOF: Multiple-organ failure; POF: Persistent organ failure; ARDS: Acute respiratory distress syndrome.

lymphocytes available to respond to a vaccine, a significant decrease in CD8 T cells, and a reduction in the T helper follicular cell response[174-176]. Unfortunately, there are currently no studies evaluating vaccination efficacy and safety in elderly patients with IBD. However, in many studies, older age has been associated with attenuated responses, with an earlier decline of the antibody title and reduction differences in the overall strength of the T-cell, although in most of

Table 5 Case reports regarding pancreatic involvement in old coronavirus disease 2019 patients

Ref.	Study design	Age (yrs)	Remarks
Meyers *et al* [151]	CR	67	COVID-19 can cause clinical AP. Typical abdominal pain radiating to the back. Increase lipase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: NA
Karimzadeh *et al*[152]	CR	65	COVID-19 presents as mild AP. Typical abdominal pain radiating to the back. Increase lipase serum level. Negative abdominal CT scan. Glasgow Acute Pancreatitis Score: 1 point
Gadiparthi *et al* [153]	CR	74	AP in a patient with COVID-19 with SARS-CoV-2 as the possible etiological agent. Typical abdominal pain radiating to the back. Increase lipase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: 1 point
Wifi *et al*[154]	CR	72	Emphasises the importance of measuring serum amylase and lipase in patients with COVID-19. Typical abdominal pain radiating to the back. Increase lipase and amylase serum levels. Negative abdominal CT scan. Glasgow Acute Pancreatitis Score: 3 points
Gonzalo-Voltas *et al*[155]	CR	76	A case of AP that could be related to COVID-19 infection. Typical abdominal pain radiating to the back. Increase amylase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: NA
Reick-Mitrisin *et al*[156]	CR	71	AP should be considered in differential abdominal pain in patients with active or recent SARS-CoV-2 infection. Typical abdominal pain. Increase lipase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: 3 points
Brikman *et al* [163]	CR	61	Unresolved abdominal pain occurring late during COVID-19 warrants a thorough workup. Typical abdominal pain. Increase lipase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: 2 points
Acherjya *et al* [157]	CR	57	Pay attention to the atypical presentations of SARS-CoV-2, including AP. Typical abdominal pain radiating to the back. Increase lipase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: 4 points
Alves *et al*[158]	CR	56	Physicians should be aware that asymptomatic or mildly gastrointestinal symptomatic patients with COVID-19 require pancreatic enzymes and even abdomen imaging to diagnose pancreatitis. Typical abdominal pain. Increase lipase and amylase serum levels. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: 3 points
Shinohara *et al* [159]	CR	58	Extrapulmonary clinical characteristics of COVID-19 remain unclear. Typical abdominal pain. Increase amylase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: NA
Kumaran *et al* [160]	CR	67	Importance of considering COVID-19 as a potential cause in patients presenting with idiopathic pancreatitis. Typical abdominal pain. Increase amylase serum level. Evidence of AP in abdominal CT scan. Glasgow Acute Pancreatitis Score: 2 points

AP: Acute pancreatitis; CR: Case reports; CT: Computed tomography; NA: Not available; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

them, the presence of IBD does not influence these findings[169,170,177,178].

In conclusion, most of the studies mentioned above agree on the potential benefit of a further vaccine booster dose in patients with IBD, especially if elderly or on specific immunosuppressive therapies, such as anti-TNF alpha or JAK inhibitors. To date, studies reporting response rates with additional doses of COVID-19 vaccine in IBD are limited; however, current data suggest a significant boost in antibody binding levels from a third vaccine dose, even during immunosuppressive therapies, but patients receiving infliximab or tofacitinib show a lower response than healthy controls[179].

Impact of SARS-CoV-2 vaccines on patients with CLD

As mentioned above, cirrhotic patients are at high risk of severe COVID-19 infection. Therefore, vaccination against SARS-CoV-2 represents a significant protective measure in patients with CLD, which must be administered as early as possible[180,181]. Moreover, due to vaccinations, patients should not discontinue any of their medications for liver disease or delay any local or regional treatments for HCC[182]. Current COVID-19 vaccines are safe, but a rare vaccine-triggered immune-mediated hepatitis is reported after COVID-19 vaccination. These events are described in the literature in association with mRNA platforms, but cases have also been described for vector-based vaccines. These cases of liver injury are sporadic and respond to corticosteroid treatment. Therefore, liver injury after vaccination should not represent a limit to further vaccination[183]. Furthermore, a Chinese multi-centric study analyzed the safety and immunogenicity of inactivated SARS-CoV-2 vaccines in patients with CLDs[184]. These vaccines are safe in patients with CLD, as there was no significant difference in adverse reactions among the non-cirrhotic CLD, compensated cirrhosis, and decompensated cirrhosis subgroups. Pain was the most common local adverse reaction, while fever was the most commonly systematic adverse reaction reported. Among laboratory findings, only three patients of 437 with CLD had significant aminopherase elevation with ALT levels > 5 ULN, all of which had elevated aminopherase at baseline. Only one of the three patients required hospitalization. Nevertheless, it is impossible to attribute his adverse reaction to the vaccine for certainty since this patient had a history of discontinuing anti-hepatitis B virus therapy before the SARS-CoV-2 vaccination. In addition, patients with CLD often present an inadequate immune response, which may cause an incomplete immediate and long-term protective response[185]. Therefore, although these vaccines are safe in patients with CLD, they do not guarantee such patients equal antibody levels if compared to healthy controls. However, the difference in the positive rate of SARS-CoV-2 neutralizing antibodies between patients with CLD and healthy control groups is statistically significant (77.3% *vs*

90.3%) despite the adjustment for age, gender, and body mass index[184]. While there was no significant difference in positive rate between non-cirrhotic CLD patients (76.8%), compensated cirrhosis patients (78.9%), and decompensated cirrhosis patients (76.7%). Chen *et al*[186] confirmed in their study that inactivated SARS-CoV-2 vaccines are safe and well tolerated in patients with severe liver disease (such as cirrhosis or HCC). However, they also stressed the necessity to assign priority to vaccine patients with severe liver disease, which may have worse antibody responses than those with non-severe CLDs. Thuluvath *et al*[187] instead, analyzed the antibody response in CLD patients after administration of 2 doses of mRNA vaccines or a single dose of viral vector vaccine. This study revealed that only 24% of patients with CLDs had poor antibody responses. More in detail, 15.8% of patients who received the vector vaccine Johnson & Johnson had a good response, and the mRNA Moderna vaccine showed a better response than the mRNA Pfizer vaccine (76.4% *vs* 64.4%). Moreover, analyzing patients' characteristics, it emerged that those with undetectable antibodies (< 0.4 U/mL) had the highest mean age (64.8 years). In line with Thuluvath *et al*[187], Bakasis *et al*[188] highlighted that CLD was not associated with mRNA vaccine hypo-responsiveness. Only 4% of patients with CLD did not respond to SARS-CoV-2 vaccination, with statistically significant differences between cirrhotic and non-cirrhotic patients. The presence of liver disease showed no correlation with antibody titers or neutralizing activity, while age was negatively correlated with neutralizing activity. According to Willuweit *et al*[189], up to 96% of patients with liver cirrhosis presented an antibody response after receiving two doses of the mRNA-based vaccine. Unfortunately, antibody titers remained relatively stable in the control group while showing a rapid and significant decrease in patients with liver cirrhosis, with any differences stratifying cirrhotic patients according to age. The studies mentioned above do not focus on the differences between safety and efficacy among age (patients under 65 and over 65 years old or following stratification of groups according to age). Moreover, the studies conducted to evaluate the safety and efficacy of SARS-CoV-2 vaccination include a limited number of older adults[190]. Therefore, considering that older adults are more inclined to develop vaccine-related adverse events[191] and the lack of older adults in specific SARS-CoV-2 vaccination studies, further research is recommended to evaluate the safety and efficacy of COVID-19 vaccines in older people.

COVID-19 vaccines and pancreatic involvement

To the best of our knowledge, no published work evaluates the COVID-19 vaccination response in older adults suffering from pancreatic diseases. On the other hand, according to Pfizer's data, only 2 cases of AP as an adverse reaction (among 38000 participants) were reported during the clinical trial of the COVID-19 mRNA vaccine[192]. Since the Pfizer-BioNTech mRNA vaccine was approved for COVID-19 infection, AP has been reported in a few case reports[193-201]. Data inferred from United Kingdom databases (up to November 2022) for the same vaccine report 21 cases of pancreatitis, 19 cases of AP, and 3 cases of necrotizing pancreatitis[202]. The National Agency for the Safety of Medicines and Health Products reports 164 cases of pancreatitis up to February 10, 2022[203]. Data released from VigiBase, the WHO's global database, show 1093 cases of pancreatitis[204]. Currently, there is no evidence supporting a direct relationship between the vaccine and AP; even assuming the existence of vaccine-related pancreatic injury, its mechanism would still be unclear[194]. Moreover, considering the high rate of idiopathic pancreatitis[205], the available data are even more challenging to analyze. Considering the higher vaccination prevalence, fewer cases of AP following vaccination may suggest the involvement of different mechanisms in developing a vaccine-related pancreatic injury[196]. The benefits of vaccination against COVID-19 are unquestionable and not disputed in this paper. However, by reporting this evidence, we aim to make all healthcare workers aware of these possible adverse effects and to highlight the importance of not underestimating any abdominal pain after vaccination. As far as we know, the current literature does not include studies evaluating the safety and efficacy of SARS-CoV-2 vaccines in patients with CP. As mentioned above[142,143], CP may be associated with an increased risk of complications from COVID-19 infection leading to worse outcomes. Thus, reducing that risk by having the vaccine would be advisable.

CONCLUSION

Literature data confirm that the digestive manifestations of COVID-19 are frequent and often impact the clinical course of affected patients. In particular, patients with pre-existing liver disease, including cirrhosis or HCC, are at increased risk for worse outcomes. On the contrary, no definitive conclusions can be drawn for patients with IBD or pancreatic diseases. As for elderly patients with digestive disorders, although the available data are limited and extrapolated from studies not designed for this specific issue, there seems to be no evidence of worse COVID-19 outcomes than those without digestive diseases. As expected, this review confirms that age represents one of the main risk factors for poorer outcomes and higher mortality for COVID-19. Moreover, considering the under-representation of older adults in SARS-CoV-2 vaccination studies, further studies are necessary to evaluate better the safety and efficacy of COVID-19 vaccines, especially in frail older people with chronic digestive diseases.

FOOTNOTES

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REFERENCES

1 **Liu YC**, Kuo RL, Shih SR. COVID-19: The first documented coronavirus pandemic in history. *Biomed J* 2020; **43**: 328-333 [PMID: 32387617 DOI: 10.1016/j.bj.2020.04.007]
2 **World Health Organization**. WHO Coronavirus (COVID-19) Dashboard. [cited 17 October 2022]. Available from: https://covid19.who.int/?gclid=CjwKCAiAlNf-
3 **Wiersinga WJ**, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* 2020; **324**: 782-793 [PMID: 32648899 DOI: 10.1001/jama.2020.12839]
4 **Struyf T**, Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Leeflang MM, Spijker R, Hooft L, Emperador D, Domen J, Tans A, Janssens S, Wickramasinghe D, Lannoy V, Horn SRA, Van den Bruel A; Cochrane COVID-19 Diagnostic Test Accuracy Group. Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19. *Cochrane Database Syst Rev* 2022; **5**: CD013665 [PMID: 35593186 DOI: 10.1002/14651858.CD013665.pub3]
5 **Chua AJ**, Charn TC, Chan EC, Loh J. Acute Olfactory Loss Is Specific for COVID-19 at the Emergency Department. *Ann Emerg Med* 2020; **76**: 550-551 [PMID: 32410763 DOI: 10.1016/j.annemergmed.2020.05.015]
6 **Carpenter CR**, Mudd PA, West CP, Wilber E, Wilber ST. Diagnosing COVID-19 in the Emergency Department: A Scoping Review of Clinical Examinations, Laboratory Tests, Imaging Accuracy, and Biases. *Acad Emerg Med* 2020; **27**: 653-670 [PMID: 32542934 DOI: 10.1111/acem.14048]
7 **Long B**, Carius BM, Chavez S, Liang SY, Brady WJ, Koyfman A, Gottlieb M. Clinical update on COVID-19 for the emergency clinician: Presentation and evaluation. *Am J Emerg Med* 2022; **54**: 46-57 [PMID: 35121478 DOI: 10.1016/j.ajem.2022.01.028]
8 **D'Amico F**, Baumgart DC, Danese S, Peyrin-Biroulet L. Diarrhea During COVID-19 Infection: Pathogenesis, Epidemiology, Prevention, and Management. *Clin Gastroenterol Hepatol* 2020; **18**: 1663-1672 [PMID: 32278065 DOI: 10.1016/j.cgh.2020.04.001]
9 **Aroniadis OC**, Wang X, Gong T, Forbes N, Yang JY, Canakis A, Elmunzer BJ, Yadav D; North American Alliance for the Study of Digestive Manifestations of Covid-19. Factors Associated with the Development of Gastrointestinal Symptoms in Patients Hospitalized with Covid-19. *Dig Dis Sci* 2022; **67**: 3860-3871 [PMID: 34751837 DOI: 10.1007/s10620-021-07286-7]
10 **Cholankeril G**, Podboy A, Aivaliotis VI, Tarlow B, Pham EA, Spencer SP, Kim D, Hsing A, Ahmed A. High Prevalence of Concurrent Gastrointestinal Manifestations in Patients With Severe Acute Respiratory Syndrome Coronavirus 2: Early Experience From California. *Gastroenterology* 2020; **159**: 775-777 [PMID: 32283101 DOI: 10.1053/j.gastro.2020.04.008]
11 **Wang Y**, Li Y, Zhang Y, Liu Y. Are gastrointestinal symptoms associated with higher risk of Mortality in COVID-19 patients? A systematic review and meta-analysis. *BMC Gastroenterol* 2022; **22**: 106 [PMID: 35255816 DOI: 10.1186/s12876-022-02132-0]
12 **Gupta A**, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, Bikdeli B, Ahluwalia N, Ausiello JC, Wan EY, Freedberg DE, Kirtane AJ, Parikh SA, Maurer MS, Nordvig AS, Accili D, Bathon JM, Mohan S, Bauer KA, Leon MB, Krumholz HM, Uriel N, Mehra MR, Elkind MSV, Stone GW, Schwartz A, Ho DD, Bilezikian JP, Landry DW. Extrapulmonary manifestations of COVID-19. *Nat Med* 2020; **26**: 1017-1032 [PMID: 32651579 DOI: 10.1038/s41591-020-0968-3]
13 **Zheng Z**, Peng F, Xu B, Zhao J, Liu H, Peng J, Li Q, Jiang C, Zhou Y, Liu S, Ye C, Zhang P, Xing Y, Guo H, Tang W. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J Infect* 2020; **81**: e16-e25 [PMID: 32335169 DOI: 10.1016/j.jinf.2020.04.021]
14 **Mueller AL**, McNamara MS, Sinclair DA. Why does COVID-19 disproportionately affect older people? *Aging (Albany NY)* 2020; **12**: 9959-9981 [PMID: 32470948 DOI: 10.18632/aging.103344]
15 **Goodman KE**, Magder LS, Baghdadi JD, Pineles L, Levine AR, Perencevich EN, Harris AD. Impact of Sex and Metabolic Comorbidities on Coronavirus Disease 2019 (COVID-19) Mortality Risk Across Age Groups: 66 646 Inpatients Across 613 U.S. Hospitals. *Clin Infect Dis* 2021; **73**: e4113-e4123 [PMID: 33337474 DOI: 10.1093/cid/ciaa1787]
16 **Chen Y**, Klein SL, Garibaldi BT, Li H, Wu C, Osevala NM, Li T, Margolick JB, Pawelec G, Leng SX. Aging in COVID-19: Vulnerability, immunity and intervention. *Ageing Res Rev* 2021; **65**: 101205 [PMID: 33137510 DOI: 10.1016/j.arr.2020.101205]
17 **Channappanavar R**, Perlman S. Age-related susceptibility to coronavirus infections: role of impaired and dysregulated host immunity. *J Clin Invest* 2020; **130**: 6204-6213 [PMID: 33085654 DOI: 10.1172/JCI144115]
18 **Papa A**, Covino M, Pizzolante F, Miele L, Lopetuso LR, Bove V, Iorio R, Simeoni B, Vetrone LM, Tricoli L, Mignini I, Schepis T, D'Alessandro A, Coppola G, Nicoletti T, Visconti E, Rapaccini G. Gastrointestinal symptoms and digestive comorbidities in an Italian cohort of patients with COVID-19. *Eur Rev Med Pharmacol Sci* 2020; **24**: 7506-7511 [PMID: 32706091 DOI: 10.26355/eurrev_202007_21923]
19 **Zou X**, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med* 2020; **14**: 185-192 [PMID: 32170560 DOI: 10.1007/s11684-020-0754-0]

20 **Lin L**, Jiang X, Zhang Z, Huang S, Fang Z, Gu Z, Gao L, Shi H, Mai L, Liu Y, Lin X, Lai R, Yan Z, Li X, Shan H. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020; **69**: 997-1001 [PMID: 32241899 DOI: 10.1136/gutjnl-2020-321013]

21 **Selva KJ**, Chung AW. Insights into how SARS-CoV2 infection induces cytokine storms. *Trends Immunol* 2022; **43**: 417-419 [PMID: 35537983 DOI: 10.1016/j.it.2022.04.007]

22 **Villapol S**. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res* 2020; **226**: 57-69 [PMID: 32827705 DOI: 10.1016/j.trsl.2020.08.004]

23 **Zhan T**, Tang Y, Han Z, Zhu Q, Tan J, Liu M, Cai Y, Huang M, Chen X, Cheng X, Deng J, Huang X, Tian X. Clinical Characteristics of 195 Cases of COVID-19 with Gastrointestinal Symptoms COVID-19 with Gastrointestinal Symptoms. *Turk J Gastroenterol* 2021; **32**: 148-154 [PMID: 33960938 DOI: 10.5152/tjg.2021.20379]

24 **Perisetti A**, Goyal H, Gajendran M, Boregowda U, Mann R, Sharma N. Prevalence, Mechanisms, and Implications of Gastrointestinal Symptoms in COVID-19. *Front Med (Lausanne)* 2020; **7**: 588711 [PMID: 33195352 DOI: 10.3389/fmed.2020.588711]

25 **Papadakis KA**, Prehn J, Nelson V, Cheng L, Binder SW, Ponath PD, Andrew DP, Targan SR. The role of thymus-expressed chemokine and its receptor CCR9 on lymphocytes in the regional specialization of the mucosal immune system. *J Immunol* 2000; **165**: 5069-5076 [PMID: 11046037 DOI: 10.4049/jimmunol.165.9.5069]

26 **Babic T**, Browning KN. The role of vagal neurocircuits in the regulation of nausea and vomiting. *Eur J Pharmacol* 2014; **722**: 38-47 [PMID: 24184670 DOI: 10.1016/j.ejphar.2013.08.047]

27 **Fang D**, Ma J, Guan J, Wang M, Song Y, Tian D, Li P. Manifestations of digestive system of hospitalized patients with coronavirus disease 2019 in Wuhan, China: a single-center descriptive study. *Chin J Digestion* 2020; 151-156

28 **Jiménez E**, Fontán-Vela M, Valencia J, Fernandez-Jimenez I, Álvaro-Alonso EA, Izquierdo-García E, Lazaro Cebas A, Gallego Ruiz-Elvira E, Troya J, Tebar-Martinez AJ, Garcia-Marina B, Peña-Lillo G, Abad-Motos A, Macaya L, Ryan P, Pérez-Butragueño M; COVID@HUIL Working Group; COVID@HUIL Working Group. Characteristics, complications and outcomes among 1549 patients hospitalised with COVID-19 in a secondary hospital in Madrid, Spain: a retrospective case series study. *BMJ Open* 2020; **10**: e042398 [PMID: 33172949 DOI: 10.1136/bmjopen-2020-042398]

29 **Vuille-Dit-Bille RN**, Liechty KW, Verrey F, Guglielmetti LC. SARS-CoV-2 receptor ACE2 gene expression in small intestine correlates with age. *Amino Acids* 2020; **52**: 1063-1065 [PMID: 32627059 DOI: 10.1007/s00726-020-02870-z]

30 **Hashimoto T**, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 2012; **487**: 477-481 [PMID: 22837003 DOI: 10.1038/nature11228]

31 **Liu Q**, Wang RS, Qu GQ, Wang YY, Liu P, Zhu YZ, Fei G, Ren L, Zhou YW, Liu L. Gross examination report of a COVID-19 death autopsy. *Fa Yi Xue Za Zhi* 2020; **36**: 21-23 [PMID: 32198987 DOI: 10.12116/j.issn.1004-5619.2020.01.005]

32 **de Souza CD**, de Arruda Magalhães AJ, Lima AJ, Nunes DN, de Fátima Machado Soares É, de Castro Silva L, Santos LG, Dos Santos Cardoso VI, Nobre YV, do Carmo RF. Clinical manifestations and factors associated with mortality from COVID-19 in older adults: Retrospective population-based study with 9807 older Brazilian COVID-19 patients. *Geriatr Gerontol Int* 2020; **20**: 1177-1181 [PMID: 33111433 DOI: 10.1111/ggi.14061]

33 **Marziliano A**, Burns E, Chauhan L, Liu Y, Makhnevich A, Zhang M, Carney MT, Dbeis Y, Lindvall C, Qiu M, Diefenbach MA, Sinvani L. Patient Factors and Hospital Outcomes Associated With Atypical Presentation in Hospitalized Older Adults With COVID-19 During the First Surge of the Pandemic. *J Gerontol A Biol Sci Med Sci* 2022; **77**: e124-e132 [PMID: 34279628 DOI: 10.1093/gerona/glab171]

34 **Ramos-Rincon JM**, Buonaiuto V, Ricci M, Martín-Carmona J, Paredes-Ruíz D, Calderón-Moreno M, Rubio-Rivas M, Beato-Pérez JL, Arnalich-Fernández F, Monge-Monge D, Vargas-Núñez JA, Acebes-Repiso G, Mendez-Bailon M, Perales-Fraile I, García-García GM, Guisado-Vasco P, Abdelhady-Kishta A, Pascual-Pérez MD, Rodríguez-Fernández-Viagas C, Montaño-Martínez A, López-Ruiz A, Gonzalez-Juarez MJ, Pérez-García C, Casas-Rojo JM, Gómez-Huelgas R; SEMI-COVID-19 Network. Clinical Characteristics and Risk Factors for Mortality in Very Old Patients Hospitalized With COVID-19 in Spain. *J Gerontol A Biol Sci Med Sci* 2021; **76**: e28-e37 [PMID: 33103720 DOI: 10.1093/gerona/glaa243]

35 **Lanthier N**, Mahiat C, Henrard S, Stärkel P, Gilard I, De Brauwer I, Cornette P, Boland B. Gastro-intestinal symptoms are associated with a lower in-hospital mortality rate in frail older patients hospitalized for COVID-19. *Acta Gastroenterol Belg* 2021; **84**: 135-136 [PMID: 33639706 DOI: 10.51821/84.1.824]

36 **Vrillon A**, Hourregue C, Azuar J, Grosset L, Boutelier A, Tan S, Roger M, Mourman V, Mouly S, Sène D, François V, Dumurgier J, Paquet C; for LRB COVID Group. COVID-19 in Older Adults: A Series of 76 Patients Aged 85 Years and Older with COVID-19. *J Am Geriatr Soc* 2020; **68**: 2735-2743 [PMID: 33045106 DOI: 10.1111/jgs.16894]

37 **Atalla E**, Zhang R, Shehadeh F, Mylona EK, Tsikala-Vafea M, Kalagara S, Henseler L, Chan PA, Mylonakis E. Clinical Presentation, Course, and Risk Factors Associated with Mortality in a Severe Outbreak of COVID-19 in Rhode Island, USA, April-June 2020. *Pathogens* 2020; **10** [PMID: 33374131 DOI: 10.3390/pathogens10010008]

38 **González González R**, Jacob J, Miró Ò, Llorens P, Jiménez S, González Del Castillo J, Burillo-Putze G, Martín A, Martín-Sánchez FJ, Lamberechts JG, Alquézar-Arbé A, Higa-Sansone L, Gayoso Martín S, Carbajosa V, Beddar Chaib F, Salido M, Marchena González MJ, Calvo López R, González Martínez F, Pavón Monzo J, Velarde Herrera DM, Niembro Valdés AP, Quero Motto E, Ferreras Amez JM, Piñera-Salmerón P; Spanish Investigators on Emergency Situations TeAm (SIESTA) Network. Incidence, Clinical Characteristics, Risk Factors, and Outcomes of Upper Gastrointestinal Bleeding in Patients With COVID-19: Results of the UMC-19-S12. *J Clin Gastroenterol* 2022; **56**: e38-e46 [PMID: 33252555 DOI: 10.1097/MCG.0000000000001465]

39 **Zhai Z**, Li C, Chen Y, Gerotziafas G, Zhang Z, Wan J, Liu P, Elalamy I, Wang C; Prevention Treatment of VTE Associated with COVID-19 Infection Consensus Statement Group. Prevention and Treatment of Venous Thromboembolism Associated with Coronavirus Disease 2019 Infection: A Consensus Statement before Guidelines. *Thromb Haemost* 2020; **120**: 937-948 [PMID: 32316065 DOI: 10.1055/s-0040-1710019]

40 **Tiwari NR**, Khatib KI, Dixit SB, Rathore PK, Melinkeri S, Ganapule A, Borawake KS, Mhatre U. Anticoagulation in COVID - 19: An Update. *J Crit Care Med (Targu Mures)* 2020; **6**: 217-223 [PMID: 33200092 DOI: 10.2478/jccm-2020-0033]

41 **Ion D**, Gherghinescu M, Andronic O, Andreescu CV, Păduraru DN, Bolocan A, Russu C, Aprodu S, Mationi G, Nicolescu C, Popa D. Prognosis Evaluation for Patients with Abdominal Trauma Using Usual Biological Parameters. *Chirurgia (Bucur)* 2021; **116**: 737-747 [PMID: 34967718 DOI: 10.21614/chirurgia.116.6.737]

42 **Dioscoridi L**, Giannetti A, Massad MT, Forti E, Pugliese F, Cintolo M, Bonato G, Rosa R, Mutignani M. A "double-hit" damage mechanism can explain self-limited GI bleeding in COVID-19 pneumonia. *Gastrointest Endosc* 2021; **93**: 1192-1193 [PMID: 33875144 DOI: 10.1016/j.gie.2020.12.022]

43 **Al-Samkari H**, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, Goodarzi K, Bendapudi PK, Bornikova L, Gupta S, Leaf DE, Kuter DJ, Rosovsky RP. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood* 2020; **136**: 489-500 [PMID: 32492712 DOI: 10.1182/blood.2020006520]

44 **Ojetti V**, Saviano A, Covino M, Acampora N, Troiani E, Franceschi F; GEMELLI AGAINST COVID-19 group. COVID-19 and intestinal inflammation: Role of fecal calprotectin. *Dig Liver Dis* 2020; **52**: 1231-1233 [PMID: 33060042 DOI: 10.1016/j.dld.2020.09.015]

45 **Ludvigsson JF**, Axelrad J, Halfvarson J, Khalili H, Larsson E, Lochhead P, Roelstraete B, Simon TG, Söderling J, Olén O. Inflammatory bowel disease and risk of severe COVID-19: A nationwide population-based cohort study in Sweden. *United European Gastroenterol J* 2021; **9**: 177-192 [PMID: 33704918 DOI: 10.1002/ueg2.12049]

46 **Amiot A**, Rahier JF, Baert F, Nahon S, Hart A, Viazis N, Biancone L, Domenech E, Reenears C, Peyrin-Biroulet L, Beaugerie L, Burisch J; I-CARE collaborator group. The Impact of COVID-19 on Patients with IBD in a Prospective European Cohort Study. *J Crohns Colitis* 2023; **17**: 37-48 [PMID: 35767639 DOI: 10.1093/ecco-jcc/jjac091]

47 **Maconi G**, Bosetti C, De Monti A, Boyapati RK, Shelton E, Piazza N, Carvalhas Gabrielli AM, Lenti MV, Bezzio C, Ricci C, Greco S, Romeo S, Giangregorio F, Gridavilla D, Tagliani F, Massari A, Pastorelli L, Di Sabatino A, Saibeni S, Alicante S, Ferretti F, Rizzardini G, Galli M, Ardizzone S. Risk of COVID 19 in patients with inflammatory bowel diseases compared to a control population. *Dig Liver Dis* 2021; **53**: 263-270 [PMID: 33483259 DOI: 10.1016/j.dld.2020.12.013]

48 **Papa A**, Gasbarrini A, Tursi A. Epidemiology and the Impact of Therapies on the Outcome of COVID-19 in Patients With Inflammatory Bowel Disease. *Am J Gastroenterol* 2020; **115**: 1722-1724 [PMID: 32826572 DOI: 10.14309/ajg.0000000000000830]

49 **Singh AK**, Jena A, Kumar-M P, Jha DK, Sharma V. Clinical presentation of COVID-19 in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Intest Res* 2022; **20**: 134-143 [PMID: 33440918 DOI: 10.5217/ir.2020.00108]

50 **Lee MH**, Li HJ, Wasuwanich P, Kim SE, Kim JY, Jeong GH, Park S, Yang JW, Kim MS, Yon DK, Lee SW, Koyanagi A, Jacob L, Kim EY, Cheon JH, Shin JI, Smith L. COVID-19 susceptibility and clinical outcomes in inflammatory bowel disease: An updated systematic review and meta-analysis. *Rev Med Virol* 2022; e2414 [PMID: 36504172 DOI: 10.1002/rmv.2414]

51 **Chen L**, Hu K, Cheng C, Hu Q, Zhang L, An T, Guo Y, Chen S, Duan G. Risk of adverse outcomes in inflammatory bowel disease patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Colorectal Dis* 2022; **37**: 2277-2289 [PMID: 36271206 DOI: 10.1007/s00384-022-04265-w]

52 **Bezzio C**, Saibeni S, Variola A, Allocca M, Massari A, Gerardi V, Casini V, Ricci C, Zingone F, Amato A, Caprioli F, Lenti MV, Viganò C, Ascolani M, Bossa F, Castiglione F, Cortelezzi C, Grossi L, Milla M, Morganti D, Pastorelli L, Ribaldone DG, Sartini A, Soriano A, Manes G, Danese S, Fantini MC, Armuzzi A, Daperno M, Fiorino G; Italian Group for the Study of Inflammatory Bowel Disease (IG-IBD). Outcomes of COVID-19 in 79 patients with IBD in Italy: an IG-IBD study. *Gut* 2020; **69**: 1213-1217 [PMID: 32354990 DOI: 10.1136/gutjnl-2020-321411]

53 **Ricciuto A**, Lamb CA, Benchimol EI, Walker GJ, Kennedy NA, Kuenzig ME, Kaplan GG, Kappelman MD, Ungaro RC, Colombel JF, Brenner EJ, Agrawal M, Reinisch W, Griffiths AM, Sebastian S. Inflammatory Bowel Disease Clinical Activity is Associated with COVID-19 Severity Especially in Younger Patients. *J Crohns Colitis* 2022; **16**: 591-600 [PMID: 34570886 DOI: 10.1093/ecco-jcc/jjab172]

54 **Tripathi K**, Godoy Brewer G, Thu Nguyen M, Singh Y, Saleh Ismail M, Sauk JS, Parian AM, Limketkai BN. COVID-19 and Outcomes in Patients With Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. *Inflamm Bowel Dis* 2022; **28**: 1265-1279 [PMID: 34718595 DOI: 10.1093/ibd/izab236]

55 **Bezzio C**, Fiorino G, Ribaldone DG, Armuzzi A, Saibeni S; IG-IBD COVID-19 Study Group. IBD Flare in the COVID-19 Pandemic: Therapy Discontinuation Is to Blame. *Inflamm Bowel Dis* 2022 [PMID: 35972338 DOI: 10.1093/ibd/izac173]

56 **Papa A**, Papa V, Lopetuso LR, Gasbarrini A, Tursi A. Covid-19 and the management of patients with inflammatory bowel disease: a practical decalogue for the post-pandemic phase. *Therap Adv Gastroenterol* 2020; **13**: 1756284820968747 [PMID: 33149764 DOI: 10.1177/1756284820968747]

57 **Sturm A**, Maaser C, Mendall M, Karagiannis D, Karatzas P, Ipenburg N, Sebastian S, Rizzello F, Limdi J, Katsanos K, Schmidt C, Jeuring S, Colombo F, Gionchetti P. European Crohn's and Colitis Organisation Topical Review on IBD in the Elderly. *J Crohns Colitis* 2017; **11**: 263-273 [PMID: 27797918 DOI: 10.1093/ecco-jcc/jjw188]

58 **Hong SJ**, Katz S. The elderly IBD patient in the modern era: changing paradigms in risk stratification and therapeutic management. *Therap Adv Gastroenterol* 2021; **14**: 17562848211023399 [PMID: 34276809 DOI: 10.1177/17562848211023399]

59 **Brenner EJ**, Ungaro RC, Gearry RB, Kaplan GG, Kissous-Hunt M, Lewis JD, Ng SC, Rahier JF, Reinisch W, Ruemmele FM, Steinwurz F, Underwood FE, Zhang X, Colombel JF, Kappelman MD. Corticosteroids, But Not TNF Antagonists, Are Associated With Adverse COVID-19 Outcomes in Patients With Inflammatory Bowel Diseases: Results From an International Registry. *Gastroenterology* 2020; **159**: 481-491.e3 [PMID: 32425234 DOI: 10.1053/j.gastro.2020.05.032]

60 **Zabana Y**, Marín-Jiménez I, Rodríguez-Lago I, Vera I, Martín-Arranz MD, Guerra I, P Gisbert J, Mesonero F, Benítez O, Taxonera C, Ponferrada-Díaz Á, Piqueras M, J Lucendo A, Caballol B, Mañosa M, Martínez-Montiel P, Bosca-Watts M, Gordillo J, Bujanda L, Manceñido N, Martínez-Pérez T, López A, Rodríguez-Gutiérrez C, García-López S, Vega P, Rivero M, Melcarne L, Calvo M, Iborra M, Barreiro de Acosta M, Sicilia B, Barrio J, Pérez Calle JL, Busquets D, Pérez-Martínez I, Navarro-Llavat M, Hernández V, Argüelles-Arias F, Ramírez Esteso F, Meijide S, Ramos L, Gomollón F, Muñoz F, Suris G, Ortiz de Zarate J, Huguet JM, Llaó J, García-Sepulcre MF, Sierra M, Durà M, Estrecha S, Fuentes Coronel A, Hinojosa E, Olivan L, Iglesias E, Gutiérrez A, Varela P, Rull N, Gilabert P, Hernández-Camba A, Brotons A, Ginard D, Sesé E, Carpio D, Aceituno M, Cabriada JL, González-Lama Y, Jiménez L, Chaparro M, López-San Román A, Alba C, Plaza-Santos R, Mena R, Tamarit-Sebastián S, Ricart E, Calafat M, Olivares S, Navarro P, Bertoletti F, Alonso-Galán H, Pajares R, Olcina P, Manzano P, Domènech E, Esteve M, On Behalf Of The Eneida Registry Of Geteccu. Risk Factors for COVID-19 in Inflammatory Bowel Disease: A National, ENEIDA-Based Case-Control Study (COVID-19-EII). *J Clin Med* 2022; **11** [PMID: 36556155 DOI: 10.3390/jcm11247540]

61 **Wetwittayakhlang P**, Albader F, Golovics PA, Hahn GD, Bessissow T, Bitton A, Afif W, Wild G, Lakatos PL. Clinical Outcomes of COVID-19 and Impact on Disease Course in Patients with Inflammatory Bowel Disease. *Can J Gastroenterol Hepatol* 2021; **2021**: 7591141 [PMID: 34858891 DOI: 10.1155/2021/7591141]

62 **Gubatan J**, Levitte S, Patel A, Balabanis T, Sharma A, Jones E, Lee B, Manohar M, Swaminathan G, Park W, Habtezion A. Prevalence, risk factors and clinical outcomes of COVID-19 in patients with a history of pancreatitis in Northern California. *Gut* 2021; **70**: 440-441 [PMID: 32493828 DOI: 10.1136/gutjnl-2020-321772]

63 **Calafat M**, González-Muñoza C, Fortuny M, Roig C, Calm A, Mombiela A, Cañete F, Bertoletti F, González-González L, Teller-Martín M, Gordillo J, Mañosa M, Garcia-Planella E, Domènech E. Impact of immunosuppressants on SARS-CoV-2 infection in elderly patients with inflammatory bowel disease. *Aging Clin Exp Res* 2021; **33**: 2355-2359 [PMID: 34164799 DOI: 10.1007/s40520-021-01917-9]

64 **Geisz M**, Ha C, Kappelman MD, Martin CF, Chen W, Anton K, Sandler RS, Long MD. Medication Utilization and the Impact of Continued

Corticosteroid Use on Patient-reported Outcomes in Older Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22**: 1435-1441 [PMID: 26978725 DOI: 10.1097/MIB.0000000000000747]

65 **Bernstein CN**, Singh H, Murthy SK, Nguyen GC, Benchimol EI, Bitton A, Kuenzig ME, Huang JG, Jones JL, Lee K, Targownik LE, Windsor JW, Mukhtar MS, Tandon P, Kaplan GG. Crohn's and Colitis Canada's 2021 Impact of COVID-19 and Inflammatory Bowel Disease in Canada: Seniors With IBD. *J Can Assoc Gastroenterol* 2021; **4**: S34-S39 [PMID: 34755037 DOI: 10.1093/jcag/gwab025]

66 **Kulkarni AV**, Kumar P, Tevethia HV, Premkumar M, Arab JP, Candia R, Talukdar R, Sharma M, Qi X, Rao PN, Reddy DN. Systematic review with meta-analysis: liver manifestations and outcomes in COVID-19. *Aliment Pharmacol Ther* 2020; **52**: 584-599 [PMID: 32638436 DOI: 10.1111/apt.15916]

67 **Mehta P**, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ; HLH Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; **395**: 1033-1034 [PMID: 32192578 DOI: 10.1016/S0140-6736(20)30628-0]

68 **Li S**, Li J, Zhang Z, Tan L, Shao T, Li M, Li X, Holmes JA, Lin W, Han M. COVID-19 induced liver function abnormality associates with age. *Aging (Albany NY)* 2020; **12**: 13895-13904 [PMID: 32721928 DOI: 10.18632/aging.103720]

69 **Nardo AD**, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int* 2021; **41**: 20-32 [PMID: 33190346 DOI: 10.1111/liv.14730]

70 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]

71 **Kumar P**, Sharma M, Kulkarni A, Rao PN. Pathogenesis of Liver Injury in Coronavirus Disease 2019. *J Clin Exp Hepatol* 2020; **10**: 641-642 [PMID: 32837092 DOI: 10.1016/j.jceh.2020.05.006]

72 **Zhao CL**, Rapkiewicz A, Maghsoodi-Deerwester M, Gupta M, Cao W, Palaia T, Zhou J, Ram B, Vo D, Rafiee B, Hossein-Zadeh Z, Dabiri B, Hanna I. Pathological findings in the postmortem liver of patients with coronavirus disease 2019 (COVID-19). *Hum Pathol* 2021; **109**: 59-68 [PMID: 33307078 DOI: 10.1016/j.humpath.2020.11.015]

73 **Xu Z**, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; **8**: 420-422 [PMID: 32085846 DOI: 10.1016/S2213-2600(20)30076-X]

74 **Ibrahim N**, Hosri J, Bteich Y, Dib A, Abou Rached A. COVID-19 and Liver Dysfunction. *Cureus* 2022; **14**: e21302 [PMID: 35186564 DOI: 10.7759/cureus.21302]

75 **Cai Q**, Huang D, Yu H, Zhu Z, Xia Z, Su Y, Li Z, Zhou G, Gou J, Qu J, Sun Y, Liu Y, He Q, Chen J, Liu L, Xu L. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; **73**: 566-574 [PMID: 32298767 DOI: 10.1016/j.jhep.2020.04.006]

76 **Săbiescu DM**, Kamal AM, Kamal CK, Alexandru DO, Mitruț P. Liver damage in the context of SARS-CoV-2. Covid-19 treatment and its effects on the liver. *J Med Life* 2022; **15**: 727-734 [PMID: 35928369 DOI: 10.25122/jml-2022-0177]

77 **Tokarczyk U**, Kaliszewski K, Kopszak A, Nowak Ł, Sutkowska-Stępień K, Sroczyński M, Sępek M, Dudek A, Diakowska D, Trocha M, Gajecki D, Gawryś J, Matys T, Maciejiczek J, Kozub V, Szalast R, Madziarski M, Zubkiewicz-Zarębska A, Letachowicz K, Kiliś-Pstrusińska K, Matera-Witkiewicz A, Pomorski M, Protasiewicz M, Sokołowski J, Adamik B, Kujawa K, Doroszko A, Madziarska K, Jankowska EA. Liver Function Tests in COVID-19: Assessment of the Actual Prognostic Value. *J Clin Med* 2022; **11** [PMID: 35956107 DOI: 10.3390/jcm11154490]

78 **Phipps MM**, Barraza LH, LaSota ED, Sobieszczyk ME, Pereira MR, Zheng EX, Fox AN, Zucker J, Verna EC. Acute Liver Injury in COVID-19: Prevalence and Association with Clinical Outcomes in a Large U.S. Cohort. *Hepatology* 2020; **72**: 807-817 [PMID: 32473607 DOI: 10.1002/hep.31404]

79 **Da BL**, Kushner T, El Halabi M, Paka P, Khalid M, Uberoi A, Lee BT, Perumalswami PV, Rutledge SM, Schiano TD, Friedman SL, Saberi B. Liver Injury in Patients Hospitalized with Coronavirus Disease 2019 Correlates with Hyperinflammatory Response and Elevated Interleukin-6. *Hepatol Commun* 2021; **5**: 177-188 [PMID: 33230491 DOI: 10.1002/hep4.1631]

80 **Pazgan-Simon M**, Serafińska S, Kukla M, Kucharska M, Zuwała-Jagiełło J, Buczyńska I, Zielińska K, Simon K. Liver Injury in Patients with COVID-19 without Underlying Liver Disease. *J Clin Med* 2022; **11** [PMID: 35054003 DOI: 10.3390/jcm11020308]

81 **Singh S**, Khan A. Clinical Characteristics and Outcomes of Coronavirus Disease 2019 Among Patients With Preexisting Liver Disease in the United States: A Multicenter Research Network Study. *Gastroenterology* 2020; **159**: 768-771.e3 [PMID: 32376408 DOI: 10.1053/j.gastro.2020.04.064]

82 **Wang Q**, Davis PB, Xu R. COVID-19 risk, disparities and outcomes in patients with chronic liver disease in the United States. *EClinicalMedicine* 2021; **31**: 100688 [PMID: 33521611 DOI: 10.1016/j.eclinm.2020.100688]

83 **Iavarone M**, D'Ambrosio R, Soria A, Triolo M, Pugliese N, Del Poggio P, Perricone G, Massironi S, Spinetti A, Buscarini E, Viganò M, Carriero C, Fagiuoli S, Aghemo A, Belli LS, Lucà M, Pedaci M, Rimondi A, Rumi MG, Invernizzi P, Bonfanti P, Lampertico P. High rates of 30-day mortality in patients with cirrhosis and COVID-19. *J Hepatol* 2020; **73**: 1063-1071 [PMID: 32526252 DOI: 10.1016/j.jhep.2020.06.001]

84 **Mallet V**, Beeker N, Bouam S, Sogni P, Pol S; Demosthenes research group. Prognosis of French COVID-19 patients with chronic liver disease: A national retrospective cohort study for 2020. *J Hepatol* 2021; **75**: 848-855 [PMID: 33992699 DOI: 10.1016/j.jhep.2021.04.052]

85 **Mohammed A**, Paranji N, Chen PH, Niu B. COVID-19 in Chronic Liver Disease and Liver Transplantation: A Clinical Review. *J Clin Gastroenterol* 2021; **55**: 187-194 [PMID: 33394628 DOI: 10.1097/MCG.0000000000001481]

86 **Magro F**, Nuzzo A, Abreu C, Libânio D, Rodriguez-Lago I, Pawlak K, Hollenbach M, Brouwer WP, Siau K. COVID-19 in gastroenterology: Where are we now? Current evidence on the impact of COVID-19 in gastroenterology. *United European Gastroenterol J* 2021; **9**: 750-765 [PMID: 34190413 DOI: 10.1002/ueg2.12115]

87 **Ekpanyapong S**, Bunchorntavakul C, Reddy KR. COVID-19 and the Liver: Lessons Learnt from the EAST and the WEST, A Year Later. *J Viral Hepat* 2022; **29**: 4-20 [PMID: 34352133 DOI: 10.1111/jvh.13590]

88 **Hoffmann C**, Gerber PA, Cavelti-Weder C, Licht L, Kotb R, Al Dweik R, Cherfane M, Bornstein SR, Perakakis N. Liver, NAFLD and COVID-19. *Horm Metab Res* 2022; **54**: 522-531 [PMID: 35468630 DOI: 10.1055/a-1834-9008]

89 **Boettler T**, Newsome PN, Mondelli MU, Maticic M, Cordero E, Cornberg M, Berg T. Care of patients with liver disease during the COVID-19 pandemic: EASL-ESCMID position paper. *JHEP Rep* 2020; **2**: 100113 [PMID: 32289115 DOI: 10.1016/j.jhepr.2020.100113]

90 **Sachdeva S**, Khandait H, Kopel J, Aloysius MM, Desai R, Goyal H. NAFLD and COVID-19: a Pooled Analysis. *SN Compr Clin Med* 2020; **2**: 2726-2729 [PMID: 33173850 DOI: 10.1007/s42399-020-00631-3]

91 **Berenguer J**, Ryan P, Rodríguez-Baño J, Jarrín I, Carratalà J, Pachón J, Yllescas M, Arriba JR; COVID-19@Spain Study Group; Fundación SEIMC-GESIDA; Hospital General Universitario Gregorio Marañón; Hospital Universitario La Paz; Hospital Infanta Leonor; Complejo

Hospitalario Virgen de la Salud; Hospital Universitario Rafael Méndez; Hospital Universitario de Cruces; Hospital de Melilla; Hospital San Eloy de Barakaldo; Hospital Universitario Central de Asturias; Hospital General Universitario de Alicante; Hospital Virgen de la Victoria; Hospital Universitario Puerto Real; EOXI Pontevedra e Salnés; Hospital de Figueres; Hospital Sant Jaume de Calella; Hospital del Mar; Hospital Virgen de la Arrixaca; Hospital de Can Misses; Hospital de Sagunto; Hospital Clínico San Cecilio; Hospital Universitario Príncipe de Asturias; Parc Sanitari Sant Joan de Déu; Hospital Nuestra Señora de Gracia; HC Marbella Internacional Hospital; Hospital La Princesa; Hospital Josep Trueta; Hospital Dos de Maig; Hospital Arnau de Vilanova-Lliria; Hospital General Universitario de Elche; Hospital Clínico Universitario de Valencia; Complejo Asistencial de Ávila; Hospital Comarcal de Alcañiz; Hospital Universitario Marqués de Valdecilla; Hospital Quiron-Salud de Torrevieja; Hospital Universitario Miguel Servet; SCIAS, Hospital de Barcelona; Fundación Hospital Universitario Alcorcón; Hospital Álvaro Cunqueiro; Complejo Asistencial Universitario de Salamanca; Hospital Universitario Severo Ochoa; Hospital CIMA-Sanitas; Hospital HLA Inmaculada; Hospital Universitario Rio Hortega; Hospital de Guadalajara; Hospital Universitario Infanta Sofía; Hospital Comarcal de Blanes; Hospital Universitari de Tarragona Joan XXIII; Hospital Universitario Basurto; Hospital Universitario de Canarias; Hospital Universitario de Gran Canaria Dr Negrín; Hospital Son Espases; Hospital Universitario de Móstoles; Complejo Hospitalario Universitario A Coruña; Hospital Costa del Sol; Hospital Clínico Universitario Lozano Blesa; Hospital Mutua de Terrassa; Hospital de la Plana; Hospital Virgen de la Concha–Complejo Asistencial de Zamora; Complejo Hospitalario Universitario Insular Materno-Infantil; Hospital de la Marina Baixa; Hospital Universitario Virgen Macarena; Hospital Universitari de Bellvitge; Hospital Universitario y Politécnico la Fe; Hospital Universitario del Vinalopó; Hospital de Sabadell (Parc Tauli); Hospital Clinic de Barcelona; Hospital Universitario de la Ribera; Fundación Jiménez Díaz; Hospital Clínico Universitario de Valladolid; Hospital Clínico San Carlos; Hospital Santa Creu i Sant Pau; Clínica Universitaria de Navarra–Campus Madrid; Hospital Son Llatzer; Hospital General de la Defensa Gómez Ulla; Hospital Universitario de Álava; Hospital Santos Reyes; Hospital Dr José Molina Orosa; Hospital Vall d'Hebrón; Hospital Universitario Rey Juan Carlos; Complejo Hospitalario Universitario Santa Lucía; Hospital Santa Bárbara; Complejo Hospitalario Universitario de Ferrol; Hospital de l'Esperit Sant; Hospital Universitario los Arcos del Mar Menor; Hospital HLA Universitario Moncloa; Hospital Virgen del Puerto; Hospital Marina Salud de Dénia; Hospital Universitario de Jerez; Hospital Reina Sofía de Tudela; Hospital Clínico Universitario de Santiago de Compostela; Hospital Universitario del Henares; Hospital Universitario Lucus Augusti; Hospital de Donostia; Hospital de Urduliz Alfredo Espinosa; Hospital de Mendaro; Hospital Juan Ramón Jiménez; Hospital de Tortosa Virgen de la Cinta; Hospital Riotinto; Hospital Vega Baja; Hospital Puerta de Hierro; Hospital Universitario de Getafe; Hospital General de la Palma; Hospital El Bierzo; Fundación Hospital de Calahorra; Hospital Alto Deba; Hospital Universitario San Juan de Alicante; Hospital de Guadarrama; Hospital Universitario de Jaén; Hospital de Mataró; Hospital de Palamós; Hospital Universitario de Valme; Clínica Universitaria de Navarra–Campus Navarra; Hospital Clínica Benidorm; Hospital Doce de Octubre; Hospital Universitario Virgen del Rocío; Hospital Universitario Ramón y Cajal; Hospital Universitario San Pedro; Hospital Quirón A Coruña; HM Sanchinarro; Hospital Francesc de Borja; Complejo Hospitalario Universitario Nuestra Señora de La Candelaria; Hospital Universitario HM Montepríncipe; Hospital Universitario HM Puerta del Sur; Hospital Universitario HM Torrelodones; Hospital Universitario HM Madrid; Hospital Don Benito-Villanueva de la Serena; Hospital de Viladecans; Centro Nacional de Epidemiología. Characteristics and predictors of death among 4035 consecutively hospitalized patients with COVID-19 in Spain. *Clin Microbiol Infect* 2020; **26**: 1525-1536 [PMID: 32758659 DOI: 10.1016/j.cmi.2020.07.024]

92 **Marjot T**, Moon AM, Cook JA, Abd-Elsalam S, Aloman C, Armstrong MJ, Pose E, Brenner EJ, Cargill T, Catana MA, Dhanasekaran R, Eshraghian A, García-Juárez I, Gill US, Jones PD, Kennedy J, Marshall A, Matthews C, Mells G, Mercer C, Perumalswami PV, Avitabile E, Qi X, Su F, Ufere NN, Wong YJ, Zheng MH, Barnes E, Barritt AS 4th, Webb GJ. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: An international registry study. *J Hepatol* 2021; **74**: 567-577 [PMID: 33035628 DOI: 10.1016/j.jhep.2020.09.024]

93 **Karcz M**, Bankey B, Schwaiberger D, Lachmann B, Papadakos PJ. Acute respiratory failure complicating advanced liver disease. *Semin Respir Crit Care Med* 2012; **33**: 96-110 [PMID: 22447264 DOI: 10.1055/s-0032-1301738]

94 **Jeon D**, Son M, Choi J. Impact of liver cirrhosis on the clinical outcomes of patients with COVID-19: a nationwide cohort study of Korea. *Korean J Intern Med* 2021; **36**: 1092-1101 [PMID: 34399573 DOI: 10.3904/kjim.2020.486]

95 **Choi J**, Han S, Kim N, Lim YS. Increasing burden of liver cancer despite extensive use of antiviral agents in a hepatitis B virus-endemic population. *Hepatology* 2017; **66**: 1454-1463 [PMID: 28628942 DOI: 10.1002/hep.29321]

96 **Kim D**, Adeniji N, Latt N, Kumar S, Bloom PP, Aby ES, Perumalswami P, Roytman M, Li M, Vogel AS, Catana AM, Wegermann K, Carr RM, Aloman C, Chen VL, Rabiee A, Sadowski B, Nguyen V, Dunn W, Chavin KD, Zhou K, Lizaola-Mayo B, Moghe A, Debes J, Lee TH, Branch AD, Viveiros K, Chan W, Chascsa DM, Kwo P, Dhanasekaran R. Predictors of Outcomes of COVID-19 in Patients With Chronic Liver Disease: US Multi-center Study. *Clin Gastroenterol Hepatol* 2021; **19**: 1469-1479.e19 [PMID: 32950749 DOI: 10.1016/j.cgh.2020.09.027]

97 **Ghazanfar H**, Kandhi S, Shin D, Muthumanickam A, Gurjar H, Qureshi ZA, Shaban M, Farag M, Haider A, Budhathoki P, Bhatt T, Ghazanfar A, Jyala A, Patel H. Impact of COVID-19 on the Gastrointestinal Tract: A Clinical Review. *Cureus* 2022; **14**: e23333 [PMID: 35464519 DOI: 10.7759/cureus.23333]

98 **Olry A**, Meunier L, Délire B, Larrey D, Horsmans Y, Le Louët H. Drug-Induced Liver Injury and COVID-19 Infection: The Rules Remain the Same. *Drug Saf* 2020; **43**: 615-617 [PMID: 32514859 DOI: 10.1007/s40264-020-00954-z]

99 **Kelly M**, O'Connor R, Townsend L, Coghlan M, Relihan E, Moriarty M, Carr B, Melanophy G, Doyle C, Bannan C, O'Riordan R, Merry C, Clarke S, Bergin C. Clinical outcomes and adverse events in patients hospitalised with COVID-19, treated with off-label hydroxychloroquine and azithromycin. *Br J Clin Pharmacol* 2021; **87**: 1150-1154 [PMID: 32687645 DOI: 10.1111/bcp.14482]

100 **Zhai G**, Li M, Wang Y, Wu J. Drug-Induced Liver Disturbance During the Treatment of COVID-19. *Front Pharmacol* 2021; **12**: 719308 [PMID: 34483929 DOI: 10.3389/fphar.2021.719308]

101 **Zampino R**, Mele F, Florio LL, Bertolino L, Andini R, Galdo M, De Rosa R, Corcione A, Durante-Mangoni E. Liver injury in remdesivir-treated COVID-19 patients. *Hepatol Int* 2020; **14**: 881-883 [PMID: 32725454 DOI: 10.1007/s12072-020-10077-3]

102 **Serviddio G**, Villani R, Stallone G, Scioscia G, Foschino-Barbaro MP, Lacedonia D. Tocilizumab and liver injury in patients with COVID-19. *Therap Adv Gastroenterol* 2020; **13**: 1756284820959183 [PMID: 33101458 DOI: 10.1177/1756284820959183]

103 **Teschke R**, Méndez-Sánchez N, Eickhoff A. Liver Injury in COVID-19 Patients with Drugs as Causatives: A Systematic Review of 996 DILI Cases Published 2020/2021 Based on RUCAM as Causality Assessment Method. *Int J Mol Sci* 2022; **23** [PMID: 35563242 DOI: 10.3390/ijms23094828]

104 **Stine JG**, Sateesh P, Lewis JH. Drug-induced liver injury in the elderly. *Curr Gastroenterol Rep* 2013; **15**: 299 [PMID: 23250699 DOI: 10.1007/s11894-012-0299-8]

105 **Gao S**, Yang Q, Wang X, Hu W, Lu Y, Yang K, Jiang Q, Li W, Song H, Sun F, Cheng H. Association Between Drug Treatments and the Incidence of Liver Injury in Hospitalized Patients With COVID-19. *Front Pharmacol* 2022; **13**: 799338 [PMID: 35387350 DOI: 10.3389/fphar.2022.799338]

106 **Sargiacomo C**, Sotgia F, Lisanti MP. COVID-19 and chronological aging: senolytics and other anti-aging drugs for the treatment or prevention of corona virus infection? *Aging (Albany NY)* 2020; **12**: 6511-6517 [PMID: 32229706 DOI: 10.18632/aging.103001]

107 **Yadav DK**, Singh A, Zhang Q, Bai X, Zhang W, Yadav RK, Zhiwei L, Adhikari VP, Liang T. Involvement of liver in COVID-19: systematic review and meta-analysis. *Gut* 2021; **70**: 807-809 [PMID: 32669289 DOI: 10.1136/gutjnl-2020-322072]

108 **Khateri S**, Mohammadi H, Khateri R, Moradi Y. The Prevalence of Underlying Diseases and Comorbidities in COVID-19 Patients; an Updated Systematic Review and Meta-analysis. *Arch Acad Emerg Med* 2020; **8**: e72 [PMID: 33134968]

109 **Spearman CW**, Aghemo A, Valenti L, Sonderup MW. COVID-19 and the liver: A 2021 update. *Liver Int* 2021; **41**: 1988-1998 [PMID: 34152690 DOI: 10.1111/liv.14984]

110 **Metawea MI**, Yousif WI, Moheb I. COVID 19 and liver: An A-Z literature review. *Dig Liver Dis* 2021; **53**: 146-152 [PMID: 32988758 DOI: 10.1016/j.dld.2020.09.010]

111 **Ji D**, Qin E, Xu J, Zhang D, Cheng G, Wang Y, Lau G. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020; **73**: 451-453 [PMID: 32278005 DOI: 10.1016/j.jhep.2020.03.044]

112 **Zhou YJ**, Zheng KI, Wang XB, Sun QF, Pan KH, Wang TY, Ma HL, Chen YP, George J, Zheng MH. Metabolic-associated fatty liver disease is associated with severity of COVID-19. *Liver Int* 2020; **40**: 2160-2163 [PMID: 32573883 DOI: 10.1111/liv.14575]

113 **Hartl L**, Haslinger K, Angerer M, Jachs M, Simbrunner B, Bauer DJM, Semmler G, Scheiner B, Eigenbauer E, Strassl R, Breuer M, Kimberger O, Laxar D, Trauner M, Mandorfer M, Reiberger T. Age-adjusted mortality and predictive value of liver chemistries in a Viennese cohort of COVID-19 patients. *Liver Int* 2022; **42**: 1297-1307 [PMID: 35412018 DOI: 10.1111/liv.15274]

114 **Ge J**, Pletcher MJ, Lai JC; N3C Consortium. Outcomes of SARS-CoV-2 Infection in Patients With Chronic Liver Disease and Cirrhosis: A National COVID Cohort Collaborative Study. *Gastroenterology* 2021; **161**: 1487-1501.e5 [PMID: 34284037 DOI: 10.1053/j.gastro.2021.07.010]

115 **Ioannou GN**, Liang PS, Locke E, Green P, Berry K, O'Hare AM, Shah JA, Crothers K, Eastment MC, Fan VS, Dominitz JA. Cirrhosis and Severe Acute Respiratory Syndrome Coronavirus 2 Infection in US Veterans: Risk of Infection, Hospitalization, Ventilation, and Mortality. *Hepatology* 2021; **74**: 322-335 [PMID: 33219546 DOI: 10.1002/hep.31649]

116 **Brozat JF**, Hanses F, Haelberger M, Stecher M, Dreher M, Tometten L, Ruethrich MM, Vehreschild JJ, Trautwein C, Borgmann S, Vehreschild MJGT, Jakob CEM, Stallmach A, Wille K, Hellwig K, Isberner N, Reuken PA, Geisler F, Nattermann J, Bruns T; LEOSS study group. COVID-19 mortality in cirrhosis is determined by cirrhosis-associated comorbidities and extrahepatic organ failure: Results from the multinational LEOSS registry. *United European Gastroenterol J* 2022; **10**: 409-424 [PMID: 35482663 DOI: 10.1002/ueg2.12232]

117 **Kandasamy S**. An unusual presentation of COVID-19: Acute pancreatitis. *Ann Hepatobiliary Pancreat Surg* 2020; **24**: 539-541 [PMID: 33234760 DOI: 10.14701/ahbps.2020.24.4.539]

118 **Schepis T**, Larghi A, Papa A, Miele L, Panzuto F, De Biase L, Annibale B, Cattani P, Rapaccini GL. SARS-CoV2 RNA detection in a pancreatic pseudocyst sample. *Pancreatology* 2020; **20**: 1011-1012 [PMID: 32498972 DOI: 10.1016/j.pan.2020.05.016]

119 **Aday U**, Gedik E, Kafadar MT, Özbek E. Acute Necrotizing Pancreatitis and Coronavirus Disease-2019 (COVID-19). *Korean J Gastroenterol* 2021; **78**: 353-358 [PMID: 34955513 DOI: 10.4166/kjg.2021.131]

120 **Tsai PH**, Lai WY, Lin YY, Luo YH, Lin YT, Chen HK, Chen YM, Lai YC, Kuo LC, Chen SD, Chang KJ, Liu CH, Chang SC, Wang FD, Yang YP. Clinical manifestation and disease progression in COVID-19 infection. *J Chin Med Assoc* 2021; **84**: 3-8 [PMID: 33230062 DOI: 10.1097/JCMA.0000000000000463]

121 **Khunti K**, Del Prato S, Mathieu C, Kahn SE, Gabbay RA, Buse JB. COVID-19, Hyperglycemia, and New-Onset Diabetes. *Diabetes Care* 2021; **44**: 2645-2655 [PMID: 34625431 DOI: 10.2337/dc21-1318]

122 **Wang F**, Wang H, Fan J, Zhang Y, Zhao Q. Pancreatic Injury Patterns in Patients With Coronavirus Disease 19 Pneumonia. *Gastroenterology* 2020; **159**: 367-370 [PMID: 32247022 DOI: 10.1053/j.gastro.2020.03.055]

123 **Samanta J**, Gupta R, Singh MP, Patnaik I, Kumar A, Kochhar R. Coronavirus disease 2019 and the pancreas. *Pancreatology* 2020; **20**: 1567-1575 [PMID: 33250089 DOI: 10.1016/j.pan.2020.10.035]

124 **Bruno G**, Fabrizio C, Santoro CR, Buccoliero GB. Pancreatic injury in the course of coronavirus disease 2019: A not-so-rare occurrence. *J Med Virol* 2021; **93**: 74-75 [PMID: 32497298 DOI: 10.1002/jmv.26134]

125 **Liu F**, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 Expression in Pancreas May Cause Pancreatic Damage After SARS-CoV-2 Infection. *Clin Gastroenterol Hepatol* 2020; **18**: 2128-2130.e2 [PMID: 32334082 DOI: 10.1016/j.cgh.2020.04.040]

126 **Stephens JR**, Wong JLC, Broomhead R, Stümpfle R, Waheed U, Patel P, Brett SJ, Soni S. Raised serum amylase in patients with COVID-19 may not be associated with pancreatitis. *Br J Surg* 2021; **108**: e152-e153 [PMID: 33793756 DOI: 10.1093/bjs/znaa168]

127 **Akkus C**, Yilmaz H, Mizrak S, Adibelli Z, Akdas O, Duran C. Development of pancreatic injuries in the course of COVID-19. *Acta Gastroenterol Belg* 2020; **83**: 585-592 [PMID: 33321015]

128 **McNabb-Baltar J**, Jin DX, Grover AS, Redd WD, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Shen L, Chan WW. Lipase Elevation in Patients With COVID-19. *Am J Gastroenterol* 2020; **115**: 1286-1288 [PMID: 32496339 DOI: 10.14309/ajg.0000000000000732]

129 **Bansal P**, Margekar SL, Suman V, Sud R, Meena S, Sharma AK, Islam SY, Gurtoo A, Agrawal A, Pangtey GS, Prakash A. Pancreatic Injury in COVID-19 Patients. *J Assoc Physicians India* 2020; **68**: 58-60 [PMID: 33247644]

130 **Rasch S**, Herner A, Schmid RM, Huber W, Lahmer T. High lipasemia is frequent in Covid-19 associated acute respiratory distress syndrome. *Pancreatology* 2021; **21**: 306-311 [PMID: 33277183 DOI: 10.1016/j.pan.2020.11.023]

131 **Barlass U**, Wiliams B, Dhana K, Adnan D, Khan SR, Mahdavinia M, Bishehsari F. Marked Elevation of Lipase in COVID-19 Disease: A Cohort Study. *Clin Transl Gastroenterol* 2020; **11**: e00215 [PMID: 32764201 DOI: 10.14309/ctg.0000000000000215]

132 **Bacaksız F**, Ebik B, Ekin N, Kılıc J. Pancreatic damage in COVID-19: Why? *Int J Clin Pract* 2021; **75**: e14692 [PMID: 34331821 DOI: 10.1111/ijcp.14692]

133 **Hunt RH**, East JE, Lanas A, Malfertheiner P, Satsangi J, Scarpignato C, Webb GJ. COVID-19 and Gastrointestinal Disease: Implications for the Gastroenterologist. *Dig Dis* 2021; **39**: 119-139 [PMID: 33040064 DOI: 10.1159/000512152]

134 **Singh RR**, Chhabra P, Kumta NA. Does Hyperlipasemia Predict Worse Clinical Outcomes in COVID-19? A Multicenter Retrospective Cohort Study. *J Clin Gastroenterol* 2022; **56**: e227-e231 [PMID: 34294655 DOI: 10.1097/MCG.0000000000001590]

135 **Inamdar S**, Benias PC, Liu Y, Sejpal DV, Satapathy SK, Trindade AJ; Northwell COVID-19 Research Consortium. Prevalence, Risk Factors, and Outcomes of Hospitalized Patients With Coronavirus Disease 2019 Presenting as Acute Pancreatitis. *Gastroenterology* 2020; **159**: 2226-2228.e2 [PMID: 32860787 DOI: 10.1053/j.gastro.2020.08.044]

136 **Karaali R**, Topal F. Evaluating the effect of SARS-Cov-2 infection on prognosis and mortality in patients with acute pancreatitis. *Am J Emerg*

Med 2021; **49**: 378-384 [PMID: 34246968 DOI: 10.1016/j.ajem.2021.06.045]
137 **Dirweesh A**, Li Y, Trikudanathan G, Mallery JS, Freeman ML, Amateau SK. Clinical Outcomes of Acute Pancreatitis in Patients With Coronavirus Disease 2019. *Gastroenterology* 2020; **159**: 1972-1974 [PMID: 32721439 DOI: 10.1053/j.gastro.2020.07.038]
138 **Pandanaboyana S**, Moir J, Leeds JS, Oppong K, Kanwar A, Marzouk A, Belgaumkar A, Gupta A, Siriwardena AK, Haque AR, Awan A, Balakrishnan A, Rawashdeh A, Ivanov B, Parmar C, M Halloran C, Caruana C, Borg CM, Gomez D, Damaskos D, Karavias D, Finch G, Ebied H, K Pine J, R A Skipworth J, Milburn J, Latif J, Ratnam Apollos J, El Kafsi J, Windsor JA, Roberts K, Wang K, Ravi K, V Coats M, Hollyman M, Phillips M, Okocha M, Sj Wilson M, A Ameer N, Kumar N, Shah N, Lapolla P, Magee C, Al-Sarireh B, Lunevicius R, Benhmida R, Singhal R, Balachandra S, Demirli Atıcı S, Jaunoo S, Dwerryhouse S, Boyce T, Charalampakis V, Kanakala V, Abbas Z, Nayar M; COVID PAN collaborative group. SARS-CoV-2 infection in acute pancreatitis increases disease severity and 30-day mortality: COVID PAN collaborative study. *Gut* 2021; **70**: 1061-1069 [PMID: 33547182 DOI: 10.1136/gutjnl-2020-323364]
139 **Ye C**, Zhang S, Zhang X, Cai H, Gu J, Lian J, Lu Y, Jia H, Hu J, Jin C, Yu G, Zhang Y, Sheng J, Yang Y. Impact of comorbidities on patients with COVID-19: A large retrospective study in Zhejiang, China. *J Med Virol* 2020; **92**: 2821-2829 [PMID: 32543710 DOI: 10.1002/jmv.26183]
140 **Miró Ò**, Llorens P, Jiménez S, Piñera P, Burillo-Putze G, Martín A, Martín-Sánchez FJ, Lamberechts J, Alquézar-Arbé A, Jacob J, Noceda J, Cano Cano MJ, Fortuny Bayarri MJ, Marín Porrino JM, Meléndez N, Pérez García C, Brasó Aznar JV, Ponce MC, Díaz Fernández E, Ejarque Martínez L, Peiró Gómez A, Tost J, Domínguez MJ, Teigell Muñoz FJ, González Del Castillo J; Spanish Investigators on Emergency Situations TeAm (SIESTA) network. A case-control emergency department-based analysis of acute pancreatitis in Covid-19: Results of the UMC-19-S(6). *J Hepatobiliary Pancreat Sci* 2021; **28**: 953-966 [PMID: 33259695 DOI: 10.1002/jhbp.873]
141 **Akarsu C**, Karabulut M, Aydin H, Sahbaz NA, Dural AC, Yegul D, Peker KD, Ferahman S, Bulut S, Dönmez T, Asar S, Yasar KK, Adas GT. Association between Acute Pancreatitis and COVID-19: Could Pancreatitis Be the Missing Piece of the Puzzle about Increased Mortality Rates? *J Invest Surg* 2022; **35**: 119-125 [PMID: 33138658 DOI: 10.1080/08941939.2020.1833263]
142 **Huang BZ**, Sidell MA, Wu BU, Setiawan VW, Chen Z, Xiang AH. Pre-Existing Pancreatitis and Elevated Risks of COVID-19 Severity and Mortality. *Gastroenterology* 2022; **162**: 1758-1760.e3 [PMID: 35149034 DOI: 10.1053/j.gastro.2022.02.005]
143 **Hadi Y**, Shah-Khan SM, Sohail AH, Jannat FRU, Syed A, Humphries CE, Daum TL, Malik A, Krafft MR, Bilal M, Thakkar S, Singh S. Su1270: Chronic pancreatitis and COVID-19: Incidence and outcomes. A multicenter research network analysis. *Gastroenterology* 2022; **162**: S564-S565
144 **Georgakopoulou VE**, Gkoufa A, Garmpis N, Makrodimitri S, Papageorgiou CV, Barlampa D, Garmpi A, Chiapoutakis S, Sklapani P, Trakas N, Damaskos C. COVID-19 and Acute Pancreatitis: A Systematic Review of Case Reports and Case Series. *Ann Saudi Med* 2022; **42**: 276-287 [PMID: 35933608 DOI: 10.5144/0256-4947.2022.276]
145 **Xin MJ**, Chen H, Luo B, Sun JB. Severe acute pancreatitis in the elderly: etiology and clinical characteristics. *World J Gastroenterol* 2008; **14**: 2517-2521 [PMID: 18442198 DOI: 10.3748/wjg.14.2517]
146 **Kara B**, Olmez S, Yalcın MS, Tas A, Ozturk NA, Sarıtaş B. Update on the effect of age on acute pancreatitis morbidity: a retrospective, single-center study. *Prz Gastroenterol* 2018; **13**: 223-227 [PMID: 30302167 DOI: 10.5114/pg.2018.75677]
147 **Quero G**, Covino M, Fiorillo C, Rosa F, Menghi R, Simeoni B, Potenza A, Ojetti V, Alfieri S, Franceschi F. Acute pancreatitis in elderly patients: a single-center retrospective evaluation of clinical outcomes. *Scand J Gastroenterol* 2019; **54**: 492-498 [PMID: 30905212 DOI: 10.1080/00365521.2019.1588369]
148 **Yu B**, Li N, Li J, Wan J, He W, Zhu Y, Lu N. The Clinical Characteristics of Acute Pancreatitis in Gerontal Patients: A Retrospective Study. *Clin Interv Aging* 2020; **15**: 1541-1553 [PMID: 32982192 DOI: 10.2147/CIA.S259920]
149 **Márta K**, Lazarescu AM, Farkas N, Mátrai P, Cazacu I, Ottóffy M, Habon T, Erőss B, Vincze À, Veres G, Czakó L, Sarlós P, Rakonczay Z, Hegyi P. Aging and Comorbidities in Acute Pancreatitis I: A Meta-Analysis and Systematic Review Based on 194,702 Patients. *Front Physiol* 2019; **10**: 328 [PMID: 31001131 DOI: 10.3389/fphys.2019.00328]
150 **Bulthuis MC**, Boxhoorn L, Beudel M, Elbers PWG, Kop MPM, van Wanrooij RLJ, Besselink MG, Voermans RP. Acute pancreatitis in COVID-19 patients: true risk? *Scand J Gastroenterol* 2021; **56**: 585-587 [PMID: 33715577 DOI: 10.1080/00365521.2021.1896776]
151 **Meyers MH**, Main MJ, Orr JK, Obstein KL. A Case of COVID-19-Induced Acute Pancreatitis. *Pancreas* 2020; **49**: e108-e109 [PMID: 33122538 DOI: 10.1097/MPA.0000000000001696]
152 **Karimzadeh S**, Manzuri A, Ebrahimi M, Huy NT. COVID-19 presenting as acute pancreatitis: Lessons from a patient in Iran. *Pancreatology* 2020; **20**: 1024-1025 [PMID: 32576441 DOI: 10.1016/j.pan.2020.06.003]
153 **Gadiparthi C**, Mohapatra S, Kanna S, Vykuntam V, Chen W. Acute pancreatitis in a patient with COVID-19: a case report. *Transl Gastroenterol Hepatol* 2021; **6**: 65 [PMID: 34805587 DOI: 10.21037/tgh-20-234]
154 **Wifi MN**, Nabil A, Awad A, Eltatawy R. COVID-induced pancreatitis: case report. *Egypt J Intern Med* 2021; **33**: 10 [PMID: 33716498 DOI: 10.1186/s43162-021-00039-y]
155 **Gonzalo-Voltas A**, Uxia Fernández-Pérez-Torres C, Baena-Díez JM. Acute pancreatitis in a patient with COVID-19 infection. *Med Clin (Engl Ed)* 2020; **155**: 183-184 [PMID: 32835111 DOI: 10.1016/j.medcle.2020.05.010]
156 **Reick-Mitrisin V**, Mukhtar K, Khan ZH. Acute Pancreatitis in a Patient With Recent History of SARS-CoV-2 Infection. *Cureus* 2022; **14**: e29032 [PMID: 36237820 DOI: 10.7759/cureus.29032]
157 **Acherjya GK**, Rahman MM, Islam MT, Alam AS, Tarafder K, Ali M, Deb SR. Acute pancreatitis in a COVID-19 patient: An unusual presentation. *Clin Case Rep* 2020; **8**: 3400-3407 [PMID: 33363941 DOI: 10.1002/ccr3.3412]
158 **Alves AM**, Yvamoto EY, Marzinotto MAN, Teixeira ACS, Carrilho FJ. SARS-CoV-2 leading to acute pancreatitis: an unusual presentation. *Braz J Infect Dis* 2020; **24**: 561-564 [PMID: 32961108 DOI: 10.1016/j.bjid.2020.08.011]
159 **Shinohara T**, Otani A, Yamashita M, Wakimoto Y, Jubishi D, Okamoto K, Kanno Y, Ikeda M, Ishigaki K, Nakai Y, Harada S, Okugawa S, Koike K, Moriya K. Acute Pancreatitis During COVID-19 Pneumonia. *Pancreas* 2020; **49**: e106-e108 [PMID: 33122537 DOI: 10.1097/MPA.0000000000001695]
160 **Kumaran NK**, Karmakar BK, Taylor OM. Coronavirus disease-19 (COVID-19) associated with acute necrotising pancreatitis (ANP). *BMJ Case Rep* 2020; **13** [PMID: 32900752 DOI: 10.1136/bcr-2020-237903]
161 **Chen T**, Dai Z, Mo P, Li X, Ma Z, Song S, Chen X, Luo M, Liang K, Gao S, Zhang Y, Deng L, Xiong Y. Clinical Characteristics and Outcomes of Older Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: A Single-Centered, Retrospective Study. *J Gerontol A Biol Sci Med Sci* 2020; **75**: 1788-1795 [PMID: 32279081 DOI: 10.1093/gerona/glaa089]
162 **Imrie CW**. Prognosis of acute pancreatitis. *Ann Ital Chir* 1995; **66**: 187-189 [PMID: 7668494]

163 **Brikman S**, Denysova V, Menzal H, Dori G. Acute pancreatitis in a 61-year-old man with COVID-19. *CMAJ* 2020; **192**: E858-E859 [PMID: 32719021 DOI: 10.1503/cmaj.201029]

164 **Jena A**, James D, Singh AK, Dutta U, Sebastian S, Sharma V. Effectiveness and Durability of COVID-19 Vaccination in 9447 Patients With IBD: A Systematic Review and Meta-Analysis. *Clin Gastroenterol Hepatol* 2022; **20**: 1456-1479.e18 [PMID: 35189387 DOI: 10.1016/j.cgh.2022.02.030]

165 **Bhurwal A**, Mutneja H, Bansal V, Goel A, Arora S, Attar B, Minacapelli CD, Kochhar G, Chen LA, Brant S, Seril D. Effectiveness and safety of SARS-CoV-2 vaccine in Inflammatory Bowel Disease patients: a systematic review, meta-analysis and meta-regression. *Aliment Pharmacol Ther* 2022; **55**: 1244-1264 [PMID: 35355306 DOI: 10.1111/apt.16913]

166 **Kennedy NA**, Lin S, Goodhand JR, Chanchlani N, Hamilton B, Bewshea C, Nice R, Chee D, Cummings JF, Fraser A, Irving PM, Kamperidis N, Kok KB, Lamb CA, Macdonald J, Mehta S, Pollok RC, Raine T, Smith PJ, Verma AM, Jochum S, McDonald TJ, Sebastian S, Lees CW, Powell N, Ahmad T; Contributors to the CLARITY IBD study. Infliximab is associated with attenuated immunogenicity to BNT162b2 and ChAdOx1 nCoV-19 SARS-CoV-2 vaccines in patients with IBD. *Gut* 2021; **70**: 1884-1893 [PMID: 33903149 DOI: 10.1136/gutjnl-2021-324789]

167 **Lin S**, Kennedy NA, Saifuddin A, Sandoval DM, Reynolds CJ, Seoane RC, Kottoor SH, Pieper FP, Lin KM, Butler DK, Chanchlani N, Nice R, Chee D, Bewshea C, Janjua M, McDonald TJ, Sebastian S, Alexander JL, Constable L, Lee JC, Murray CD, Hart AL, Irving PM, Jones GR, Kok KB, Lamb CA, Lees CW, Altmann DM, Boyton RJ, Goodhand JR, Powell N, Ahmad T; CLARITY IBD study. Antibody decay, T cell immunity and breakthrough infections following two SARS-CoV-2 vaccine doses in inflammatory bowel disease patients treated with infliximab and vedolizumab. *Nat Commun* 2022; **13**: 1379 [PMID: 35296643 DOI: 10.1038/s41467-022-28517-z]

168 **McMahan K**, Yu J, Mercado NB, Loos C, Tostanoski LH, Chandrashekar A, Liu J, Peter L, Atyeo C, Zhu A, Bondzie EA, Dagotto G, Gebre MS, Jacob-Dolan C, Li Z, Nampanya F, Patel S, Pessaint L, Van Ry A, Blade K, Yalley-Ogunro J, Cabus M, Brown R, Cook A, Teow E, Andersen H, Lewis MG, Lauffenburger DA, Alter G, Barouch DH. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* 2021; **590**: 630-634 [PMID: 33276369 DOI: 10.1038/s41586-020-03041-6]

169 **Xu AM**, Li D, Ebinger JE, Mengesha E, Elyanow R, Gittelman RM, Chapman H, Joung S, Botwin GJ, Pozdnyakova V, Debbas P, Mujukian A, Prostko JC, Frias EC, Stewart JL, Horizon AA, Merin N, Sobhani K, Figueiredo JC, Cheng S, Kaplan IM, McGovern DPB, Merchant A, Melmed GY, Braun J. Differences in SARS-CoV-2 Vaccine Response Dynamics Between Class-I- and Class-II-Specific T-Cell Receptors in Inflammatory Bowel Disease. *Front Immunol* 2022; **13**: 880190 [PMID: 35464463 DOI: 10.3389/fimmu.2022.880190]

170 **Li D**, Xu A, Mengesha E, Elyanow R, Gittelman RM, Chapman H, Prostko JC, Frias EC, Stewart JL, Pozdnyakova V, Debbas P, Mujukian A, Horizon AA, Merin N, Joung S, Botwin GJ, Sobhani K, Figueiredo JC, Cheng S, Kaplan IM, McGovern DPB, Merchant A, Melmed GY, Braun J. The T-Cell Response to SARS-CoV-2 Vaccination in Inflammatory Bowel Disease is Augmented with Anti-TNF Therapy. *Inflamm Bowel Dis* 2022; **28**: 1130-1133 [PMID: 35397000 DOI: 10.1093/ibd/izac071]

171 **Tabesh E**, Soheilipour M, Rezaeisadrabadi M, Zare-Farashbandi E, Mousavi-Roknabadi RS. Comparison the effects and side effects of Covid-19 vaccination in patients with inflammatory bowel disease (IBD): a systematic scoping review. *BMC Gastroenterol* 2022; **22**: 393 [PMID: 35987619 DOI: 10.1186/s12876-022-02460-1]

172 **Botwin GJ**, Li D, Figueiredo J, Cheng S, Braun J, McGovern DPB, Melmed GY. Adverse Events After SARS-CoV-2 mRNA Vaccination Among Patients With Inflammatory Bowel Disease. *Am J Gastroenterol* 2021; **116**: 1746-1751 [PMID: 34047304 DOI: 10.14309/ajg.0000000000001342]

173 **Derhovanessian E**, Pawelec G. Vaccination in the elderly. *Microb Biotechnol* 2012; **5**: 226-232 [PMID: 21880118 DOI: 10.1111/j.1751-7915.2011.00283.x]

174 **Soiza RL**, Scicluna C, Thomson EC. Efficacy and safety of COVID-19 vaccines in older people. *Age Ageing* 2021; **50**: 279-283 [PMID: 33320183 DOI: 10.1093/ageing/afaa274]

175 **Collier DA**, Ferreira IATM, Kotagiri P, Datir RP, Lim EY, Touizer E, Meng B, Abdullahi A; CITIID-NIHR BioResource COVID-19 Collaboration, Elmer A, Kingston N, Graves B, Le Gresley E, Caputo D, Bergamaschi L, Smith KGC, Bradley JR, Ceron-Gutierrez L, Cortes-Acevedo P, Barcenas-Morales G, Linterman MA, McCoy LE, Davis C, Thomson E, Lyons PA, McKinney E, Doffinger R, Wills M, Gupta RK. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* 2021; **596**: 417-422 [PMID: 34192737 DOI: 10.1038/s41586-021-03739-1]

176 **Karamese M**, Tutuncu EE. The effectiveness of inactivated SARS-CoV-2 vaccine (CoronaVac) on antibody response in participants aged 65 years and older. *J Med Virol* 2022; **94**: 173-177 [PMID: 34427924 DOI: 10.1002/jmv.27289]

177 **Kappelman MD**, Weaver KN, Zhang X, Dai X, Watkins R, Adler J, Dubinsky MC, Kastl A, Bousvaros A, Strople JA, Cross RK, Higgins PDR, Ungaro RC, Bewtra M, Bellaguarda EA, Farraye FA, Boccieri ME, Firestine A, Chun KY, Fernando M, Bastidas M, Zikry M, Long MD. Factors Affecting Initial Humoral Immune Response to SARS-CoV-2 Vaccines Among Patients With Inflammatory Bowel Diseases. *Am J Gastroenterol* 2022; **117**: 462-469 [PMID: 35029167 DOI: 10.14309/ajg.0000000000001619]

178 **Classen JM**, Muzalyova A, Nagl S, Fleischmann C, Ebigbo A, Römmele C, Messmann H, Schnoy E. Antibody Response to SARS-CoV-2 Vaccination in Patients with Inflammatory Bowel Disease: Results of a Single-Center Cohort Study in a Tertiary Hospital in Germany. *Dig Dis* 2022; **40**: 719-727 [PMID: 34915480 DOI: 10.1159/000521343]

179 **Alexander JL**, Liu Z, Muñoz Sandoval D, Reynolds C, Ibraheim H, Anandabaskaran S, Saifuddin A, Castro Seoane R, Anand N, Nice R, Bewshea C, D'Mello A, Constable L, Jones GR, Balarajah S, Fiorentino F, Sebastian S, Irving PM, Hicks LC, Williams HRT, Kent AJ, Linger R, Parkes M, Kok K, Patel KV, Teare JP, Altmann DM, Goodhand JR, Hart AL, Lees CW, Boyton RJ, Kennedy NA, Ahmad T, Powell N; VIP study investigators. COVID-19 vaccine-induced antibody and T-cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose (VIP): a multicentre, prospective, case-control study. *Lancet Gastroenterol Hepatol* 2022; **7**: 1005-1015 [PMID: 36088954 DOI: 10.1016/S2468-1253(22)00274-6]

180 **Cornberg M**, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. *J Hepatol* 2021; **74**: 944-951 [PMID: 33563499 DOI: 10.1016/j.jhep.2021.01.032]

181 **Fix OK**, Blumberg EA, Chang KM, Chu J, Chung RT, Goacher EK, Hameed B, Kaul DR, Kulik LM, Kwok RM, McGuire BM, Mulligan DC, Price JC, Reau NS, Reddy KR, Reynolds A, Rosen HR, Russo MW, Schilsky ML, Verna EC, Ward JW, Fontana RJ; AASLD COVID-19 Vaccine Working Group. American Association for the Study of Liver Diseases Expert Panel Consensus Statement: Vaccines to Prevent Coronavirus Disease 2019 Infection in Patients With Liver Disease. *Hepatology* 2021; **74**: 1049-1064 [PMID: 33577086 DOI: 10.1002/hep.31751]

182 **Sripongpun P**, Pinpathomrat N, Bruminhent J, Kaewdech A. Coronavirus Disease 2019 Vaccinations in Patients With Chronic Liver Disease

and Liver Transplant Recipients: An Update. *Front Med (Lausanne)* 2022; **9**: 924454 [PMID: 35814781 DOI: 10.3389/fmed.2022.924454]

183 **Marjot T**, Eberhardt CS, Boettler T, Belli LS, Berenguer M, Buti M, Jalan R, Mondelli MU, Moreau R, Shouval D, Berg T, Cornberg M. Impact of COVID-19 on the liver and on the care of patients with chronic liver disease, hepatobiliary cancer, and liver transplantation: An updated EASL position paper. *J Hepatol* 2022; **77**: 1161-1197 [PMID: 35868584 DOI: 10.1016/j.jhep.2022.07.008]

184 **Ai J**, Wang J, Liu D, Xiang H, Guo Y, Lv J, Zhang Q, Li J, Zhang X, Li Q, Liang J, Guo X, Feng Y, Liu L, Qin W, Wang X, Rao W, Tian Q, Zhang Y, Xie F, Jiang S, Yan Y, Qiu Y, Wu H, Hou Z, Zhang N, Zhang A, Ji J, Yang J, Huang J, Zhao Z, Gu Y, Bian L, Zhang Z, Zou S, Ji H, Ge G, Du X, Hou A, Zhu Y, Cong Q, Xu J, Zu H, Wang Y, Yan Z, Yan X, BianBa Y, Ci Q, Zhang L, Yang S, Gao X, Zhong L, He S, Liu C, Huang Y, Liu Y, Xu D, Zhu Q, Xu X, Lv M, Zhang W, Qi X. Safety and Immunogenicity of SARS-CoV-2 Vaccines in Patients With Chronic Liver Diseases (CHESS-NMCID 2101): A Multicenter Study. *Clin Gastroenterol Hepatol* 2022; **20**: 1516-1524.e2 [PMID: 34942370 DOI: 10.1016/j.cgh.2021.12.022]

185 **Noor MT**, Manoria P. Immune Dysfunction in Cirrhosis. *J Clin Transl Hepatol* 2017; **5**: 50-58 [PMID: 28507927 DOI: 10.14218/JCTH.2016.00056]

186 **Chen Z**, Zhang Y, Song R, Wang L, Hu X, Li H, Cai D, Hu P, Shi X, Ren H. Waning humoral immune responses to inactivated SARS-CoV-2 vaccines in patients with severe liver disease. *Signal Transduct Target Ther* 2022; **7**: 174 [PMID: 35654782 DOI: 10.1038/s41392-022-01032-9]

187 **Thuluvath PJ**, Robarts P, Chauhan M. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. *J Hepatol* 2021; **75**: 1434-1439 [PMID: 34454993 DOI: 10.1016/j.jhep.2021.08.008]

188 **Bakasis AD**, Bitzogli K, Mouziouras D, Pouliakis A, Roumpoutsou M, Goules AV, Androutsakos T. Antibody Responses after SARS-CoV-2 Vaccination in Patients with Liver Diseases. *Viruses* 2022; **14** [PMID: 35215801 DOI: 10.3390/v14020207]

189 **Willuweit K**, Frey A, Passenberg M, Korth J, Saka N, Anastasiou OE, Möhlendick B, Schütte A, Schmidt H, Rashidi-Alavijeh J. Patients with Liver Cirrhosis Show High Immunogenicity upon COVID-19 Vaccination but Develop Premature Deterioration of Antibody Titers. *Vaccines (Basel)* 2022; **10** [PMID: 35335009 DOI: 10.3390/vaccines10030377]

190 **Voysey M**, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, Angus B, Baillie VL, Barnabas SL, Bhorat QE, Bibi S, Briner C, Cicconi P, Collins AM, Colin-Jones R, Cutland CL, Darton TC, Dheda K, Duncan CJA, Emary KRW, Ewer KJ, Fairlie L, Faust SN, Feng S, Ferreira DM, Finn A, Goodman AL, Green CM, Green CA, Heath PT, Hill C, Hill H, Hirsch I, Hodgson SHC, Izu A, Jackson S, Jenkin D, Joe CCD, Kerridge S, Koen A, Kwatra G, Lazarus R, Lawrie AM, Lelliott A, Libri V, Lillie PJ, Mallory R, Mendes AVA, Milan EP, Minassian AM, McGregor A, Morrison H, Mujadidi YF, Nana A, O'Reilly PJ, Padayachee SD, Pittella A, Plested E, Pollock KM, Ramasamy MN, Rhead S, Schwarzbold AV, Singh N, Smith A, Song R, Snape MD, Sprinz E, Sutherland RK, Tarrant R, Thomson EC, Török ME, Toshner M, Turner DPJ, Vekemans J, Villafana TL, Watson MEE, Williams CJ, Douglas AD, Hill AVS, Lambe T, Gilbert SC, Pollard AJ; Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397**: 99-111 [PMID: 33306989 DOI: 10.1016/S0140-6736(20)32661-1]

191 **Liang CK**, Lee WJ, Peng LN, Meng LC, Hsiao FY, Chen LK. COVID-19 Vaccines in Older Adults: Challenges in Vaccine Development and Policy Making. *Clin Geriatr Med* 2022; **38**: 605-620 [PMID: 35868676 DOI: 10.1016/j.cger.2022.03.006]

192 **US Food Drug Aaministration**. Vaccines and Related Biological Products Advisory Committee. [cited 15 October 2022]. Available from: https://www.fda.gov/advisory-committees/blood-vaccines-and-other-biologics/vaccines-and-related-biological-products-advisory-committee

193 **Parkash O**, Sharko A, Farooqi A, Ying GW, Sura P. Acute Pancreatitis: A Possible Side Effect of COVID-19 Vaccine. *Cureus* 2021; **13**: e14741 [PMID: 34084669 DOI: 10.7759/cureus.14741]

194 **Dey RK**, Ilango H, Bhatta S, Shaheed A, Dole S, Zooshan A, Faisham M, Murad M. Acute pancreatitis in pregnancy following COVID-19 vaccine: a case report. *J Med Case Rep* 2022; **16**: 354 [PMID: 36175940 DOI: 10.1186/s13256-022-03607-0]

195 **Ozaka S**, Kodera T, Ariki S, Kobayashi T, Murakami K. Acute pancreatitis soon after COVID-19 vaccination: A case report. *Medicine (Baltimore)* 2022; **101**: e28471 [PMID: 35029194 DOI: 10.1097/MD.0000000000028471]

196 **Cacdac R**, Jamali A, Jamali R, Nemovi K, Vosoughi K, Bayraktutar Z. Acute pancreatitis as an adverse effect of COVID-19 vaccination. *SAGE Open Med Case Rep* 2022; **10**: 2050313X221131169 [PMID: 36313269 DOI: 10.1177/2050313X221131169]

197 **Kantar A**, Seminara M, Odoni M, Dalla Verde I. Acute Mild Pancreatitis Following COVID-19 mRNA Vaccine in an Adolescent. *Children (Basel)* 2021; **9** [PMID: 35053654 DOI: 10.3390/children9010029]

198 **Kalra RK**, Jayadeep S, Ball AL. Acute Pancreatitis in an Adolescent Following COVID Vaccination. *Clin Pediatr (Phila)* 2022; **61**: 236-240 [PMID: 35081801 DOI: 10.1177/00099228211067678]

199 **Patel AH**, Amin R, Lalos AT. Acute liver injury and IgG4-related autoimmune pancreatitis following mRNA-based COVID-19 vaccination. *Hepatol Forum* 2022; **3**: 97-99 [PMID: 36177105 DOI: 10.14744/hf.2022.2022.0019]

200 **Walter T**, Connor S, Stedman C, Doogue M. A case of acute necrotising pancreatitis following the second dose of Pfizer-BioNTech COVID-19 mRNA vaccine. *Br J Clin Pharmacol* 2022; **88**: 1385-1386 [PMID: 34423463 DOI: 10.1111/bcp.15039]

201 **Cieślewicz A**, Dudek M, Krela-Kaźmierczak I, Jabłecka A, Lesiak M, Korzeniowska K. Pancreatic Injury after COVID-19 Vaccine-A Case Report. *Vaccines (Basel)* 2021; **9** [PMID: 34205898 DOI: 10.3390/vaccines9060576]

202 COVID-19 mRNA Pfizer- BioNTech vaccine analysis print. [cited 15 October 2022]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1072043/COVID-19_mRNA_Pfizer-_BioNTech_vaccine_analysis_print.pdf

203 Enquête de pharmacovigilance du vaccin Pfizer-BioNTech Comirnaty. [cited 15 October 2022]. Available from: https://ansm.sante.fr/uploads/2021/10/22/20211021-covid-19-vaccins-pfizer-focus-1-2.pdf

204 **World Health Organization**. VigiAccess. [cited 17 October 2022]. Available from: https://www.vigiaccess.org/

205 **Guda NM**, Trikudanathan G, Freeman ML. Idiopathic recurrent acute pancreatitis. *Lancet Gastroenterol Hepatol* 2018; **3**: 720-728 [PMID: 30215363 DOI: 10.1016/S2468-1253(18)30211-5]

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REVIEW

Irritable bowel syndrome: Epidemiology, overlap disorders, pathophysiology and treatment

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Abstract

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disease with a significant impact on patients' quality of life and a high socioeconomic burden. And the understanding of IBS has changed since the release of the Rome IV diagnosis in 2016. With the upcoming Rome V revision, it is necessary to review the results of IBS research in recent years. In this review of IBS, we can highlight future concerns by reviewing the results of IBS research on epidemiology, overlap disorders, pathophysiology, and treatment over the past decade and summarizing the latest research.

Key Words: Irritable bowel syndrome; Overlap; Pathophysiology; Treatment

Core Tip: Irritable bowel syndrome (IBS) is a physical and mental illness that is becoming more prevalent, and its impact on society is expanding. Understanding of IBS has changed since the release of the Rome IV diagnosis in 2016, and this paper reviews the literature from the past decade to find that research around the brain-gut axis, diet, and gut microbiota are at the forefront of IBS. Moreover, as the research on the physiopathology of IBS has advanced, the treatment model has become more refined, which has important clinical implications.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional disease, and the changes that it causes in bowel function and abdominal pain seriously affect the patient's normal life and work. It mainly affects young and female individuals, and it tends to overlap with other functional gastrointestinal diseases (FGIDs) and cause a huge burden to life and society's economy[1,2]. Prevalence varies greatly between countries because of differences in food, culture, and diagnosis. The Rome Foundation Global Study[3] coverage across the country reported that the overall prevalence of IBS was 3.8% in Rome IV and 10.1% in Rome III. The Rome IV criteria, based on symptoms that have undergone a change in dynasty, suggest that the pathogenesis of IBS is associated with gut-brain interactions, which may be an overlapping pathogenesis between FGIDs. Based on the results of Rome IV, many studies have been performed, so it is necessary to summarize their findings. The aim of this study is to summarize IBS from the perspectives of epidemiology, disease overlap, pathological mechanisms, diagnosis, and treatment, focusing on disease overlap, pathological mechanisms, and treatment.

REGIONALIZATION SHOULD BE EMPHASIZED IN EPIDEMIOLOGY

The prevalence of IBS varies widely between different countries. In 2017, the Rome Foundation working group reviewed related work and showed that the prevalence of IBS varied from 1.1% (France and Iran) to 35.5% (Mexico), and the prevalence in Asia is also uneven[4-6]. It might be that many previous surveys did not use uniform diagnostic criteria or the same methodology, with geography, culture, and population being the reasons for different prevalences, and thus the included studies were heterogeneous. The goal of determining the global prevalence of IBS is still inaccurate[7]. Therefore, we discuss the epidemiology of IBS in different continents in recent years.

The Rome Foundation Global Epidemiological Study organized a study using Rome IV in 33 countries and Our analysis discovered that the prevalence rates in Europe and the United States were comparable, while those in Asia and Australia were marginally lower[7] (Figure 1). Egypt had the highest prevalence rate of internet surveyed countries[8]. As well as that, representative researches have also been carried out in various countries in recent years and reported that the prevalence of IBS was 5.2% (Rome IV), 5.9% (Rome III), and 6.98% (Rome IV) in Gibraltar, the United Kingdom[9], Hangzhou, China[10] and Latin America[11], respectively. Based on population survey data in the United States, Canada, and the United Kingdom, the results revealed the Rome III IBS rate was roughly twice as high as the Rome IV rate[12]. Overall, there is a clear predominance in the prevalence in Africa, and the prevalence based on Rome IV diagnosis is similar in the United States and Europe. However, prevalence varies widely between Europe and Asia, especially in Asian countries surveyed by using the internet and questionnaires. In the past, most studies have shown a higher prevalence of IBS in women[13]. Interestingly, IBS is equally common in men and women in Asia[14-16]. The highest prevalence was observed in the educated, the wealthy, students and younger individuals[17]. It also declined with age[1,18,19]. Through years of research and analysis, it was determined that estimating a pooled global prevalence of IBS was unlikely to be feasible, so regionalization should be emphasized in future research.

NEW INSIGHTS INTO THE OVERLAP OF IBS

Rome criteria for IBS based on symptoms were the most recognized, and the overlap of FGIDs was gradually valued by Roman criteria over time (Figure 2). Now, Rome IV suggests that the pathologies exist in the gastrointestinal tract on a continuum instead of as separate disorders, and overlap may be a natural clinical symptom of FGIDs[20]. Likewise, 54127 adults from 26 countries participated in an internet survey and discovered that 68.3% had symptom overlap in both gastrointestinal regions and 2.3% had esophageal, gastroduodenal, bowel, and anorectal overlap[21]. Overall, by reviewing the overlap of IBS and other diseases, it was found that there was obvious overlap between IBS and FGIDs, and anxiety and depression were their common characteristics, which verified the vital position of the central nervous system and brain-gut axis in the pathological mechanism of FGIDs. Therefore, the overlapping pattern and pathology of FGIDs are something that should be studied in depth.

Functional dyspepsia

Functional dyspepsia (FD) and IBS are the most prevalent FGIDs, postprandial fullness, early satiation, epigastric pain, and epigastric burning are the main symptoms of FD. The global prevalence of FD varies from 10%-20%[22]. Clinical studies have identified not only overlap between FD and IBS[23-25], but also the most common overlapping characteristics. In the overlap between FD and IBS-D (diarrhea), abdominal pain, bloating, and diarrhea are prominent. However, in the overlap between FD and IBS-C (constipation), abdominal fullness and constipation are prominent.

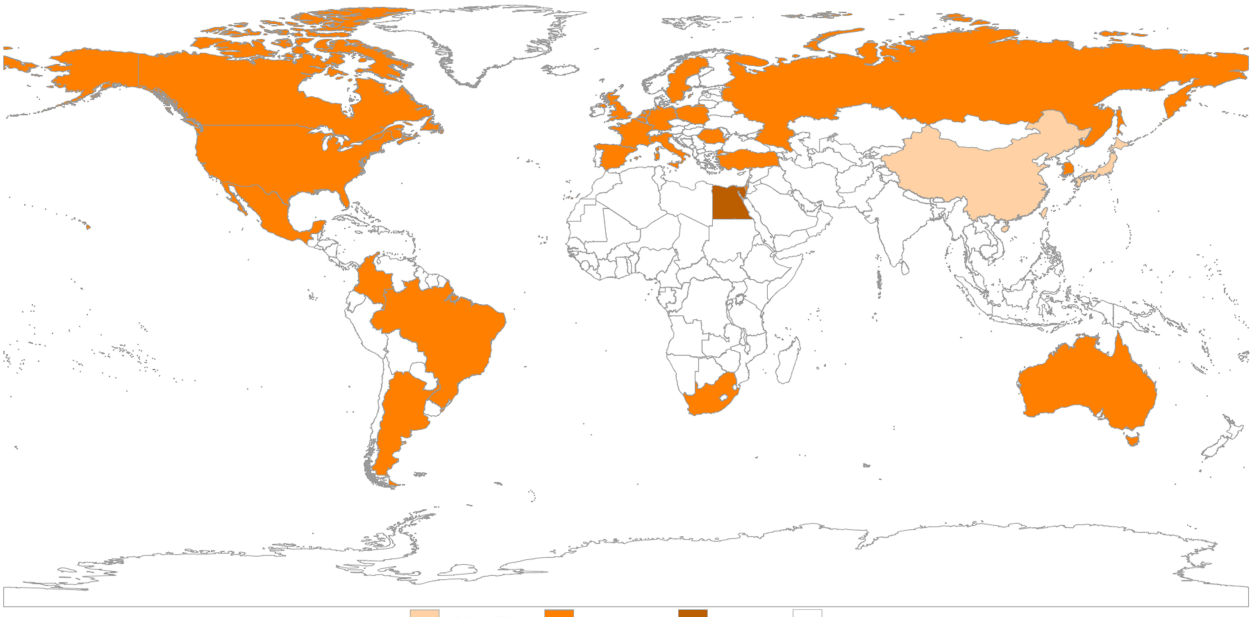

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Figure 1 Prevalence of irritable bowel syndrome by Rome IV. Prevalence of an Internet survey conducted by the Rome Foundation in multiple centers worldwide based on Rome IV. Asia, 1.3%-4.7%; Europe, 3.5%-5.9%; America, 3.5%-5.3%; Australia, 3.5%; Egypt, 7.6%; and South Africa, 5.9%.

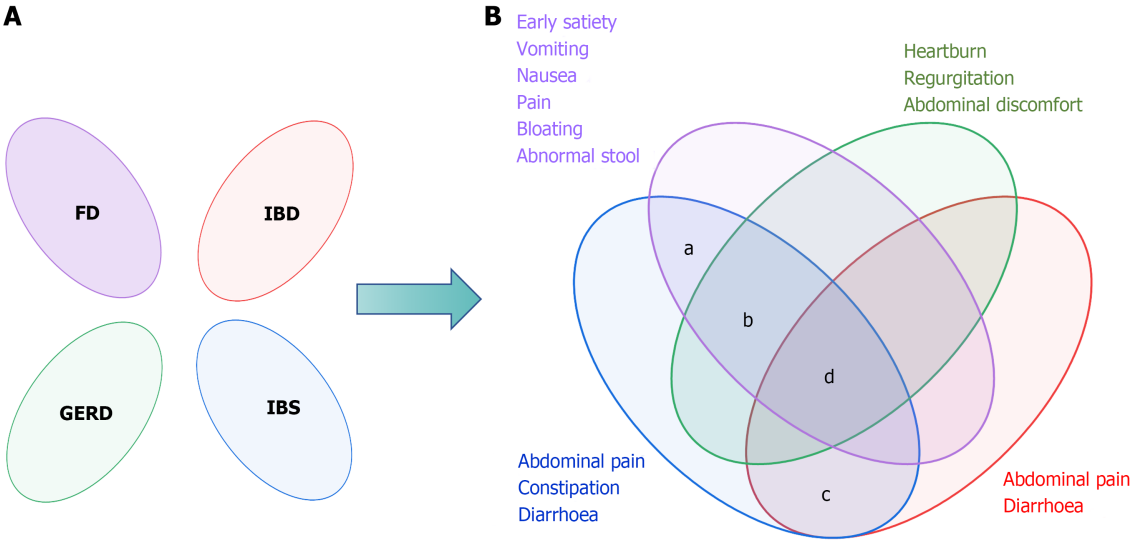

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Figure 2 The Rome Foundation's views on the overlap of functional gastrointestinal diseases. A: Rome I considered the functional bowel disorders to be independent diseases; B: Rome II and Rome III recognized there was overlap between functional gastrointestinal diseases. a: The prevalence of overlap between irritable bowel syndrome (IBS) and functional dyspepsia was 55.3%; b: The overlap between IBS and gastroesophageal reflux disease ranged from 3%-79% in the questionnaire and 10%-74% when diagnosed by endoscopy; c: A 2020 meta-analysis showed that the pooled prevalence of IBS-type symptoms was 32.5%; d: Only 2.3% had esophageal, gastroduodenal, bowel, and anorectal overlap. FD: Functional dyspepsia; IBD: Inflammatory bowel diseases; GERD: Gastroesophageal reflux disease; IBS: Irritable bowel syndrome.

In a longitudinal follow-up study published in 2022, 807 individuals (Rome IV) were included, 446 (55.3%) of whom had overlapping IBS and FD, which showed that patients with overlapping IBS and FD had more severe symptoms and were more likely to have depression and anxiety[26,27]. Furthermore, a prospective study in South Korea in 2019-2020 reported the same conclusion; moreover, women with overlap of IBS and FD experienced more severe gastrointestinal and depression symptoms than men. Interestingly, an Australian study showed no relationship between gender and overlap[25,28]. There seems to be a distinct overlap between IBS, FD, and gastroesophageal reflux disease (GERD), in which it is easier to merge psychological morbidity and sleep disturbance[29]. Although age, gender, and IBS subtype were not correlated with overlap[25], the pathogenesis analysis of IBS and FD indicates that psychological factors are linked to the overlap of IBS and FD. Therefore, the diagnosis of IBS or FD should be considered in terms of each other,

especially when encountering some anxious, severe symptoms.

GERD

GERD is a condition in which stomach contents reflux and cause uncomfortable symptoms[30], which present with regurgitation, heartburn, or being asymptomatic. Then, GERD is divided into three phenotypic presentations: Nonerosive reflux disease, erosive esophagitis (RE), and Barrett's esophagus (BE), with prevalence rates of 60%-70%, 30%, and 6%-8%, respectively[31]. Before Rome IV, a small number of studies had shown overlap between GERD and FGIDs[32], and IBS is a risk factor for GERD[33]. But now, the Rome Foundation considers overlap between FGIDs to be a trend. In patients with overlapping GERD and IBS, acid reflux and heartburn may present with abdominal pain or discomfort, and visceral hypersensitivity and gastrointestinal motility disorders may be coexisting mechanisms. However, the prevalence of patients with GERD and IBS (different criteria) varies greatly, and the overlap between IBS and GERD ranges from 3% to 79% based on the questionnaire and 10% to 74% when diagnosed by endoscopy[34]. In 2016, an Italian study with 697 heartburn patients found that cases of IBS overlapping with GERD/hypersensitive esophagus (HE) and overlapping functional heartburn (FH) were 147/454 (33%) and 187/243 (77%), respectively[35]. Besides, there is a higher risk of possible overlap between FGIDs.

Inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) are common inflammatory bowel diseases (IBDs). CD is characterized by chronic or nocturnal diarrhea, abdominal pain, and weight loss, whereas UC is characterized by bloody diarrhea with rectal urgency and tenesmus[36,37]. Although some biomarkers are used to distinguish between IBS and IBD, there is also overlap between them. Patients with overlapping IBD and IBS are prone to diarrhea and abdominal pain, which can be serious. Besides, a 2020 meta-analysis showed that the pooled prevalence of IBS-type symptoms among patients with IBD was 32.5%[38]. IBS-D is related to gut infections, and the gut microbiome and the intestinal barrier are bridges that connect them. Thus, IBS-D is a common diagnosis in patients with chronic diarrhea following chronic infection[39,40]. Overall, IBD and IBS can be different stages of the same disease. Therefore, the overlapping disease characteristics of IBS and IBD should not be ignored when the patient has a history of intestinal infection. At the same time, it is necessary to prevent IBS when diagnosing IBD.

Other

A follow-up study in the US performed an analysis of data from 655 adults to compare the degree of overlap between chronic overlapping pain conditions (COPCs). Surprisingly, IBS is the most common COPC other than headache. Furthermore, 63% of IBS cases have one or more COPCs, and 53% of IBS cases reported pain in ≥ 3 non abdominal areas [41]. Therefore, when there is chronic physical and abdominal pain, IBS overlap should not be ignored[42]. It was observed that IBS and nonceliac gluten sensitivity had significant symptom overlap, and their physiology and pathology were not clear[43]. Moreover, there is overlap between adolescents with endometriosis and IBS[44], and the overlap between IBS and endometriosis may have the same pathogenesis; specific mechanisms need to be further explored.

PATHOPHYSIOLOGY

In the past, IBS was thought to be a functional disorder that could not be explained by organic disease or a clear etiology [45]. With the increasing research on IBS and the update of the Rome criteria, the view on the pathophysiological mechanisms of IBS has changed from functional to brain-gut interaction. The aim of this article is to review the pathophysiology from clinical studies and basic research on IBS after Rome IV.

Clinical studies

Recurrent abdominal discomfort, abdominal pain, and altered bowel habits are the core clinical symptoms of patients with IBS, and clinical studies on pathogenesis show that the microbiome, gastrointestinal endocrine cells, visceral hypersensitivity, and gastrointestinal motility disorders , are observed in IBS patients and are the direct causes of abdominal discomfort, abdominal pain, or diarrhea. It was discovered through experiments that the levels of colonic mucosal Takeda G protein-coupled receptor 5 protein expression, short-chain fatty acid (SCFA), fecal bile acids (FBA)[46, 47], tryptophan (aryl hydrocarbon receptor kynurenine pathways), and methane gas production[47,48] were higher in patients with IBS than in healthy control (HC), and metabolites such as SCFA and bile acids are mainly associated with gastrointestinal malabsorption; there are differences between IBS subtypes, and neurotransmitters cause abdominal pain through the brain-gut axis and center. DuPont *et al*[49], recorded intestinal transport in 46 patients with IBS using a wireless pH/pressure recording capsule and found a delayed gastric emptying time in 35/46 (76%) IBS patients. And abnormal colonic transit and disorders of evacuation are important physiopathologies in patients with IBS, leading to constipation, bloating, and abdominal pain. Furthermore, abnormal oroanal transit time (OATT) was associated with hydrogen and methane concentrations, and more rapid OATT was associated with a higher severity of abdominal discomfort, rumbling, and nausea[48]. In addition, gut endocrine cells are scattered throughout the gastrointestinal tract and have sensory microvilli that sense gut pressure and gut contents[50-52], and when the gut lumen is stimulated by food[53], and microbial metabolism, the cells release hormones into the lamina propria to act mainly through paracrine and afferent and efferent synaptic transmission[54-56]. And studies found that histamine, 5-HT, glutamate, and noradrenalin strengthen visceral pain, and γ-aminobutyric acid reduces gastrointestinal motility.

Abdominal pain in IBS patients has been shown to be associated with structural features of the brain. Rectal stimulation seems to activate the anterior cingulate cortex, prefrontal cortex, insula, thalamus, and cerebellum, and is higher in patients with IBS[57]. A study[58] of female IBS patients included 216 female IBS patients and 138 women serving as HC. In comparison to HC, patients with IBS had an increase in gray matter volume and cortical thickness in the primary and secondary somatosensory cortex and subcortical regions; however, the volume, surface, and cortical thickness of the gray matter in the posterior insula and superior frontal gyrus were reduced. Moreover, abdominal pain caused by rectal dilation is linked to the thicker left primary somatosensory cortex (Figure 3).

Animal experiments

Visceral hypersensitivity and gut barrier disruption have been shown to be mediated *via* corticotropin-releasing factor (CRF)-Toll-like receptor 4 (TLR4)-proinflammatory cytokine signaling in animal experiments[59,60]. In addition, Nozu *et al*[61] conducted experiments based on IBS model rats and discovered that apelin activates CRF and TLR4, which may create a vicious cycle of proinflammatory cytokine signaling, which is a key pathway for the pathological mechanism of IBS. Then, the disruption of the gut barrier leads to an increase in lipopolysaccharides (LPS) and proinflammatory cytokines, which is a vital pathological mechanism that causes abdominal pain in patients with IBS[62]. And there are some new developments, such as a study using NanoString mRNA measurement of colonic neuroimmune gene expression and founding that the expression of the gene Trpv1 was higher in Gnotobiotic mice from patients with IBS and comorbid anxiety; moreover, it was associated with visceral hypersensitivity and anxiety[63]. Besides, decreasing miR-199 caused visceral hypersensitivity and augmented visceral pain in patients with IBS through translational upregulation of TRPV1[64]. Both activating BDNF-TrkB-PKMζ signaling in the thoracolumbar spinal cord of rats to increase synaptic activity and activating TLR4 trigger the release of pro-inflammatory cytokine afferent nerves and can cause visceral hypersensitivity[65,66]. Moreover, recent studies have shown that abnormal mast cell structure or function is a potential mechanism for visceral hypersensitivity in IBS[67], and post-IBS with gut microbial disorders leading to IBS and signaling pathways are also associated with visceral hypersensitivity[67,68]. In addition, an excellent review by Tozlu *et al*[69]. indicated that the number of mucosal eosinophils increased substantially more in patients with post-IBD IBS-D than in patients with active IBD, there was a reaction to the removal of allergic foods during treatment, and intestinal inflammation in patients with IBS was associated with food allergic reactions. Peptide YY (PYY) is localized in endocrine cells and regulates gut motility and visceral sensitivity by releasing and modulating serotonin[70] (Figure 4).

Microbiome

Koloski *et al*[71,72] discovered that higher baseline levels of anxiety and depression were significant predictors of developing IBS, and two prospective studies found that functional gastrointestinal symptoms preceded the mood disorder in two-thirds of patients. Dinan *et al*[73,74] have suggested that disturbances in the gut microbiota can affect brain function, behavior, and cognition, and the theory has developed into the microbiota-gut-brain axis, which is an important basis for the influence of gut microbes as well as neurotransmitters on IBS. The microbial diversity and abundance of stool in patients with IBS were altered compared to those in HC, with a decrease in *Coli*, *Lactobacilli*, *Collinsella*, and *Bifidobacteria* and an increase in *Enterobacteria*, *Coli*, anaerobes, *Escherichia coli*, *Ruminococcus gnavus*, and Bacteroides in patients with IBS. And a higher proportion of Bacteroides and *Allisonella* in patients with IBS-M[75,76]. In addition a study[77] used 16S rRNA metagenomic sequencing and performed phylogenetic investigation of communities by reconstruction of unobserved states to analyze fecal samples from control (n = 12) and IBS-D patients (n = 7) and reported that in patients with IBS, the abundances of *Sutterellaceae*, *Acidaminococcaceae,* and *Desulfovibrionaceae* were significantly increased, and those of *Clostridiaceae, Leuconostocaceae, Enterococcaceae, Peptostreptococcaceae*, and *Lachnospiraceae* were significantly decreased; moreover, secondary bile acid biosynthesis was decreased, and the citrate cycle was increased. Moreover, a study[78] used proton nuclear magnetic resonance spectroscopy and shotgun metagenomic sequencing to analyze fecal metabolites and the gut microbiome (IBS patients =142 and HC =120). It reported that the gut microbial diversity of IBS (Simpson's evenness metric) was drastically lower than that of HC, and metabolomics found that the mechanism of IBS was related to 5-HT.

TREATMENTS

The diagnosis of IBS is based on symptoms ranging from the Manning criteria to the Rome criteria, and the most widely used diagnostic criteria are the Rome IV[79]. Research around Rome IV has revealed that there are many important biomarkers that guide the differential diagnosis and symptomatic treatment of IBS that may be taken into account. According to Vijayvargiya *et al*[80], FBA and fecal fat are potential biomarkers for IBS-D and IBS-C. Total FBA, chenodeoxycholic acid (CDCA), cholic acid (CA), and primary bile acids were significantly higher in patients with IBS-D than in healthy patients or patients with IBS-C. In contrast, deoxycholic acid (DCA) and combined DCA and CDCA (secretory) bile acids were significantly lower in patients with IBS-C than in HC and patients with IBS-D. Combining fasting serum 7α-hydroxy-4-cholesten-3-one and primary bile acids or fecal bile acid concentrations in stool samples is a simple, low-cost diagnostic for bile acid diarrhea (BAD). Circulating resolvin D1 (RvD1) and c-reactive protein (CRP) are inflammatory markers in patients with IBS-C; patients with IBS-C have higher CRP and lower RvD1 concentrations than HC[81]. Furthermore, radiopaque markers and scintigraphy can be used to assess transit function, and rectal sensation to balloon distension can be used to assess visceral hypersensitivity[82]. All of the ancillary tests listed above can be used to further identify the cause and guide medication use if the first-line medication is ineffective.

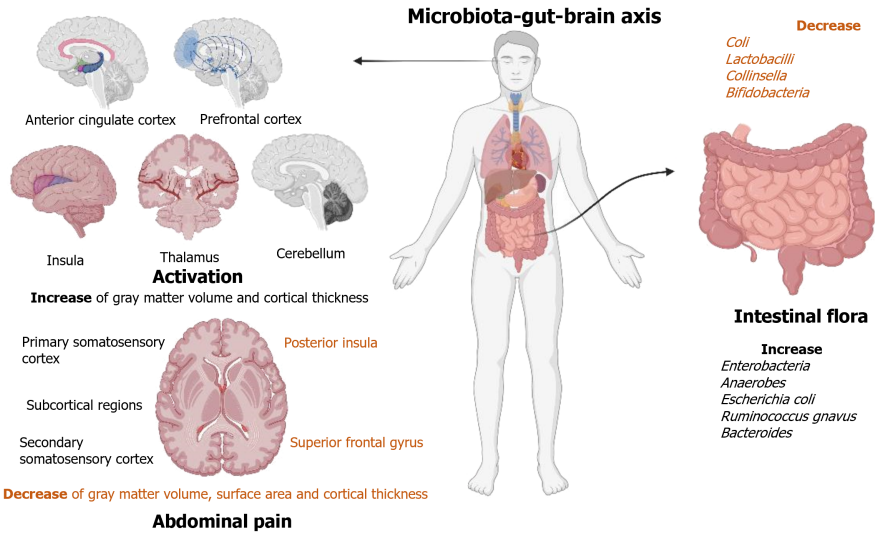

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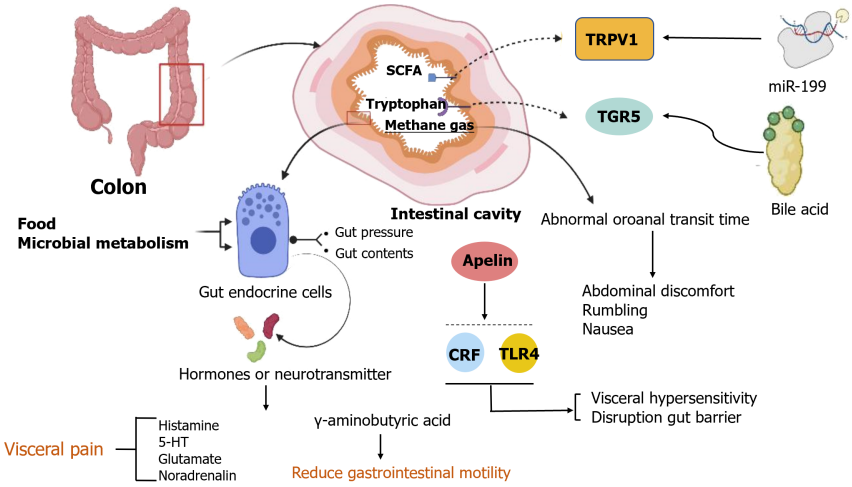
Figure 3 Research progresses on the mechanism of action of the microbiota-gut-brain axis. Brain changes in patients with irritable bowel syndrome (IBS) are associated with abdominal pain. They have higher activation of the anterior cingulate cortex, prefrontal cortex, insula, thalamus, and cerebellum. They also mainly showed an increase of gray matter volume and cortical thickness in the primary somatosensory cortex, secondary somatic sensory cortex, and subcortical regions and a decrease of gray matter volume, surface area, and cortical thickness in the posterior insula and superior frontal gyrus. Specific changes in the intestinal flora of patients with IBS. The number of *Coli*, *Lactobacilli*, *Collinsella*, and *Bifidobacteria* in IBS patients decreased, while the number of *Enterobacteria*, *Anaerobes*, *Escherichia coli*, *Ruminococcus gnavus*, and *Bacteroides* increased.



DOI: 10.3748/wjg.v29.i26.4120

Figure 4 Pathology of irritable bowel syndrome in the intestinal. Food and microbial metabolism stimulate the gut's endocrine cells to release hormones and neurotransmitters, leading to visceral pain and reducing gastrointestinal motility. Apelin, corticotropin-releasing factor, and Toll-like receptor 4-proinflammatory cytokine signaling lead to visceral hypersensitivity and disruption of the gut barrier. The concentrations of hydrogen and methane are related to abnormal oroanal transit time (OATT), and a more rapid OATT was associated with a higher severity of abdominal discomfort, rumbling, and nausea. Decreasing miR-199 caused visceral hypersensitivity and augmented visceral pain in patients with irritable bowel syndrome (IBS) through translational upregulation of TRPV1. Colonic mucosal protein expression and faecal bile acids were correlated with the symptom severity of IBS-D patients. CRF: Corticotropin-releasing factor; TLR4: Toll-like receptor 4.

Patients with mild IBS first choose education, diet, and lifestyle interventions as prerequisites, combined with first-line therapeutic drugs. If first-line treatment is ineffective, clinical judgment combined with ancillary tests is required to select appropriate second-line drugs and non-pharmacological interventions (Figure 5). Furthermore, patients with psychological problems can be assessed using psychological questionnaires, emphasizing doctor-patient communication for emotional relief, and using tricyclic antidepressant (TCA) or selective serotonin reuptake inhibitor (SSRI) medications.

Lifestyle intervention therapy

Stress reduction, appropriate exercise, and a special diet are the main non-pharmacological treatments for preventing induction; likewise, the publication of the British Gastroenterological Society guidelines[83] in 2021 and the updated guidelines from the American College of Gastroenterology (ACG) in 2022 emphasized that dietary counseling should be regarded as a first-line treatment option. A low-FODMAP (fermentable oligosaccharides, disaccharides, monosac-

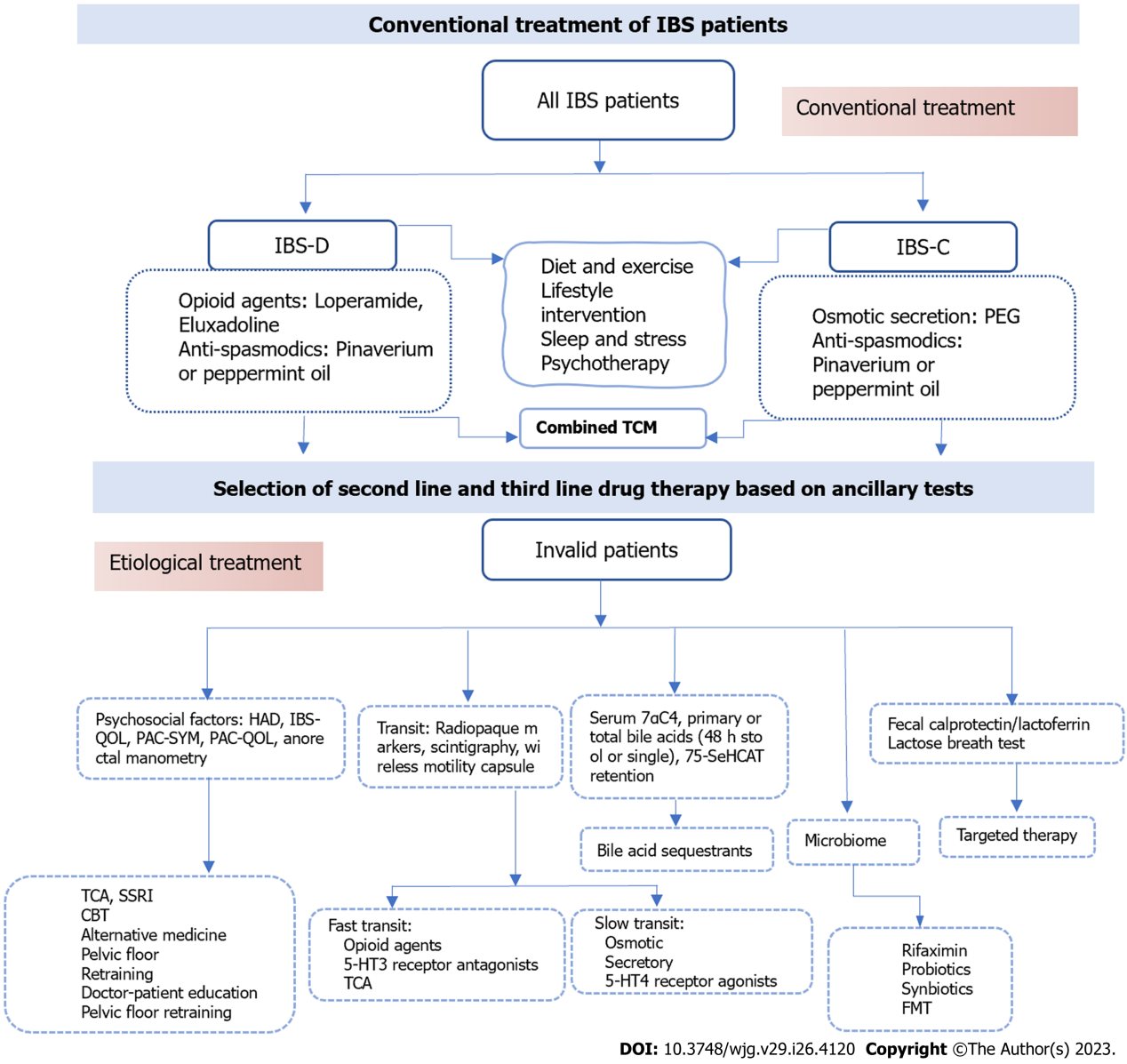


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Figure 5 The conventional treatment and further treatment of irritable bowel syndrome patients. TCM: Traditional Chinese treatment; CBT: Cognitive behavioral treatment; HAD: Hospital Anxiety and Depression scale; IBS-QOL: Irritable Bowel Syndrome-Quality of Life Questionnaire; PAC-QOL: Patient Assessment of Constipation-Quality of Life Questionnaire; PAC-SYM: PAC-Symptoms Questionnaire; 7αC4: 7α-hydroxy-4-cholesten-3-one; 75-SeHCAT: 75-selenium homocholic acid taurine; TCA: Tricyclic Antidepressants; SSRI: Selective Serotonin Reuptake Inhibitors; IBS: Irritable bowel syndrome; IBS-C: IBS-constipation; IBS-D: IBS-diarrhea.

charides, and polyols, LFD) diet is currently the most recommended and effective diet for IBS intervention[84]. And FODMAP induces symptom generation in IBS based on the gut-brain axis[85]. The ACG suggests that the LFD diet be implemented in three steps: (1) A period of strict restriction (lasting no longer than 4-6 wk); (2) reintroduction of FODMAP foods; and (3) personalization based on reintroduction results[86]. The short-term efficacy and safety of LFD compared to a Western diet and conventional diet in relieving IBS patients are definite[87]; of course, a regular diet is the foundation. Gluten-free foods and dietary fiber are other currently approved diets for patients with IBS[88,89]. IBS-D patients benefit more from LFD than IBS-C patients, while fiber diets such as psyllium fiber are more effective in IBS-C patients[90]. Garg[91], professor, proposed the "FEED" method, in which ample daily psyllium fiber (25 g) and sufficient water (500 mL), along with elevation of the feet and exercises of the abdominal muscles while sitting on the toilet, can help IBS-D symptoms. In contrast, lactose, sorbitol, fructose, xylitol, mannitol, fat, alcohol, insoluble fibers, and fizzy drinks increase pain and flatulence and should be avoided by patients with IBS[92-94].

Cognitive behavioral treatment

Since IBS is a gastrointestinal physical disorder that often fluctuates with stress, the Rome working team strongly recommends brain-gut axis behavior therapies as part of the treatment of DGBI disorders such as IBS[95]. Including hypnotherapy, dynamic psychotherapy, and relaxation therapy[96] can improve abdominal pain, standard of living, and psychological symptoms in patients with IBS and can reduce health care costs. Although some patients are unable to

receive psychotherapy, recent studies have shown that cognitive behavioral treatment (CBT) of hypnotherapy is a potential and affordable treatment. A study included 436 patients with IBS (Rome III) who were followed up at 2 wk and 3, 6, 9 and 12 mo after the end of specific CBT treatment, and the results showed that not only did CBT improve symptoms, but the improvement usually extended up to 12 mo after treatment[97,98]. Additionally, gut-directed hypnotherapy (GHT) can also improve the symptoms of IBS by affecting gastrointestinal motility and visceral sensitivity [99,100]. Overall, most views support the ideal that it works because it is based on the brain-gut axis. GHT is beneficial in directly reducing the discomfort of IBS and refractory IBS as well as improving quality of life and health, and the efficacy is sustained. Moreover, it can reduce anxiety and depression, but its mechanism is largely unclear[101]. The mechanism of hypnotherapy is related to the brain-gut axis, but current research on the microbiome has not provided definitive results[102]. What is certain is that hypnotherapy works by regulating the autonomic nervous system (ANS). The vagus nerve is related to the brain-gut axis and can coordinate gastrointestinal functions, and there seems to be potential in studying the role of the vagus nerve[102,103].

Pharmacological treatment

IBS-D: The ACG published guidelines for IBS-D conditional recommendations in 2022[104] include the three drugs eluxadoline, rifaximin, and alosetron (moderate certainty), which can relieve or assist abdominal pain and stools, but there are adverse effects and contraindications. Loperamide (very low certainty) can relieve diarrhea, but there is no evidence that it improves abdominal discomfort. TCA and antispasmodics have low certainty. Moreover, SSRIs are recommended against use (low certainty) (Table 1).

IBS-C: The first-line therapy for IBS-C are bulking agents and osmotic laxatives. The ACG published guidelines for IBS-C [105] and recommended them in 2022, including a strong recommendation for linaclotide (high certainty) and conditional recommendations for tenapanor, plecanatide, tegaserod, and lubiprostone (moderate certainty); polyethylene glycol laxatives, TCA, and antispasmodics have low certainty. The panel made a conditional recommendation against the use of SSRIs (low certainty). Chloride channel activators and guanylate cyclase activators are recommended for global IBS with constipation symptoms[106]. However, adverse effects of diarrhea may occur (Table 1).

Pain: Antispasmodics, including anticholinergic and calcium-blocking drugs, which can relieve pain and improve bowel movements, remain the first choice for abdominal pain in IBS[107]. Such as cimetropium/dicyclomine, peppermint oil, pinaverium, and trimebutine have clear benefits on abdominal pain and symptom scores[108]. By reviewing relevant RCTs[109-112], it was shown that most samples were small and of moderate quality. Overall, limited to short-term treatment Antidepressants can improve pain through central nervous system action, but clinical trials are scarce and the limitation of adverse events is uncertain. Although pinaverium was the most commonly used drug for the treatment of abdominal pain with a rapid onset of action and the improvement in abdominal pain was greater than that of bowel movements, its efficacy was less significant than that of placebo after one week. Whereas otilonium bromide (OB) significantly has a longer onset of action than pinaverium but is more suitable for patients with diarrhea. Moreover, drotaverine has a slow onset of action and is more suitable for the later stages of IBS. Although peppermint oil has been shown to be effective, it has many adverse events (heartburn or GERD symptoms, belching, headache, *etc.*). Finally, TCA and SSRIs have been shown to be effective, but SSRI adverse events are more numerous, and TCA is recommended for patients with significant anxiety or abdominal pain[113,114] (Table 1).

Traditional Chinese treatment

Traditional Chinese treatment: Traditional Chinese treatment (TCM) prescriptions Traditional Chinese medicine treatments, including prescriptions, acupuncture, and moxibustion, are the main treatments for IBS, although they are still complementary treatments that have been found to have great potential through research. Its possible mechanisms of action are mainly through regulating the enteric nervous system, improving gastrointestinal motility, reducing visceral hypersensitivity, regulating intestinal flora, and regulating the immune system to alleviate IBS[115].

A RCT designed in China, enrolling 216 patients with IBS who were assigned to the control group that took the Chang'an I Recipe or placebo group, reported that the Chang'an I Recipe outperformed the placebo in the treatment of IBS-D with no major side effects[116]. Furthermore, 60 IBS patients were enrolling in a study and were divided into the control group (n = 20) and the treatment group (n = 40), which were given oral pinaverium bromide tablets and Tongxie Yaofang decoction on the basis of conventional treatment, respectively. And the results reported that the flavored Tongxie Yaofang had a significant effect on the symptoms of patients with IBS-D[117], and improving the gut microbiome, alleviating visceral hypersensitivity, regulating 5-HT level in patients, and inhibiting colonic contraction are mechanisms for the treatment of IBS[118-120]. Moreover, Tongxie Anchang Decoction improves IBS by reducing visceral hypersensitivity, reversing mast cell infiltration, and regulating 5-HT[118,121]. And Xiang Sha Liu Jun Zi Decoction reduced the mean diarrhea score of IBS patients[122]. Finally, the Fuzi-Lizhong pill can impact bacterial diversity in the gut and regulate inflammation and immune system to treat IBS-D[123].

Acupuncture and moxibustion: The therapeutic effects of acupuncture are recognized worldwide, although its mechanisms of action are still being further explored. Acupuncture has a bright future in IBS and FGIDs, yet there remain controversies that need to be further explored. A randomized trial of 344 patients with IBS in the acupuncture group and 175 in the pinaverium bromide group reported that the acupuncture group was more effective than the control group, and the effect lasted up to 12 wk[124]. Besides, 126 patients with IBS-D (liver stagnation and spleen deficiency) were randomly assigned to one of three groups: A herb-separated moxibustion group (n = 42, applied to Jinsuo (GV 8)-eight-diagram points), a Western medication group (n = 42), and a Chinese herbal medication group (n = 42), and the results

Table 1 Summary of irritable bowel syndrome medications

Type	Mechanism of action	Example	Appearing dose	Efficient	
IBS-D					
Opioid agents	Inhibits secretion, transit	Loperamide	4 mg tid	Unknown for IBS; effective for diarrhea	First-line
		Eluxadoline	75 mg or 100 mg bid	Effective for FDA composite: 100 mg: OR, 0.87 (95%CI: 0.83-0.91); 75 mg: OR, 0.89 (95%CI: 0.84-0.94). RCTs: Effective for diarrhea and composite diarrhea + pain; not pain alone	
Bile acid sequestrants	Bind to bile acids	Cholestyramine	4 g bid	Unknown: effective in open-label studies; ineffective in 1, single-center RCT	
		Colestipol	-		
		Colesevelam	32 mg bid		
Antibiotic	Anti-inflammatory	Rifaximin	550 mg tid	Effective: In 2012 SRMA: Global: OR 1.57 (1.22 to 2.01); Bloating: OR 1.55 (1.23 to 1.96); In 2020 SRMA: FDA composite: OR: 0.92 (0.86 to 0.98) Global OR: 0.91 (0.77, 1.07)	Second-line
5-HT3 Receptor antagonists	Delays colonic transit and reduces visceral pain	Alosetron	0.5-1 mg qd or 1 mg bid	Effective: Global RR 1.60 (1.49 to 1.72); Pain RR 1.30 (1.22 to 1.39); FDA composite: OR 0.69 (0.60 to 0.80)	Third-line
		Ramosetron	2.5 μg qd		
IBS-C					
Osmotic	Osmotic secretion	PEG3350	-	Effective: improves SBMs, CSBMs, consistency straining but not pain, bloating or incomplete evacuation	First-line
Secretory	Increased Cl- and water secretion	Lubiprostone	8 μg bid	Effective: Lubiprostone 8 μg RR: 0.85 (0.78 to 0.96) for FDA endpoint	Second-line
		Linaclotide	290 mg qd	Effective: Adequate relief IBS: RR 1.95 (1.3 to 2.9); Abdo pain: RR 1.58 (1.02 to 2.46) RR 0.81 (0.76 to 0.86) for 290 μg for FDA endpoint	
		Plecanatide	3 mg/6 mg qd	Effective: Using FDA endpoint 6 mg: RR 0.87 (0.81 to 0.94); 3 mg RR 0.88 (0.82 to 0.94)	
Anti-absorptive	NHE3 inhibitor stimulates Na+, water secretion	Tenapanor	15mg bid	Effective at 50 mg 2/d; NNT, 7-9 for complete SBM and combined complete SBM ≥ 30% pain reduction; 11 for abdominal pain reduction > 30% alone	Third-line
Pain					
Anti-spasmodics	Inhibition of muscarinic Ach receptors or block Ca++ channels, relaxation of GI smooth muscle	Pinaverium	50 mg tid	May be effective: OR, 0.68 (95%CI: 0.57-0.71); overall NNT, 5	First-line
		Otilonium	20/40/80 mg tid		
		Hyoscine	-		
		Peppermint Oil	182 mg	Effective: OR, 0.43 (95%CI: 0.32-0.59); Global: RR 2.23 (95%CI: 1.78-2.81); overall NNT, 2.5; RCT of sustained release formulation: decrease pain, bloat, urgency but not total IBS scores	
Antidepressants	Psychological, acting on the CNS	TCA	-	Effective: OR, 0.67 (95%CI: 0.58-0.77); for global: OR, 0.62 (95%CI: 0.43-0.88); NNT, 4 for abdominal pain	Second-line
		SSRI	-		

Adapted from Camilleri *et al*[82]. Ach: Acetylcholine; 5-HT3: Serotonin type 3; IBS: Irritable bowel syndrome; IBS-C: Constipation; IBS-D: Diarrhea; IBS-M: Mixed symptoms; NHE3: Sodium-hydrogen exchanger 3; OR: Odds ratio; PEG 3350: Polyethylene glycol 3350; RCT: Randomized clinical trial; RR: Relative risk; Rx: Prescription; SSRI: Selective serotonin reuptake inhibitor; SBM: Spontaneous bowel movement; SRMA: Systematic review and meta-analysis; TCA: Tricyclic antidepressant.

showed that the TCM symptom score, gastrointestinal symptom score, and IBS-SSS score were significantly reduced in the moxibustion group[125]. Overall, Pishu (BL 20), Zhongwan (RN 12), and Zusanli (ST 36) are acupuncture points commonly used in clinical practice, and acupuncture and moxibustion have few side effects[93].

Microbial therapy

Probiotics: Probiotics can relieve bloating, intestinal gas, and IBS symptoms, and in addition, studies have shown that probiotics (*Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, and *Streptococcus*) can significantly relieve the overall symptoms of diarrhea and IBS-D[126,127]. However, the role of probiotics is controversial; a small number of studies have discovered that probiotics have no effect on bloating or abdominal pain[128,129]. A RCT included 389 patients with IBS. The control group was treated with oral probiotics for 6 wk. The final results showed that the treatment effect of probiotics was not superior to placebo when all IBS subtypes were included, but the analysis found a higher percentage of sustained responders in the probiotic group than in the placebo group in IBS-D[130]. Although there is a contradiction in the current evidence, analyzing it objectively resolved the contradictions and also demonstrated that probiotics have great potential for the treatment of IBS, especially in patients with IBS-D[131].

Prebiotics and synbiotics: Prebiotics and synbiotics, the collaboration of prebiotics and probiotics, become synbiotics, which have beneficial effects on the gastrointestinal tract by regulating the diversity and activity of intestinal microorganisms and protecting the integrity of the intestinal mucosa[132,133]. A RCT reported, that compared to placebo, synbiotics treatment over an 8-wk period (*Lactobacillus* and *Bifidobacterium* probiotic strains and short-chain fructooligosaccharides; colony-forming units (CFU) per sachet was five billion, bid) significantly improved overall symptoms of IBS, flatulence (P = 0.028), and bowel habits (P = 0.028). It is recommended to try probiotics for 12 wk and observe the efficacy[83], have benefits in improving IBS-D.

Fecal microbiota transplantation: Fecal microbiota transplantation (FMT) for *Clostridium difficile* infection has shown good efficacy in improving intestinal flora[134], and although studies on FMT for IBS are scarce and results remain controversial, the overall results suggest a positive trend for FMT for IBS. A RCT included 135 IBS patients randomly assigned to receive their own stool, 30 g FMT, or 60 g FMT, with response rates of 23.6%, 76.9%, and 89.1%, respectively [135]. Moreover, a recent study has found that FMT not only improves the symptoms of IBS patients but also improves depression and anxiety[93]. However, the studies of Madsen, A.M.A. *et al*[136] and Browne *et al*[137] reported that FMT capsules have no clinical benefit on abdominal pain, stool frequency, or stool form in IBS patients and have no benefit. Therefore, the clinical practice and clinical effect of FMT in the field of gut microbiomes and IBS need to be further verified and explored.

CONCLUSION

IBS-related studies have shown a downward trend in IBS dysfunction, and the brain-gut axis is gradually becoming more prominent in IBS research by reviewing the related research of the last decade. With the help of convenient laboratory tests, a new diagnosis and treatment model was formed based on the complex etiology and clinical combination of IBS, and the above has positive implications for a new understanding of IBS.

It is concluded that a pooled global prevalence of IBS is unlikely to be meaningful and that future research should focus more on regionalization. The definition of IBS has been updated with the discovery of overlapping symptoms and advances in research on IBS pathogenesis, and its definition tends to suggest that FGIDs are a group of disorders with the same pathogenesis, such as the brain-gut axis and visceral hypersensitivity. Therefore, in the future, we should pay more attention to the influence of the brain-gut axis and the central nervous system on the entire gastrointestinal tract and understand FGIDs as a whole. IBS is a dysfunctional disease, but in the absence of simple and inexpensive screening tests for many biological markers, patients such as those with BAD are still included in IBS-D. These technologies still need further validation and dissemination. It is possible that an updated Rome criteria will exclude them from the IBS diagnosis as technology advances. Furthermore, studies of IBS-related dietary interventions, such as LFD, special diets for IBS-C, and foods with gastrointestinal allergies, as well as the gut microenvironment and the brain-gut axis, are the hot spots of research on gut inflammation and the gut barrier.

Long-term treatment brings economic pressure and psychological burden, and for patients for whom conventional treatment is ineffective, further search for etiology should be done with the help of adjuvant examinations, and appropriate second-line treatment or psychotherapy should be chosen. In recent years, non-pharmacological treatment and Chinese medicine have been favored by IBS patients, but the current treatment should be further improved in order to facilitate the development of alternative medicine, with lifestyle, diet, and acupressure as routine interventions. It is important to note that lifestyle and CBT only relieve the symptoms and frequency of IBS; they do not improve the quality of life. Moreover, the involvement of microbiota in the brain-gut axis is widely recognized and studied, and RCTs related to intestinal flora have yielded encouraging results. However, current studies of microbiota are mostly related to IBS-D and have limitations. Let's look forward to more clarity on the treatment and management of IBS.

FOOTNOTES

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REFERENCES

1 **Yang W**, Yang X, Cai X, Zhou Z, Yao H, Song X, Zhao T, Xiong P. The Prevalence of Irritable Bowel Syndrome Among Chinese University Students: A Systematic Review and Meta-Analysis. *Front Public Health* 2022; **10**: 864721 [PMID: 35493361 DOI: 10.3389/fpubh.2022.864721]
2 **Roisinblit KC**. Irritable bowel syndrome in women. *J Midwifery Womens Health* 2013; **58**: 15-24; quiz 116 [PMID: 23279011 DOI: 10.1111/j.1542-2011.2012.00200.x]
3 **Sperber AD**, Bangdiwala SI, Drossman DA, Ghoshal UC, Simren M, Tack J, Whitehead WE, Dumitrascu DL, Fang X, Fukudo S, Kellow J, Okeke E, Quigley EMM, Schmulson M, Whorwell P, Archampong T, Adibi P, Andresen V, Benninga MA, Bonaz B, Bor S, Fernandez LB, Choi SC, Corazziari ES, Francisconi C, Hani A, Lazebnik L, Lee YY, Mulak A, Rahman MM, Santos J, Setshedi M, Syam AF, Vanner S, Wong RK, Lopez-Colombo A, Costa V, Dickman R, Kanazawa M, Keshteli AH, Khatun R, Maleki I, Poitras P, Pratap N, Stefanyuk O, Thomson S, Zeevenhooven J, Palsson OS. Worldwide Prevalence and Burden of Functional Gastrointestinal Disorders, Results of Rome Foundation Global Study. *Gastroenterology* 2021; **160**: 99-114.e3 [PMID: 32294476 DOI: 10.1053/j.gastro.2020.04.014]
4 **Black CJ**, Ford AC. Global burden of irritable bowel syndrome: trends, predictions and risk factors. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 473-486 [PMID: 32296140 DOI: 10.1038/s41575-020-0286-8]
5 **Sperber AD**, Dumitrascu D, Fukudo S, Gerson C, Ghoshal UC, Gwee KA, Hungin APS, Kang JY, Minhu C, Schmulson M, Bolotin A, Friger M, Freud T, Whitehead W. The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a Rome Foundation working team literature review. *Gut* 2017; **66**: 1075-1082 [PMID: 26818616 DOI: 10.1136/gutjnl-2015-311240]
6 **Rahman MM**, Mahadeva S, Ghoshal UC. Epidemiological and clinical perspectives on irritable bowel syndrome in India, Bangladesh and Malaysia: A review. *World J Gastroenterol* 2017; **23**: 6788-6801 [PMID: 29085223 DOI: 10.3748/wjg.v23.i37.6788]
7 **Sperber AD**. Epidemiology and Burden of Irritable Bowel Syndrome: An International Perspective. *Gastroenterol Clin North Am* 2021; **50**: 489-503 [PMID: 34304784 DOI: 10.1016/j.gtc.2021.04.001]
8 **Galica AN**, Galica R, Dumitrascu DL. Epidemiology of Irritable Bowel Syndrome in Albania. *J Gastrointestin Liver Dis* 2021; **30**: 334-338 [PMID: 34551031 DOI: 10.15403/jgld-3828]
9 **Kovács DB**, Szekely A, Hubai AG, Palsson O. Prevalence, epidemiology and associated healthcare burden of Rome IV irritable bowel syndrome and functional dyspepsia in the adult population of Gibraltar. *BMJ Open Gastroenterol* 2022; **9** [PMID: 35953148 DOI: 10.1136/bmjgast-2022-000979]
10 **Long Y**, Huang Z, Deng Y, Chu H, Zheng X, Yang J, Zhu Y, Fried M, Fox M, Dai N. Prevalence and risk factors for functional bowel disorders in South China: a population based study using the Rome III criteria. *Neurogastroenterol Motil* 2017; **29** [PMID: 27412422 DOI: 10.1111/nmo.12897]
11 **Pontet Y**, Olano C. [Irritable bowel syndrome prevalence in Latin America]. *Rev Gastroenterol Peru* 2021; **41**: 144-149 [PMID: 34978550]
12 **Palsson OS**, Whitehead W, Törnblom H, Sperber AD, Simren M. Prevalence of Rome IV Functional Bowel Disorders Among Adults in the United States, Canada, and the United Kingdom. *Gastroenterology* 2020; **158**: 1262-1273.e3 [PMID: 31917991 DOI: 10.1053/j.gastro.2019.12.021]
13 **Lovell RM**, Ford AC. Effect of gender on prevalence of irritable bowel syndrome in the community: systematic review and meta-analysis. *Am J Gastroenterol* 2012; **107**: 991-1000 [PMID: 22613905 DOI: 10.1038/ajg.2012.131]
14 **Gwee KA**, Lu CL, Ghoshal UC. Epidemiology of irritable bowel syndrome in Asia: something old, something new, something borrowed. *J Gastroenterol Hepatol* 2009; **24**: 1601-1607 [PMID: 19788601 DOI: 10.1111/j.1440-1746.2009.05984.x]
15 **Gwee KA**. Irritable bowel syndrome in developing countries--a disorder of civilization or colonization? *Neurogastroenterol Motil* 2005; **17**: 317-324 [PMID: 15916618 DOI: 10.1111/j.1365-2982.2005.00627.x]
16 **Ghoshal UC**, Singh R. Frequency and risk factors of functional gastro-intestinal disorders in a rural Indian population. *J Gastroenterol Hepatol* 2017; **32**: 378-387 [PMID: 27262283 DOI: 10.1111/jgh.13465]
17 **Husain N**, Chaudhry IB, Jafri F, Niaz SK, Tomenson B, Creed F. A population-based study of irritable bowel syndrome in a non-Western population. *Neurogastroenterol Motil* 2008; **20**: 1022-1029 [PMID: 18492027 DOI: 10.1111/j.1365-2982.2008.01143.x]

18 **Devanarayana NM**, Rajindrajith S, Pathmeswaran A, Abegunasekara C, Gunawardena NK, Benninga MA. Epidemiology of irritable bowel syndrome in children and adolescents in Asia. *J Pediatr Gastroenterol Nutr* 2015; **60**: 792-798 [PMID: 26000888 DOI: 10.1097/MPG.0000000000000714]

19 **AlButaysh OF**, AlQuraini AA, Almukhaitah AA, Alahmdi YM, Alharbi FS. Epidemiology of irritable bowel syndrome and its associated factors in Saudi undergraduate students. *Saudi J Gastroenterol* 2020; **26**: 89-93 [PMID: 32031159 DOI: 10.4103/sjg.SJG_459_19]

20 **Drossman DA**. Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features and Rome IV. *Gastroenterology* 2016 [PMID: 27144617 DOI: 10.1053/j.gastro.2016.02.032]

21 **Sperber AD**, Freud T, Aziz I, Palsson OS, Drossman DA, Dumitrascu DL, Fang X, Fukudo S, Ghoshal UC, Kellow J, Khatun R, Okeke E, Quigley EMM, Schmulson M, Simren M, Tack J, Whitehead WE, Whorwell P, Bangdiwala SI. Greater Overlap of Rome IV Disorders of Gut-Brain Interactions Leads to Increased Disease Severity and Poorer Quality of Life. *Clin Gastroenterol Hepatol* 2022; **20**: e945-e956 [PMID: 34052391 DOI: 10.1016/j.cgh.2021.05.042]

22 **Madisch A**, Andresen V, Enck P, Labenz J, Frieling T, Schemann M. The Diagnosis and Treatment of Functional Dyspepsia. *Dtsch Arztebl Int* 2018; **115**: 222-232 [PMID: 29669681 DOI: 10.3238/arztebl.2018.0222]

23 **Agréus L**, Svärdsudd K, Nyrén O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. *Gastroenterology* 1995; **109**: 671-680 [PMID: 7657095 DOI: 10.1016/0016-5085(95)90373-9]

24 **Whitehead WE**, Gibbs NA, Li Z, Drossman DA. Is functional dyspepsia just a subset of the irritable bowel syndrome? *Baillieres Clin Gastroenterol* 1998; **12**: 443-461 [PMID: 9890081 DOI: 10.1016/s0950-3528(98)90017-3]

25 **von Wulffen M**, Talley NJ, Hammer J, McMaster J, Rich G, Shah A, Koloski N, Kendall BJ, Jones M, Holtmann G. Overlap of Irritable Bowel Syndrome and Functional Dyspepsia in the Clinical Setting: Prevalence and Risk Factors. *Dig Dis Sci* 2019; **64**: 480-486 [PMID: 30368683 DOI: 10.1007/s10620-018-5343-6]

26 **Barberio B**, Yiannakou Y, Houghton LA, Black CJ, Savarino EV, Ford AC. Overlap of Rome IV Irritable Bowel Syndrome and Functional Dyspepsia and Effect on Natural History: A Longitudinal Follow-Up Study. *Clin Gastroenterol Hepatol* 2022; **20**: e89-e101 [PMID: 33839276 DOI: 10.1016/j.cgh.2021.04.011]

27 **Choi YJ**, Kim N, Yoon H, Shin CM, Park YS, Kim JW, Kim YS, Lee DH, Jung HC. Overlap between irritable bowel syndrome and functional dyspepsia including subtype analyses. *J Gastroenterol Hepatol* 2017; **32**: 1553-1561 [PMID: 28160607 DOI: 10.1111/jgh.13756]

28 **Nam K**, Kim N, Song HJ, Baik GH, Choi SC, Kim HJ, Lee JY, Park KS, Park SY, Park SJ. Gender difference in the overlap of irritable bowel syndrome and functional dyspepsia: a prospective nationwide multicenter study in Korea. *J Gastroenterol* 2021; **56**: 537-546 [PMID: 33687537 DOI: 10.1007/s00535-021-01775-2]

29 **Jones MP**, Shah A, Walker MM, Koloski NA, Holtmann G, Talley NJ. Overlap of heartburn, functional dyspepsia, and irritable bowel syndrome in a population sample: Prevalence, temporal stability, and associated comorbidities. *Neurogastroenterol Motil* 2022; **34**: e14349 [PMID: 35293084 DOI: 10.1111/nmo.14349]

30 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R; Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-20; quiz 1943 [PMID: 16928254 DOI: 10.1111/j.1572-0241.2006.00630.x]

31 **Fass R**, Boeckxstaens GE, El-Serag H, Rosen R, Sifrim D, Vaezi MF. Gastro-oesophageal reflux disease. *Nat Rev Dis Primers* 2021; **7**: 55 [PMID: 34326345 DOI: 10.1038/s41572-021-00287-w]

32 **Rasmussen S**, Jensen TH, Henriksen SL, Haastrup PF, Larsen PV, Søndergaard J, Jarbøl DE. Overlap of symptoms of gastroesophageal reflux disease, dyspepsia and irritable bowel syndrome in the general population. *Scand J Gastroenterol* 2015; **50**: 162-169 [PMID: 25525975 DOI: 10.3109/00365521.2014.983157]

33 **Lovell RM**, Ford AC. Prevalence of gastro-esophageal reflux-type symptoms in individuals with irritable bowel syndrome in the community: a meta-analysis. *Am J Gastroenterol* 2012; **107**: 1793-801; quiz 1802 [PMID: 23032982 DOI: 10.1038/ajg.2012.336]

34 **de Bortoli N**, Tolone S, Frazzoni M, Martinucci I, Sgherri G, Albano E, Ceccarelli L, Stasi C, Bellini M, Savarino V, Savarino EV, Marchi S. Gastroesophageal reflux disease, functional dyspepsia and irritable bowel syndrome: common overlapping gastrointestinal disorders. *Ann Gastroenterol* 2018; **31**: 639-648 [PMID: 30386113 DOI: 10.20524/aog.2018.0314]

35 **de Bortoli N**, Frazzoni L, Savarino EV, Frazzoni M, Martinucci I, Jania A, Tolone S, Scagliarini M, Bellini M, Marabotto E, Furnari M, Bodini G, Russo S, Bertani L, Natali V, Fuccio L, Savarino V, Blandizzi C, Marchi S. Functional Heartburn Overlaps With Irritable Bowel Syndrome More Often than GERD. *Am J Gastroenterol* 2016; **111**: 1711-1717 [PMID: 27644732 DOI: 10.1038/ajg.2016.432]

36 **Torres J**, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet* 2017; **389**: 1741-1755 [PMID: 27914655 DOI: 10.1016/S0140-6736(16)31711-1]

37 **Glick LR**, Cifu AS, Feld L. Ulcerative Colitis in Adults. *JAMA* 2020; **324**: 1205-1206 [PMID: 32857108 DOI: 10.1001/jama.2020.11583]

38 **Fairbrass KM**, Costantino SJ, Gracie DJ, Ford AC. Prevalence of irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease in remission: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 1053-1062 [PMID: 33010814 DOI: 10.1016/S2468-1253(20)30300-9]

39 **Müller M**, Willén R, Stotzer PO. Colonoscopy and SeHCAT for investigation of chronic diarrhea. *Digestion* 2004; **69**: 211-218 [PMID: 15205569 DOI: 10.1159/000079150]

40 **Klem F**, Wadhwa A, Prokop LJ, Sundt WJ, Farrugia G, Camilleri M, Singh S, Grover M. Prevalence, Risk Factors, and Outcomes of Irritable Bowel Syndrome After Infectious Enteritis: A Systematic Review and Meta-analysis. *Gastroenterology* 2017; **152**: 1042-1054.e1 [PMID: 28069350 DOI: 10.1053/j.gastro.2016.12.039]

41 **Slade GD**, Greenspan JD, Fillingim RB, Maixner W, Sharma S, Ohrbach R. Overlap of Five Chronic Pain Conditions: Temporomandibular Disorders, Headache, Back Pain, Irritable Bowel Syndrome, and Fibromyalgia. *J Oral Facial Pain Headache* 2020; **34**: s15-s28 [PMID: 32975538 DOI: 10.11607/ofph.2581]

42 **Üçüncü MZ**, Çoruh Akyol B, Toprak D. The early diagnosis of fibromyalgia in irritable bowel syndrome patients. *Med Hypotheses* 2020; **143**: 110119 [PMID: 32721811 DOI: 10.1016/j.mehy.2020.110119]

43 **Rej A**, Sanders DS. The overlap of irritable bowel syndrome and noncoeliac gluten sensitivity. *Curr Opin Gastroenterol* 2019; **35**: 199-205 [PMID: 30762700 DOI: 10.1097/MOG.0000000000000517]

44 **DiVasta AD**, Zimmerman LA, Vitonis AF, Fadayomi AB, Missmer SA. Overlap Between Irritable Bowel Syndrome Diagnosis and Endometriosis in Adolescents. *Clin Gastroenterol Hepatol* 2021; **19**: 528-537.e1 [PMID: 32184183 DOI: 10.1016/j.cgh.2020.03.014]

45 **Holtmann GJ**, Ford AC, Talley NJ. Pathophysiology of irritable bowel syndrome. *Lancet Gastroenterol Hepatol* 2016; **1**: 133-146 [PMID:

28404070 DOI: 10.1016/S2468-1253(16)30023-1]
46 **Waseem MR**, Shin A, Siwiec R, James-Stevenson T, Bohm M, Rogers N, Wo J, Waseem L, Gupta A, Jarrett M, Kadariya J, Xu H. Associations of Fecal Short Chain Fatty Acids With Colonic Transit, Fecal Bile Acid, and Food Intake in Irritable Bowel Syndrome. *Clin Transl Gastroenterol* 2023; **14**: e00541 [PMID: 36227781 DOI: 10.14309/ctg.0000000000000541]
47 **Wei W**, Wang H, Zhang Y, Niu B, Chen S, Zhang W, Yao S. Faecal bile acids and colonic bile acid membrane receptor correlate with symptom severity of diarrhoea-predominant irritable bowel syndrome: A pilot study. *Dig Liver Dis* 2021; **53**: 1120-1127 [PMID: 34053874 DOI: 10.1016/j.dld.2021.04.022]
48 **Algera JP**, Colomier E, Melchior C, Hreinsson JP, Midenfjord I, Clevers E, Simrén M, Törnblom H. Associations between postprandial symptoms, hydrogen and methane production, and transit time in irritable bowel syndrome. *Neurogastroenterol Motil* 2023; **35**: e14482 [PMID: 36221245 DOI: 10.1111/nmo.14482]
49 **DuPont AW**, Jiang ZD, Harold SA, Snyder N, Galler GW, Garcia-Torres F, DuPont HL. Motility abnormalities in irritable bowel syndrome. *Digestion* 2014; **89**: 119-123 [PMID: 24503633 DOI: 10.1159/000356314]
50 **Sandström O**, El-Salhy M. Ageing and endocrine cells of human duodenum. *Mech Ageing Dev* 1999; **108**: 39-48 [PMID: 10366038 DOI: 10.1016/s0047-6374(98)00154-7]
51 **Lee J**, Cummings BP, Martin E, Sharp JW, Graham JL, Stanhope KL, Havel PJ, Raybould HE. Glucose sensing by gut endocrine cells and activation of the vagal afferent pathway is impaired in a rodent model of type 2 diabetes mellitus. *Am J Physiol Regul Integr Comp Physiol* 2012; **302**: R657-R666 [PMID: 22160540 DOI: 10.1152/ajpregu.00345.2011]
52 **Cani PD**, Everard A, Duparc T. Gut microbiota, enteroendocrine functions and metabolism. *Curr Opin Pharmacol* 2013; **13**: 935-940 [PMID: 24075718 DOI: 10.1016/j.coph.2013.09.008]
53 **Mazzawi T**, El-Salhy M. Effect of diet and individual dietary guidance on gastrointestinal endocrine cells in patients with irritable bowel syndrome (Review). *Int J Mol Med* 2017; **40**: 943-952 [PMID: 28849091 DOI: 10.3892/ijmm.2017.3096]
54 **Gershon MD**, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 2007; **132**: 397-414 [PMID: 17241888 DOI: 10.1053/j.gastro.2006.11.002]
55 **Bertrand PP**, Bertrand RL. Serotonin release and uptake in the gastrointestinal tract. *Auton Neurosci* 2010; **153**: 47-57 [PMID: 19729349 DOI: 10.1016/j.autneu.2009.08.002]
56 **Bertrand PP**. The cornucopia of intestinal chemosensory transduction. *Front Neurosci* 2009; **3**: 48 [PMID: 20582275 DOI: 10.3389/neuro.21.003.2009]
57 **Nisticò V**, Rossi RE, D'Arrigo AM, Priori A, Gambini O, Demartini B. Functional Neuroimaging in Irritable Bowel Syndrome: A Systematic Review Highlights Common Brain Alterations With Functional Movement Disorders. *J Neurogastroenterol Motil* 2022; **28**: 185-203 [PMID: 35189600 DOI: 10.5056/jnm21079]
58 **Grinsvall C**, Ryu HJ, Van Oudenhove L, Labus JS, Gupta A, Ljungberg M, Törnblom H, Mayer EA, Simrén M. Association between pain sensitivity and gray matter properties in the sensorimotor network in women with irritable bowel syndrome. *Neurogastroenterol Motil* 2021; **33**: e14027 [PMID: 33174312 DOI: 10.1111/nmo.14027]
59 **Nozu T**, Okumura T. Corticotropin-releasing factor receptor type 1 and type 2 interaction in irritable bowel syndrome. *J Gastroenterol* 2015; **50**: 819-830 [PMID: 25962711 DOI: 10.1007/s00535-015-1086-8]
60 **Nozu T**, Miyagishi S, Nozu R, Takakusaki K, Okumura T. Lipopolysaccharide induces visceral hypersensitivity: role of interleukin-1, interleukin-6, and peripheral corticotropin-releasing factor in rats. *J Gastroenterol* 2017; **52**: 72-80 [PMID: 27075754 DOI: 10.1007/s00535-016-1208-y]
61 **Nozu T**, Miyagishi S, Ishioh M, Takakusaki K, Okumura T. Peripheral apelin mediates visceral hypersensitivity and impaired gut barrier in a rat irritable bowel syndrome model. *Neuropeptides* 2022; **94**: 102248 [PMID: 35526468 DOI: 10.1016/j.npep.2022.102248]
62 **Dlugosz A**, Nowak P, D'Amato M, Mohammadian Kermani G, Nyström J, Abdurahman S, Lindberg G. Increased serum levels of lipopolysaccharide and antiflagellin antibodies in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2015; **27**: 1747-1754 [PMID: 26387872 DOI: 10.1111/nmo.12670]
63 **Constante M**, De Palma G, Lu J, Jury J, Rondeau L, Caminero A, Collins SM, Verdu EF, Bercik P. Saccharomyces boulardii CNCM I-745 modulates the microbiota-gut-brain axis in a humanized mouse model of Irritable Bowel Syndrome. *Neurogastroenterol Motil* 2021; **33**: e13985 [PMID: 32955166 DOI: 10.1111/nmo.13985]
64 **Zhou Q**, Yang L, Larson S, Basra S, Merwat S, Tan A, Croce C, Verne GN. Decreased miR-199 augments visceral pain in patients with IBS through translational upregulation of TRPV1. *Gut* 2016; **65**: 797-805 [PMID: 25681400 DOI: 10.1136/gutjnl-2013-306464]
65 **Nozu T**, Miyagishi S, Nozu R, Ishioh M, Takakusaki K, Okumura T. EMA401, an angiotensin II type 2 receptor antagonist blocks visceral hypersensitivity and colonic hyperpermeability in rat model of irritable bowel syndrome. *J Pharmacol Sci* 2021; **146**: 121-124 [PMID: 34030794 DOI: 10.1016/j.jphs.2021.04.001]
66 **Fan F**, Tang Y, Dai H, Cao Y, Sun P, Chen Y, Chen A, Lin C. Blockade of BDNF signalling attenuates chronic visceral hypersensitivity in an IBS-like rat model. *Eur J Pain* 2020; **24**: 839-850 [PMID: 31976585 DOI: 10.1002/ejp.1534]
67 **Hasler WL**, Grabauskas G, Singh P, Owyang C. Mast cell mediation of visceral sensation and permeability in irritable bowel syndrome. *Neurogastroenterol Motil* 2022; **34**: e14339 [PMID: 35315179 DOI: 10.1111/nmo.14339]
68 **Chen Z**, Liu Y, Wu X, Lin W, Liu Z, Huang Y, Chen Y, Tang Y, Chen A, Lin C. Spinal CircKcnk9 Regulates Chronic Visceral Hypersensitivity of Irritable Bowel Syndrome. *J Pain* 2023; **24**: 463-477 [PMID: 36257575 DOI: 10.1016/j.jpain.2022.10.007]
69 **Tozlu M**, Cash B, Younes M, Ertan A. Dilemma in post-IBD patients with IBS-D symptoms: A 2020 overview. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 5-8 [PMID: 32990090 DOI: 10.1080/17474124.2021.1829469]
70 **El-Salhy M**, Hatlebakk JG, Hausken T. Possible role of peptide YY (PYY) in the pathophysiology of irritable bowel syndrome (IBS). *Neuropeptides* 2020; **79**: 101973 [PMID: 31727345 DOI: 10.1016/j.npep.2019.101973]
71 **Koloski NA**, Jones M, Kalantar J, Weltman M, Zaguirre J, Talley NJ. The brain--gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study. *Gut* 2012; **61**: 1284-1290 [PMID: 22234979 DOI: 10.1136/gutjnl-2011-300474]
72 **Koloski NA**, Jones M, Talley NJ. Evidence that independent gut-to-brain and brain-to-gut pathways operate in the irritable bowel syndrome and functional dyspepsia: a 1-year population-based prospective study. *Aliment Pharmacol Ther* 2016; **44**: 592-600 [PMID: 27444264 DOI: 10.1111/apt.13738]
73 **Dinan TG**, Cryan JF. Gut-brain axis in 2016: Brain-gut-microbiota axis - mood, metabolism and behaviour. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 69-70 [PMID: 28053341 DOI: 10.1038/nrgastro.2016.200]

74 **Dinan TG**, Cryan JF. Brain-Gut-Microbiota Axis and Mental Health. *Psychosom Med* 2017; **79**: 920-926 [PMID: 28806201 DOI: 10.1097/PSY.0000000000000519]

75 **Sabo CM**, Dumitrascu DL. Microbiota and the irritable bowel syndrome. *Minerva Gastroenterol (Torino)* 2021; **67**: 377-384 [PMID: 34515452 DOI: 10.23736/S2724-5985.21.02923-5]

76 **Mishima Y**, Ishihara S. Molecular Mechanisms of Microbiota-Mediated Pathology in Irritable Bowel Syndrome. *Int J Mol Sci* 2020; **21** [PMID: 33212919 DOI: 10.3390/ijms21228664]

77 **Lee SM**, Kim N, Yoon H, Kim YS, Choi SI, Park JH, Lee DH. Compositional and Functional Changes in the Gut Microbiota in Irritable Bowel Syndrome Patients. *Gut Liver* 2021; **15**: 253-261 [PMID: 32457278 DOI: 10.5009/gnl19379]

78 **Mujagic Z**, Kasapi M, Jonkers DM, Garcia-Perez I, Vork L, Weerts ZZRM, Serrano-Contreras JI, Zhernakova A, Kurilshikov A, Scotcher J, Holmes E, Wijmenga C, Keszthelyi D, Nicholson JK, Posma JM, Masclee AA. Integrated fecal microbiome-metabolome signatures reflect stress and serotonin metabolism in irritable bowel syndrome. *Gut Microbes* 2022; **14**: 2063016 [PMID: 35446234 DOI: 10.1080/19490976.2022.2063016]

79 **Mearin F**, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. *Gastroenterology* 2016 [PMID: 27144627 DOI: 10.1053/j.gastro.2016.02.031]

80 **Vijayvargiya P**, Camilleri M, Burton D, Busciglio I, Lueke A, Donato LJ. Bile and fat excretion are biomarkers of clinically significant diarrhoea and constipation in irritable bowel syndrome. *Aliment Pharmacol Ther* 2019; **49**: 744-758 [PMID: 30740753 DOI: 10.1111/apt.15106]

81 **Karatay E**, Utku ÖG. Serum resolvin D1 levels as a marker of inflammation in constipation dominant irritable bowel syndrome. *Turk J Gastroenterol* 2020; **31**: 113-119 [PMID: 32141819 DOI: 10.5152/tjg.2020.19751]

82 **Camilleri M**, Boeckxstaens G. Irritable bowel syndrome: treatment based on pathophysiology and biomarkers. *Gut* 2023; **72**: 590-599 [PMID: 36307180 DOI: 10.1136/gutjnl-2022-328515]

83 **Vasant DH**, Paine PA, Black CJ, Houghton LA, Everitt HA, Corsetti M, Agrawal A, Aziz I, Farmer AD, Eugenicos MP, Moss-Morris R, Yiannakou Y, Ford AC. British Society of Gastroenterology guidelines on the management of irritable bowel syndrome. *Gut* 2021; **70**: 1214-1240 [PMID: 33903147 DOI: 10.1136/gutjnl-2021-324598]

84 **Black CJ**, Staudacher HM, Ford AC. Efficacy of a low FODMAP diet in irritable bowel syndrome: systematic review and network meta-analysis. *Gut* 2022; **71**: 1117-1126 [PMID: 34376515 DOI: 10.1136/gutjnl-2021-325214]

85 **Wu J**, Masuy I, Biesiekierski JR, Fitzke HE, Parikh C, Schofield L, Shaikh H, Bhagwanani A, Aziz Q, Taylor SA, Tack J, Van Oudenhove L. Gut-brain axis dysfunction underlies FODMAP-induced symptom generation in irritable bowel syndrome. *Aliment Pharmacol Ther* 2022; **55**: 670-682 [PMID: 35166384 DOI: 10.1111/apt.16812]

86 **Chey WD**, Hashash JG, Manning L, Chang L. AGA Clinical Practice Update on the Role of Diet in Irritable Bowel Syndrome: Expert Review. *Gastroenterology* 2022; **162**: 1737-1745.e5 [PMID: 35337654 DOI: 10.1053/j.gastro.2021.12.248]

87 **Schumann D**, Klose P, Lauche R, Dobos G, Langhorst J, Cramer H. Low fermentable, oligo-, di-, mono-saccharides and polyol diet in the treatment of irritable bowel syndrome: A systematic review and meta-analysis. *Nutrition* 2018; **45**: 24-31 [PMID: 29129233 DOI: 10.1016/j.nut.2017.07.004]

88 **Zannini E**, Arendt EK. Low FODMAPs and gluten-free foods for irritable bowel syndrome treatment: Lights and shadows. *Food Res Int* 2018; **110**: 33-41 [PMID: 30029704 DOI: 10.1016/j.foodres.2017.04.001]

89 **Ford AC**, Moayyedi P, Chey WD, Harris LA, Lacy BE, Saito YA, Quigley EMM; ACG Task Force on Management of Irritable Bowel Syndrome. American College of Gastroenterology Monograph on Management of Irritable Bowel Syndrome. *Am J Gastroenterol* 2018; **113**: 1-18 [PMID: 29950604 DOI: 10.1038/s41395-018-0084-x]

90 **Di Rosa C**, Altomare A, Terrigno V, Carbone F, Tack J, Cicala M, Guarino MPL. Constipation-Predominant Irritable Bowel Syndrome (IBS-C): Effects of Different Nutritional Patterns on Intestinal Dysbiosis and Symptoms. *Nutrients* 2023; **15** [PMID: 37049488 DOI: 10.3390/nu15071647]

91 **Garg P**. Inflammation in Irritable Bowel Syndrome (IBS): Role of Psyllium Fiber Supplementation in Decreasing Inflammation and Physiological Management of IBS. *Turk J Gastroenterol* 2021; **32**: 108-110 [PMID: 33893774 DOI: 10.5152/tjg.2020.20229]

92 **Saha L**. Irritable bowel syndrome: pathogenesis, diagnosis, treatment, and evidence-based medicine. *World J Gastroenterol* 2014; **20**: 6759-6773 [PMID: 24944467 DOI: 10.3748/wjg.v20.i22.6759]

93 **Bonetto S**, Fagoonee S, Battaglia E, Grassini M, Saracco GM, Pellicano R. Recent advances in the treatment of irritable bowel syndrome. *Pol Arch Intern Med* 2021; **131**: 709-715 [PMID: 34463082 DOI: 10.20452/pamw.16067]

94 **Sunderland R**. Irritable bowel syndrome in adults: symptoms, treatment and management. *Nurs Stand* 2017; **31**: 52-63 [PMID: 28224836]

95 **Keefer L**, Ballou SK, Drossman DA, Ringstrom G, Elsenbruch S, Ljótsson B. A Rome Working Team Report on Brain-Gut Behavior Therapies for Disorders of Gut-Brain Interaction. *Gastroenterology* 2022; **162**: 300-315 [PMID: 34529986 DOI: 10.1053/j.gastro.2021.09.015]

96 **Ford AC**, Lacy BE, Harris LA, Quigley EMM, Moayyedi P. Effect of Antidepressants and Psychological Therapies in Irritable Bowel Syndrome: An Updated Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2019; **114**: 21-39 [PMID: 30177784 DOI: 10.1038/s41395-018-0222-5]

97 **Lackner JM**, Jaccard J, Radziwon CD, Firth RS, Gudleski GD, Hamilton F, Katz LA, Keefer L, Krasner SS, Ma CX, Sitrin MD, Brenner DM. Durability and Decay of Treatment Benefit of Cognitive Behavioral Therapy for Irritable Bowel Syndrome: 12-Month Follow-Up. *Am J Gastroenterol* 2019; **114**: 330-338 [PMID: 30429592 DOI: 10.1038/s41395-018-0396-x]

98 **Lalouni M**, Ljótsson B, Bonnert M, Ssegonja R, Benninga M, Bjureberg J, Högström J, Sahlin H, Simrén M, Feldman I, Hedman-Lagerlöf E, Serlachius E, Olén O. Clinical and Cost Effectiveness of Online Cognitive Behavioral Therapy in Children With Functional Abdominal Pain Disorders. *Clin Gastroenterol Hepatol* 2019; **17**: 2236-2244.e11 [PMID: 30502501 DOI: 10.1016/j.cgh.2018.11.043]

99 **Prior A**, Colgan SM, Whorwell PJ. Changes in rectal sensitivity after hypnotherapy in patients with irritable bowel syndrome. *Gut* 1990; **31**: 896-898 [PMID: 2387513 DOI: 10.1136/gut.31.8.896]

100 **Whorwell PJ**, Houghton LA, Taylor EE, Maxton DG. Physiological effects of emotion: assessment *via* hypnosis. *Lancet* 1992; **340**: 69-72 [PMID: 1352012 DOI: 10.1016/0140-6736(92)90394-i]

101 **Peter J**, Fournier C, Keip B, Rittershaus N, Stephanou-Rieser N, Durdevic M, Dejaco C, Michalski M, Moser G. Intestinal Microbiome in Irritable Bowel Syndrome before and after Gut-Directed Hypnotherapy. *Int J Mol Sci* 2018; **19** [PMID: 30453528 DOI: 10.3390/ijms19113619]

102 **De Benedittis G**. Hypnobiome: A New, Potential Frontier of Hypnotherapy in the Treatment of Irritable Bowel Syndrome-A Narrative Review of the Literature. *Int J Clin Exp Hypn* 2022; **70**: 286-299 [PMID: 35792903 DOI: 10.1080/00207144.2022.2094269]

103 **DeBenedittis G**, Cigada M, Bianchi A, Signorini MG, Cerutti S. Autonomic changes during hypnosis: a heart rate variability power spectrum analysis as a marker of sympatho-vagal balance. *Int J Clin Exp Hypn* 1994; **42**: 140-152 [PMID: 8200716 DOI: 10.1080/00207149408409347]

104 **Lembo A**, Sultan S, Chang L, Heidelbaugh JJ, Smalley W, Verne GN. AGA Clinical Practice Guideline on the Pharmacological Management of Irritable Bowel Syndrome With Diarrhea. *Gastroenterology* 2022; **163**: 137-151 [PMID: 35738725 DOI: 10.1053/j.gastro.2022.04.017]

105 **Chang L**, Sultan S, Lembo A, Verne GN, Smalley W, Heidelbaugh JJ. AGA Clinical Practice Guideline on the Pharmacological Management of Irritable Bowel Syndrome With Constipation. *Gastroenterology* 2022; **163**: 118-136 [PMID: 35738724 DOI: 10.1053/j.gastro.2022.04.016]

106 **Lacy BE**, Pimentel M, Brenner DM, Chey WD, Keefer LA, Long MD, Moshiree B. ACG Clinical Guideline: Management of Irritable Bowel Syndrome. *Am J Gastroenterol* 2021; **116**: 17-44 [PMID: 33315591 DOI: 10.14309/ajg.0000000000001036]

107 **Qin D**, Tao QF, Huang SL, Chen M, Zheng H. Eluxadoline Versus Antispasmodics in the Treatment of Irritable Bowel Syndrome: An Adjusted Indirect Treatment Comparison Meta-analysis. *Front Pharmacol* 2022; **13**: 757969 [PMID: 35281934 DOI: 10.3389/fphar.2022.757969]

108 **Ruepert L**, Quartero AO, de Wit NJ, van der Heijden GJ, Rubin G, Muris JW. Bulking agents, antispasmodics and antidepressants for the treatment of irritable bowel syndrome. *Cochrane Database Syst Rev* 2011; **2011**: CD003460 [PMID: 21833945 DOI: 10.1002/14651858.CD003460.pub3]

109 **Zheng L**, Lai Y, Lu W, Li B, Fan H, Yan Z, Gong C, Wan X, Wu J, Huang D, Wang Y, Mei Y, Li Z, Jiang Z, Liu X, Ye J, Yang Y, Huang H, Xiao J. Pinaverium Reduces Symptoms of Irritable Bowel Syndrome in a Multicenter, Randomized, Controlled Trial. *Clin Gastroenterol Hepatol* 2015; **13**: 1285-1292.e1 [PMID: 25632806 DOI: 10.1016/j.cgh.2015.01.015]

110 **Zheng L**, Lu W, Xiao Q, Lai Y, Fan H, Sun Y, Huang D, Wang Y, Li Z, Jiang Z, Liu X, Zhang L, Zuo D, Shou Z, Tang Q, Huang H, Yang Y, Tang Z, Xiao J. Assessing the post-treatment therapeutic effect of pinaverium in irritable bowel syndrome: a randomized controlled trial. *Sci Rep* 2021; **11**: 13894 [PMID: 34230526 DOI: 10.1038/s41598-021-92990-7]

111 **Chmielewska-Wilkoń D**, Reggiardo G, Egan CG. Otilonium bromide in irritable bowel syndrome: a dose-ranging randomized double-blind placebo-controlled trial. *World J Gastroenterol* 2014; **20**: 12283-12291 [PMID: 25232263 DOI: 10.3748/wjg.v20.i34.12283]

112 **Rai RR**, Nijhawan S. Comparative evaluation of efficacy and safety of drotaverine versus mebeverine in irritable bowel syndrome: A randomized double-blind controlled study. *Saudi J Gastroenterol* 2021; **27**: 136-143 [PMID: 33642357 DOI: 10.4103/sjg.SJG_266_20]

113 **Weerts ZZRM**, Masclee AAM, Witteman BJM, Clemens CHM, Winkens B, Brouwers JRBJ, Frijlink HW, Muris JWM, De Wit NJ, Essers BAB, Tack J, Snijkers JTW, Bours AMH, de Ruiter-van der Ploeg AS, Jonkers DMAE, Keszthelyi D. Efficacy and Safety of Peppermint Oil in a Randomized, Double-Blind Trial of Patients With Irritable Bowel Syndrome. *Gastroenterology* 2020; **158**: 123-136 [PMID: 31470006 DOI: 10.1053/j.gastro.2019.08.026]

114 **Xie C**, Tang Y, Wang Y, Yu T, Jiang L, Lin L. Efficacy and Safety of Antidepressants for the Treatment of Irritable Bowel Syndrome: A Meta-Analysis. *PLoS One* 2015; **10**: e0127815 [PMID: 26252008 DOI: 10.1371/journal.pone.0127815]

115 **Chen GR**, Xie XF, Peng C. Treatment of Irritable Bowel Syndrome by Chinese Medicine: A Review. *Chin J Integr Med* 2023; **29**: 377-384 [PMID: 34546535 DOI: 10.1007/s11655-021-3521-4]

116 **Tang XD**, Lu B, Li ZH, Wei W, Meng LN, Li BS, Tang ZP, Gao R, Wang FY, Lu F, Bian LQ, Zhao YP, Wang P, Zhang YQ. Therapeutic Effect of Chang'an I Recipe (I) on Irritable Bowel Syndrome with Diarrhea: A Multicenter Randomized Double-Blind Placebo-Controlled Clinical Trial. *Chin J Integr Med* 2018; **24**: 645-652 [PMID: 27487786 DOI: 10.1007/s11655-016-2596-9]

117 **He S**, Lin Q, Huang J, Zheng L, Lai J, Chen C. Effect and Mechanism of Flavored Tongxie Yaofang Decoction for Diarrheal Irritable Bowel Syndrome under Intestinal Microecology. *Evid Based Complement Alternat Med* 2022; **2022**: 3904571 [PMID: 35966738 DOI: 10.1155/2022/3904571]

118 **Tan X**, Pei W, Xie C, Wang Z, Liu J, Cheng Y, Mao T, Shi L, Zhao X, Lu Q, Sun Z, Kou F, Jiang H, Li J. Tongxie Anchang Decoction Relieves Visceral Hypersensitivity in Diarrhea-Predominant Irritable Bowel Syndrome Rats by Regulating the NGF/TrkA Signaling Pathway. *Evid Based Complement Alternat Med* 2021; **2021**: 6679348 [PMID: 34239591 DOI: 10.1155/2021/6679348]

119 **Yang C**, Zhang SS, Li XL, Wang ZF, Zhao LQ. Inhibitory effect of TongXie-YaoFang formula on colonic contraction in rats. *World J Gastroenterol* 2015; **21**: 2912-2917 [PMID: 25780288 DOI: 10.3748/wjg.v21.i10.2912]

120 **Li J**, Cui H, Cai Y, Lin J, Song X, Zhou Z, Xiong W, Zhou H, Bian Y, Wang L. Tong-Xie-Yao-Fang Regulates 5-HT Level in Diarrhea Predominant Irritable Bowel Syndrome Through Gut Microbiota Modulation. *Front Pharmacol* 2018; **9**: 1110 [PMID: 30323765 DOI: 10.3389/fphar.2018.01110]

121 **Tan X**, Pei W, Xie C, Wang Z, Mao T, Zhao X, Kou F, Lu Q, Sun Z, Xue X, Li J. Network Pharmacology Identifies the Mechanisms of Action of Tongxie Anchang Decoction in the Treatment of Irritable Bowel Syndrome with Diarrhea Predominant. *Evid Based Complement Alternat Med* 2020; **2020**: 2723705 [PMID: 33281910 DOI: 10.1155/2020/2723705]

122 **Shih YS**, Tsai CH, Li TC, Lai HC, Wang KT, Liao WL, Hsieh CL. The effect of Xiang-Sha-Liu-Jun-Zi tang (XSLJZT) on irritable bowel syndrome: A randomized, double-blind, placebo-controlled trial. *J Ethnopharmacol* 2019; **238**: 111889 [PMID: 31009707 DOI: 10.1016/j.jep.2019.111889]

123 **Zhen Z**, Xia L, You H, Jingwei Z, Shasha Y, Xinyi W, Wenjing L, Xin Z, Chaomei F. An Integrated Gut Microbiota and Network Pharmacology Study on Fuzi-Lizhong Pill for Treating Diarrhea-Predominant Irritable Bowel Syndrome. *Front Pharmacol* 2021; **12**: 746923 [PMID: 34916934 DOI: 10.3389/fphar.2021.746923]

124 **Pei L**, Geng H, Guo J, Yang G, Wang L, Shen R, Xia S, Ding M, Feng H, Lu J, Li J, Liu L, Shu Y, Fang X, Wu X, Wang X, Weng S, Ju L, Chen X, Shen H, Sun J. Effect of Acupuncture in Patients With Irritable Bowel Syndrome: A Randomized Controlled Trial. *Mayo Clin Proc* 2020; **95**: 1671-1683 [PMID: 32499125 DOI: 10.1016/j.mayocp.2020.01.042]

125 **Hao LJ**, Shi ZM. [Therapeutic effect of herb-separated moxibustion at Jinsuo (GV 8)-eight-diagram points on diarrhea-type irritable bowel syndrome of liver stagnation and spleen deficiency]. *Zhongguo Zhen Jiu* 2020; **40**: 702-706 [PMID: 32648391 DOI: 10.13703/j.0255-2930.20190621-k0007]

126 **Ki Cha B**, Mun Jung S, Hwan Choi C, Song ID, Woong Lee H, Joon Kim H, Hyuk J, Kyung Chang S, Kim K, Chung WS, Seo JG. The effect of a multispecies probiotic mixture on the symptoms and fecal microbiota in diarrhea-dominant irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. *J Clin Gastroenterol* 2012; **46**: 220-227 [PMID: 22157240 DOI: 10.1097/MCG.0b013e31823712b1]

127 **Dai C**, Zheng CQ, Jiang M, Ma XY, Jiang LJ. Probiotics and irritable bowel syndrome. *World J Gastroenterol* 2013; **19**: 5973-5980 [PMID: 24106397 DOI: 10.3748/wjg.v19.i36.5973]

128 **Sun YY**, Li M, Li YY, Li LX, Zhai WZ, Wang P, Yang XX, Gu X, Song LJ, Li Z, Zuo XL, Li YQ. The effect of Clostridium butyricum on symptoms and fecal microbiota in diarrhea-dominant irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. *Sci Rep* 2018; **8**: 2964 [PMID: 29445178 DOI: 10.1038/s41598-018-21241-z]

129 **Wang Y**, Chen N, Niu F, Li Y, Guo K, Shang X, E F, Yang C, Yang K, Li X. Probiotics therapy for adults with diarrhea-predominant irritable bowel syndrome: a systematic review and meta-analysis of 10 RCTs. *Int J Colorectal Dis* 2022; **37**: 2263-2276 [PMID: 36251040 DOI: 10.1007/s00384-022-04261-0]

130 **Mack I**, Schwille-Kiuntke J, Mazurak N, Niesler B, Zimmermann K, Mönnikes H, Enck P. A Nonviable Probiotic in Irritable Bowel Syndrome: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Study. *Clin Gastroenterol Hepatol* 2022; **20**: 1039-1047.e9 [PMID: 34214682 DOI: 10.1016/j.cgh.2021.06.028]

131 **Jung K**, Kim A, Lee JH, Cho D, Seo J, Jung ES, Kang HJ, Roh J, Kim W. Effect of Oral Intake of Lactiplantibacillus plantarum APsulloc 331261 (GTB1(TM)) on Diarrhea-Predominant Irritable Bowel Syndrome: A Randomized, Double-Blind, Placebo-Controlled Study. *Nutrients* 2022; **14** [PMID: 35631156 DOI: 10.3390/nu14102015]

132 **Gu Q**, Yin Y, Yan X, Liu X, Liu F, McClements DJ. Encapsulation of multiple probiotics, synbiotics, or nutrabiotics for improved health effects: A review. *Adv Colloid Interface Sci* 2022; **309**: 102781 [PMID: 36209686 DOI: 10.1016/j.cis.2022.102781]

133 **You S**, Ma Y, Yan B, Pei W, Wu Q, Ding C, Huang C. The promotion mechanism of prebiotics for probiotics: A review. *Front Nutr* 2022; **9**: 1000517 [PMID: 36276830 DOI: 10.3389/fnut.2022.1000517]

134 **Kelly CR**, Khoruts A, Staley C, Sadowsky MJ, Abd M, Alani M, Bakow B, Curran P, McKenney J, Tisch A, Reinert SE, Machan JT, Brandt LJ. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. *Ann Intern Med* 2016; **165**: 609-616 [PMID: 27547925 DOI: 10.7326/M16-0271]

135 **El-Salhy M**, Hatlebakk JG, Gilja OH, Bråthen Kristoffersen A, Hausken T. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut* 2020; **69**: 859-867 [PMID: 31852769 DOI: 10.1136/gutjnl-2019-319630]

136 **Madsen AMA**, Halkjær SI, Christensen AH, Günther S, Browne PD, Kallemose T, Hansen LH, Petersen AM. The effect of faecal microbiota transplantation on abdominal pain, stool frequency, and stool form in patients with moderate-to-severe irritable bowel syndrome: results from a randomised, double-blind, placebo-controlled study. *Scand J Gastroenterol* 2021; **56**: 761-769 [PMID: 34000958 DOI: 10.1080/00365521.2021.1915375]

137 **Browne PD**, Cold F, Petersen AM, Halkjær SI, Christensen AH, Günther S, Hestbjerg Hansen L. Engraftment of strictly anaerobic oxygen-sensitive bacteria in irritable bowel syndrome patients following fecal microbiota transplantation does not improve symptoms. *Gut Microbes* 2021; **13**: 1-16 [PMID: 34074214 DOI: 10.1080/19490976.2021.1927635]

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REVIEW

Peroxisome proliferator-activated receptors as targets to treat metabolic diseases: Focus on the adipose tissue, liver, and pancreas

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Abstract

The world is experiencing reflections of the intersection of two pandemics: Obesity and coronavirus disease 2019. The prevalence of obesity has tripled since 1975 worldwide, representing substantial public health costs due to its comorbidities. The adipose tissue is the initial site of obesity impairments. During excessive energy intake, it undergoes hyperplasia and hypertrophy until overt inflammation and insulin resistance turn adipocytes into dysfunctional cells that send lipotoxic signals to other organs. The pancreas is one of the organs most affected by obesity. Once lipotoxicity becomes chronic, there is an increase in insulin secretion by pancreatic beta cells, a surrogate for type 2 diabetes mellitus (T2DM). These alterations threaten the survival of the pancreatic islets, which tend to become dysfunctional, reaching exhaustion in the long term. As for the liver, lipotoxicity favors lipogenesis and impairs beta-oxidation, resulting in hepatic steatosis. This silent disease affects around 30% of the worldwide population and can evolve into end-stage liver disease. Although therapy for hepatic steatosis remains to be defined, peroxisome proliferator-activated receptors (PPARs) activation copes with T2DM management. Peroxisome PPARs are transcription factors found at the intersection of several metabolic pathways, leading to insulin resistance relief, improved thermogenesis, and expressive hepatic steatosis mitigation by increasing mitochondrial beta-oxidation. This review aimed to update the potential of PPAR agonists as targets to treat meta-

bolic diseases, focusing on adipose tissue plasticity and hepatic and pancreatic remodeling.

Key Words: Obesity; Insulin resistance; Peroxisome proliferator-activated receptors; Pancreas; Hepatic steatosis; Adipose tissue

Core Tip: The world faces a pandemic of obesity and metabolic diseases. Peroxisome proliferator-activated receptors' (PPARs') target genes regulate several metabolic pathways, alleviating obesity and its metabolic impairments. PPARα exerts relevant anti-inflammatory, anti-steatotic, and pro-thermogenic effects, collaborating with weight loss and insulin resistance alleviation. PPARγ is useful for glycemic management, albeit with caution due to side effects after its total activation. PPAR β/δ is not clinically used owing to a pro-tumorigenic profile. However, Pan-PPAR or dual-PPAR agonists can retain PPARβ /δ or partial PPARγ activation benefits and configure promising approaches to treat metabolic diseases alone or in combination with other drug classes.

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INTRODUCTION

The unprecedented obesity rates have become an immense public health problem due to increased costs to treat comorbidities like diabetes, cancer, osteoarticular diseases, and cardiovascular events[1]. Most obesity cases stem from a chronic positive energy balance, where surplus energy intake surpasses the energy expenditure (EE), sending excessive energy to the adipose tissue[2,3].

The white adipose tissue (WAT) is a buffer against excess energy from the diet and first undergoes hyperplasia and hypertrophy and preserves its different types of cell composition[4,5]. As this process continues, preadipocytes become rare, and hypertrophy predominates until the adipocyte reaches its maximal capacity to enlarge, parallel to a rarefaction of vascularization and the high amount of proinflammatory immune cells within the stromal vascular fraction of the WAT[6,7]. At this stage, inflammation and insulin resistance activates lipolysis to allow the storage of the continuing excessive energy from the diet at the expense of diverting the non-esterified fatty acids (NEFAs) from lipolysis to organs not specialized to store lipids like the liver and the pancreas (lipotoxicity), triggering steatosis[8,9].

Hepatic and pancreatic steatosis compromises the physiology of these organs, collaborating with the progression to end-stage liver diseases and diabetes[10-12]. Thus, the scientific community seeks for strategies to mitigate the deleterious effects of obesity and its comorbidities, which affects the worldwide population regardless of economic income or age. The peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors linked to the cellular metabolism of lipids, carbohydrates, proteins, and cell proliferation, existing in three isoforms: PPARα, PPARβ/δ, and PPARγ and emerged in recent decades as a strategy to treat obesity and its complications[13].

PPARs are binding-dependent transcription factors that regulate gene expression by specifically binding to PPAR-responsive elements (PPREs). Each receptor heterodimerizes with the retinoid X receptor (RXR, where X can be α, β/δ, or γ) and binds to its respective PPRE, forming a structure that will recognize specific DNA sequences (AGGTCA) for the transcription of their target genes. This PPAR mechanism of action is known as trans-activation. Moreover, PPARs can regulate gene expression independently of binding to PPREs, through the mechanism of trans-repression. There is a crosstalk between PPARs and other transcription factors that regulate their gene expression, and most of the anti-inflammatory effects of PPARs stem from this mechanism[14,15]. Figure 1 summarizes PPARs mechanisms of action.

PPARs regulate countless metabolic pathways after activation by endogenous ligands, such as fatty acids and their derivatives or synthetic agonists, which trigger a conformational change to interact with transcriptional coactivators[16,17]. The PPARα isoform expression is high in the liver, muscle, and heart, and its activation, according to previous studies, suggests that this receptor participates in lipid metabolism. PPARγ is mainly expressed in white and brown adipose tissue (BAT), being responsible, among other functions, for adipogenesis. Finally, PPARβ/δ has a wide body distribution with described roles in fatty acids oxidation in muscle and general energy regulation[13].

PPARs exert their physiological activities by activating the transcription of their target genes and, in this way, regulate lipid metabolism, glucose homeostasis, cell differentiation, obesity, and cancer. Furthermore, PPARs may directly participate in immune responses and inflammation mechanisms[18].

Considering that PPARs are found at the intersection of several metabolic pathways, influencing glucose homeostasis, adiposity, and mitochondrial function in the liver, this review aimed to update the potential of PPAR agonists as targets to treat metabolic diseases, focusing on adipose tissue plasticity, hepatic, and pancreatic remodeling.

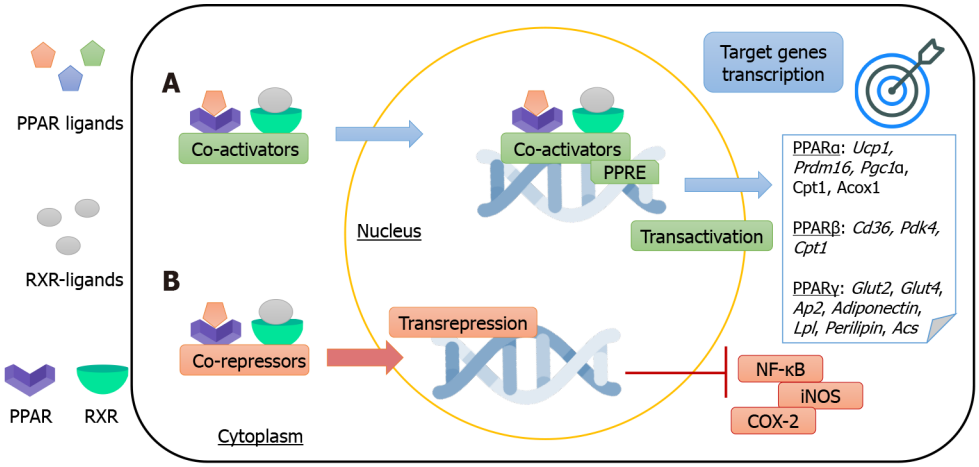

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Figure 1 Peroxisome proliferator-activated receptors mechanisms of action. Proliferator-activated receptors (PPARs) are ligand-activated transcription factors that regulate gene expression through two different mechanisms. A: PPAR-retinoid X receptor heterodimers bind to DNA-specific sequences called peroxisome proliferator-response elements to trigger target genes transcription (transactivation). PPARs' target genes include thermogenic [*Ucp1*, *PPAR-gamma coactivator 1 alpha* (*Pgc1α*), and *PR domain containing 16* (*Prdm16*)], mitochondrial and peroxisomal [*carnitine palmitoyltransferase I (Cpt1)*, peroxisomal acyl-coenzyme A oxidase (*Acox*), and pyruvate dehydrogenase kinase 4 (*Pdk4*)], anti-inflammatory (*Adiponectin*), insulin-sensitizing [glucose transporter 2 (*Glut2*), *Glut4*], and adipocyte metabolism [*Cd36*, adipocyte protein 2 (*Ap2*), lipoprotein lipase (*Lpl*), *Perilipin*, and acetyl CoA synthetase (*Acs*)] genes; B: PPARs regulate gene expression through transrepression, a DNA-independent mechanism. For example, PPARs inhibits the activity of nuclear factor kappa b and enzymes, yielding anti-inflammatory effects. Abbreviations: Uncoupling protein 1, *Pgc1α*, *Prdm16*, *Cpt1*, peroxisomal *Acox*, *Pdk4*, *Glut*, *Ap2*, *Lpl*, and *Acs*, inducible nitric oxide synthase, nuclear factor kappa B, and ciclooxigenase-2. PPARs: Peroxisome proliferator-activated receptors; RXR: Retinoid X receptor; PPRE: PPAR-responsive elements; NF-κB: Nuclear factor kappa B; iNOS: Inducible nitric oxide synthase; COX-2: Ciclooxigenase-2.

ADIPOSE TISSUE PHYSIOLOGY AND PLASTICITY

In mammals, the adipose organ comprises two types of tissues: WAT and BAT. Depending on the adipose tissue origin (for example, gonadal or subcutaneous), differences occur in its lipolytic or lipogenic capacity[19].

The white adipocytes are the only cells specialized in storing lipids without compromising their functional integrity. They have the necessary enzymatic machinery to synthesize triacylglycerol (TAG) when the energy supply is abundant and to mobilize them through lipolysis when there is an energy deficit[20].

The autonomic nervous system acts directly on the adipose tissue. The sympathetic nervous system promotes catabolic actions (lipolysis) *via* adrenergic stimulation, which activates the hormone-sensitive lipase (HSL) enzyme[21]. Conversely, the parasympathetic nervous system yields anabolism by stimulating insulin secretion, increasing glucose uptake, and activating lipogenesis[22].

Regarding the mature white adipocyte characteristics, it stores TAG in a single large lipid droplet that occupies the central portion of the cell, promoting displacement of the nucleus to the periphery and its consequent flattening. The single lipid droplet occupies 85%-95% of the cell volume, characterizing the white adipocyte as unilocular[23,24].

WAT, described as the chief energy reservoir in mammals, has a mesenchymal origin and is composed of adipocytes and the stromal vascular fraction (the main constituent of pre-adipocytes, immune cells, and fibroblasts); together, adipocytes and the stromal vascular fraction produce the extracellular matrix to maintain the structural and functional integrity of the tissue[25,26].

For a long time, the WAT was a secondary structure whose characteristic was the reservoir of large amounts of fat in the form of TAG. There was a lack of attention to its participation in body weight and food intake control. As a result of the discovery of the ability of WAT to secrete substances with biological effects like leptin in 1994, known collectively as adipokines, WAT emerged as a major endocrine organ[27,28].

Conversely, BAT has a smaller adipocyte than WAT, exhibiting several cytoplasmic lipid droplets of different sizes (multilocular), relatively abundant cytoplasm, spherical and slightly eccentric nucleus, and numerous mitochondria that produce energy by oxidizing acids[29]. The brownish BAT coloration stems from its high mitochondrial content, whose uncoupling protein 1 (UCP1) is one of the primary markers for brown adipocyte identification[30,31].

The brown adipocytes specialize in performing nonshivering thermogenesis (NST), by which the UCP1 acts as an alternative channel in the inner mitochondrial membrane to the proton gradient in the intermembrane space return to the mitochondrial matrix without resulting in ATP synthesis. Instead, the chemical energy is released as heat, enhancing the body temperature and EE, a promising target to treat obesity through negative energy balance[32,33].

Brown adipocytes originate from myocyte progenitor cells (myogenic lineage) and express the myogenic factor 5. *Prdm16* controls a bidirectional cell fate switching between skeletal myoblasts and brown adipose cells. Loss of the PR domain containing 16 (*Prdm16*) in brown fat precursors causes loss of brown fat characteristics and promotes muscle differentiation[34]. *Prdm16* induces a complete program of brown fat differentiation, including the expression of PPAR-gamma coactivator 1α (*Pgc-1α*) and *Ucp-1*. *Pgc-1α* is an essential transcriptional coactivator for NST and mitochondrial biogenesis[35].

Although BAT total mass in mammals is small, previous research has already shown that its adequate stimulation (cold exposure, specific drugs, some food compounds, or physical activity) could quadruple the EE of an animal, parallel to an increase in tissue perfusion[36-38]. Thus, understanding the damage obesity causes to BAT morphology and physiology can help to develop strategies to treat obesity.

Recently, experimental studies reported that diet-induced obesity (DIO) results in glucose intolerance, functional BAT hypoxia, and structural whitening. Brown adipocytes in obese animals reduce their thermogenic capacity and assume a white phenotype, becoming unilocular[39,40]. The molecular mechanisms that lead to reduced BAT activity in obesity and its physiological implications are under investigation, and altered PPAR expression may have a role in the whitening phenomenon as *Pparα* expression declines in whitened BAT[41]. Compared to WAT, BAT is more extensively vascularized, and vascular endothelial growth factor-A (VEGFA)-dependent angiogenesis is pivotal for the thermogenic response[42]. The hypoxic microenvironment in BAT, due to obesity, has been shown to lead to an accelerated loss of mitochondria, and as oxidative capacity is lost, lipid droplets accumulate, generating the white phenotype[39].

Since obesity is a public health problem in developed and developing countries, the world urges us to find new approaches to treat or prevent this metabolic disease and its comorbidities. WAT plasticity towards an intermediary adipocyte between white and brown, the beige adipocyte, may emerge as a suitable strategy to fight the obesity pandemic [43].

Beige adipocytes are found in the subcutaneous WAT (sWAT) and exhibit a brown-like adipocyte phenotype (multilocular). In the basal state, beige adipocytes act like white adipocytes, but under the right stimulus, they can acquire intermediate mitochondrial content and perform thermogenesis, a process called browning[44]. *Prdm16* is essential to the browning phenomenon once its down-regulation turns a beige adipocyte into a white one, showing that browning is reversible and morphophysiological changes rely on adequate PRDM16 expression and active mitochondrial biogenesis machinery to increase mitochondrial content and thermogenic capacity[45,46].

Beige adipocytes originate from mature white adipocytes [low expression of the cluster of differentiation 137 (*Cd137*)], which under specific stimulation, acquire a brown-like phenotype, or even from a beige pre-adipocyte (high *Cd137*), which differentiates into a multilocular cell capable of performing thermogenesis. The latter originates from a lineage different from the WAT[47,48]. Although the beige adipocytes have a lower thermogenic activity than the brown adipocytes, their presence is linked to a metabolically healthy phenotype in humans[49], reducing the chances of developing type 2 diabetes mellitus (T2DM), hepatic steatosis, and other metabolic constraints.

The metabolic benefits of beige/brown adipocytes presence also stem from their endocrine role in secreting the batokines. Like the adipokines from the WAT, batokines act in autocrine, paracrine, and endocrine fashions to provide increased neurovascular supply to BAT, favoring thermogenesis and inhibiting whitening besides exerting anti-inflammatory effects, favoring browning, and influencing glucose homeostasis[50]. Figure 2 depicts white and BAT plasticity under obesogenic and thermogenic stimuli.

The recent literature suggests that less than 100 g of adipose tissue in an adult human with thermogenic activity can prevent 4 kg of fat accumulation per year[51], stimulating the search for drugs that trigger browning while inhibiting whitening, relevant for metabolic disease control.

PPARS ROLE IN THE ADIPOSE TISSUE PLASTICITY

PPARα was the first receptor discovered, mapped to chromosome 22q12-13.1 in humans, found in metabolically active tissues such as the liver, kidney, heart, skeletal muscle, and brown fat[52,53]. PPARα has polyunsaturated fatty acids and leukotrienes as natural ligands, which are inflammatory mediators; as pharmacological ligands, the family of hypolipidemic drugs, fibrates, is considered an accessible PPARα agonist[54].

The participation of PPARα in the thermogenic pathway involves directing NEFAs, generated after adrenergic stimulation, to the β-oxidation instead of cellular efflux. β-adrenergic stimulus mobilizes most of NEFA in normal conditions, and this mobilization is related to the inflammatory response and the reduction of cell function in the long term. The chronic stimulation of the beta-3 adrenergic receptor upregulates PPARα expression, increasing adipocytes' oxidative capacity[55].

Natural or pharmacological ligands mainly control the expression of genes involved in lipid metabolism. If the concentration of fatty acids increases, PPARα is activated and absorbs the oxidized forms of these acids. During the influx of fatty acids, the transcription of PPARα-regulated genes increases, and the systems are activated[55,56]. Thus, PPARα functions as a lipid sensor and controls energy combustion.

A previous study showed that treatment with WY14643 (PPARα agonist) exerted lipolytic effects with reduced fat mass and increased whole-body fat oxidation. These results demonstrate a novel role for PPARα activation in beta-adrenergic regulation of adipose tissue lipolysis[57]. In addition, treatment with WY-14643 has already shown effects in reducing hepatic steatosis, serum insulin, and inflammation in adipose tissue, and these changes are generally observed in obesity onset[58,59].

In BAT, PPARα stimulates lipid oxidation and thermogenesis in synergy with PGC1α[60]. In this scenario, a study demonstrated that the activation of PPARα by a pharmacological agent (fenofibrate) activated NST (and mitochondrial biogenesis in the BAT of obese mice fed a high-fat diet[61]. Subsequently, the chronic intake of a high-fat diet caused BAT whitening, and the treatment with WY14643 mitigated this morphophysiological impairment through anti-inflammatory signals and enhanced VEGFA, resulting in increased thermogenesis. In the same experiment, the high-fructose diet did not trigger whitening, but the WY14643 also attenuated the histological changes caused by excessive dietary fructose[62].

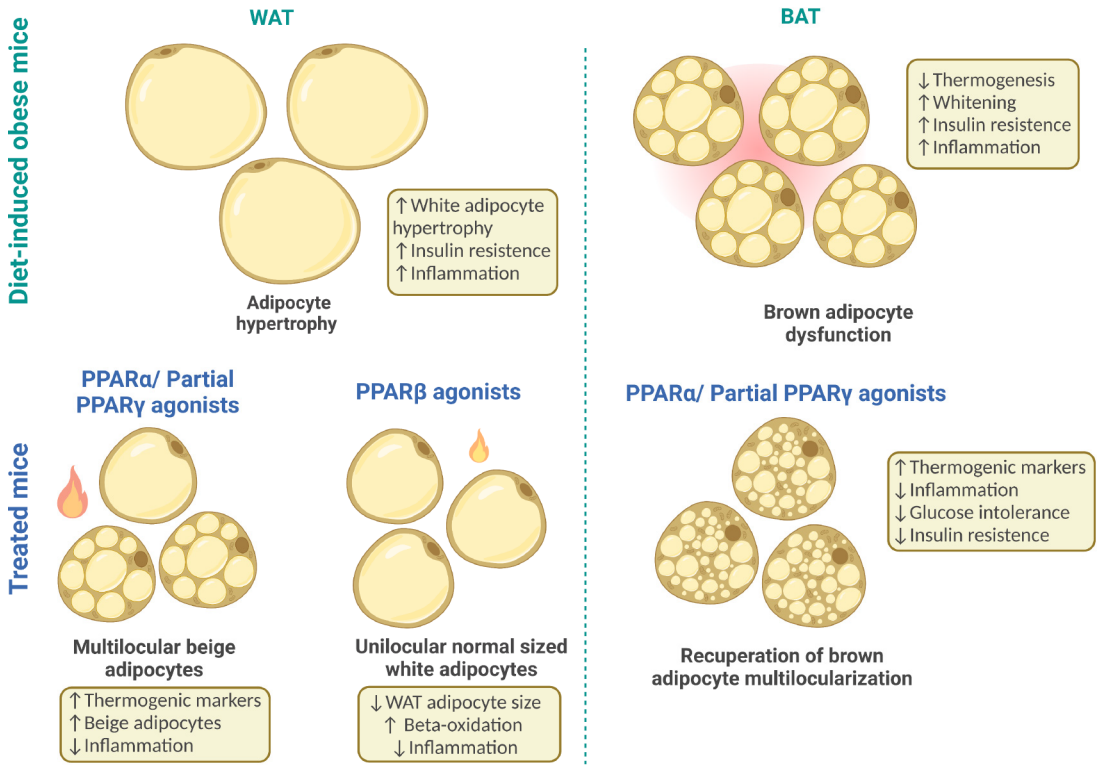

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Figure 2 Adipose tissue plasticity and peroxisome proliferator-activated receptor effects. Under an obesogenic diet, the white adipocytes undergo hypertrophy and profound change in adipokine profile release, leading to growing insulin resistance and low-grade inflammation. Conversely, under adequate stimuli [peroxisome proliferator-activated receptors α (PPARα) and dual PPARα/γ], the subcutaneous white adipose tissue can also undergo browning and form beige adipocytes, an intermediate between white and brown adipocytes with lower thermogenic capacity than brown adipocytes but whose presence points to metabolic homeostasis. Finally, the brown adipose tissue, which has the most potent thermogenic capacity, can express a whitened phenotype when exposed to a lipotoxic milieu. Whitening entails decreased vascularization and pro-inflammatory signals characterizing brown adipocyte dysfunction. In contrast, PPARα and dual PPARα/γ treatments counter whitening, increasing thermogenesis through anti-inflammatory and proangiogenic effects. Made with Biorender (www.biorender.com). PPARs: Peroxisome proliferator-activated receptors; WAT: White adipose tissue; BAT: Brown adipose tissue. Created by BioRender.

PPARα is the isoform that induces the browning of sWAT more abundantly. Fenofibrate has yielded expressive browning in DIO mice, with an irisin-*Pgc1α*-*Prdm16* interaction driven by PPARα stimulating thermogenesis and mitigating insulin resistance and inflammation, countering obesity[63]. Confirming these results, the PPARα agonist WY14643 yielded *Cd137*/*Prdm16*/*Ucp1*+ beige adipocytes, whereas the PPARβ/δ agonist GW0742 reduced sWAT adipocyte size and increased beta-oxidation without browning induction[59]. PPARβ/δ activation by GW501516 countered adipocyte hypertrophy through suppression of angiotensin-converting enzyme (ACE)/angiotensin II receptor type 1 (AT1r) axis and downstream potent anti-inflammatory effects[64].

PPARγ received much attention since the mid-1990s as the molecular target of thiazolidinediones (TZDs) or glitazones, a class of insulin-sensitizing and antidiabetic drugs[65]. PPARγ, a transcription factor from the nuclear receptor family, plays a significant role in lipid and glucose metabolism regulation[66]. WAT and BAT, large intestine, and spleen express PPARγ. However, its expression is much higher in adipocytes[67,68].

Ligand-activated PPARγ induces adipocyte differentiation, stimulates mitochondrial biogenesis, and inhibits the production of pro-inflammatory mediators[68]. In addition, PPARγ activated in adipocytes ensures a balanced and adequate secretion of adipokines (adiponectin and leptin), mediators of insulin action in peripheral tissues[69].

In WAT, PPARγ is pivotal to lipid accumulation. In contrast, PPARγ activation in BAT induces the expression of genes related to the thermogenic program, including *Pgc1α* and *Ucp1*. PPARγ is crucial for brown adipocyte differentiation, but additional transcription factors, including PRDM16, are required to activate the thermogenic program[70].

The PPARγ1 isoform is expressed in almost all cells, while PPARγ2 is mainly limited to adipose tissue. However, PPARγ2 is a more potent transcriptional activator[71]. Both PPARγ1 and PPARγ2 are essential for adipogenesis and insulin sensitivity control. However, PPARγ2 is the isoform upregulated in response to nutrient intake and obesity[72,73].

TZDs, synthetic PPARγ ligands, are antidiabetic drugs with potent insulin-sensitizing effects that confer long-term glycemic control[74]. However, its clinical use has been contested due to side effects such as weight gain, edema, and bone fractures[75]. The increase in body weight after TZDs administration is due to PPARγ-dependent WAT expansion [76] and fluid retention caused by PPARγ activation in the renal collecting ducts[77].

TZDs improve peripheral insulin sensitivity and have a spectrum of anti-inflammatory properties, including a reduction in plasma inflammatory markers and adipose tissue macrophages[72,78]. In WAT, TZDs promote adipocyte differentiation, insulin action, and the formation of beige adipocytes[66]. In BAT, TZDs activate thermogenic activity[79].

The dual PPARα/γ agonist tesaglitazar reversed iBAT whitening through gut dysbiosis and ultrastructure improvements[80]. Intestinal PPARα activation suppresses postprandial hyperlipidemia by increasing the fatty acid oxidation of intestinal epithelial cells[81]. In addition, intestinal PPARα activation reduces cholesterol esterification, suppresses chylomicron production, and increases enterocytes' HDL synthesis[82]. These observations pave a new way to treat metabolic diseases through the modulation of gut microbiota by PPARs and the consequent gut-adipose tissue stimulation of thermogenesis due to anti-inflammatory, angiogenic, and high beta-adrenergic signaling[80]. Figure 2 shows the main PPAR effects on the adipose tissue.

PANCREAS MORPHOPHYSIOLOGY AND OBESITY OUTCOMES

The human pancreas is a mixed gland made up of five anatomical divisions: Head (fitted with the duodenum), uncinate process, neck, body, and tail (nearby the spleen); measures 15-25 cm in length; and weighs 100 g to 150 g in a healthy adult[83]. The inferior pancreaticoduodenal artery, the superior pancreaticoduodenal artery, and the splenic artery are responsible for its perfusion, and the pancreatic plexus, celiac ganglia, and vagus nerve innervate this gland[84,85].

The pancreas has endocrine and exocrine functions, encompassing four structural components: The exocrine portion, constituted by acinar and ductal cells; the endocrine region formed by the islet cells; the blood vessels; and the extracellular space[84]. The exocrine portion corresponds to 85% of the pancreas volume, comprehending a ductal system like a bunch of grapes with a blinded end. Each acinus corresponds to a grape and secretes pancreatic juice enzymes that flow into bicarbonate-secreting ductal epithelial cells[83,84]. After passing through accessory ducts, these enzymes reach the main pancreatic duct that connects with the bile duct in the ampulla of Vater in the duodenum. The exocrine pancreatic secretion (amylase, lipase, and zymogens) helps to digest proteins, fats, and carbohydrates, being secreted by the autonomic nervous system stimulation in the presence of food in the duodenum and the consequent release of secretin, cholecystokinin, and other hormones by the enteroendocrine cells[83].

The endocrine portion of the pancreas comprises mini-organs called islets of Langerhans, corresponding to 1%-2% of the total pancreatic volume. Pancreatic islets have a spherical shape and secrete hormones related to glucose homeostasis. There are five islet cell types: Alpha cells (α cells, produce glucagon), beta cells (β cells, insulin), delta cells (δ cells, somatostatin), PP cells (also known as F cells, pancreatic polypeptide-containing cells), and epsilon cells (ε cells, ghrelin). Neuroendocrine, endocrine, paracrine, and endocrine mechanisms influence islet secretion. Therefore, overactivation or inactivation of their regulatory pathways can drastically impact the metabolism, causing metabolic diseases[86-88].

The most prevalent cell types in pancreatic islets are alpha and beta cells. In mice, alpha cells are restricted to the islet periphery, while the beta cells found in the core of the islet are vastly innervated and comprehend 60%-80% of the total islet mass. In humans, alpha and beta cells are interspersed all around the islets, with beta cells comprising 50%-75% of the islet mass with a sparse innervation. The remaining islet cell types do not differ significantly between men and mice. Even though there are cytoarchitectural differences regarding the islets of these species, the dynamics of islet remodeling in obesity and T2DM are similar and propitiates relevant translational studies[87].

Obesity entails an impaired glucose-stimulated insulin secretion (GSIS) triggered by the adipoinsular axis deregulation due to increased demand for insulin release. The resistance to insulin action relates to hyperleptinemia, prompted by the inflamed hypertrophied WAT[89,90]. Under an insulin-resistant state, the glucose fails to enter the pancreatic beta cell, resulting in a high demand for insulin to keep euglycemia. Islet hypertrophy and hypersecretion are commonplace features in DIO mice as the expansion of alpha and beta cell masses cope with normal glycemic levels maintenance at the beginning of glucose homeostasis impairments[91-93].

Insulin resistance (IR) precedes the T2DM diagnosis by around ten years[94]. Initially, the compensatory hyperinsulinemia prompts normal fasting glucose levels (IR). As adiposity evolves, hyperinsulinemia cannot cope with adequate glycemic control. At this point, raised plasma insulin and glucose concentrations coexist, characterizing glucose intolerance[91,95]. Glucose-intolerant mice exhibit hyperglycemia, alpha cells infiltrated to the islet core, besides a disrupted GSIS, with endoplasmic reticulum (ER) stress and loss of beta cell polarity, compromising its secretion and threatening islet survival[96,97].

Glucotoxicity (hyperglycemia) elicits ER stress, which leads to proinsulin misfolding and accumulation, a hallmark of beta cell dysfunction and T2DM onset[98]. Beta cell dysfunction originates from continuing islet hypertrophy and hypersecretion (lipotoxicity), impaired GSIS, beta cell ER stress (glucolipotoxicity), and downregulation of proliferative markers like the pancreatic duodenal homeobox-1 (PDX1)[96,99]. Compromised beta cell proliferation, increased apoptosis rate, and dedifferentiation result in beta cell failure[100]. T2DM is linked to a decreased beta cell mass (-22%-63%) and begins when beta cell mass cannot compensate for the high demand for insulin to sustain glucose homeostasis [101].

As a multi-hormonal disease, T2DM entails decreased beta cell mass coupled with the loss of incretin effect. Incretins are gut-derived hormones that enhance insulin secretion and sensitivity after meal ingestion, participating in the glycemic control at the postprandial state. Chronic high-fat diets lead to loss of the incretin glucagon-like peptide-1 (GLP-1) capacity to enhance beta cell responsiveness to glucose and proliferation, resulting in beta cell exhaustion[102,103]. Loss of incretin effect occurs even with normal GLP-1 and gastric inhibitory polypeptide levels in obese subjects, suggesting a possible resistance to their actions like described for insulin and leptin[104].

To avoid the progression of IR towards T2DM onset, therapies that could promote pancreatic alpha cell transdifferentiation into beta cells to maintain glycemic control are promising approaches to tackle beta cell exhaustion and reduced beta cell mass[105]. In this context, PPARs may be therapeutic targets for glycemic homeostasis.

PPARS EFFECTS ON PANCREATIC REMODELING AND ISLET PRESERVATION

PPARs regulate the expression of several target genes and allow the proper functioning of pathways linked to energy metabolism. An imbalance of these pathways can lead to the development of T2DM and other disorders[106,107]. Thus, PPARs are relevant therapeutic targets for the clinical management of diabetes.

Numerous drugs have been used and developed for the treatment of hyperlipidemia and T2DM such as PPARα agonists (fibrates, *e.g.*, fenofibrate, bezafibrate and clofibrate) and PPARγ agonists (TZDs, *e.g.*, troglitazone, rosiglitazone, pioglitazone and ciglitazone). PPARβ/δ does not have a role well-established in glucose metabolism, but it is known to improve insulin sensitivity by facilitating fatty acid oxidation in some tissues and reducing glucose oxidation[108,109].

PPARα plays an essential role in glucose homeostasis and regulates enzymes and proteins involved in glucose synthesis in the fasting state[110]. In addition, it stimulates pancreatic beta cells and increases fatty acid oxidation and GSIS[111]. Fenofibrate and fish oil countered islet hypertrophy and increased adiponectin levels in diabetic KK mice [112], mitigating lipotoxicity-induced beta cell dysfunction by inhibiting the nuclear factor kappa B (NF-κB) and reducing macrophage migration[113]. In agreement, fenofibrate exerted anti-inflammatory and antiapoptotic effects besides enhancing islet innervation in young non-obese diabetic mice, providing adequate glucose handling, and preventing diabetes-associated diseases[114].

Conversely, Fenofibrate long-term use (12 wk, 100 mg/kg) disrupted beta cell function due to increased NF-κB and inducible nitric oxide synthase islet expression in monosodium glutamate-induced obese rats[115]. An *in vitro* study showed that PPARα activation by WY14643 or bezafibrate (pan-PPAR agonist) enhances GSIS. However, long-term treatments contribute to beta cell dysfunction owing to overstimulation[116]. In a clinical study, bezafibrate showed that parameters related to insulin resistance were attenuated during the follow-up period, while the placebo group showed an increase in this parameter over two years[117].

PPARγ, in turn, controls glucose homeostasis as it increases glucose transporter (GLUT4) expression and its translocation in adipocytes, causing adipose remodeling by reducing visceral fat deposits while selectively increasing sWAT, resulting in a possible insulin sensitization scenario[118,119]. Furthermore, studies have shown that PPARγ activation increases adipocytes' fatty acid uptake, reducing lipotoxic damage to insulin-sensitive tissues[120], increasing glucose uptake by skeletal muscle, and consequent peripheral reduction[120,121].

PPARγ agonists are the most developed antidiabetic and insulin-sensitizing agents to date. Studies have shown that TZDs (or glitazones) bind specifically to the ligand binding domain (LBD) of recombinant PPARγ but not to PPARα or PPARβ/δ. These agents also selectively stimulate the activity of the PPARγ gene promoter and modulate the expression of several of its target genes[122]. TZDs class increases glucose catabolism and reduces its hepatic production[123,124], sensitizes cells to insulin, improves insulin sensitivity and action[125,126], and promotes pre-adipocyte differentiation along with lipogenesis, which favors a reduction in NEFAs concentrations, attributed to increased glucose utilization and reduced gluconeogenesis.

Human and animal studies indicate that the TZDs (total PPARγ agonists) rosiglitazone and pioglitazone improve hyperglycemia by reversing insulin resistance and improving insulin sensitivity. In addition, both agents also show significant effects on plasma lipoprotein lipids in humans, although pioglitazone results in a relatively better lipid profile than rosiglitazone[127,128]. Of note, the clinical use of rosiglitazone was discouraged owing to the unwanted effects of fluid retention, arterial vasodilation, endothelial alterations[129,130], increased risk of bone fracture and congestive heart failure[131], and weight gain in both animal models and humans due to the master regulatory role of PPARγ in adipogenesis[66].

Pioglitazone (15 mg/kg per mouse) yielded healthy pancreatic islets in db/db mice, enhancing insulin and NK6 homeobox 1 expression and restoring islet function in this mice model[132]. The islet preservation due to PPARγ activation by pioglitazone encompasses anti-inflammatory effects and alleviation of ER stress[133]. Pioglitazone and vildagliptin (DPP-4 inhibitor) combination maximized pioglitazone's effects on suppressed inflammation and oxidative stress in male rats[134]. In agreement with this, pioglitazone plus sitagliptin enhanced alpha and beta cell functions at the postprandial state in T2DM subjects[135].

Telmisartan, a partial PPARγ agonist and angiotensin receptor blocker, reduces serum insulin levels and mitigates insulin resistance without affecting serum adiponectin levels[136]. The experimental background shows that telmisartan reduced islet hypertrophy, with adequate glycemic control and normalized alpha and beta cell mass in DIO mice. These effects were maximized by telmisartan combination with sitagliptin, reverting oral glucose intolerance and normalizing body mass in this DIO model[93]. Telmisartan combination with linagliptin promoted islet preservation by suppressing oxidative stress[137].

The beneficial effects of telmisartan on islet cytoarchitecture featured ultrastructural remodeling, with polarized cells and mature insulin granules reestablished after the treatment[97]. Telmisartan elicited enhanced islet vascularization, enhanced PDX1 and GLP-1 islet expression plasma concentrations, ameliorating GSIS and preserving pancreatic islets through reduced apoptosis rate and macrophage infiltration[96]. Due to improved islet proliferation capacity, telmisartan might be a candidate for beneficial islet cell transdifferentiation to slow insulin resistance to T2DM onset progression. Figure 3 summarizes the main effects of PPARα and partial PPAR γ agonists on the endocrine pancreas.

Drugs with specificity for at least two PPAR isoforms (*e.g.*, dual PPARα/γ or pan-PPAR agonists) could be more effective and have relatively fewer undesirable side effects compared with currently used agonists with specificity for a single PPAR isoform[138]. In this context, a series of dual PPARα/γ agonists, named glitazars, was designed to combine total PPARα and partial PPARγ activation, retaining the insulin-sensitizing property of PPARγ target genes with the beneficial effects of PPARα activation on energy metabolism[139,140]. However, muraglitazar showed many of the same side effects (weight gain and edema) in patients[141,142], having glitazars development halted due to safety concerns.

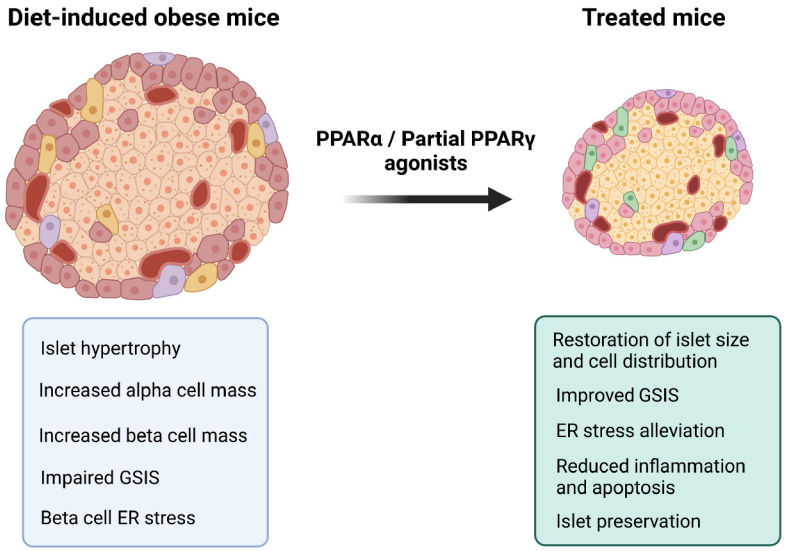

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Figure 3 Beneficial effects of Peroxisome proliferator-activated receptor agonist on islet remodelling. Diet-induced obese mice exhibit enlarged pancreatic islets, with are prone to exhaustion in the long run due to glucolipotoxicity. Peroxisome proliferator-activated receptor α (PPARα) and partial PPARγ activation are promising targets to counter glucolipotoxicity, yielding islet preservation. Made with Biorender (www.biorender.com). PPARs: Peroxisome proliferator-activated receptors; GSIS: Glucose-stimulated insulin secretion; ER: Endoplasmic reticulum. Created by BioRender.

Research is ongoing to develop new PPAR agonists without side effects. Since PPARs orchestrate many physiological processes, agonists may play beneficial or non-beneficial effects. So, PPAR actions on pancreatic islets need elucidation to support the development of new drugs with more assertive outcomes.

LIVER MORPHOPHYSIOLOGY AND OBESITY IMPLICATIONS

The liver is a complex organ that concentrates on several physiological processes as macronutrient metabolism, immune system support, fatty acid, and cholesterol homeostasis, besides the degradation of xenobiotic compounds and many medications[143]. It is the largest solid glandular organ in the body. With versatile endocrine and exogenous functions, the liver is considered paramount for metabolic activities[144]. It has a remarkably uniform anatomical structure composed of the regular arrangement of lobules that are the functional unit of the liver. The base of the lobule is composed of hepatocytes, the most prevalent cells of the liver[145]. Hepatocytes arranged between the sinusoids along the portal-central axis demonstrate heterogeneity concerning their biochemical and physiological functions[146]. Additional cell types within the liver include cholangiocytes, Kupffer cells, stellate cells, and endothelial cells, with specialized functions[147].

The liver is enriched with cell organelles to fulfill its metabolic functions. It is one of the organs with the highest number and density of mitochondria, which interact with other organelles such as ER, lipid deposits (LDs), and lysosomes[148,149]. In the gastrointestinal tract, food digestion generates metabolic substrates (glucose, fatty acids, and amino acids) that are absorbed by the bloodstream and conducted to the liver through the portal vein circulation system. In the postprandial state, glucose forwards to glycogen storage, and the excess undergoes *de novo* lipogenesis (DNL) in the liver. DNL forms new TAG from acetyl-CoA or malonyl-CoA[150,151].

In mammals, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS)-an enzyme intricately regulated by several nuclear receptors (NRs, *e.g.*, PPARα, PPARγ, and the bile acid receptor/farnesoid X receptor) catalyze fatty acid production. NRs are also relevant mediators of insulin signaling, whereas DNL occurs under anabolic conditions, linking glucose and lipid metabolism. Further support of this link encompasses insulin stimulation of FAS expression. In hepatocytes, NEFAs are esterified with glycerol-3-phosphate to synthesize TAG. TAG is stored in LDs within hepatocytes or secreted into the circulation as very low-density lipoprotein (VLDL) particles. In the fasted state or during exercise, fuel substrates (glucose and TAG) are delivered from the liver into the circulation and metabolized by muscle, adipose tissue, and other extrahepatic tissues[150,151]. However, the increased intake of dietary lipids, associated with upregulated DNL process, causes lipotoxicity and the abnormal accumulation of lipids, often consistent with insulin resistance in steatotic livers, which is related to disturbed ER protein folding homeostasis in hepatocytes[152,153].

The ER in hepatocytes can adapt to extracellular and intracellular changes, allowing the maintenance of essential hepatic metabolic functions. However, several alterations can disturb the ER homeostasis of hepatocytes, contributing to the dysregulation of hepatic lipid metabolism and liver diseases. Consequently, ER includes a progressively conserved pathway called unfolded protein response (UPR) to control liver protein and lipid homeostasis[154]. UPR decreases secretory protein quantity, increases ER protein folding (chaperone and foldase transcription), and enhances deletion capacity by stimulating autophagy and ER-related degradation[152]. Thus, ER stress is a cellular stress in which the demand for folding newly synthesized proteins exceeds ER capacity to fold or to correct it through UPR, generating

unfolded protein accumulation[155].

The existence of three transmembrane-ER proteins is understood to reveal and transduce the ER stress response: Protein kinase RNA-like ER kinase (PERK), inositol-requiring protein-1α (IRE-1α) and activating transcription factor 6 (ATF6). Activation of ATF6 and PERK elicit the expression of the C/EBP homologous protein (CHOP), whereas IRE-1α induces the activation of the N-terminal C-Jun kinase and also generates an improved form of X-box binding protein-1 (XBP1), the spliced XBP1, which attempts to the depletion of misfolded ER proteins through chaperone, folding proteins, and ERAD components encoding[156].

However, when ER stress is intense to the point that damage is irreversible, the ER cannot restore its function. Hence, inflammatory responses trigger cell death pathways[157]. There is strong communication between ER homeostasis perturbations and mitochondrial dysfunction in the onset of obesity and metabolic syndrome. The mitochondrion organelle plays an essential role in hepatic cellular redox, lipid metabolism, and cell death regulation[158], and genes related to mitochondrial fatty acid β-oxidation are mostly transcriptionally regulated by PPARα[159]. Fatty acids input into the mitochondria requires carnitine palmitoyltransferase 1a (*Cpt1a*) (a PPARα target gene), located in the outer mitochondrial membrane. PPARα activation leads to the transcription of *Cpt1a*, associated with fatty acid oxidation in mitochondria, peroxisomes (acyl-CoA oxidase, ACOX), and cytochrome P450 4A family[160].

Mitochondrial dysfunction is associated with acute and chronic liver diseases, and relevant information indicates that mitophagy, a selective form of autophagy (catabolic process, which manifests itself by selective sequestration of mitochondria by autophagosomes, phagophores, a double membrane that isolates autophagic components)[161] of dysfunctional/excessive mitochondria, plays a pivotal role in liver physiology and pathophysiology[158]. There are specificities and mechanisms mediating mitophagy, many of them mediated by the FUN14 domain containing 1 (FUNDC1), a mitochondrial outer membrane protein tightly regulated at transcriptional and post-transcriptional levels. NRF1 (nuclear respiratory factor 1) and PGC1α, the major transcription factor and related cofactor in mitochondrial biogenesis, beneficially adjust FUNDC1 expression and enhance mitophagy[162]. Therefore, mitophagy favors mitochondrial remodeling, and PPARs are implicated in this pathway[163]. Figure 4 summarizes liver alterations under lipotoxicity in obesity.

PPARS IN THE LIVER

Functional studies have demonstrated that PPARα governs hepatic expression of genes involved in nearly all aspects of lipid metabolism, including fatty acid uptake, elongation and desaturation, activation and binding of intracellular fatty acids, formation and degradation of TGs and lipid droplets, and metabolism from plasma lipoproteins[164,165]. The constitutive activity of mitochondrial β-oxidation was significantly reduced in the liver of mice lacking the *Pparα* gene (*Pparα* null mice)[166]. *Pparα* knockout mice fed a high-fat diet increased markers of oxidative stress, inflammation, and cell death[167].

Thus, PPARα has been the target of hypolipidemic drugs from the fibrate family, under investigation for treating non-alcoholic fatty liver disease (NAFLD). PPARα upregulates fatty acid β-oxidation and lipolysis, upregulating the expression of several genes involved in lipid metabolism (*Pgc1α* and *Cpt1a*), leading to less fat accumulation in NAFLD [168]. *Pgc1α* modulates the expression of key metabolic enzymes involved in gluconeogenesis, fatty acid oxidation, and oxidative phosphorylation in the liver through their functional interaction with PPARα[169]. Mitochondrial β-oxidation of long-chain fatty acids is regulated by CPT1a, an enzyme physiologically inhibited by malonyl-CoA, a glucose-derived metabolite and an intermediate in DNL synthesis[170].

Administration of Wy-14643, one of the most potent PPARα agonists, decreased serum insulin, rescued hyperglycemia, and suppressed carbohydrate response element binding protein (ChREBP), mitigating steatosis and hepatic damage. The reductions in ChREBP and FAS activity likely reflect the diminished stimulatory effects of glucose on ChREBP and as a substrate (AcylCoA) for fatty acid synthesis[171]. Moreover, WY-14643 markedly reduced hepatic steatosis in high-fat-fed C57BL/6 mice by augmenting the volume density of mitochondria per area of liver tissue and downregulating hepatic gluconeogenesis and DNL[58]. More recently, WY-14643 treated gut dysbiosis in high-fat and high-fructose-fed-mice, exerting antisteatotic effects through diminished endotoxemic inflammatory inputs to the liver, showing that PPARα mitigates liver steatosis by acting on multiple hits that trigger this outcome[172,173].

Fibrates are a less potent but clinically relevant class of PPARα agonists compared to Wy-14643, which have also been evaluated in experimental models and human studies[174]. *In vivo* experiments, treatment with pemafibrate, a selective PPARα agonist, identify PPARα as a pharmacological, sexually dimorphic target primarily related to related gene functions to lipid homeostasis, with the female liver being much more responsive to pemafibrate than the male liver[175]. The potency of synthetic PPARα agonists may differ from receptor to species as measured using the PPARα-GAL4 transactivation system, *i.e.*, fenofibrate (mouse receptor, EC50 = 18000 nM *vs* human receptor, EC50 = 30000 nM), bezafibrate (EC50 = 90000 nM *vs* 50000 nM, respectively) and Wy14643 (EC50 = 630 nM *vs* 5000 nM, respectively)[176].

In contrast, several studies have provided evidence that hepatic PPARγ expression markedly increases in many models of obesity (lipoatrophy and hyperphagic obesity), insulin resistance, and diabetes with varying degrees of steatosis[177]. PPARγ expression in the liver is low under healthy conditions but increases as steatosis develops in rodents[178]; this effect is not seen in humans[75,179]. They have tremendous potential in patients with NAFLD because they promote preadipocyte differentiation into adipocytes and may induce fat redistribution from visceral sites such as the liver and muscle to peripheral subcutaneous adipose tissue, increase circulating adiponectin levels, and improve insulin sensitivity [180].

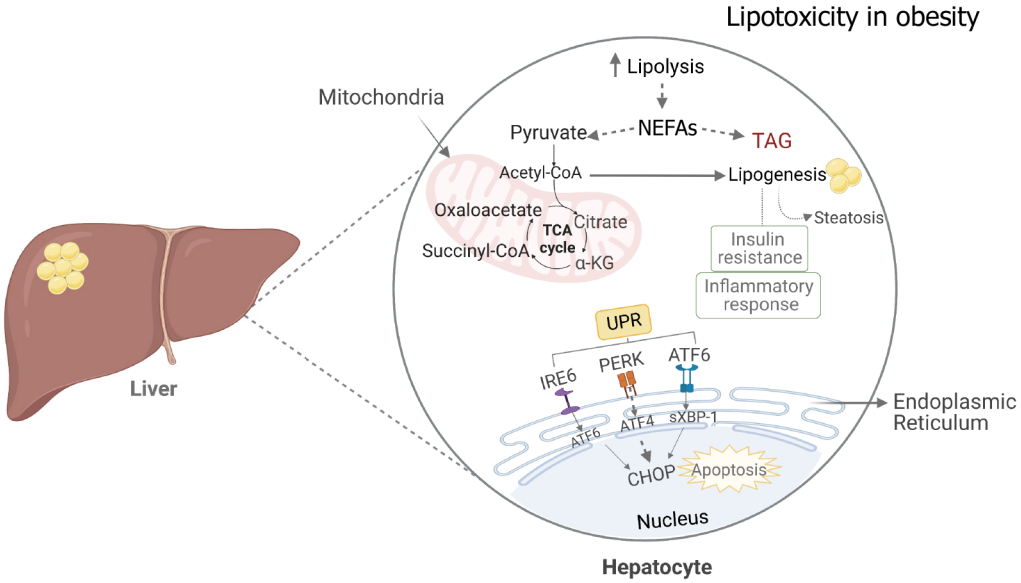

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Figure 4 Schematic illustrates hepatic lipotoxicity caused by obesity. There is an increase in lipolysis, which triggers the intracellular accumulation of fatty acids (FAs) within hepatocytes, resulting in a delay in mitochondrial beta-oxidation followed by an increase in lipogenesis, favoring the deposition of lipid droplets in the hepatic parenchyma, culminating in hepatic steatosis. Concomitantly, there is a reduction in insulin signaling, potentiating inflammation, and endoplasmic reticulum (ER) stress. ER stress is detected by the unfolded protein response-IRE1, PERK, and activating transcription factor 6 (ATF6) in the ER membrane. If it is impossible to reverse the unfolded proteins, ATF4 promotes transcription of the C/element binding protein homologous protein, a transcription factor that induces apoptosis. Made with Biorender (www.biorender.com). TAG: Triacylglycerol; UPR: Unfolded protein response; UPR: Unfolded protein response; PERK: Protein kinase RNA-like ER kinase; ATF: Activating transcription factor 6; IRE6: CHOP: C/EBP homologous protein; NEFAs: Non-esterified fatty acids. Created by BioRender.

PPARγ agonists, such as the TZDs, stimulate genes that favor the storage of TAG, thus lowering circulating free fatty acid concentrations[181]. In humans, one-year treatment with pioglitazone (at low dosage) significantly improved liver steatosis, inflammation, and systemic and local adipose tissue insulin resistance in patients with T2D. A decrease in sterol regulatory element-binding transcription factor 1c (SREBP-1c) expression by pioglitazone has a primary effect in reducing hepatic steatosis[182]. Pooled results suggest that acetaldehyde produced from ethanol metabolism may increase the synthesis of the mature SREBP-1 protein, which increases hepatic lipogenesis, thus leading to the development of fatty liver[183,184].

Administration of the PPARγ agonist rosiglitazone (5 mg/kg, daily, gavage) accelerated regression of liver fibrosis as associated with increased expression of PPARγ in mice, with similar findings in human hepatic stellate cells (HSCs). Furthermore, *in vitro*, GATA binding protein 6 (GATA6)-deficient HSCs exhibit a defect in inactivation, suggesting that GATA6 and PPARγ agonists can be used to drive the inactivation of HSCs/myofibroblasts and could compose a combination strategy to halt liver fibrosis in patients[185]. On the other hand, some studies associate TZD therapy with an average weight gain of 4 kg to 5 kg[186,187]. Weight gain combines adiposity and fluid retention as the increased peripheral edema observed during the pre-marketing clinical trials of pioglitazone and rosiglitazone. Increased vascular permeability through high vascular endothelial growth factor secretion and decreased systemic vascular resistance are non-kidney factors that contribute to this edema[188,189].

Recently, dual PPAR-α/γ agonists emerged as a strategy to lessen undesirable side effects related to PPAR-γ agonism. In a randomized controlled clinical trial, Saroglitazar (PPAR-α/γ agonist, 4 mg) significantly improved alanine transaminase, liver fat content, insulin resistance, and atherogenic dyslipidemia in participants with NAFLD/nonalcoholic steatohepatitis (NASH)[190]. In an *in vitro* study on HepG2 cells, saroglitazar prevented HSC activation from quiescent cells to highly proliferative and fibrogenic cells. Pro-inflammatory cytokines, upregulated in NAFLD, can activate hepatic stellate cells, causing increased collagen deposition that initiates fibrogenesis. In turn, the use of saroglitazar reduced the expression of pro-inflammatory [tumor necrosis factor-alpha (*Tnfα*), interleukin (IL)-1β, and IL-6] and pro-fibrogenic (monocyte chemoattractant protein-1, transforming growth factor beta, collagen type I alpha 1 chain, and α-smooth muscle actin) genes in HSC[191]. Evidence is growing in favor of dual PPARα/γ agonism as a candidate for the treatment of NAFLD/NASH due to findings of improvements in all components responsible for these conditions.

In agreement with PPARα beneficial effects on the liver, PPARβ/δ possesses anti-inflammatory effects in the liver by inhibiting NF-κB activity by directly binding to its subunit p65[192-195]. The PPAR-β/δ agonist GW0742 Led to the modulation of the inflammatory response induced by NF-κB in rats, reducing the release of pro-inflammatory cytokines and neutrophils infiltration into the liver[193,196]. During the induction of inflammatory responses, the inactivated PPAR β/δ participates in the activation of NF-κB p65. Activation by ligand PPARβ/δ results in a lack of this cooperation, and consequently, activation of PPARβ/δ interferes with the function of NF-κB p65. As a result, inflammatory responses caused by a high concentration of glucose, activation of the receptor for TNF-α, IL-1β, or activation of TLR4 are reduced [197]. Along with the anti-inflammatory effects, GW0742 has recently mitigated hepatic steatosis through attenuating

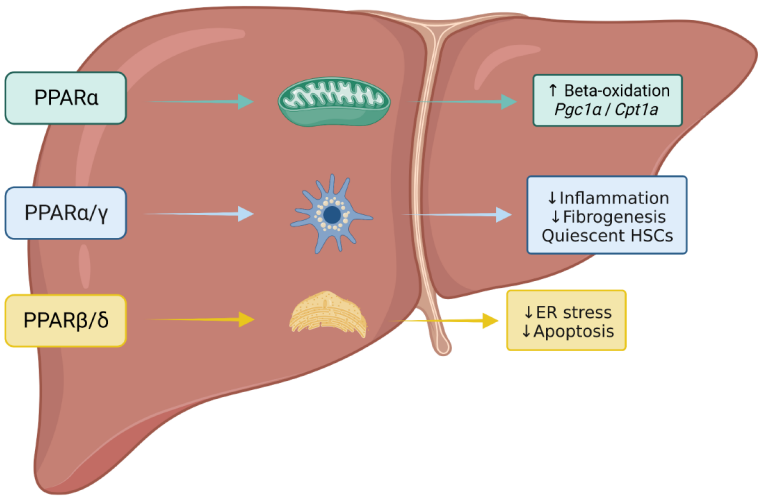

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Figure 5 Beneficial effects of Peroxisome proliferator-activated receptor activation on the liver. Peroxisome proliferator-activated receptor α (PPARα) activation mitigates hepatic steatosis through the activation of genes related to mitochondrial biogenesis and beta-oxidation, such as *Pgc1α* and *Cpt1a*. Dual PPARα/γ agonists emerge as promising candidates to treat nonalcoholic steatohepatitis once this approach reduces hepatic stellate cells activation, keeping their quiescent stage and impeding fibrogenesis. PPARβ/δ/δ activation entails antisteatotic effects through alleviating the hepatic endoplasmic reticulum stress, inflammation, and apoptosis. Made with Biorender (www.biorender.com). PPAR: Peroxisome proliferator-activated receptor; *Pgc1α*: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; *Cpt1a*: Carnitine palmitoyltransferase 1a; HSC: Hepatic stellate cell; ER: Endoplasmic reticulum. Created by BioRender.

hepatic ER stress (reduced p-eIF2α/ATF4/CHOP expression), yielding anti-apoptotic signals and favored beta-oxidation over lipogenesis in high-fat-fed mice[198].

Despite this, the contribution of PPARβ/δ to hepatic lipid metabolism is still controversial. PPAR-β/δ null mice on a high-fat diet showed an increased rate of hepatic VLDL production and an increase in the plasma VLDL apoB48, apoE, apoAI, and apoAII levels, as well as a reduction in hepatic lipid stores[178]. However, the other potent PPARβ/δ agonist, GW501516, increased the expression of the lipogenic enzyme ACC2 and consequently increased the hepatic TAG content in db/db mice[192,199]. The role that PPAR-β/δ has on liver metabolism is not defined as there is not a PPAR-β/δ agonist available to the population[200]. Figure 5 depicts the beneficial effects of PPAR activation on the liver. Table 1 shows the main mechanisms and endpoints of PPAR agonists on experimental, *in vitro*, and clinical backgrounds.

CONCLUSION

Overall, there is no doubt that PPARs are promising therapeutic targets for metabolic syndrome, insulin resistance, dyslipidemia, and NAFLD/NASH. However, more research, improvement, and testing are needed to apply PPAR-targeted agents to human metabolic diseases with increased safety and efficacy. Much as PPARs agonists are widely prescribed to treat dyslipidemia, hypertension, and T2DM, dual agonists are promising in the context of obesity due to the combination of anti-inflammatory, lipid oxidation, insulin-sensitizing, and thermogenesis activation, which prevails from one of the isoforms actions and might be highlighted with suitable modulation of their combined activation. Considering that the jury is still out on defined drug therapy for obesity and NAFLD (one of obesity's more prevalent comorbidity), PPARs entail a potent target to reach adequate control of the glucolipotoxicity that trigger metabolic diseases, whether as part of a combination of different PPARs isoforms or combined with agents from other drug classes.

Table 1 Summary of evidence on PPARs agonists on metabolic outcomes in clinical and experimental studies

Model	PPAR agonist	Effect/mechanism	Ref.
HF diet fed C57BL/6 mice (14 wk)	WY14643 (3.0 mg/kg BM)	Decreased body mass and increased hepatic beta-oxidation	Barbosa-da-Silva *et al* [57], 2015
HF diet fed C57BL/6 mice (14 wk)	WY14643 (2.5 mg/kg BM)	Decreased insulin, hepatic steatosis, and enhanced mitochondria per area of liver tissue	Veiga *et al*[58], 2017
HF diet fed C57BL/6 mice (14 wk)	WY14643 (2.5 mg/kg BM)	Browning of subcutaneous WAT, increased thermogenesis	Rachid *et al*[59], 2018
HF diet or high-fructose-fed C57BL/6 mice (17 wk)	WY14643 (3.5 mg/kg BM)	Reduced whitening in HF-fed mice *via* increased VEGFA and reduced inflammation	Miranda *et al*[62], 2020

HF diet fed C57BL/6 mice (15 wk)	Fenofibrate (100.0 mg/kg BM)	Increased irisin-*Pgc1α*-*Prdm16* and induced thermogenic beige adipocytes	Rachid *et al*[63], 2015
High-fructose diet-fed C57BL/6 mice (11 wk)	GW501516 (3.0 mg/kg/d)	Potent anti-inflammatory effects that reversed adipocyte hypertrophy	Magliano *et al*[64], 2015
Knockout mice for *Pparγ* in collecting duct	Rosiglitazone (320.0 mg/kg diet)	PPARγ regulates sodium transport in the collecting ducts and mediates the rosiglitazone-induced edema	Zhang *et al*[77], 2005
Human subcutaneous white adipose tissue biopsy	Pioglitazone (45 mg daily for two months)	Pioglitazone reduced CD68 and MCP-1 expression in adipose tissue, improving insulin sensitivity	Di Gregorio *et al*[78], 2005
HF diet fed C57BL/6 mice (16 wk)	Tesaglitazar (4 mg/kg BM)	PPARα/γ synergism treated dysbiosis and favored thermogenesis	Miranda *et al*[80], 2023
Human jejunal biopsies	Cells treated with GW7647 (600 nM) or GFT505 (1 μM) for 18 h	Intestinal PPARα activation induces HDL production	Colin *et al*[82], 2013
Knockout mice	25.0% fish oil in the diet	Increased adiponectin, improved glucose metabolism, and islet hypertrophy	Nakasatomi *et al*[112], 2018
Non-obese diabetic mice	0.1% fenofibrate in the diet	Anti-inflammatory, antiapoptotic effects, and enhanced islet innervation, ameliorating glucose handling	Holm *et al*[114], 2019
Monosodium glutamate induced obese rats	100 mg/kg fenofibrate for 12 wk	Long-term treatment disrupted beta cell function due to increased NF-κB and iNOS expression	Liu *et al*[115], 2011
Rat pancreatic islets *in vitro*	300 microM bezafibrate for 8 h	Bezafibrate enhanced GSIS through *Pparα* activation in short-term culture. Long-term culture caused beta cell dysfunction due to overstimulation	Yoshikawa *et al*[116], 2001
Diabetic subjects	400 mg bezafibrate for two years	Bezafibrate avoided the progressive decline of beta cell function and insulin resistance increase	Tenenbaum *et al*[117], 2007
Subjects diagnosed with type 2 diabetes and dyslipidemia	Pioglitazone 30 mg/d for 12 wk and 45 mg/d for additional 12 wk; rosiglitazone 4 mg/d for 12 wk and 8 mg/d for additional 12 wk	Pioglitazone had better effects regarding improvements in triglycerides, HDL cholesterol, LDL particle concentration, and LDL particle size	Goldberg *et al*[127], 2005
db/db mice	Pioglitazone 15 mg/kg BM for 18 d	Restoration of pancreatic islet function with increased expression of insulin and NK6 Homeobox 1 expression	Collier *et al*[132], 2021
Knockout-Ay mice	High-fat diet plus pioglitazone 0.02% for 6 wk	Islet preservation through ER stress and inflammation alleviation	Hong *et al*[133], 2018
Diabetic Wistar rats (low streptozotocin dose)	10 mg/kg pioglitazone or vildagliptin or their combination for 4 wk	Vildagliptin maximized pioglitazone effects on inflammation and oxidative stress attenuation	Refaat *et al*[134], 2016
Type 2 diabetic patients	Sitagliptin 100 mg/d or Pioglitazone 30 mg/d or their combination for 12 wk	Both drugs exerted complementary effects on blood glucose control	Alba *et al*[135], 2013
Non-diabetic hypertensive subjects	Telmisartan (80 mg/d) for 6 wk	Telmisartan enhanced insulin sensitivity in hypertensive patients independent of adiponectin induction	Benndorf *et al*[136], 2006
High-fat diet-fed mice (16 wk)	Telmisartan (5 mg/kg BM) alone or in combination with sitagliptin (1 g/kg BM) or metformin (300 mg/kg BM)	Treated animals exhibited marked mitigation of hepatic steatosis and islet hypertrophy; telmisartan combination with sitagliptin normalized alpha and beta cell mass	Souza-Mello *et al*[93], 2010; Souza-Mello *et al*[97], 2011
High-fat diet-fed mice (15 wk)	Telmisartan (10 mg/kg)	Amelioration of endocrine pancreas structure and function, with enhanced islet vascularization and reduced apoptosis rate	Graus-Nunes *et al*[96], 2017
db/db mice	Telmisartan (3 mg/kg BM), linagliptin (3 mg/kg BM) or their combination for eight weeks	Combined therapy provided better results than the monotherapies on glucose homeostasis, islet cell functions, and structure *via* reduced oxidative stress	Zhao *et al*[137], 2016
High-fat-fed foz/foz obese/diabetic mice for 16 wk	WY14643 (0.1% w/w) for 10 d or 20 d	PPARα activation mitigated steatosis, and hepatocyte ballooning, besides reducing NF-κB and JNK activation. Persistent adipose-derived MCP1 enhanced levels may limit its property to treat NASH	Larter *et al*[171], 2012
High-fructose diet-fed mice (17 wk)	WY14643 (3.5 mg/kg BM) or linagliptin (15.0 mg/kg BM) or their combination	The WY14643 monotherapy or its combination with linagliptin-treated dysbiosis, controlling endotoxemia and mitigating liver steatosis	Silva-Veiga *et al*[173], 2020
Type 2 diabetic patients	Metformin 2 g/d + Pioglitazone (15 mg/d, 30 mg/d, or 45 mg/d)	All pioglitazone doses exerted similar effects, with mitigation of liver steatosis and inflammation, with improved systemic insulin resistance	Della Pepa *et al*[182], 2021
CCl4-injured mice	Rosiglitazone (5 mg/kg BM) for two weeks	Rosiglitazone blocked liver fibrosis progression through the down-regulation of fibrogenic genes and HSCs inactivation	Liu *et al*[185], 2020
Patients with	Saroglitazar 1 mg, 2 mg, or 4 mg for 16	Saroglitazar 4 mg significantly mitigated insulin resistance	Gawrieh *et al*[190],

NAFLD/NASH	wk	and atherogenic dyslipidemia	2021
HF diet fed mice (14 wk)	GW0742 (1 mg/kg BM) for four weeks	PPARβ/δ mitigated hepatic steatosis through improved insulin resistance and ER stress alleviation	Silva-Veiga *et al* [198], 2018

HF: High-fructose; NAFLD: Non-alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PPAR: Peroxisome proliferator-activated receptors; WAT: White adipose tissue; ER: Endoplasmic reticulum; NF-κB: Noncanonical nuclear factor-kappaB; HSCs: Hepatic stellate cells; iNOS: Inducible nitric oxide synthase; GSIS: Glucose-stimulated insulin secretion; HDL: High-density cholesterol; LDL: Low-density cholesterol.

FOOTNOTES

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REFERENCES

1 **GBD 2015 Obesity Collaborators**, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, Marczak L, Mokdad AH, Moradi-Lakeh M, Naghavi M, Salama JS, Vos T, Abate KH, Abbafati C, Ahmed MB, Al-Aly Z, Alkerwi A, Al-Raddadi R, Amare AT, Amberbir A, Amegah AK, Amini E, Amrock SM, Anjana RM, Ärnlöv J, Asayesh H, Banerjee A, Barac A, Baye E, Bennett DA, Beyene AS, Biadgilign S, Biryukov S, Bjertness E, Boneya DJ, Campos-Nonato I, Carrero JJ, Cecilio P, Cercy K, Ciobanu LG, Cornaby L, Damtew SA, Dandona L, Dandona R, Dharmaratne SD, Duncan BB, Eshrati B, Esteghamati A, Feigin VL, Fernandes JC, Fürst T, Gebrehiwot TT, Gold A, Gona PN, Goto A, Habtewold TD, Hadush KT, Hafezi-Nejad N, Hay SI, Horino M, Islami F, Kamal R, Kasaeian A, Katikireddi SV, Kengne AP, Kesavachandran CN, Khader YS, Khang YH, Khubchandani J, Kim D, Kim YJ, Kinfu Y, Kosen S, Ku T, Defo BK, Kumar GA, Larson HJ, Leinsalu M, Liang X, Lim SS, Liu P, Lopez AD, Lozano R, Majeed A, Malekzadeh R, Malta DC, Mazidi M, McAlinden C, McGarvey ST, Mengistu DT, Mensah GA, Mensink GBM, Mezgebe HB, Mirrakhimov EM, Mueller UO, Noubiap JJ, Obermeyer CM, Ogbo FA, Owolabi MO, Patton GC, Pourmalek F, Qorbani M, Rafay A, Rai RK, Ranabhat CL, Reinig N, Safiri S, Salomon JA, Sanabria JR, Santos IS, Sartorius B, Sawhney M, Schmidhuber J, Schutte AE, Schmidt MI, Sepanlou SG, Shamsizadeh M, Sheikhbahaei S, Shin MJ, Shiri R, Shiue I, Roba HS, Silva DAS, Silverberg JI, Singh JA, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadese F, Tedla BA, Tegegne BS, Terkawi AS, Thakur JS, Tonelli M, Topor-Madry R, Tyrovolas S, Ukwaja KN, Uthman OA, Vaezghasemi M, Vasankari T, Vlassov VV, Vollset SE, Weiderpass E, Werdecker A, Wesana J, Westerman R, Yano Y, Yonemoto N, Yonga G, Zaidi Z, Zenebe ZM, Zipkin B, Murray CJL. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med* 2017; **377**: 13-27 [PMID: 28604169 DOI: 10.1056/NEJMoa1614362]

2 **Betz MJ**, Enerbäck S. Human Brown Adipose Tissue: What We Have Learned So Far. *Diabetes* 2015; **64**: 2352-2360 [PMID: 26050667 DOI: 10.2337/db15-0146]

3 **Tseng YH**, Cypess AM, Kahn CR. Cellular bioenergetics as a target for obesity therapy. *Nat Rev Drug Discov* 2010; **9**: 465-482 [PMID: 20514071 DOI: 10.1038/nrd3138]

4 **Gustafson B**, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. *Trends Endocrinol Metab* 2015; **26**: 193-200 [PMID: 25703677 DOI: 10.1016/j.tem.2015.01.006]

5 **Gustafson B**, Smith U. Regulation of white adipogenesis and its relation to ectopic fat accumulation and cardiovascular risk. *Atherosclerosis* 2015; **241**: 27-35 [PMID: 25957567 DOI: 10.1016/j.atherosclerosis.2015.04.812]

6 **Cildir G**, Akıncılar SC, Tergaonkar V. Chronic adipose tissue inflammation: all immune cells on the stage. *Trends Mol Med* 2013; **19**: 487-500 [PMID: 23746697 DOI: 10.1016/j.molmed.2013.05.001]

7 **Gustafson B**, Hammarstedt A, Hedjazifar S, Smith U. Restricted adipogenesis in hypertrophic obesity: the role of WISP2, WNT, and BMP4. *Diabetes* 2013; **62**: 2997-3004 [PMID: 23970518 DOI: 10.2337/db13-0473]

8 **DeFronzo RA**. Dysfunctional fat cells, lipotoxicity and type 2 diabetes. *Int J Clin Pract Suppl* 2004; 9-21 [PMID: 16035392 DOI: 10.1111/j.1368-504x.2004.00389.x]

9 **Longo M**, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, Beguinot F, Miele C. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *Int J Mol Sci* 2019; **20** [PMID: 31085992 DOI: 10.3390/ijms20092358]

10 **Polyzos SA**, Kountouras J, Mantzoros CS. Adipose tissue, obesity and non-alcoholic fatty liver disease. *Minerva Endocrinol* 2017; **42**: 92-108 [PMID: 27711029 DOI: 10.23736/S0391-1977.16.02563-3]

11 **Poitout V**, Robertson RP. Minireview: Secondary beta-cell failure in type 2 diabetes--a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 2002; **143**: 339-342 [PMID: 11796484 DOI: 10.1210/endo.143.2.8623]

12 **Saponaro C**, Gaggini M, Carli F, Gastaldelli A. The Subtle Balance between Lipolysis and Lipogenesis: A Critical Point in Metabolic Homeostasis. *Nutrients* 2015; **7**: 9453-9474 [PMID: 26580649 DOI: 10.3390/nu7115475]

13 **Harrington WW**, S Britt C, G Wilson J, O Milliken N, G Binz J, C Lobe D, R Oliver W, C Lewis M, M Ignar D. The Effect of PPARalpha, PPARdelta, PPARgamma, and PPARpan Agonists on Body Weight, Body Mass, and Serum Lipid Profiles in Diet-Induced Obese AKR/J Mice. *PPAR Res* 2007; **2007**: 97125 [PMID: 17710237 DOI: 10.1155/2007/97125]

14 **Li M**, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. *Mol Cell Biol* 2000; **20**: 4699-4707 [PMID: 10848596 DOI: 10.1128/mcb.20.13.4699-4707.2000]

15 **Delerive P**, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, Fruchart JC, Tedgui A, Haegeman G, Staels B. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem* 1999; **274**: 32048-32054 [PMID: 10542237 DOI: 10.1074/jbc.274.45.32048]

16 **Dubois V**, Eeckhoute J, Lefebvre P, Staels B. Distinct but complementary contributions of PPAR isotypes to energy homeostasis. *J Clin Invest* 2017; **127**: 1202-1214 [PMID: 28368286 DOI: 10.1172/JCI88894]

17 **Corrales P**, Vidal-Puig A, Medina-Gómez G. PPARs and Metabolic Disorders Associated with Challenged Adipose Tissue Plasticity. *Int J Mol Sci* 2018; **19** [PMID: 30037087 DOI: 10.3390/ijms19072124]

18 **Moraes LA**, Piqueras L, Bishop-Bailey D. Peroxisome proliferator-activated receptors and inflammation. *Pharmacol Ther* 2006; **110**: 371-385 [PMID: 16168490 DOI: 10.1016/j.pharmthera.2005.08.007]

19 **Fonseca-Alaniz MH**, Takada J, Alonso-Vale MI, Lima FB. Adipose tissue as an endocrine organ: from theory to practice. *J Pediatr (Rio J)* 2007; **83**: S192-S203 [PMID: 17989837 DOI: 10.2223/JPED.1709]

20 **Ahima RS**, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab* 2000; **11**: 327-332 [PMID: 10996528 DOI: 10.1016/s1043-2760(00)00301-5]

21 **Pénicaud L**, Cousin B, Leloup C, Lorsignol A, Casteilla L. The autonomic nervous system, adipose tissue plasticity, and energy balance. *Nutrition* 2000; **16**: 903-908 [PMID: 11054595 DOI: 10.1016/s0899-9007(00)00427-5]

22 **Kreier F**, Fliers E, Voshol PJ, Van Eden CG, Havekes LM, Kalsbeek A, Van Heijningen CL, Sluiter AA, Mettenleiter TC, Romijn JA, Sauerwein HP, Buijs RM. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat--functional implications. *J Clin Invest* 2002; **110**: 1243-1250 [PMID: 12417562 DOI: 10.1172/jci15736]

23 **Giralt M**, Villarroya F. White, brown, beige/brite: different adipose cells for different functions? *Endocrinology* 2013; **154**: 2992-3000 [PMID: 23782940 DOI: 10.1210/en.2013-1403]

24 **Lidell ME**, Betz MJ, Enerbäck S. Two types of brown adipose tissue in humans. *Adipocyte* 2014; **3**: 63-66 [PMID: 24575372 DOI: 10.4161/adip.26896]

25 **Wronska A**, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. *Acta Physiol (Oxf)* 2012; **205**: 194-208 [PMID: 22226221 DOI: 10.1111/j.1748-1716.2012.02409.x]

26 **Divoux A**, Clément K. Architecture and the extracellular matrix: the still unappreciated components of the adipose tissue. *Obes Rev* 2011; **12**: e494-e503 [PMID: 21366833 DOI: 10.1111/j.1467-789X.2010.00811.x]

27 **Frederich RC**, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1995; **1**: 1311-1314 [PMID: 7489415 DOI: 10.1038/nm1295-1311]

28 **Lee MW**, Lee M, Oh KJ. Adipose Tissue-Derived Signatures for Obesity and Type 2 Diabetes: Adipokines, Batokines and MicroRNAs. *J Clin Med* 2019; **8** [PMID: 31208019 DOI: 10.3390/jcm8060854]

29 **Nedergaard J**, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2007; **293**: E444-E452 [PMID: 17473055 DOI: 10.1152/ajpendo.00691.2006]

30 **Langin D**. Recruitment of brown fat and conversion of white into brown adipocytes: strategies to fight the metabolic complications of obesity? *Biochim Biophys Acta* 2010; **1801**: 372-376 [PMID: 19782764 DOI: 10.1016/j.bbalip.2009.09.008]

31 **Cannon B**, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004; **84**: 277-359 [PMID: 14715917 DOI: 10.1152/physrev.00015.2003]

32 **Lowell BB**, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature* 2000; **404**: 652-660 [PMID: 10766252 DOI: 10.1038/35007527]

33 **Nedergaard J**, Golozoubova V, Matthias A, Asadi A, Jacobsson A, Cannon B. UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim Biophys Acta* 2001; **1504**: 82-106 [PMID: 11239487 DOI: 10.1016/s0005-2728(00)00247-4]

34 **Timmons JA**, Wennmalm K, Larsson O, Walden TB, Lassmann T, Petrovic N, Hamilton DL, Gimeno RE, Wahlestedt C, Baar K, Nedergaard J, Cannon B. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci U S A* 2007; **104**: 4401-4406 [PMID: 17360536 DOI: 10.1073/pnas.0610615104]

35 **Seale P**, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM. Transcriptional control of brown fat determination by PRDM16. *Cell Metab* 2007; **6**: 38-54 [PMID: 17618855 DOI: 10.1016/j.cmet.2007.06.001]

36 **Nedergaard J**, Bengtsson T, Cannon B. Three years with adult human brown adipose tissue. *Ann N Y Acad Sci* 2010; **1212**: E20-E36 [PMID: 21375707 DOI: 10.1111/j.1749-6632.2010.05905.x]

37 **Smith RE**, Hock RJ. Brown fat: thermogenic effector of arousal in hibernators. *Science* 1963; **140**: 199-200 [PMID: 13989560 DOI: 10.1126/science.140.3563.199]

38 **Smith RE**. Thermoregulatory and adaptive behavior of brown adipose tissue. *Science* 1964; **146**: 1686-1689 [PMID: 14224520 DOI: 10.1126/science.146.3652.1686]

39 **Shimizu I**, Aprahamian T, Kikuchi R, Shimizu A, Papanicolaou KN, MacLauchlan S, Maruyama S, Walsh K. Vascular rarefaction mediates

whitening of brown fat in obesity. *J Clin Invest* 2014; **124**: 2099-2112 [PMID: 24713652 DOI: 10.1172/JCI71643]

40 **Roberts-Toler C**, O'Neill BT, Cypess AM. Diet-induced obesity causes insulin resistance in mouse brown adipose tissue. *Obesity (Silver Spring)* 2015; **23**: 1765-1770 [PMID: 26242777 DOI: 10.1002/oby.21134]

41 **Rangel-Azevedo C**, Santana-Oliveira DA, Miranda CS, Martins FF, Mandarim-de-Lacerda CA, Souza-Mello V. Progressive brown adipocyte dysfunction: Whitening and impaired nonshivering thermogenesis as long-term obesity complications. *J Nutr Biochem* 2022; **105**: 109002 [PMID: 35346828 DOI: 10.1016/j.jnutbio.2022.109002]

42 **Xue Y**, Petrovic N, Cao R, Larsson O, Lim S, Chen S, Feldmann HM, Liang Z, Zhu Z, Nedergaard J, Cannon B, Cao Y. Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. *Cell Metab* 2009; **9**: 99-109 [PMID: 19117550 DOI: 10.1016/j.cmet.2008.11.009]

43 **Concha F**, Prado G, Quezada J, Ramirez A, Bravo N, Flores C, Herrera JJ, Lopez N, Uribe D, Duarte-Silva L, Lopez-Legarrea P, Garcia-Diaz DF. Nutritional and non-nutritional agents that stimulate white adipose tissue browning. *Rev Endocr Metab Disord* 2019; **20**: 161-171 [PMID: 31020455 DOI: 10.1007/s11154-019-09495-y]

44 **Wu J**, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012; **150**: 366-376 [PMID: 22796012 DOI: 10.1016/j.cell.2012.05.016]

45 **Harms MJ**, Ishibashi J, Wang W, Lim HW, Goyama S, Sato T, Kurokawa M, Won KJ, Seale P. Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. *Cell Metab* 2014; **19**: 593-604 [PMID: 24703692 DOI: 10.1016/j.cmet.2014.03.007]

46 **Spiegelman BM**. Banting Lecture 2012: Regulation of adipogenesis: toward new therapeutics for metabolic disease. *Diabetes* 2013; **62**: 1774-1782 [PMID: 23704518 DOI: 10.2337/db12-1665]

47 **Sanchez-Gurmaches J**, Guertin DA. Adipocyte lineages: tracing back the origins of fat. *Biochim Biophys Acta* 2014; **1842**: 340-351 [PMID: 23747579 DOI: 10.1016/j.bbadis.2013.05.027]

48 **Long JZ**, Svensson KJ, Tsai L, Zeng X, Roh HC, Kong X, Rao RR, Lou J, Lokurkar I, Baur W, Castellot JJ Jr, Rosen ED, Spiegelman BM. A smooth muscle-like origin for beige adipocytes. *Cell Metab* 2014; **19**: 810-820 [PMID: 24709624 DOI: 10.1016/j.cmet.2014.03.025]

49 **Min SY**, Kady J, Nam M, Rojas-Rodriguez R, Berkenwald A, Kim JH, Noh HL, Kim JK, Cooper MP, Fitzgibbons T, Brehm MA, Corvera S. Human 'brite/beige' adipocytes develop from capillary networks, and their implantation improves metabolic homeostasis in mice. *Nat Med* 2016; **22**: 312-318 [PMID: 26808348 DOI: 10.1038/nm.4031]

50 **Martins FF**, Souza-Mello V, Aguila MB, Mandarim-de-Lacerda CA. Brown adipose tissue as an endocrine organ: updates on the emerging role of batokines. *Horm Mol Biol Clin Investig* 2022 [PMID: 36288452 DOI: 10.1515/hmbci-2022-0044]

51 **Virtanen KA**, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009; **360**: 1518-1525 [PMID: 19357407 DOI: 10.1056/NEJMoa0808949]

52 **Sher T**, Yi HF, McBride OW, Gonzalez FJ. cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry* 1993; **32**: 5598-5604 [PMID: 7684926 DOI: 10.1021/bi00072a015]

53 **Braissant O**, Foufelle F, Scotto C, Dauça M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996; **137**: 354-366 [PMID: 8536636 DOI: 10.1210/endo.137.1.8536636]

54 **Vamecq J**, Latruffe N. Medical significance of peroxisome proliferator-activated receptors. *Lancet* 1999; **354**: 141-148 [PMID: 10408502 DOI: 10.1016/s0140-6736(98)10364-1]

55 **Li P**, Zhu Z, Lu Y, Granneman JG. Metabolic and cellular plasticity in white adipose tissue II: role of peroxisome proliferator-activated receptor-alpha. *Am J Physiol Endocrinol Metab* 2005; **289**: E617-E626 [PMID: 15941786 DOI: 10.1152/ajpendo.00010.2005]

56 **Rösch S**, Ramer R, Brune K, Hinz B. Prostaglandin E2 induces cyclooxygenase-2 expression in human non-pigmented ciliary epithelial cells through activation of p38 and p42/44 mitogen-activated protein kinases. *Biochem Biophys Res Commun* 2005; **338**: 1171-1178 [PMID: 16256948 DOI: 10.1016/j.bbrc.2005.10.051]

57 **Barbosa-da-Silva S**, Souza-Mello V, Magliano DC, Marinho Tde S, Aguila MB, Mandarim-de-Lacerda CA. Singular effects of PPAR agonists on nonalcoholic fatty liver disease of diet-induced obese mice. *Life Sci* 2015; **127**: 73-81 [PMID: 25748419 DOI: 10.1016/j.lfs.2015.02.003]

58 **Veiga FMS**, Graus-Nunes F, Rachid TL, Barreto AB, Mandarim-de-Lacerda CA, Souza-Mello V. Anti-obesogenic effects of WY14643 (PPAR-alpha agonist): Hepatic mitochondrial enhancement and suppressed lipogenic pathway in diet-induced obese mice. *Biochimie* 2017; **140**: 106-116 [PMID: 28711683 DOI: 10.1016/j.biochi.2017.07.003]

59 **Rachid TL**, Silva-Veiga FM, Graus-Nunes F, Bringhenti I, Mandarim-de-Lacerda CA, Souza-Mello V. Differential actions of PPAR-α and PPAR-β/δ on beige adipocyte formation: A study in the subcutaneous white adipose tissue of obese male mice. *PLoS One* 2018; **13**: e0191365 [PMID: 29351550 DOI: 10.1371/journal.pone.0191365]

60 **Seale P**. Transcriptional Regulatory Circuits Controlling Brown Fat Development and Activation. *Diabetes* 2015; **64**: 2369-2375 [PMID: 26050669 DOI: 10.2337/db15-0203]

61 **Rachid TL**, Penna-de-Carvalho A, Bringhenti I, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. PPAR-α agonist elicits metabolically active brown adipocytes and weight loss in diet-induced obese mice. *Cell Biochem Funct* 2015; **33**: 249-256 [PMID: 25959716 DOI: 10.1002/cbf.3111]

62 **Miranda CS**, Silva-Veiga F, Martins FF, Rachid TL, Mandarim-De-Lacerda CA, Souza-Mello V. PPAR-α activation counters brown adipose tissue whitening: a comparative study between high-fat- and high-fructose-fed mice. *Nutrition* 2020; **78**: 110791 [PMID: 32682271 DOI: 10.1016/j.nut.2020.110791]

63 **Rachid TL**, Penna-de-Carvalho A, Bringhenti I, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. Fenofibrate (PPARalpha agonist) induces beige cell formation in subcutaneous white adipose tissue from diet-induced male obese mice. *Mol Cell Endocrinol* 2015; **402**: 86-94 [PMID: 25576856 DOI: 10.1016/j.mce.2014.12.027]

64 **Magliano DC**, Penna-de-Carvalho A, Vazquez-Carrera M, Mandarim-de-Lacerda CA, Aguila MB. Short-term administration of GW501516 improves inflammatory state in white adipose tissue and liver damage in high-fructose-fed mice through modulation of the renin-angiotensin system. *Endocrine* 2015; **50**: 355-367 [PMID: 25854303 DOI: 10.1007/s12020-015-0590-1]

65 **Lehmann JM**, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 1995; **270**: 12953-12956 [PMID: 7768881 DOI: 10.1074/jbc.270.22.12953]

66 **Ahmadian M**, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM. PPARγ signaling and metabolism: the good, the bad and the future. *Nat Med* 2013; **19**: 557-566 [PMID: 23652116 DOI: 10.1038/nm.3159]

67 **Stuhlmeier KM**, Kao JJ, Bach FH. Arachidonic acid influences proinflammatory gene induction by stabilizing the inhibitor-kappaBalpha/nuclear factor-kappaB (NF-kappaB) complex, thus suppressing the nuclear translocation of NF-kappaB. *J Biol Chem* 1997; **272**: 24679-24683 [PMID: 9305938 DOI: 10.1074/jbc.272.39.24679]

68 **Korbecki J**, Baranowska-Bosiacka I, Gutowska I, Chlubek D. Cyclooxygenase pathways. *Acta Biochim Pol* 2014; **61**: 639-649 [PMID: 25343148]

69 **Lawrence T**, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat Rev Immunol* 2011; **11**: 750-761 [PMID: 22025054 DOI: 10.1038/nri3088]

70 **Inagaki T**, Sakai J, Kajimura S. Transcriptional and epigenetic control of brown and beige adipose cell fate and function. *Nat Rev Mol Cell Biol* 2016; **17**: 480-495 [PMID: 27251423 DOI: 10.1038/nrm.2016.62]

71 **Consoli A**, Devangelio E. Thiazolidinediones and inflammation. *Lupus* 2005; **14**: 794-797 [PMID: 16218490 DOI: 10.1191/0961203305lu2223oa]

72 **Staels B**, Fruchart JC. Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes* 2005; **54**: 2460-2470 [PMID: 16046315 DOI: 10.2337/diabetes.54.8.2460]

73 **Fritsche K**. Fatty acids as modulators of the immune response. *Annu Rev Nutr* 2006; **26**: 45-73 [PMID: 16848700 DOI: 10.1146/annurev.nutr.25.050304.092610]

74 **Kahn SE**, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G; ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; **355**: 2427-2443 [PMID: 17145742 DOI: 10.1056/NEJMoa066224]

75 **Gross B**, Pawlak M, Lefebvre P, Staels B. PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. *Nat Rev Endocrinol* 2017; **13**: 36-49 [PMID: 27636730 DOI: 10.1038/nrendo.2016.135]

76 **Lefterova MI**, Haakonsson AK, Lazar MA, Mandrup S. PPARγ and the global map of adipogenesis and beyond. *Trends Endocrinol Metab* 2014; **25**: 293-302 [PMID: 24793638 DOI: 10.1016/j.tem.2014.04.001]

77 **Zhang H**, Zhang A, Kohan DE, Nelson RD, Gonzalez FJ, Yang T. Collecting duct-specific deletion of peroxisome proliferator-activated receptor gamma blocks thiazolidinedione-induced fluid retention. *Proc Natl Acad Sci U S A* 2005; **102**: 9406-9411 [PMID: 15956187 DOI: 10.1073/pnas.0501744102]

78 **Di Gregorio GB**, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* 2005; **54**: 2305-2313 [PMID: 16046295 DOI: 10.2337/diabetes.54.8.2305]

79 **Nedergaard J**, Petrovic N, Lindgren EM, Jacobsson A, Cannon B. PPARgamma in the control of brown adipocyte differentiation. *Biochim Biophys Acta* 2005; **1740**: 293-304 [PMID: 15949696 DOI: 10.1016/j.bbadis.2005.02.003]

80 **Miranda CS**, Silva-Veiga FM, Fernandes-da-Silva A, Guimarães Pereira VR, Martins BC, Daleprane JB, Martins FF, Souza-Mello V. Peroxisome proliferator-activated receptors-alpha and gamma synergism modulate the gut-adipose tissue axis and mitigate obesity. *Mol Cell Endocrinol* 2023; **562**: 111839 [PMID: 36581062 DOI: 10.1016/j.mce.2022.111839]

81 **Kimura R**, Takahashi N, Goto T, Murota K, Kawada T. Activation of peroxisome proliferator-activated receptor-α (PPARα) in proximal intestine improves postprandial lipidemia in obese diabetic KK-Ay mice. *Obes Res Clin Pract* 2013; **7**: e353-e360 [PMID: 24455763 DOI: 10.1016/j.orcp.2013.05.005]

82 **Colin S**, Briand O, Touche V, Wouters K, Baron M, Pattou F, Hanf R, Tailleux A, Chinetti G, Staels B, Lestavel S. Activation of intestinal peroxisome proliferator-activated receptor-α increases high-density lipoprotein production. *Eur Heart J* 2013; **34**: 2566-2574 [PMID: 22843443 DOI: 10.1093/eurheartj/ehs227]

83 **Leung PS**. Overview of the pancreas. *Adv Exp Med Biol* 2010; **690**: 3-12 [PMID: 20700834 DOI: 10.1007/978-90-481-9060-7_1]

84 **Atkinson MA**, Campbell-Thompson M, Kusmartseva I, Kaestner KH. Organisation of the human pancreas in health and in diabetes. *Diabetologia* 2020; **63**: 1966-1973 [PMID: 32894306 DOI: 10.1007/s00125-020-05203-7]

85 **Bornman P**. The Pancreas: An Integrated Textbook of Basic Science, Medicine and Surgery. 2nd Ed. Oxford: Oxford University Press, 2008

86 **Cabrera O**, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A* 2006; **103**: 2334-2339 [PMID: 16461897 DOI: 10.1073/pnas.0510790103]

87 **Steiner DJ**, Kim A, Miller K, Hara M. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. *Islets* 2010; **2**: 135-145 [PMID: 20657742 DOI: 10.4161/isl.2.3.11815]

88 **Noguchi GM**, Huising MO. Integrating the inputs that shape pancreatic islet hormone release. *Nat Metab* 2019; **1**: 1189-1201 [PMID: 32694675 DOI: 10.1038/s42255-019-0148-2]

89 **Vickers MH**, Reddy S, Ikenasio BA, Breier BH. Dysregulation of the adipoinsular axis -- a mechanism for the pathogenesis of hyperleptinemia and adipogenic diabetes induced by fetal programming. *J Endocrinol* 2001; **170**: 323-332 [PMID: 11479129 DOI: 10.1677/joe.0.1700323]

90 **Martínez-Sánchez N**. There and Back Again: Leptin Actions in White Adipose Tissue. *Int J Mol Sci* 2020; **21** [PMID: 32839413 DOI: 10.3390/ijms21176039]

91 **Cerf ME**. High fat diet modulation of glucose sensing in the beta-cell. *Med Sci Monit* 2007; **13**: RA12-RA17 [PMID: 17179917]

92 **Cerf ME**. Beta Cell Physiological Dynamics and Dysfunctional Transitions in Response to Islet Inflammation in Obesity and Diabetes. *Metabolites* 2020; **10** [PMID: 33182622 DOI: 10.3390/metabo10110452]

93 **Souza-Mello V**, Gregório BM, Cardoso-de-Lemos FS, de Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. *Clin Sci (Lond)* 2010; **119**: 239-250 [PMID: 20415664 DOI: 10.1042/CS20100061]

94 **Pennings N**, Jaber J, Ahiawodzi P. Ten-year weight gain is associated with elevated fasting insulin levels and precedes glucose elevation. *Diabetes Metab Res Rev* 2018; **34**: e2986 [PMID: 29392827 DOI: 10.1002/dmrr.2986]

95 **Janikiewicz J**, Hanzelka K, Kozinski K, Kolczynska K, Dobrzyn A. Islet β-cell failure in type 2 diabetes--Within the network of toxic lipids. *Biochem Biophys Res Commun* 2015; **460**: 491-496 [PMID: 25843796 DOI: 10.1016/j.bbrc.2015.03.153]

96 **Graus-Nunes F**, Marinho TS, Barbosa-da-Silva S, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. Differential effects of angiotensin receptor blockers on pancreatic islet remodelling and glucose homeostasis in diet-induced obese mice. *Mol Cell Endocrinol* 2017; **439**: 54-64 [PMID: 27780713 DOI: 10.1016/j.mce.2016.10.021]

97 **Souza-Mello V**, Gregório BM, Relvas-Lucas B, da Silva Faria T, Aguila MB, Mandarim-de-Lacerda CA. Pancreatic ultrastructural enhancement due to telmisartan plus sitagliptin treatment in diet-induced obese C57BL/6 mice. *Pancreas* 2011; **40**: 715-722 [PMID: 21602737 DOI: 10.1097/MPA.0b013e3182153922]

98 **Fernandes-da-Silva A**, Miranda CS, Santana-Oliveira DA, Oliveira-Cordeiro B, Rangel-Azevedo C, Silva-Veiga FM, Martins FF, Souza-Mello V. Endoplasmic reticulum stress as the basis of obesity and metabolic diseases: focus on adipose tissue, liver, and pancreas. *Eur J Nutr* 2021; **60**: 2949-2960 [PMID: 33742254 DOI: 10.1007/s00394-021-02542-y]

99 **Sun J**, Cui J, He Q, Chen Z, Arvan P, Liu M. Proinsulin misfolding and endoplasmic reticulum stress during the development and progression of diabetes. *Mol Aspects Med* 2015; **42**: 105-118 [PMID: 25579745 DOI: 10.1016/j.mam.2015.01.001]

100 **Cerf ME**. High fat programming of beta-cell failure. *Adv Exp Med Biol* 2010; **654**: 77-89 [PMID: 20217495 DOI: 10.1007/978-90-481-3271-3_5]

101 **Cho JH**, Kim JW, Shin JA, Shin J, Yoon KH. β-cell mass in people with type 2 diabetes. *J Diabetes Investig* 2011; **2**: 6-17 [PMID: 24843456 DOI: 10.1111/j.2040-1124.2010.00072.x]

102 **Nauck MA**, Meier JJ. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *Lancet Diabetes Endocrinol* 2016; **4**: 525-536 [PMID: 26876794 DOI: 10.1016/S2213-8587(15)00482-9]

103 **Holst JJ**, Knop FK, Vilsbøll T, Krarup T, Madsbad S. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. *Diabetes Care* 2011; **34** Suppl 2: S251-S257 [PMID: 21525464 DOI: 10.2337/dc11-s227]

104 **Knop FK**, Aaboe K, Vilsbøll T, Vølund A, Holst JJ, Krarup T, Madsbad S. Impaired incretin effect and fasting hyperglucagonaemia characterizing type 2 diabetic subjects are early signs of dysmetabolism in obesity. *Diabetes Obes Metab* 2012; **14**: 500-510 [PMID: 22171657 DOI: 10.1111/j.1463-1326.2011.01549.x]

105 **Katahira T**, Miyatsuka T, Miura M, Suzuki L, Himuro M, Nishida Y, Satoh H, Watada H. Conversion of pancreatic α cells into insulin-producing cells modulated by β-cell insufficiency and supplemental insulin administration. *Biochem Biophys Res Commun* 2020; **521**: 178-183 [PMID: 31653346 DOI: 10.1016/j.bbrc.2019.10.100]

106 **Fruchart JC**. Peroxisome proliferator-activated receptor-alpha (PPARalpha): at the crossroads of obesity, diabetes and cardiovascular disease. *Atherosclerosis* 2009; **205**: 1-8 [PMID: 19386311 DOI: 10.1016/j.atherosclerosis.2009.03.008]

107 **Jay MA**, Ren J. Peroxisome proliferator-activated receptor (PPAR) in metabolic syndrome and type 2 diabetes mellitus. *Curr Diabetes Rev* 2007; **3**: 33-39 [PMID: 18220654 DOI: 10.2174/157339907779802067]

108 **Gilde AJ**, van der Lee KA, Willemsen PH, Chinetti G, van der Leij FR, van der Vusse GJ, Staels B, van Bilsen M. Peroxisome proliferator-activated receptor (PPAR) alpha and PPARbeta/delta, but not PPARgamma, modulate the expression of genes involved in cardiac lipid metabolism. *Circ Res* 2003; **92**: 518-524 [PMID: 12600885 DOI: 10.1161/01.Res.0000060700.55247.7c]

109 **Planavila A**, Laguna JC, Vázquez-Carrera M. Nuclear factor-kappaB activation leads to down-regulation of fatty acid oxidation during cardiac hypertrophy. *J Biol Chem* 2005; **280**: 17464-17471 [PMID: 15728586 DOI: 10.1074/jbc.M414220200]

110 **Patsouris D**, Mandard S, Voshol PJ, Escher P, Tan NS, Havekes LM, Koenig W, März W, Tafuri S, Wahli W, Müller M, Kersten S. PPARalpha governs glycerol metabolism. *J Clin Invest* 2004; **114**: 94-103 [PMID: 15232616 DOI: 10.1172/JCI20468]

111 **Lefebvre P**, Chinetti G, Fruchart JC, Staels B. Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J Clin Invest* 2006; **116**: 571-580 [PMID: 16511589 DOI: 10.1172/jci27989]

112 **Nakasatomi M**, Kim H, Arai T, Hirako S, Shioda S, Iizuka Y, Sakurai K, Matsumoto A. Fish oil and fenofibrate inhibit pancreatic islet hypertrophy, and improve glucose and lipid metabolic dysfuntions with different ways in diabetic KK mice. *Obes Res Clin Pract* 2018; **12**: 29-38 [PMID: 27130153 DOI: 10.1016/j.orcp.2016.03.012]

113 **Zheng S**, Ren X, Han T, Chen Y, Qiu H, Liu W, Hu Y. Fenofibrate attenuates fatty acid-induced islet β-cell dysfunction and apoptosis *via* inhibiting the NF-κB/MIF dependent inflammatory pathway. *Metabolism* 2017; **77**: 23-38 [PMID: 28941594 DOI: 10.1016/j.metabol.2017.09.001]

114 **Holm LJ**, Haupt-Jorgensen M, Giacobini JD, Hasselby JP, Bilgin M, Buschard K. Fenofibrate increases very-long-chain sphingolipids and improves blood glucose homeostasis in NOD mice. *Diabetologia* 2019; **62**: 2262-2272 [PMID: 31410530 DOI: 10.1007/s00125-019-04973-z]

115 **Liu SN**, Liu Q, Li LY, Huan Y, Sun SJ, Shen ZF. Long-term fenofibrate treatment impaired glucose-stimulated insulin secretion and up-regulated pancreatic NF-kappa B and iNOS expression in monosodium glutamate-induced obese rats: is that a latent disadvantage? *J Transl Med* 2011; **9**: 176 [PMID: 21999347 DOI: 10.1186/1479-5876-9-176]

116 **Yoshikawa H**, Tajiri Y, Sako Y, Hashimoto T, Umeda F, Nawata H. Effects of bezafibrate on beta-cell function of rat pancreatic islets. *Eur J Pharmacol* 2001; **426**: 201-206 [PMID: 11527545 DOI: 10.1016/s0014-2999(01)01204-3]

117 **Tenenbaum H**, Behar S, Boyko V, Adler Y, Fisman EZ, Tanne D, Lapidot M, Schwammenthal E, Feinberg M, Matas Z, Motro M, Tenenbaum A. Long-term effect of bezafibrate on pancreatic beta-cell function and insulin resistance in patients with diabetes. *Atherosclerosis* 2007; **194**: 265-271 [PMID: 16970952 DOI: 10.1016/j.atherosclerosis.2006.08.005]

118 **Semple RK**, Chatterjee VK, O'Rahilly S. PPAR gamma and human metabolic disease. *J Clin Invest* 2006; **116**: 581-589 [PMID: 16511590 DOI: 10.1172/jci28003]

119 **Berthiaume M**, Sell H, Lalonde J, Gélinas Y, Tchernof A, Richard D, Deshaies Y. Actions of PPARgamma agonism on adipose tissue remodeling, insulin sensitivity, and lipemia in absence of glucocorticoids. *Am J Physiol Regul Integr Comp Physiol* 2004; **287**: R1116-R1123 [PMID: 15256367 DOI: 10.1152/ajpregu.00339.2004]

120 **Armoni M**, Harel C, Bar-Yoseph F, Milo S, Karnieli E. Free fatty acids repress the GLUT4 gene expression in cardiac muscle *via* novel response elements. *J Biol Chem* 2005; **280**: 34786-34795 [PMID: 16096283 DOI: 10.1074/jbc.M502740200]

121 **Panunti B**, Fonseca V. Effects of PPAR gamma agonists on cardiovascular function in obese, non-diabetic patients. *Vascul Pharmacol* 2006; **45**: 29-35 [PMID: 16777491 DOI: 10.1016/j.vph.2005.11.013]

122 **Tontonoz P**, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem* 2008; **77**: 289-312 [PMID: 18518822 DOI: 10.1146/annurev.biochem.77.061307.091829]

123 **Nagashima K**, Lopez C, Donovan D, Ngai C, Fontanez N, Bensadoun A, Fruchart-Najib J, Holleran S, Cohn JS, Ramakrishnan R, Ginsberg HN. Effects of the PPARgamma agonist pioglitazone on lipoprotein metabolism in patients with type 2 diabetes mellitus. *J Clin Invest* 2005; **115**: 1323-1332 [PMID: 15841215 DOI: 10.1172/JCI23219]

124 **Rhee EJ**, Oh KW, Lee WY, Kim SY, Oh ES, Baek KH, Kang MI, Kim SW. Effects of two common polymorphisms of peroxisome proliferator-activated receptor-gamma gene on metabolic syndrome. *Arch Med Res* 2006; **37**: 86-94 [PMID: 16314192 DOI: 10.1016/j.arcmed.2005.04.008]

125 **Barnett AH**. Redefining the role of thiazolidinediones in the management of type 2 diabetes. *Vasc Health Risk Manag* 2009; **5**: 141-151 [PMID: 19436665 DOI: 10.2147/vhrm.s4664]

126 **Jandeleit-Dahm KA**, Calkin A, Tikellis C, Thomas M. Direct antiatherosclerotic effects of PPAR agonists. *Curr Opin Lipidol* 2009; **20**: 24-29 [PMID: 19133407 DOI: 10.1097/mol.0b013e32831f1b18]

127 **Goldberg RB**, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA, Tan MH, Khan MA, Perez AT, Jacober SJ; GLAI Study Investigators. A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 2005; **28**: 1547-1554 [PMID: 15983299 DOI: 10.2337/diacare.28.7.1547]

128 **Deeg MA**, Buse JB, Goldberg RB, Kendall DM, Zagar AJ, Jacober SJ, Khan MA, Perez AT, Tan MH; GLAI Study Investigators. Pioglitazone and rosiglitazone have different effects on serum lipoprotein particle concentrations and sizes in patients with type 2 diabetes and dyslipidemia. *Diabetes Care* 2007; **30**: 2458-2464 [PMID: 17595355 DOI: 10.2337/dc06-1903]

129 **Nesto RW**, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, Le Winter M, Porte D, Semenkovich CF, Smith S, Young LH, Kahn R; American Heart Association; American Diabetes Association. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. October 7, 2003. *Circulation* 2003; **108**: 2941-2948 [PMID: 14662691 DOI: 10.1161/01.Cir.0000103683.99399.7e]

130 **Sotiropoulos KB**, Clermont A, Yasuda Y, Rask-Madsen C, Mastumoto M, Takahashi J, Della Vecchia K, Kondo T, Aiello LP, King GL. Adipose-specific effect of rosiglitazone on vascular permeability and protein kinase C activation: novel mechanism for PPARgamma agonist's effects on edema and weight gain. *FASEB J* 2006; **20**: 1203-1205 [PMID: 16672634 DOI: 10.1096/fj.05-4617fje]

131 **Yang T**, Soodvilai S. Renal and vascular mechanisms of thiazolidinedione-induced fluid retention. *PPAR Res* 2008; **2008**: 943614 [PMID: 18784848 DOI: 10.1155/2008/943614]

132 **Collier JJ**, Batdorf HM, Merrifield KL, Martin TM, White U, Ravussin E, Burk DH, Cooley CR, Karlstad MD, Burke SJ. Pioglitazone Reverses Markers of Islet Beta-Cell De-Differentiation in db/db Mice While Modulating Expression of Genes Controlling Inflammation and Browning in White Adipose Tissue from Insulin-Resistant Mice and Humans. *Biomedicines* 2021; **9** [PMID: 34572374 DOI: 10.3390/biomedicines9091189]

133 **Hong SW**, Lee J, Cho JH, Kwon H, Park SE, Rhee EJ, Park CY, Oh KW, Park SW, Lee WY. Pioglitazone Attenuates Palmitate-Induced Inflammation and Endoplasmic Reticulum Stress in Pancreatic β-Cells. *Endocrinol Metab (Seoul)* 2018; **33**: 105-113 [PMID: 29589392 DOI: 10.3803/EnM.2018.33.1.105]

134 **Refaat R**, Sakr A, Salama M, El Sarha A. Combination of Vildagliptin and Pioglitazone in Experimental Type 2 Diabetes in Male Rats. *Drug Dev Res* 2016; **77**: 300-309 [PMID: 27520857 DOI: 10.1002/ddr.21324]

135 **Alba M**, Ahrén B, Inzucchi SE, Guan Y, Mallick M, Xu L, O'Neill EA, Williams-Herman DE, Kaufman KD, Goldstein BJ. Sitagliptin and pioglitazone provide complementary effects on postprandial glucose and pancreatic islet cell function. *Diabetes Obes Metab* 2013; **15**: 1101-1110 [PMID: 23782502 DOI: 10.1111/dom.12145]

136 **Benndorf RA**, Rudolph T, Appel D, Schwedhelm E, Maas R, Schulze F, Silberhorn E, Böger RH. Telmisartan improves insulin sensitivity in nondiabetic patients with essential hypertension. *Metabolism* 2006; **55**: 1159-1164 [PMID: 16919533 DOI: 10.1016/j.metabol.2006.04.013]

137 **Zhao S**, Chan LK, Chen L, Cheng TW, Klein T, Leung PS. Combination of Telmisartan and Linagliptin Preserves Pancreatic Islet Cell Function and Morphology in db/db Mice. *Pancreas* 2016; **45**: 584-592 [PMID: 26495783 DOI: 10.1097/MPA.0000000000000505]

138 **Willson TM**, Brown PJ, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. *J Med Chem* 2000; **43**: 527-550 [PMID: 10691680 DOI: 10.1021/jm990554g]

139 **Wallenius K**, Kjellstedt A, Thalén P, Löfgren L, Oakes ND. The PPAR α / γ Agonist, Tesaglitazar, Improves Insulin Mediated Switching of Tissue Glucose and Free Fatty Acid Utilization In Vivo in the Obese Zucker Rat. *PPAR Res* 2013; **2013**: 305347 [PMID: 24285952 DOI: 10.1155/2013/305347]

140 **Harrity T**, Farrelly D, Tieman A, Chu C, Kunselman L, Gu L, Ponticiello R, Cap M, Qu F, Shao C, Wang W, Zhang H, Fenderson W, Chen S, Devasthale P, Jeon Y, Seethala R, Yang WP, Ren J, Zhou M, Ryono D, Biller S, Mookhtiar KA, Wetterau J, Gregg R, Cheng PT, Hariharan N. Muraglitazar, a novel dual (alpha/gamma) peroxisome proliferator-activated receptor activator, improves diabetes and other metabolic abnormalities and preserves beta-cell function in db/db mice. *Diabetes* 2006; **55**: 240-248 [PMID: 16380499]

141 **Sun Y**, Alexander SP, Kendall DA, Bennett AJ. Cannabinoids and PPARalpha signalling. *Biochem Soc Trans* 2006; **34**: 1095-1097 [PMID: 17073758 DOI: 10.1042/BST0341095]

142 **Mittra S**, Sangle G, Tandon R, Sharma S, Roy S, Khanna V, Gupta A, Sattigeri J, Sharma L, Priyadarsiny P, Khattar SK, Bora RS, Saini KS, Bansal VS. Increase in weight induced by muraglitazar, a dual PPARalpha/gamma agonist, in db/db mice: adipogenesis/or oedema? *Br J Pharmacol* 2007; **150**: 480-487 [PMID: 17211457 DOI: 10.1038/sj.bjp.0707000]

143 **Trefts E**, Gannon M, Wasserman DH. The liver. *Curr Biol* 2017; **27**: R1147-R1151 [PMID: 29112863 DOI: 10.1016/j.cub.2017.09.019]

144 **Mahadevan V**. Anatomy of the liver. *Surgery (Oxford)* 2020; **38**: 427-431 [DOI: 10.1016/j.mpsur.2014.10.004]

145 **Gebhardt R**, Matz-Soja M. Liver zonation: Novel aspects of its regulation and its impact on homeostasis. *World J Gastroenterol* 2014; **20**: 8491-8504 [PMID: 25024605 DOI: 10.3748/wjg.v20.i26.8491]

146 **Jungermann K**, Sasse D. Heterogeneity of liver parenchymal cells. *Trends Biochem Sci* 1978; **3**: 198-202 [DOI: 10.1016/S0968-0004(78)91764-4]

147 **Vasconcellos R**, Alvarenga ÉC, Parreira RC, Lima SS, Resende RR. Exploring the cell signalling in hepatocyte differentiation. *Cell Signal* 2016; **28**: 1773-1788 [PMID: 27555287 DOI: 10.1016/j.cellsig.2016.08.011]

148 **Wang M**, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature* 2016; **529**: 326-335 [PMID: 26791723 DOI: 10.1038/nature17041]

149 **Nassir F**. NAFLD: Mechanisms, Treatments, and Biomarkers. *Biomolecules* 2022; **12** [PMID: 35740949 DOI: 10.3390/biom12060824]

150 **Rui L**. Energy metabolism in the liver. *Compr Physiol* 2014; **4**: 177-197 [PMID: 24692138 DOI: 10.1002/cphy.c130024]

151 **Bechmann LP**, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. *J Hepatol* 2012; **56**: 952-964 [PMID: 22173168 DOI: 10.1016/j.jhep.2011.08.025]

152 **Lebeaupin C**, Vallée D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J Hepatol* 2018; **69**: 927-947 [PMID: 29940269 DOI: 10.1016/j.jhep.2018.06.008]

153 **Magliano DC**, Bargut TC, de Carvalho SN, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. Peroxisome proliferator-activated receptors-alpha and gamma are targets to treat offspring from maternal diet-induced obesity in mice. *PLoS One* 2013; **8**: e64258 [PMID: 23700465 DOI: 10.1371/journal.pone.0064258]

154 **Walter P**, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011; **334**: 1081-1086 [PMID: 22116877 DOI: 10.1126/science.1209038]

155 **Oyadomari S**, Araki E, Mori M. Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. *Apoptosis* 2002; **7**: 335-345 [PMID: 12101393 DOI: 10.1023/a:1016175429877]

156 **Malhi H**, Kaufman RJ. Endoplasmic reticulum stress in liver disease. *J Hepatol* 2011; **54**: 795-809 [PMID: 21145844 DOI: 10.1016/j.jhep.2010.11.005]

157 **Kim YS**, Kim SG. Endoplasmic reticulum stress and autophagy dysregulation in alcoholic and non-alcoholic liver diseases. *Clin Mol Hepatol* 2020; **26**: 715-727 [PMID: 32951410 DOI: 10.3350/cmh.2020.0173]

158 **Ma X**, McKeen T, Zhang J, Ding WX. Role and Mechanisms of Mitophagy in Liver Diseases. *Cells* 2020; **9** [PMID: 32244304 DOI: 10.3390/cells9040837]

159 **Su Q**, Baker C, Christian P, Naples M, Tong X, Zhang K, Santha M, Adeli K. Hepatic mitochondrial and ER stress induced by defective PPAR α signaling in the pathogenesis of hepatic steatosis. *Am J Physiol Endocrinol Metab* 2014; **306**: E1264-E1273 [PMID: 24735884 DOI: 10.1152/ajpendo.00438.2013]

160 **Badmus OO**, Hillhouse SA, Anderson CD, Hinds TD, Stec DE. Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): functional analysis of lipid metabolism pathways. *Clin Sci (Lond)* 2022; **136**: 1347-1366 [PMID: 36148775 DOI: 10.1042/CS20220572]

161 **Zhang T**, Liu Q, Gao W, Sehgal SA, Wu H. The multifaceted regulation of mitophagy by endogenous metabolites. *Autophagy* 2022; **18**: 1216-1239 [PMID: 34583624 DOI: 10.1080/15548627.2021.1975914]

162 **Liu L**, Li Y, Wang J, Zhang D, Wu H, Li W, Wei H, Ta N, Fan Y, Liu Y, Wang X, Pan X, Liao X, Zhu Y, Chen Q. Mitophagy receptor FUNDC1 is regulated by PGC-1α/NRF1 to fine tune mitochondrial homeostasis. *EMBO Rep* 2021; **22**: e50629 [PMID: 33554448 DOI: 10.15252/embr.202050629]

163 **Ke PY**. Mitophagy in the Pathogenesis of Liver Diseases. *Cells* 2020; **9** [PMID: 32235615 DOI: 10.3390/cells9040831]

164 **Kersten S**. Integrated physiology and systems biology of PPARα. *Mol Metab* 2014; **3**: 354-371 [PMID: 24944896 DOI: 10.1016/j.molmet.2014.02.002]

165 **Kersten S**, Stienstra R. The role and regulation of the peroxisome proliferator activated receptor alpha in human liver. *Biochimie* 2017; **136**: 75-84 [PMID: 28077274 DOI: 10.1016/j.biochi.2016.12.019]

166 **Aoyama T**, Peters JM, Iritani N, Nakajima T, Furihata K, Hashimoto T, Gonzalez FJ. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J Biol Chem* 1998; **273**: 5678-5684 [PMID: 9488698 DOI: 10.1074/jbc.273.10.5678]

167 **Abdelmegeed MA**, Yoo SH, Henderson LE, Gonzalez FJ, Woodcroft KJ, Song BJ. PPARalpha expression protects male mice from high fat-induced nonalcoholic fatty liver. *J Nutr* 2011; **141**: 603-610 [PMID: 21346097 DOI: 10.3945/jn.110.135210]

168 **Yoo J**, Jeong IK, Ahn KJ, Chung HY, Hwang YC. Fenofibrate, a PPARα agonist, reduces hepatic fat accumulation through the upregulation of TFEB-mediated lipophagy. *Metabolism* 2021; **120**: 154798 [PMID: 33984335 DOI: 10.1016/j.metabol.2021.154798]

169 **Lin J**, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jäger S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* 2004; **119**: 121-135 [PMID: 15454086 DOI: 10.1016/j.cell.2004.09.013]

170 **Weber M**, Mera P, Casas J, Salvador J, Rodríguez A, Alonso S, Sebastián D, Soler-Vázquez MC, Montironi C, Recalde S, Fucho R, Calderón-Domínguez M, Mir JF, Bartrons R, Escola-Gil JC, Sánchez-Infantes D, Zorzano A, Llorente-Cortes V, Casals N, Valentí V, Frühbeck G, Herrero L, Serra D. Liver CPT1A gene therapy reduces diet-induced hepatic steatosis in mice and highlights potential lipid biomarkers for human NAFLD. *FASEB J* 2020; **34**: 11816-11837 [PMID: 32666604 DOI: 10.1096/fj.202000678R]

171 **Larter CZ**, Yeh MM, Van Rooyen DM, Brooling J, Ghatora K, Farrell GC. Peroxisome proliferator-activated receptor-α agonist, Wy 14,643, improves metabolic indices, steatosis and ballooning in diabetic mice with non-alcoholic steatohepatitis. *J Gastroenterol Hepatol* 2012; **27**: 341-350 [PMID: 21929649 DOI: 10.1111/j.1440-1746.2011.06939.x]

172 **Silva-Veiga FM**, Miranda CS, Vasques-Monteiro IML, Souza-Tavares H, Martins FF, Daleprane JB, Souza-Mello V. Peroxisome proliferator-activated receptor-alpha activation and dipeptidyl peptidase-4 inhibition target dysbiosis to treat fatty liver in obese mice. *World J Gastroenterol* 2022; **28**: 1814-1829 [PMID: 35633911 DOI: 10.3748/wjg.v28.i17.1814]

173 **Silva-Veiga FM**, Miranda CS, Martins FF, Daleprane JB, Mandarim-de-Lacerda CA, Souza-Mello V. Gut-liver axis modulation in fructose-fed mice: a role for PPAR-alpha and linagliptin. *J Endocrinol* 2020; **247**: 11-24 [PMID: 32698143 DOI: 10.1530/JOE-20-0139]

174 **Liss KH**, Finck BN. PPARs and nonalcoholic fatty liver disease. *Biochimie* 2017; **136**: 65-74 [PMID: 27916647 DOI: 10.1016/j.biochi.2016.11.009]

175 **Dille M**, Nikolic A, Wahlers N, Fahlbusch P, Jacob S, Hartwig S, Lehr S, Kabra D, Klymenko O, Al-Hasani H, Kotzka J, Knebel B. Long-term adjustment of hepatic lipid metabolism after chronic stress and the role of FGF21. *Biochim Biophys Acta Mol Basis Dis* 2022; **1868**: 166286 [PMID: 34624498 DOI: 10.1016/j.bbadis.2021.166286]

176 **Pawlak M**, Lefebvre P, Staels B. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* 2015; **62**: 720-733 [PMID: 25450203 DOI: 10.1016/j.jhep.2014.10.039]

177 **Han L**, Shen WJ, Bittner S, Kraemer FB, Azhar S. PPARs: regulators of metabolism and as therapeutic targets in cardiovascular disease. Part II: PPAR-β/δ and PPAR-γ. *Future Cardiol* 2017; **13**: 279-296 [PMID: 28581362 DOI: 10.2217/fca-2017-0019]

178 **Gavrilova O**, Haluzik M, Matsusue K, Cutson JJ, Johnson L, Dietz KR, Nicol CJ, Vinson C, Gonzalez FJ, Reitman ML. Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. *J Biol Chem* 2003; **278**: 34268-34276 [PMID: 12805374 DOI: 10.1074/jbc.M300043200]

179 **Francque S**, Verrijken A, Caron S, Prawitt J, Paumelle R, Derudas B, Lefebvre P, Taskinen MR, Van Hul W, Mertens I, Hubens G, Van Marck E, Michielsen P, Van Gaal L, Staels B. PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. *J Hepatol* 2015; **63**: 164-173 [PMID: 25703085 DOI: 10.1016/j.jhep.2015.02.019]

180 **Mundi MS**, Velapati S, Patel J, Kellogg TA, Abu Dayyeh BK, Hurt RT. Evolution of NAFLD and Its Management. *Nutr Clin Pract* 2020; **35**: 72-84 [PMID: 31840865 DOI: 10.1002/ncp.10449]

181 **Janani C**, Ranjitha Kumari BD. PPAR gamma gene--a review. *Diabetes Metab Syndr* 2015; **9**: 46-50 [PMID: 25450819 DOI: 10.1016/j.dsx.2014.09.015]

182 **Della Pepa G**, Russo M, Vitale M, Carli F, Vetrani C, Masulli M, Riccardi G, Vaccaro O, Gastaldelli A, Rivellese AA, Bozzetto L. Pioglitazone even at low dosage improves NAFLD in type 2 diabetes: clinical and pathophysiological insights from a subgroup of the

TOSCA.IT randomised trial. *Diabetes Res Clin Pract* 2021; **178**: 108984 [PMID: 34311022 DOI: 10.1016/j.diabres.2021.108984]

183 **Ahn HY**, Kim HH, Hwang JY, Park C, Cho BY, Park YJ. Effects of Pioglitazone on Nonalcoholic Fatty Liver Disease in the Absence of Constitutive Androstane Receptor Expression. *PPAR Res* 2018; **2018**: 9568269 [PMID: 30363699 DOI: 10.1155/2018/9568269]

184 **You M**, Matsumoto M, Pacold CM, Cho WK, Crabb DW. The role of AMP-activated protein kinase in the action of ethanol in the liver. *Gastroenterology* 2004; **127**: 1798-1808 [PMID: 15578517 DOI: 10.1053/j.gastro.2004.09.049]

185 **Liu X**, Xu J, Rosenthal S, Zhang LJ, McCubbin R, Meshgin N, Shang L, Koyama Y, Ma HY, Sharma S, Heinz S, Glass CK, Benner C, Brenner DA, Kisseleva T. Identification of Lineage-Specific Transcription Factors That Prevent Activation of Hepatic Stellate Cells and Promote Fibrosis Resolution. *Gastroenterology* 2020; **158**: 1728-1744.e14 [PMID: 31982409 DOI: 10.1053/j.gastro.2020.01.027]

186 **Richter B**, Bandeira-Echtler E, Bergerhoff K, Clar C, Ebrahim SH. Rosiglitazone for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2007; **2007**: CD006063 [PMID: 17636824 DOI: 10.1002/14651858.CD006063.pub2]

187 **Richter B**, Bandeira-Echtler E, Bergerhoff K, Clar C, Ebrahim SH. Pioglitazone for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2006; **2006**: CD006060 [PMID: 17054272 DOI: 10.1002/14651858.CD006060.pub2]

188 **Lebovitz HE**. Thiazolidinediones: the Forgotten Diabetes Medications. *Curr Diab Rep* 2019; **19**: 151 [PMID: 31776781 DOI: 10.1007/s11892-019-1270-y]

189 **Horita S**, Nakamura M, Satoh N, Suzuki M, Seki G. Thiazolidinediones and Edema: Recent Advances in the Pathogenesis of Thiazolidinediones-Induced Renal Sodium Retention. *PPAR Res* 2015; **2015**: 646423 [PMID: 26074951 DOI: 10.1155/2015/646423]

190 **Gawrieh S**, Noureddin M, Loo N, Mohseni R, Awasty V, Cusi K, Kowdley KV, Lai M, Schiff E, Parmar D, Patel P, Chalasani N. Saroglitazar, a PPAR-α/γ Agonist, for Treatment of NAFLD: A Randomized Controlled Double-Blind Phase 2 Trial. *Hepatology* 2021; **74**: 1809-1824 [PMID: 33811367 DOI: 10.1002/hep.31843]

191 **Jain MR**, Giri SR, Bhoi B, Trivedi C, Rath A, Rathod R, Ranvir R, Kadam S, Patel H, Swain P, Roy SS, Das N, Karmakar E, Wahli W, Patel PR. Dual PPARα/γ agonist saroglitazar improves liver histopathology and biochemistry in experimental NASH models. *Liver Int* 2018; **38**: 1084-1094 [PMID: 29164820 DOI: 10.1111/Liv.13634]

192 **Wang Y**, Nakajima T, Gonzalez FJ, Tanaka N. PPARs as Metabolic Regulators in the Liver: Lessons from Liver-Specific PPAR-Null Mice. *Int J Mol Sci* 2020; **21** [PMID: 32192216 DOI: 10.3390/ijms21062061]

193 **Zingarelli B**, Piraino G, Hake PW, O'Connor M, Denenberg A, Fan H, Cook JA. Peroxisome proliferator-activated receptor {delta} regulates inflammation *via* NF-{kappa}B signaling in polymicrobial sepsis. *Am J Pathol* 2010; **177**: 1834-1847 [PMID: 20709805 DOI: 10.2353/ajpath.2010.091010]

194 **Alvarez-Guardia D**, Palomer X, Coll T, Serrano L, Rodríguez-Calvo R, Davidson MM, Merlos M, El Kochairi I, Michalik L, Wahli W, Vázquez-Carrera M. PPARβ/δ activation blocks lipid-induced inflammatory pathways in mouse heart and human cardiac cells. *Biochim Biophys Acta* 2011; **1811**: 59-67 [PMID: 21070867 DOI: 10.1016/j.bbalip.2010.11.002]

195 **Wahli W**, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol Metab* 2012; **23**: 351-363 [PMID: 22704720 DOI: 10.1016/j.tem.2012.05.001]

196 **Botta M**, Audano M, Sahebkar A, Sirtori CR, Mitro N, Ruscica M. PPAR Agonists and Metabolic Syndrome: An Established Role? *Int J Mol Sci* 2018; **19** [PMID: 29662003 DOI: 10.3390/ijms19041197]

197 **Korbecki J**, Bobiński R, Dutka M. Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. *Inflamm Res* 2019; **68**: 443-458 [PMID: 30927048 DOI: 10.1007/s00011-019-01231-1]

198 **Silva-Veiga FM**, Rachid TL, de Oliveira L, Graus-Nunes F, Mandarim-de-Lacerda CA, Souza-Mello V. GW0742 (PPAR-beta agonist) attenuates hepatic endoplasmic reticulum stress by improving hepatic energy metabolism in high-fat diet fed mice. *Mol Cell Endocrinol* 2018; **474**: 227-237 [PMID: 29580823 DOI: 10.1016/j.mce.2018.03.013]

199 **Lee CH**, Olson P, Hevener A, Mehl I, Chong LW, Olefsky JM, Gonzalez FJ, Ham J, Kang H, Peters JM, Evans RM. PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A* 2006; **103**: 3444-3449 [PMID: 16492734 DOI: 10.1073/pnas.0511253103]

200 **Souza-Mello V**. Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver disease. *World J Hepatol* 2015; **7**: 1012-1019 [PMID: 26052390 DOI: 10.4254/wjh.v7.i8.1012]

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MINIREVIEWS

Schistosomal (bilharzial) polyps: Travel through the colon and beyond

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Abstract

Schistosomiasis (bilharziasis) is a major neglected tropical disease. It is endemic in many tropical and subtropical communities. Schistosomal polyps (S. polyps) are not uncommon presentation of this infection. Although the colon is the most commonly affected organ, many other organs are affected. S. polyps are associated with a variable range of morbidity independent of the Schistosomal infection. S. polyps are frequently described in endemic areas and increasingly reported in non-endemic areas mainly among immigrants and visitors to the endemic areas. This review aimed to increase awareness of practitioners, especially gastroenterologists, for this peculiar type of polyps caused by this neglected infection hence improving patient outcomes. Web-based search of different databases was conducted for the literature focusing the development of S. polyps in the colon and other organs with analysis of the clinical manifestations, diagnosis and treatment. The following key words were used in the search, "Schistosomiasis" OR "Bilharziasis" AND "Polyps" OR "Polyp" AND "Colon" OR "Small intestine" OR " Duodenum" OR " Stomach" OR "Esophagus" OR " Gallbladder" OR" Pharynx" OR "Larynx" OR "Trachea" OR "Urinary bladder" OR " Ureter" OR "Renal Pelvis" OR "Urethra". All publication types including case reports, case series, original research, and review articles were retrieved and analyzed. S.

polyps are not infrequent presentation of acute or chronic Schistosomal infection. S. polyps are described in many organs including the bowel, genitourinary tract, skin, gallbladder and the larynx. Presentation of S. polyps is variable and depends on the site, number as well as the polyp size. The relationship of *S. polyps* to malignant transformation is a matter of discussion. Presence of S. polyps is sometimes the only manifestation of Schistosomiasis. Small polyps can be treated medically with praziquantel, while large accessible polyps are amendable for endoscopic excision through different polyp resection techniques. However, huge, complicated, non-accessible and suspicious polyps are indicated for surgical management or advanced endoscopic resection when appropriate. Clinicians and endoscopists should be aware about these facts when treating patients living in, immigrated from or visiting endemic areas.

Key Words: Schistosomiasis; Bilharziasis; *Schistosomal polyps*; Colon; Praziquantel

Core Tip: Schistosomiasis is associated with a wide range of pathological lesions including development of polyps. Colon is the commonest site for polyp development, however polyps are reported in many organs including urinary bladder, ureters, larynx, duodenum, small intestine, gallbladder, anus, uterine cervix and external genitalia. Schistosomal polyps are associated with a wide range of morbidity according to the polyp site, size and number. The malignant potential of these polyps is a hot point of discussion. Although small sized polyps can regress with medical therapy using praziquantel, large accessible polyps can be retrieved endoscopically. Complicated, huge and inaccessible polyps can be treated surgically.

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INTRODUCTION

Schistosomiasis is a major neglected tropical disease. It is endemic in many geographical regions mainly in Africa, Latin America and Asia**.** Many data have been published from endemic areas and also from areas where the infection is not likely to occur with diverse clinical presentations. In non-endemic areas the infection is reported among immigrants or visitors to endemic areas[1,2].

This parasitic infestation was described in early human history, with evidence of infection reported in the ancient Egyptian papers and probably other old civilizations. The infection was characterized in the 19th century by the German pathologist Theodor Bilharz; that is why it is named after him as "Bilharziasis". There are many species of the parasite. Human infection is likely caused by five species, namely *Schistosoma Mansoni, S. Haematobium, S. Japonicum, S. Intercalatum and S. Mekongi*[1]. It is obvious that, the infection is linked to water supplies because of the snail intermediate host settles in the water canals and hence agricultural communities are the ultimate victims of the infestations, although persons who came in contact with infected water are prone also to catch the infection.

The clinical manifestation of this infection is either acute or chronic. The acute manifestations are related to the invasion of the human body by the cercarial invading stage through the skin, migration within the body and the early stage of ovi-position. Chronic manifestations are related to the establishment of adult worms and trapping of the deposited ova within the tissues and consequently granuloma formation[1]. The acute presentations include constitutional manifestations with fever, myalgia, urticarial rashes and with ovi-position the manifestations will change to hematuria (urinary Schistosomiasis), diarrhea with blood (intestinal Schistosomiasis), and chronic blood loss; manifested as anemia[1,3]. In chronic cases, development of fibrosis is the hallmark of the disease and this results in devastating sequelae including portal hypertension, hepatic peri-portal fibrosis, and splenomegaly for intestinal Schistosomiasis, while urinary Schistosomiasis is associated with obstructive lesions, increased incidence of stone formation, chronic urinary tract infections, and urinary bladder malignancy[4].

The current review aimed to increase awareness of practitioners, especially gastroenterologists, for this peculiar type of polyps caused by this neglected infection and hence improve patient outcomes.

LITERATURE SEARCH

Web-based search of different databases was conducted, including PubMed/MEDLINE, Cochrane library, Web of Science, Ovid, Science Direct, Scopus, Directory of Open Access Journals, EBSCO HOST, ProQuest, Institute for Scientific Information, EBESCO, Egyptian knowledge bank, Google scholar, *Reference Citation Analysis* (https://www.referencecitationanalysis.com/) and the Research Gate, for relevant articles focusing on the development of Schistosomal polyps (S.

polyps) in the colon and other organs with analysis of the clinical manifestations, diagnosis and treatment. The following key words were used in the search, "Schistosomiasis" OR "Bilharziasis" AND "Polyps" OR "Polyp" AND " Colon" OR "Small intestine" OR " Duodenum" OR " Stomach" OR "Esophagus" OR " Gallbladder" OR" Pharynx" OR "Larynx" OR "Trachea" OR "Urinary bladder" OR " Ureter" OR "Renal Pelvis" OR "Urethra". All publication types including case reports, case series, original research, and review articles were retrieved and analyzed.

PATHOGENESIS OF S. POLYPS

The development of polyps in general is linked to hollow organs or organs with contact to space; this enables external growth. Consequently, polyps are frequently reported in the bowel rather than other organs. The final shelters for adults Schistosomes are the intestinal and pelvic venous plexuses whatever the species are and hence the development of S. polyps in the hollow organs drained by this plexuses is not strange, *e.g.*, colon and urinary bladder, while development of polyps in other organs, *e.g.*, skin is considered ectopic. Furthermore, S. polyps may develop in atypical sites, *e.g.*, duodenum, that are not drained by the intestinal or pelvic veins.

The development of polyps in different stages

Stage of ova deposition and trapping: The eggs laid by the adult parasite had a characteristic spine that helps it to dig and find way out, *e.g.*, through the colon mucosa to be released with the stool. The initial step in polyp formation starts when Bilharzial eggs are deposited and trapped within the superficial layers of submucosa. The submucosal connective tissue is delicate and not superficially bound by firmer tissue. Consequently, accumulation of large amounts of eggs, reactive cellular debris and vascular granulation tissue do occur. In the submucosa, the trapped ova produce a TH2 cell-mediated inflammatory response with many cellular and chemical elements being recruited to the area and granuloma formation begins. The hallmark of the granuloma is infiltration by eosinophils (eosinophilic granuloma)[1], however, during this exudative stage of granuloma formation, other inflammatory cells are seen including the epithelioid macrophages and mononuclear cells[1,5].

Stage of proliferation: Within the granuloma necrosis occur[5]. Healing of the developed necrotic foci is associated with fibrous connective tissue formation. Furthermore, the adjacent muscularis mucosa becomes hypertrophied[1,5]. The fibrous tissue in the submucosa and the hypertrophied muscularis mucosa form a barrier hindering the ova transit from the veins to the lumen, hence more ova are entrapped. Sometimes, the adult worms either alone or matted are seen lodged and obstructing small venules within the polyp[5].

Stage of growth and protrusion: The granuloma formation is the basic pathology unit of chronic Schistosomiasis. Ova entrapment induces a foreign body reaction with progressive inflammation and fibrosis (chronic granuloma). As this process continues, a nodule is formed that elevates the hypertrophied muscularis mucosa and mucosa to form the polyp [6]. The polyp's mucosa harbors many goblet cells which secrete large amounts of mucus and this matches the abdominal pain and passage of mucus with stool seen among those patients. Furthermore, the delicate, and highly vascular nature of the polyp facilitates bleeding with the passage of stools[1,5-7].

This previously described mechanism of fibrosis and ova entrapment explain why the Schistosomal eggs are concentrated within the polyps more frequently than in the surrounding mucosa and submucosa[8]. Furthermore, non-inflammatory polyps associated with Schistosomiasis including adenomatous, hamartomatous, and hyperplastic polyps have been reported[1,9].

Stage of fibrosis: When the infection became chronic, the developed granulomas are infiltrated with fibroblasts, and fibrosis does occur[2,10]. Consequently, the polyps may become fibrotic. Small polyps especially when medically treated with praziquantel (PZQ) can then regress in size[11].

SCHISTOSOMAL COLONIC POLYPS

Site

The most common site for the development of S. polyps is the left colon, mainly recto-sigmoid region[3,12] because mesenteric (especially inferior mesenteric) and pelvic venous plexuses are the final habitat of the adult parasite. However, no part of the colon is immune against development of S. polyps. The polyps were described in all parts of the colon mainly in the rectosigmoid[12], but also were described in the caecum[13], ileocaecal valve[14], appendix[15], ascending colon[16], transverse colon[17] as well as the descending colon[12], some reports documented the distribution of S. polyps all through the large bowel starting from the caecum up to the anus during the course of heavy infestations in endemic areas[18].

Number

Schistosomiasis in the endemic areas, is associated with development of multiple polyps and multiplicity correlates with the density of infection[3,18]. However, there are many reports that Schistosomiasis can manifest by single colon polyp discovered either incidentally during colonoscopy or complicated[13,16,19] even without any clinical manifestations suggestive of Schistosomal infection.

Size

Schistosomiasis is usually associated with small-sized polyps[1,3,12]. However, large-sized S. polyps have been described in many case reports[12,16,18,19]. We reported earlier[20] that, no part of the colon was immune against development of large S. polyps. Many cases presented with solitary S. polyps[13,16,19,21] even in absence of any Schistosomiasis-related colon inflammation[9,13,16,19,21].

Atypical presentations and complications

Schistosomal colonic polyps had a wide range of atypical presentations. Elbatee *et al*[19], Alyhari *et al*[7], and Al-Zubaidi *et al*[22], described colo-rectal cancer (CRC) like presentation due to huge polyp size, abnormal polyp morphology or both, respectively. Furthermore, Smith *et al*[23], described diffuse colonic polypoid masses with dysensteric features, a picture endoscopically indistinguishable from familial polyposis and severe ulcerative colitis. Polyp-like lesion in the appendix manifestated as acute appendicitis was reported in a Chinese woman by Zhu *et al*[15], while pan-colonic inflammation and polyposis were described in Kenia by Bosire *et al*[18].

S. POLYPS BEYOND THE COLON

Urinary bladder polyps due to *S. Haematobium* have long been described and development of polyps correlates with heaviness of the infection in endemic areas[23]. Urinary bladder S. polyps were also reported among visitors to endemic areas[24]. Urinary S. polyps are not limited to urinary bladder, they were described within the ureters with a prevalence of 5.9% (30/511) in a large Egyptian study[25]. Due to the anatomical constraints, ureteric S. polyps tend to be small[25].

The atypical sites of S. polyps (Table 1) were described not infrequently in the literature. As early as in 1951, Gilges [26], described a skin polyp in the clitoris area of an African child. Furthermore, Schistosomal vulvual polyps have been described as vulvual swelling in 9- and 11-year-old girls from endemic areas in Senegal[27] and Nigera[28] respectively.

An ectopic cervical polyp was reported in Puerto Rico by File *et al*[29], despite the light Schistosomal infection. While Eladl *et al*[30] reported an endocervical polyp containing granulomas rich in viable eggs of *S. Hematobium* in a 43-year-old Egyptian woman who manifested with vaginal bleeding.

A slowly growing anal polyp containing Schistosomal ova as well as adult worms was described in a young Brazilian adult by Raso *et al*[5], the parasites probably migrated to the anus through the veins of hemorrhoidal plexus and it was associated with troublesome mass lesion with intense pruritus.

Duodenal polyps due to schistosomiasis have been reported in the literature although not common. In the duodenum it is discovered during endoscopic evaluation for obscure anemia and/or abdominal pain. The described duodenal S. polyps are either small, multiple and sessile[31] or solitary and large[32]. One case of dull abdominal pain was diagnosed in the United States with Schistosomiasis complicating a huge duodenal Peutz-Jeghers hamartomatous polyp[9].

The small intestine is infrequently affected with S. polyps and due to its narrow lumen, which is usually characterized by obstructive manifestations and diagnosis is established either at laparotomy or more frequently retrograde through histopathologic assessment. Small bowel obstruction due to huge polyp/polypoid mass at the ileocaecl valave[14] was described among visitors as well as residents of endemic areas[33] and small bowel obstruction is the usual presentation of such cases.

Gallbladder S. polyps are infrequently reported in the literature, the developed polyps are usually small in size and grew in the fundus, hence obstructive presentation is not likely and it is usually manifest (if any) as right upper quadrant pain. Both urinary (*S. Haematobium*) and intestinal Schistosomiasis (*S. Mansoni* and *S. Japonicum*) have been associated with gallbladder Schistosomiasis[34,35].

Larynx is less commonly affected during the course of Schistosomiasis. Toppozada described S. polyp involving the right vocal cord of a 25-year-old Egyptian male presented with 5-mo progressive hoarseness of voice, surgical microscopic excision and pathological examination showed granuloma with terminal sine ova[36].

S. POLYPS AND MALIGNANT TRANSFORMATION

The malignant potential of S. polyps is debatable. Although the relationship of urinary Schistosomiasis to malignancy is well established, the direct tumorigenic impact of intestinal S. polyps is questionable.

The bowel S. polyps have been implicated in the development of CRC and liver (HCC) cancers. The evidence supporting the malignant potential of intestinal Schistosomiasis is in favor of *S. Japonicum* rather than *S. Mansoni*. The chronic inflammatory state induced by ovi-position in the submucosa and other tissues have been proposed as the potential mechanism linking *S. Japonicum* in the Far East to the development of CRC and liver cancer[37].

So far the link between *S. Mansoni* and CRC has been viewed as no more than an epidemiological association and most published literatures deny the precancerous potential of the *S. Mansoni*-associated lesions including the polyps[38]. However, the mood of clinicians is spoiled by the emerging evidence incriminating *S. Mansoni* as a potential carcinogen. A large Egyptian study proposed an association between *S. Mansoni* infection and CRC relying on the increased levels of carcinoembryonic antigen within Bilharzial polyps' tissue[39]. In addition, one case report[40] retrieved Schistosome ova from the CRC specimens during histologic examination. Parasitism is associated with DNA repair defects with resultant genome instability, a commonly reported anomaly in CRC[38]. Furthermore, an emerging evidence through biomolecular mechanisms suggests an association between *S. Mansoni* and human carcinogenesis mainly for HCC and CRC and this is

Table 1 Summary of atypical and ectopic schistosomal polyps

Ref.	Site	Size	Presentation	Source
Raso *et al*[5], 2013	Anus	Large (2.5 cm)	Swelling with pain and intense pruritus	Endemic area
Gilges[26], 1951	External genitalia (clitoris)	Not mentioned	Mass like	Report from endemic area
Eladl *et al*[30], 2012	Cervix	Large sized (3 mc)	Vaginal bleeding	Endemic area
File *et al*[29], 1998	Cervix	Large	Gynecologic	Common area of spread. Adult worms within the polyp
Dioussé *et al*[27], 2016	Vulva	Large	Mass like	Endemic area
Sahabi and Rabiu [28], 2017	Vulva	Large	Mass like	Endemic area
Gonzalez *et al*[9], 2021	Duodenum	Huge (≥ 4 cm)	Dull abdominal pain	Resident of non-endemic area with a history of travel to endemic areas
Altonbary *et al*[31], 2014	Duodenum	Diminutive (≤ 4 mm)	Abdominal pain and generalized lymphadenopathy	Endemic area
Thatcher *et al*[32], 1984	Duodenum	Large	Isolated polyp	Non-endemic area
Lamyman *et al*[14], 2006	Ileocaecal valve area	Large	Intestinal obstruction	Visitor to endemic areas
Ali *et al*[34], 2021	Gallbladder fundus	Small 6 mm	Vague Abdominal pain	Patient from endemic area
Ghimire *et al*[35], 2020	Gallbladder fundus	Diminutive 3 mm	Right hypochndrial pain	Non-endemic areas
Toppozada[36], 1985	Larynx, right vocal cord	Large	Hoarseness of voice	Endemic area

likely through many egg-related agents and Th2-immune mechanisms[41]. A single recent report described for the first time a concomitant infection with *S. Mekongi* and rectal cancer[42].

The association of *S. Haematobium* and the urinary bladder squamous cell cancer is well established[43] and that is why *S. Haematobium* is classified as group 1 definitive biological carcinogenic. This association is probable with all forms of urinary bladder- induced Schistosomal pathology including chronic bladder wall inflammation and irritation by the deposited ova, the induced ulcers, sandy patches and also to the developed S. polyps[44]. There is no clear evidence in the literature linking intestinal Schistosomiasis to bladder tumors and vice versa[9].

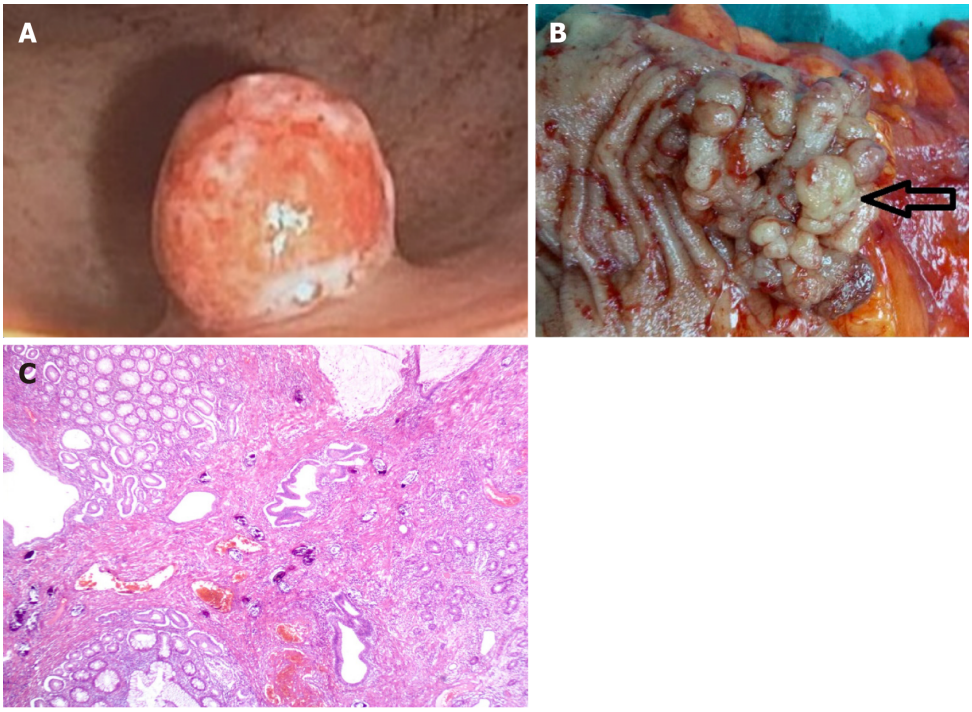
DIFFERENTIAL DIAGNOSIS

The severe forms of intestinal Schistosomiasis are characterized by the presence of multiple variable sized polyps against a background of diffuse mucosal affection showing mucosal erythema, and ulcerations, a picture mistaken with inflammatory bowel disease[3]. S. polyps of the colon are usually located in the recto-sigmoid region[12] and tend to be small[3, 12] (Figure 1A) and dark grey in color with surface ulceration, however other conditions with multiple colonic polyps should be considered in the differential diagnosis.

The solitary forms of S. polyps should be differentiated from other polyps according to the location. In fact, there is no characteristic morphology for S. polyps[45]. The huge polyps are commonly mistaken as malignant polyps (Figure 1B) and in such cases diagnosis is achieved through histopathology either by biopsy or after excision[19,21,22].

DIAGNOSIS

Diagnosis of polyps of the Schistosomal origin is sometimes challenging. There are no peculiar endoscopic morphologic features of S. polyps in comparison to other types of polyps and high index of suspicion is required especially in endemic areas. However, most cases are diagnosed either through endoscopic biopsy from the polyps or retrograde upon histopathology examination of removed polyps where the characteristic eosinophilic granulomas with the pathogenomic ova (Figure 2) of the parasite are seen [12-19,22] and infrequently the mature parasites may also be seen embedded within the background tissue of the excised polyps[22,29] or is seen lodged within thrombosed veins inside the polyp[5].



DOI: 10.3748/wjg.v29.i26.4156

Figure 1 Schistosomal polyp. A: Small Schistosomal polyp located in the rectum. Note its dark red color with minute surface ulcerations; B: Surgical specimen of huge lobulated Schistosomal polyp morphologically and radiological confused with colo-rectal cancer; C: Schistosomal polyp with noticeable mucoid hyperplasia.

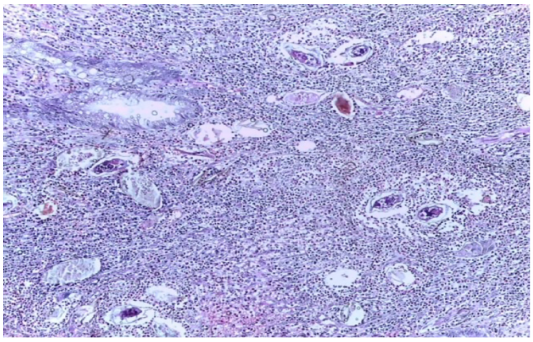


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Figure 2 Active schistosomal granuloma; multiple schistosomal eggs in the submucosa surrounded by cellular infiltrate composed of eosinophils, lymphocytes, plasma cells and macrophages.

Linking the developed polyps, whatever its location, with active intestinal or urinary Schistosomiasis is not always achievable. In many cases described in the literature, identification of Bilharzial ova in fresh stool or urine samples taken from the same cases was not possible[9,16,31]. Furthermore, S. polyps may be the only clue of a Bilharzial infection[19,31].

Barium enema was used frequently to diagnose Bilharzial polyps of the bowel[46] in endemic areas before the era of endoscopy. However, currently the role of contrast study in diagnosis of intestinal or urinary Schistosomiasis is not likely to replace the standard methods for direct ova detection in stool or urine samples or detection of the ova with its characteristic granuloma on endoscopic or surgical specimens due to low sensitivity and specificity of these contrast studies[47].

Upon histologic examination, the polyp had a stalk of fibrous connective tissue that project from the submucosa into the lumen and is covered with mucosa. The overlying mucosa harbor distorted glands showing variable degrees of mucoid activity (Figure 1C), mucinous degeneration, and adenomatous hyperplasia. The covering mucosa frequently had focal areas of ulceration. Larger areas of ulceration may be replaced by granulation tissue. Mononuclear cells, eosinophils, and few polymorphonuclear leucocytes infiltrate the mucosa[48].

The peculiar granuloma had a centrally located ova (viable and/or nonviable) surrounded by cellular infiltrate characteristically with eosinophils, mononuclear cells and multinucleated histiocytes (Figure 2)[1,5], chronic granulomas are surrounded by fibroblasts (Figure 3). The supporting tissue is composed of fibrous connective tissue and muscle derived from the muscularis mucosa. Blood vessels may be present in large numbers but diminish with progression of fibrosis [48].

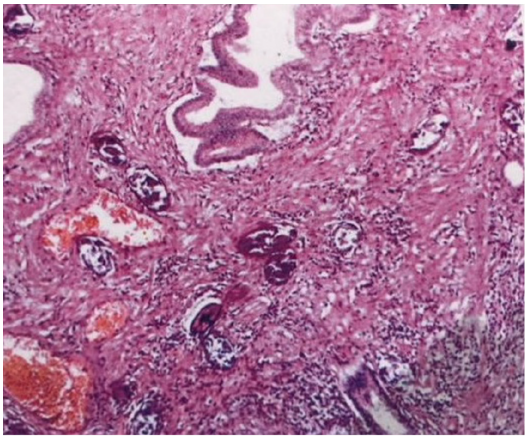

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Figure 3 Schistosomal granuloma infiltrated by fibroblasts with dense submucosal fibrosis.

TREATMENT

Medical treatment

Currently, PZQ is the recommended first-line medical therapy for Schistosomiasis. The drug is active and effective in treatment of all Schistosome species with no reports of resistance[49]. Small-sized polyps can be cured with PZQ therapy alone. The dose is 40-60 mg/kg, it is given as a single dose after a meal[1] and can be divided over one day[3,9], and can be repeated[7]. The side effects associated with PZQ are usually mild and include nausea, vomiting, abdominal cramps and allergic reactions. S. polyps are exceptional types of polyps that can regress with medical treatment[11] especially when they are small and are not associated with manifestations necessitating excision. All cases associated with large or ectopic polyps after being excised either endoscopically or surgically were followed by treatment with PZQ irrespective of the patients' active intestinal or urinary Schistosomiasis state[9,13,19,34].

Endoscopic treatment

Large polyps are indicated for excision. Endoscopic excision for S. polyps have been reported thoroughly in the literature as early as the 80s of the last century[50,51] with the early time of endoscopy. S. polyps were excised with different techniques including cold snare for small polyps, standard direct hot polypectomy for larger polyps whatever the site is the duodenum[9], or the colon[3]. Hot polypectomy after application of endoloop[52], or clips[13] has been associated with good outcomes. While advanced endoscopic resection techniques including endoscopic mucosal resection[21,22] and endoscopic submucosal dissection[53] were also used for endoscopic removal of large S. polyps. All endoscopic resection techniques were used with accepted safety profile. The adverse events reported were not different from those reported with other types of polyps[3,9,13,16,22]. Resection of S. polyps combined with medical treatment using PZQ was successful [3,9,13,16,22,48]. Endoscopic resection of urinary S. polyps is less frequently performed in comparison to bowel and was associated with acceptable success rates[24].

Surgical treatment

Surgical excision is not the standard of care in treatment of S. polyps. However, surgery is usually the ultimate solution for challenging situations including cases presented with bowel obstruction[14,33], or when the lesion is morphologically or radiologically suspected to be malignant[7,12,19] and also for lesions in ectopic areas including the anus[5], cervix[30] and the external genitalia[27,28]. Atypical sites of S. polyps may require special surgical approaches, *e.g.*, in the larynx, the surgical microscope is essential to excise the polyp[36], while in other situations, *e.g.*, gallbladder, the organ is excised with the polyp en bloc[34]. All cases managed by surgery, similar to endoscopic management, should be followed by PZQ therapy.

Outcomes of treatment

The outcome of S. polyps achieved by the above mentioned treatments depend on many factors. First, the number of lesions detected. For single lesions excision (either endoscopic or surgical) is usually curative, while multiple lesions may require more rounds of excision[3]. Second, patients' residency; patients residing in endemic areas are susceptible to re-infection and hence should follow the general preventive measures together with PZQ therapy that sometimes given as chemoprophylaxis or as mass treatment campaigns even without testing for the presence of the parasite[54], while patients out of the endemic areas a single course of PZQ is usually sufficient for treatment. Third, the location of the polyps, some polyps are accessible for endoscopic treatment, such as polyps in the colon, and urinary bladder, while other lesions, *e.g.*, gallbladder are not. Last, presentation of the lesions, such as lesions presented with acute manifestations including bowel obstruction, surgery usually the corner stone of treatment and in such cases diagnosis is often achieved retrograde[14,33].

CONCLUSION

Schistosomiasis is a neglected tropical disease and its prevalence is no more limited to endemic areas. S. polyps are not infrequent presentation of acute or chronic Schistosomal infection. S. polyps are described in many organs including the bowel, genitourinary tract, skin, gallbladder and the larynx. Presentation of S. polyps is variable and depends on the site, number as well as the polyp size. The relationship of S. polyps to malignant transformation is a matter of discussion. Small polyps can be treated medically with PZQ, while large accessible polyps are amendable for endoscopic excision through different polyp resection techniques. However, huge, complicated and suspicious polyps are indicated for surgical management or advanced endoscopic resection if appropriate. Clinicians and endoscopists should be aware about these facts when treating patients living in, immigrated from or visiting endemic areas.

FOOTNOTES

Author contributions: Emara MH, Mahros AM, Radwan MI, and Rasheda AMA developed concept of the study, and retrieved the evidence; Elbatae H, Emara MH, Abdelrazik O, and Elazab M searched the literature; Elbatae H, Emara MH, Abdelrazik O, Elazab M, Mahros AM, and Radwan MI analyzed the evidence; Emara MH, Rasheda AMA, Elazab M, and Mahros AM drafted the article; All authors revised the article.

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REFERENCES

1 **Elbaz T**, Esmat G. Hepatic and intestinal schistosomiasis: review. *J Adv Res* 2013; **4**: 445-452 [PMID: 25685451 DOI: 10.1016/j.jare.2012.12.001]
2 **Hams E**, Aviello G, Fallon PG. The schistosoma granuloma: friend or foe? *Front Immunol* 2013; **4**: 89 [PMID: 23596444 DOI: 10.3389/fimmu.2013.00089]
3 **Abd El-Latif N**, Bedewy E. Bilharzial Colonic Polyps: Case Presentation. *Afro-Egyptian J Infect Endemic Dis* 2020; **10**: 233-236 [DOI: 10.21608/aeji.2020.29738.1079]
4 **Barsoum RS**. Urinary schistosomiasis: review. *J Adv Res* 2013; **4**: 453-459 [PMID: 25685452 DOI: 10.1016/j.jare.2012.08.004]
5 **Raso P**, Sander EM, Raso LA, Andrade Filho Jde S. Anal polyp caused by Schistosoma mansoni. *Rev Soc Bras Med Trop* 2013; **46**: 252-254 [PMID: 23740071 DOI: 10.1590/0037-8682-1681-2013]
6 **Mostafa I**. Schistosomal colonic polyposis. *Gastrointest Endosc* 1997; **46**: 584-587 [PMID: 9434242]
7 **Alyhari Q**, Ahmed F, Al Shaibani H, Al Kubati M, Alhadi A. Huge Colonic Granuloma of Schistosomiasis Mimicked Cancer in a 10-Years-Old Child: A Case Report. *Int Med Case Rep J* 2022; **15**: 379-384 [PMID: 35903496 DOI: 10.2147/IMCRJ.S372186]
8 **Cao J**, Liu WJ, Xu XY, Zou XP. Endoscopic findings and clinicopathologic characteristics of colonic schistosomiasis: a report of 46 cases. *World J Gastroenterol* 2010; **16**: 723-727 [PMID: 20135720 DOI: 10.3748/wjg.v16.i6.723]
9 **Gonzalez A**, Gupta K, Rodriguez S, Wadhwa V, Bejarano P, Charles R. Unique Case of a Hamartomatous Duodenal Polyp Associated With Intestinal Schistosomiasis. *ACG Case Rep J* 2021; **8**: e00485 [PMID: 33532510 DOI: 10.14309/crj.0000000000000485]
10 **Pesce JT**, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, Smith AM, Thompson RW, Cheever AW, Murray PJ, Wynn TA. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog* 2009; **5**: e1000371 [PMID: 19360123 DOI: 10.1371/journal.ppat.1000371]
11 **Nebel OT**, el-Masry NA, Castell DO, Farid Z, Fornes MF, Sparks HA. Schistosomal disease of the colon: a reversible form of polyposis. *Gastroenterology* 1974; **67**: 939-943 [PMID: 4426495]
12 **Ahmed MH**, Emara MH, Elfert AA, El-Saka AM, Abd-Elsalam S, Yousef M. Persistent Colonic Schistosomiasis among Symptomatic Rural Inhabitants in the Egyptian Nile Delta. *Mediterr J Hematol Infect Dis* 2021; **13**: e2021033 [PMID: 34007421 DOI: 10.4084/MJHID.2021.033]
13 **Orare K**, Mohamed A, Thakkar M, Rajula A, Gatheru J. Colonic polyps: a rare clinical manifestation of schistosomiasis (a case report). *Pan Afr Med J* 2022; **42**: 187 [PMID: 36212935 DOI: 10.11604/pamj.2022.42.187.34227]
14 **Lamyman MJ**, Noble DJ, Narang S, Dehalvi N. Small bowel obstruction secondary to intestinal schistosomiasis. *Trans R Soc Trop Med Hyg* 2006; **100**: 885-887 [PMID: 16443245 DOI: 10.1016/j.trstmh.2005.10.013]

15 **Zhu XL**, Song JZ, Yu WY, Hua LQ, Zhang ML. Intestinal schistosomiasis masquerading as intestinal polyps. *BMC Infect Dis* 2021; **21**: 434 [PMID: 33964884 DOI: 10.1186/s12879-021-06125-4]

16 **Issa I**, Osman M, Aftimos G. Schistosomiasis manifesting as a colon polyp: a case report. *J Med Case Rep* 2014; **8**: 331 [PMID: 25296942 DOI: 10.1186/1752-1947-8-331]

17 **Lin CT**, Chen CY, Jao SW, Wu CC. An unusual pedunculated polyp of the transverse colon associated with schistosomiasis. *Endoscopy* 2010; **42** Suppl 2: E160 [PMID: 20556713 DOI: 10.1055/s-0029-1244142]

18 **Bosire F**, Orwa I, Mwachiro M, Parker R, Whit R. Schistosoma Mansoni Proctocolitis with Polyposis. *Ann African Sury* 2018; **15**: 81-85

19 **Elbatee HE**, Emara MH, Zaghloul MS, Ahmed MH, Radwan MI, Seif AS, Elmoghazy MB, Elkashef WF. Huge bilharzial polyp mimicking colon cancer. *JGH Open* 2020; **4**: 280-283 [PMID: 32280778 DOI: 10.1002/jgh3.12181]

20 **Emara MH**, Ahmed MH, Mahros AM, Amer IF, Elkerdawy MA. No part of the colon is immune from large Bilharzial polyps. *Eur J Gastroenterol Hepatol* 2020; **32**: 896-897 [PMID: 32472818 DOI: 10.1097/MEG.0000000000001727]

21 **Farooq A**, Hussain A, Singh C, Kadkhodayan K, Arain M, Hasan MK, Yang D. Large Colon Polyp as the Only Manifestation of Chronic Schistosomiasis Infection. *ACG Case Rep J* 2022; **9**: e00889 [PMID: 36237280 DOI: 10.14309/crj.0000000000000889]

22 **Al-Zubaidi AM**, Bashanfer GA, Alqannas MH, Al Atawi AS. Colonic Polyps an Unusual Manifestation of Schistosomiasis. *Am J Case Rep* 2020; **21**: e923177 [PMID: 32764533 DOI: 10.12659/AJCR.923177]

23 **Smith JH**, Torky H, Kelada AS, Farid Z. Schistosomal polyposis of the urinary bladder. *Am J Trop Med Hyg* 1977; **26**: 85-88 [PMID: 842788 DOI: 10.4269/ajtmh.1977.26.85]

24 **Dessyn JF**, Duquenne S, Hoarau G. Incidental pseudolymphomatous bladder inflammatory polyp revealing urinary schistosomiasis. *Int J Infect Dis* 2016; **53**: 39-40 [PMID: 27810520 DOI: 10.1016/j.ijid.2016.10.022]

25 **Saad SM**, Hanafy HM. Bilharzial (schistosomal) polypi of ureter. *Urology* 1974; **4**: 85-89 [PMID: 21322991 DOI: 10.1016/0090-4295(74)90114-9]

26 **Gilges W**. Skin polyp-only symptom of bilharzia infection. *East Afr Med J* 1951; **28**: 288 [PMID: 14872783]

27 **Dioussé P**, Dione H, Bammo M, Dial C, Thiam M, Diamé AA, Diop BM, Ka MM. Vulvar bilharzia on nine-year old girl: A case report. *Int J Case Rep Images* 2016; **7**: 724-728

28 **Sahabi MS**, Rabiu FA. Unusual presentation of schistosomiasis as vulval polyp. *Obs Gyne Review: J Obstet Gynecol* 2017; **3**: 33-37 [DOI: 10.17511/joog.2017.i04.01]

29 **File S**, Francheschini AB, Fernandez-Santiago A. Short report: A case of ectopic schistosomiasis in Puerto Rico with some observations on the biology of the parasite. *Am J Trop Med Hyg* 1998; **58**: 671-672 [PMID: 9598459 DOI: 10.4269/ajtmh.1998.58.671]

30 **Eladl AE**, Shebl AM, Elkashef WF, Zalata KR. Bilharzial endocervical polyp. *Indian J Pathol Microbiol* 2012; **55**: 377-378 [PMID: 23032837 DOI: 10.4103/0377-4929.101750]

31 **Altonbary A**, Bahgat M, Elkashef W. Bilharzial granuloma of the duodenum. *OA Case Rep* 2014; **3**: 62

32 **Thatcher BS**, Fleischer D, Rankin GB, Petras R. Duodenal schistosomiasis diagnosed by endoscopic biopsy of an isolated polyp. *Am J Gastroenterol* 1984; **79**: 927-929 [PMID: 6507419]

33 **Gul R**, Khalid K, Al-Rajhi MF, Bismar HA. Acute small bowel obstruction due to bilharziasis. *Saudi Med J* 2005; **26**: 1624-1626 [PMID: 16228068]

34 **Ali GA**, Goravey W, Al-Bozom I, Al Maslamani MA, Abdel Hadi H. Schistosoma gallbladder polyp masquerading as a neoplasm: Rare case report and literature review. *Clin Case Rep* 2021; **9**: e04420 [PMID: 34267906 DOI: 10.1002/ccr3.4420]

35 **Ghimire PG**, Ghimire P. Gallbladder schistosomiasis - a rare presentation as gallbladder polyp: a case report. *Radiol Case Rep* 2020; **15**: 1394-1397 [PMID: 32636981 DOI: 10.1016/j.radcr.2020.06.013]

36 **Toppozada HH**. Laryngeal bilharzia. *J Laryngol Otol* 1985; **99**: 1039-1041 [PMID: 4056583 DOI: 10.1017/s0022215100098145]

37 **Hamid HKS**. Schistosoma japonicum-Associated Colorectal Cancer: A Review. *Am J Trop Med Hyg* 2019; **100**: 501-505 [PMID: 30560774 DOI: 10.4269/ajtmh.18-0807]

38 **H Salim OE**, Hamid HK, Mekki SO, Suleiman SH, Ibrahim SZ. Colorectal carcinoma associated with schistosomiasis: a possible causal relationship. *World J Surg Oncol* 2010; **8**: 68 [PMID: 20704754 DOI: 10.1186/1477-7819-8-68]

39 **Abdulla H**, Khalil RY, Ibrahim MS, Elaish N, Abdulaziz A. The relationship between colonic Bilharzial polyposzs and colorectal cancer, Sci. *Med J ESCME* 1996; **8**: 129-140

40 **Herman AM**, Kishe A, Babu H, Shilanaiman H, Tarmohamed M, Lodhia J, Amsi P, Pyuza J, Mremi A, Mwasamwaja A, Nyindo M, Chilonga K, Msuya D. Colorectal cancer in a patient with intestinal schistosomiasis: a case report from Kilimanjaro Christian Medical Center Northern Zone Tanzania. *World J Surg Oncol* 2017; **15**: 146 [PMID: 28768520 DOI: 10.1186/s12957-017-1217-1]

41 **von Bülow V**, Lichtenberger J, Grevelding CG, Falcone FH, Roeb E, Roderfeld M. Does Schistosoma Mansoni Facilitate Carcinogenesis? *Cells* 2021; **10** [PMID: 34440754 DOI: 10.3390/cells10081982]

42 **Burky M**, Trembath D, Bookhout C. Rectal carcinoma arising in a patient with intestinal and hepatic schistosomiasis due to Schistosoma mekongi. *IDCases* 2022; **27**: e01383 [PMID: 35036322 DOI: 10.1016/j.idcr.2022.e01383]

43 **Mostafa MH**, Sheweita SA, O'Connor PJ. Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev* 1999; **12**: 97-111 [PMID: 9880476 DOI: 10.1128/CMR.12.1.97]

44 **Ishida K**, Hsieh MH. Understanding Urogenital Schistosomiasis-Related Bladder Cancer: An Update. *Front Med (Lausanne)* 2018; **5**: 223 [PMID: 30159314 DOI: 10.3389/fmed.2018.00223]

45 **Ye C**, Tan S, Jiang L, Li M, Sun P, Shen L, Luo H. Endoscopic characteristics and causes of misdiagnosis of intestinal schistosomiasis. *Mol Med Rep* 2013; **8**: 1089-1093 [PMID: 23969514 DOI: 10.3892/mmr.2013.1648]

46 **El-Masry NA**, Farid Z, Kilpatrick ME, Watten RH. Role of the barium enema in schistosomal colonic polyposis. *East Afr Med J* 1982; **59**: 465-468 [PMID: 7160324]

47 **Shebel HM**, Elsayes KM, Abou El Atta HM, Elguindy YM, El-Diasty TA. Genitourinary schistosomiasis: life cycle and radiologic-pathologic findings. *Radiographics* 2012; **32**: 1031-1046 [PMID: 22786992 DOI: 10.1148/rg.324115162]

48 **Mostafa IM**, Zakaria S, Khalil A, El-Kaluoby A. The effect of medical treatment and endoscopic polypectomy on clinicopathological, immunological and endoscopic aspects of schistosomal colonic polyposis in Egypt. In: Proceedings of the world congresses of gastroenterology 1990. Australia: Sydney; 1990: 1260

49 **Vale N**, Gouveia MJ, Rinaldi G, Brindley PJ, Gärtner F, Correia da Costa JM. Praziquantel for Schistosomiasis: Single-Drug Metabolism Revisited, Mode of Action, and Resistance. *Antimicrob Agents Chemother* 2017; **61** [PMID: 28264841 DOI: 10.1128/AAC.02582-16]

50 **Thakeb F**, El-Kalouby AH, Ibrahim IM, Hunter S, Zakaria S. Colonoscopic polypectomy in management of schistosomal colonic polyposis. *Egypt J Bilh* 1980; **7**: 49-56

51 **Bessa SM**, Helmy I, El-Kharadly Y. Colorectal schistosomiasis. Endoscopic polypectomy. *Dis Colon Rectum* 1983; **26**: 772-774 [PMID: 6641458 DOI: 10.1007/BF02554745]

52 **Ahmed S**, Babiker F, Elshafie A, Alhedaithy M, Yousif OE, Toulaymat M. Unusual Presentations of Intestinal Schistosomiasis. *Dr. Sulaiman Al Habib Med J* 2020; **2**: 33-37

53 **Probst A**, Schaller T, Ebigbo A, Messmann H. Colonic schistosomiasis and early rectal cancer: coincidence or causal relationship? *Endoscopy* 2014; **46** Suppl 1: E671 [PMID: 25526419 DOI: 10.1055/s-0034-1390863]

54 **Kabuyaya M**, Chimbari MJ, Mukaratirwa S. Efficacy of praziquantel treatment regimens in pre-school and school aged children infected with schistosomiasis in sub-Saharan Africa: a systematic review. *Infect Dis Poverty* 2018; **7**: 73 [PMID: 29986763 DOI: 10.1186/s40249-018-0448-x]

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MINIREVIEWS

Mortality from chronic liver disease: Recent trends and impact of the COVID-19 pandemic

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Abstract

Prepandemic time trends in mortality from chronic liver disease (CLD) differed according to specific cause of death (decreasing for liver cirrhosis, stable or increasing for liver cancer), etiology (increasing for nonalcoholic fatty liver disease, generally decreasing for other etiologies), and world region (decreasing in areas with the highest burden of hepatitis B virus, increasing in Eastern Europe and other countries). The coronavirus disease 2019 (COVID-19) pandemic affected mortality of patients with CLD both directly, with a higher risk for severe illness and death depending on age, stage and etiology of the disease, and indirectly, through social isolation and loss of support, harmful drinking, and difficulties in access to care. Nevertheless, only sparse data are available on variations in CLD as a cause of death during the pandemic. In the USA, in 2020-2021 a growth in mortality was registered for all liver diseases, more marked for alcoholic liver disease, especially among young people aged 25-44 years and in selected ethnic groups. COVID-19 related deaths accounted only for a minor part of the excess. Further data from mortality registers of other countries are warranted, preferably adopting the so-called multiple cause-of-death approach, and extended to deaths attributed to viral hepatitis and liver cancer.

Key Words: Mortality; Multiple causes of death; COVID-19; Chronic liver disease; Liver

cirrhosis; Liver cancer

Core tip: Preliminary data on causes of death during the coronavirus disease 2019 pandemic suggest that mortality from chronic liver disease (CLD) increased especially in countries where the alcoholic etiology was predominant, or with a prepandemic growing trend in mortality from alcoholic liver disease. Population-based studies on the direct and indirect effects of the pandemic on CLD mortality are strongly warranted. Analyses adopting the multiple cause-of-death approach might be better suited to fully investigate the impact of the pandemic on complex long-term pre-existing trends.

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INTRODUCTION

According to a systematic analysis of the Global Burden of Disease (GBD) Study 2017, deaths from cirrhosis and other chronic liver diseases (CLDs) were estimated at 1.32 million in 2017, compared to 889 000 in 1990. However, the corresponding age-standardized rate decreased from 21.0 to 16.5 per 100000 population. Rates decreased or remained constant in all regions except for Eastern Europe and Central Asia. Globally, age-standardized death rates associated to all main etiologies (alcohol, hepatitis B, and hepatitis C) declined, except for nonalcoholic steatohepatitis[1].

However, interpretation of mortality data on CLD poses several challenges. Cirrhosis mortality captures only a limited fraction of deaths due to CLD. In the USA, by adopting the standard definition for CLD mortality, a 38% decrease in rates was observed from 1979 to 2008; if other liver-related causes of death including viral hepatitis and liver cancer were examined, rates remained essentially unchanged[2]. Liver cirrhosis mortality dropped, whereas death rates for primary liver cancer were stable in Northeastern Italy in 1995-2010[3], and increased in China in 1987-2016[4]. A more comprehensive assessment on mortality from CLD can also be achieved by analyses not limited to the underlying cause of death, but extended to all conditions mentioned in death certificates. The so-called multiple cause-of-death approach can unveil rapidly changing trends limited to specific etiologies within selected birth cohorts. As an example, a rise in mortality related to hepatitis C virus (HCV) was registered in the USA in 2003-2013; most deaths were confined to the 1945-1965 birth cohort, affected by the highest prevalence of HCV infection[5]. A similar growth in HCV-related mortality within a specific birth cohort was observed in Northern Italy[6]. After 2014, time trends in the USA changed and mortality related to HCV started to decline, whereas death rates related to alcoholic liver disease and nonalcoholic fatty liver disease (NAFLD) were on the rise[7].

In the most recent years, patients with CLD represented a vulnerable population during the coronavirus disease 2019 (COVID-19) pandemic due to immune dysregulation and coagulopathy among those with advanced disease, and coexisting comorbidity, including obesity, diabetes and cardiovascular diseases. Notably, baseline liver disease stage and alcoholic liver disease were demonstrated to be independent risk factors for death from COVID-19[8]. Nevertheless, to date, population-based data on trends in CLD as a cause of death during the pandemic are lacking, except for an increase in mortality from liver cirrhosis reported from the USA already in its early phases[9]. We review here the available evidence on the impact of the pandemic on pre-existing time trends in mortality from CLD.

MORTALITY TRENDS BEFORE THE PANDEMIC

In 2017, there were 2.14 million estimated liver-related deaths; liver cirrhosis and liver cancer accounted for 61.7% and 38.3%, respectively. Between 2012 and 2017 the global age-standardized death rate increased (annual percent change, APC = 0.51%, 0.05%-0.98%) for liver cancer and decreased (APC = 0.70%, 1.01% to 0.40%) for liver cirrhosis. In Asia, the most common cause was hepatitis B virus (HBV) infection (highest in East Asia), whereas HCV was the most common cause in high-income Asia Pacific and Middle East and North Africa (MENA), and alcohol-related liver disease in Central Asia. Incidence and mortality associated with NAFLD increased globally, and this increase was recorded in almost every region worldwide[10].

Hepatitis B is the leading etiology for liver cancer mortality and the third largest contributor to deaths from cirrhosis. HBV-related diseases resulted in 555 000 global deaths in 2019; the number of HBV-related deaths increased between 1990 and 2019. By contrast, age-standardized death rates declined globally and in all world regions. Meanwhile, there was a 31.3% decrease in the prevalence of HBV at all ages[11]. Mortality trends related to hepatitis C are less pronounced; in 2010-2019 the decline in mortality was larger for acute hepatitis C than for HCV-related cirrhosis, with no significant change for HCV-related liver cancer[12].

A number of studies addressed global trends in liver cancer incidence and mortality. According to the GBD 2015 study, globally, HBV accounted for 33% of a total of 810 000 liver cancer deaths, alcohol for 30%, and HCV for 21%. The highest share for HBV was observed in Western sub-Saharan Africa, East Asia, and Andean Latin America; that for HCV in high-income Asia Pacific, MENA, Central and Southern Latin America, and Western Europe; and that for alcohol in Central and Eastern Europe. From 1999 to 2015, age-standardized mortality rates declined substantially in regions with high liver cancer burden such as East Asia and sub-Saharan Africa, probably due to the effect of primary liver cancer prevention through HBV vaccination[13]. These trends parallel those in liver cancer incidence. The burden in highly endemic regions has been partially alleviated due to improved control of viral hepatitis, especially among young and middle-aged people. By contrast, an unfavorable trend was observed in most developed countries and in older populations[14]. In fact, the most significant increases in incidence were generally observed in countries with a high sociodemographic index, including the UK and the USA[15]. Nevertheless, chronic HBV and HCV remain important risk factors for liver cancer. Based on the GLOBOCAN database, the overall increasing trend in incidence and mortality from liver cancer was confirmed among older male subjects, and in countries with a higher prevalence of HCV-related liver cancer. The increasing prevalence of alcohol consumption and obesity may have contributed to this epidemiological scenario in high-income countries[16].

As regards mortality from liver cirrhosis, analyses of the GBD 2017 study showed that deaths due to cirrhosis constituted 2.4% of total deaths globally. Central Asia had the highest age-standardized death rate, followed by Sub-Saharan Africa. Central Asia and Eastern Europe were the only two regions in which mortality rates significantly increased during 1990-2017; in both, prevalent cases were predominantly caused by alcohol-related liver disease. At the national level, the highest mortality rate was registered in Egypt, whereas the steepest growth was observed in Lithuania. Deaths from cirrhosis in males were mostly attributed to hepatitis B (31.5%), alcohol (27.3%), and hepatitis C (25.5%); the corresponding percentages in females were 24.0%, 20.6%, and 26.7%[1].

In view of the above, prepandemic global patterns in CLD mortality differed according to cause of death (liver cirrhosis or liver cancer), etiology, and world region (Figure 1). Furthermore, even within a single country, different patterns of mortality could be registered according to complex interactions between demographic characteristics (sex, age group, ethnicity, and socioeconomic status) and etiology of liver disease. Mortality from CLD overall, and especially that from alcoholic liver disease, is in fact strongly associated with socioeconomic status[17]. Different trends in CLD mortality by etiology have been investigated in the USA by means of the multiple cause-of-death approach. Overall, an increase in mortality from cirrhosis and hepatocellular carcinoma has been registered in the prepandemic period[18]. However, drastically different trends were observed according to etiology. HCV-related mortality increased from 2007 to 2014 and sharply declined in 2014-2016, after the introduction of direct-acting antiviral therapies; meanwhile, a decrease in HBV-related mortality, and an increase in alcoholic liver disease and NAFLD mortality was observed in 2007-2016. Minorities were disproportionately affected, especially non-Hispanic blacks by HCV-related mortality and Asians by HBV-related mortality[7]. An increase in mortality related to NAFLD was confirmed by other authors in the same period, especially among older subjects, females, non-Hispanic whites, and American Indian/Alaskan Natives[19].

CLD AND COVID-19

Although COVID-19 primarily affects the respiratory system, causing pneumonia and acute respiratory distress syndrome in severe cases, it can also result in multiple extrapulmonary complications. The pathogenesis of extrapulmonary damage in patients with COVID-19 is probably multifactorial, involving both the direct effects of SARS-CoV-2 and the indirect mechanisms associated with the host inflammatory response[20]. Research has shown that COVID-19 can cause liver injury due to inflammation, which can lead to liver damage or failure, particularly in people with pre-existing liver conditions such as cirrhosis. People with COVID-19 who have pre-existing liver conditions may be at a higher risk for severe illness and death[21]. For these reasons, they should always continue to follow their treatment plan and keep in close contact with their healthcare provider.

Etiology of liver disease and COVID-19 impact

It is reported that liver diseases of viral etiology are not associated *per se* with the severity or outcome of COVID-19, and this finding has been consistent across Asia, Europe and the USA. According to recent data from the European Reference Network for Rare Liver Diseases, autoimmune liver diseases and the related immunosuppressive therapy do not represent a specific risk factor for COVID-19, and the risk, as with other etiologies, is determined by the stage of cirrhosis [22]. Several studies highlighted the presence of severe complications in patients with SARS-CoV-2 infection and metabolic-syndrome-associated comorbidities, including NAFLD. The shared genetic influence between COVID-19 susceptibility and NAFLD, the sex-linked and the liver single-cell differential expression level of diverse transcripts and biological pathways might uncover shared disease mechanisms that explain the severe complications and increased in-hospital mortality risk associated with comorbidities, including NAFLD[23]. Lockdown, economic hardship, and the psychological impact of the pandemic all had a detrimental effect on people with liver disease, including poorer metabolic control in people with metabolic syndrome and fatty liver disease. This likely had deleterious consequences on the liver and cardiovascular outcomes of people with NAFLD, particularly those with advanced liver disease. Mortality has been shown to increase in people with alcohol-associated liver disease[24].

The role of cirrhosis

Prospective data from multicenter studies confirmed that patients with cirrhosis, particularly those who are

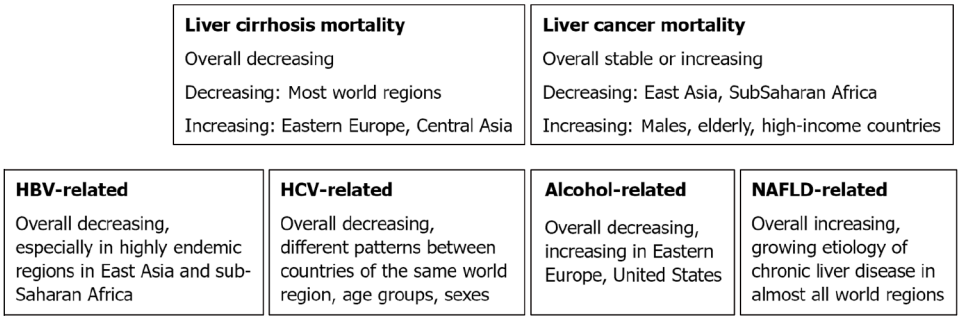

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Figure 1 Trends in mortality from liver diseases before the pandemic, by cause of death and etiology. HBV: Hepatitis B virus; HCV: Hepatitis C virus; NAFLD: Nonalcoholic fatty liver disease.

decompensated, are at a higher risk of hospitalization, ventilation and death than those without CLD. Older age and cirrhosis severity, as assessed by Child-Pugh class, are the most important predictors of mortality[25]. Overall mortality in patients with cirrhosis following SARS-CoV-2 infection was 32% in a large registry cohort of 729 predominantly hospitalized patients with CLD across 29 countries, with case-fatality rates incrementally increasing with each Child-Pugh class (CLD without cirrhosis 8%; class A 19%; class B 35%; class C 51%). The multivariable analysis of factors associated with death demonstrated persisting positive associations with age [odds ratio (OR) 1.02; 95% confidence interval (CI) 1.01-1.04; $P = 0.011$], the different stages of cirrhosis compared with CLD without cirrhosis (Child-Pugh class A, OR 1.90; 95%CI: 1.03-3.52; $P = 0.040$; class B, OR 4.14; 95%CI: 2.4-7.65; $P < 0.001$; and class C, OR 9.32; 95%CI: 4.80-18.08; $P < 0.001$), and alcoholic liver disease (OR 1.79; 95%CI: 1.03-3.13; $P = 0.040$)[8].

Patients with CLD and especially cirrhosis, have multiple mechanisms of immune dysfunction that can lead to increased susceptibility to infection and an aberrant inflammatory response during infection, collectively known as cirrhosis-associated immune dysfunction[26]. This immune dysfunction includes reduced components of the complement system, macrophage activation, impaired lymphocyte and neutrophil function, Toll-like receptor upregulation, and intestinal dysbiosis[27].

The role of vaccination

By April 2022, more than half of the world's population had received at least one vaccine dose, with real-world data showing that vaccination is generally safe and significantly reduces mortality. However, the initial high efficacy against infection has decreased following the emergence of new SARS-CoV-2 variants. Vaccine efficacy is particularly low against the Omicron variant, although fortunately it still confers considerable protection against severe COVID-19[28,29]. Although vaccines and previous infections largely protect against severe clinical courses of COVID-19, particularly in the current phase of the pandemic, which is dominated by sublineages of the SARS-CoV-2 Omicron variant, there is still a significant unmet medical need for therapeutics to treat severe disease in unvaccinated or older patients and patients with chronic disease conditions[30]. The impact of liver disease severity on vaccine efficacy should be convened to patients, so that they are aware of their increased risk and continue to take personal protective measures[31].

CLD AS A CAUSE OF DEATH DURING THE COVID-19 PANDEMIC

Between 2012 and 2019, rates of alcohol-specific deaths in the UK remained stable, whereas during the pandemic years, they rose from 11.8 per 100000 in 2019 to 14.8 in 2021. Alcoholic liver disease accounted for 78% of alcohol-specific mortality: the number of registered deaths grew from 5840 in 2019 to 7518 in 2021. This rise was attributed to increased alcohol consumption during the pandemic. Although alcoholic liver disease takes many years to develop, a further increase in alcohol intake among high-risk consumers can lead to rises in mortality in a short period of time, from what is known as acute-on-chronic liver failure[32].

In Minnesota, USA, an increase in both alcoholic liver disease and cirrhosis/other CLD as the underlying cause of death was registered in 2020 with respect to 2018-2019[33]. The growth in deaths from alcoholic liver disease as the underlying cause was confirmed at the national level[34]. According to analyses of national US multiple cause-of-death data, during the first year of the COVID-19 pandemic, the pre-existing growing trend in alcoholic liver disease mortality further accelerated, with the quarterly increase rising from 1.1% to 11.2%. Similarly, the quarterly increase in NAFLD grew from 1.9% to 6.6%. Mortality related to HBV and HCV, previously declining in the prepandemic period, remained stable[35]. Similar analyses were extended to 2021 data from the USA, confirming the increase through the second year of the pandemic for all liver diseases. This was more marked for alcoholic liver disease, especially among the young population aged 25-44 years and in selected ethnic groups such as American Indian/Alaskan Natives. The decline in HCV-related mortality observed since 2014 slowed down[36]. Years of potential life lost from premature deaths (< 65 years) attributable to cirrhosis listed as one of the causes of death in US certificates grew by 20.7% in 2020 and by 29.5% in 2021. COVID-19-related deaths accounted only for a minor part of such excess. Again, the increase was more pronounced for the alcoholic etiology, consistent with growing alcohol sales in the USA during the pandemic[37]. Patients with

alcoholic liver disease might have disproportionally suffered from a surge in harmful drinking, social isolation, and loss of familial and social support, along with barriers to access to outpatient clinics and rehabilitation services. Another study of US national multiple cause-of-death data reported a marked increase in mortality from CLD and/or cirrhosis among decedents with mention of diabetes, specifically associated with increased mortality from NAFLD and alcohol-related liver diseases[38].

By contrast in Spain, no major change in the number of deaths from CLD in 2020 compared to the 2018-2019 average was observed, based both on the underlying cause of death (0.9%) and on multiple cause-of-death data (+3.1%)[39].

In view of the paucity of published evidence outside the USA, we retrieved mortality rates for liver cirrhosis (International Classification of Diseases-10th Revision codes K70 and K74) from the World Health Organization (WHO) mortality database (https://platform.who.int/mortality). Table 1 shows countries with data updated at least until 2020; few had data available for the year 2021. In 2020 a large increase was registered in the USA (+18% with respect to 2019) and the UK (+16%). Among the other most populous countries, rates in the Americas were almost unchanged (Mexico, Brazil and Argentina) or declined (Peru and Colombia). European countries showed no increase (Spain) or only a limited mortality increase (Germany and Poland). Rates decreased in Malaysia and showed only minor growth in other Asian countries (Kazakhstan, Japan and Korea) and in Australia. Among less populous countries, a marked increase was registered in some Eastern European countries: Estonia, Lithuania, and in 2021 Latvia, Czechia and North Macedonia. A more comprehensive evaluation will however be possible when complete 2021 data become available, in consideration of how the pandemic involved different world regions in subsequent phases (*e.g.*, Central and Eastern Europe were more severely affected in 2021 compared to 2020). Additional nationwide data not included in the WHO database can be retrieved from institutional websites. As an example, in Italy age-adjusted mortality rates from liver cirrhosis, fibrosis and chronic hepatitis in 2020 showed a 3% reduction with respect to 2019, which is consistent with the decreasing trend observed in recent years (http://dati.istat.it/). Mortality from liver cirrhosis more closely parallels mortality from alcoholic liver disease with respect to that associated with other etiologies, and analyses including codes for viral hepatitis and liver cancer are warranted[3]. Lastly, also due to coding rules set by WHO[40], COVID-19 itself may have acted as a competing condition for the selection as the underlying cause of death in patients affected by pre-existing chronic diseases[35].

CONCLUSION

A large variation in trends of mortality from CLD was observed at the global level before 2020. Based on the few available published reports, the COVID-19 pandemic had a different impact depending on the local epidemiological context. Probably, mortality from CLD increased, especially in areas where the alcoholic etiology was predominant, or where a growing trend in mortality from alcoholic liver disease was already in place. Therefore, population-based studies on the direct and the indirect effects of the COVID-19 pandemic on CLD mortality are strongly warranted. Analyses adopting the multiple cause-of-death approach, and extended to deaths attributed to viral hepatitis and liver cancer, might be better suited to fully investigate the impact of the pandemic on complex long-term pre-existing trends.

Table 1 Age-standardized mortality rates (×100000) from cirrhosis, World Health Origination mortality database

	2021	2020	2019	2018	2017	2016	2015	2014	2013	2012
Antigua-Barbuda		3.4	2.7	4.5	3.6	0.9	2.9	6.8	1.9	10.1
Argentina		6.7	6.6	7.0	7.2	8.3	7.9	6.8	6.5	6.5
Armenia	10.9	12.2	10.6	12.0	16.0	19.5	16.6	17.1	14.9	16.5
Australia	4.6	4.3	4.1	4.0	4.0	3.7	4.1	3.9	3.9	3.3
Austria	8.6	8.5	8.1	7.9	8.4	9.0	9.5	9.4	9.9	10.7
Bosnia-Herzegovina		5.4	5.0	4.8	5.1	5.6		5.9		
Brazil		8.0	8.1	8.4	8.8	9.2	9.3	9.4	9.8	10.0
Bulgaria		15.6	15.8	14.3	14.5	14.8	14.4	13.4	13.1	13.8
Chile		9.7	9.4	9.9	11.3	12.4	11.7	11.8	12.3	13.5
Colombia		4.6	5.1	5.1	5.1	5.4	5.2	5.1	4.8	4.8
Costa Rica		5.2	6.9	6.2	6.3	6.3	6.5	6.1	6.2	5.2
Croatia		11.4	13.0		12.5	12.1	12.4	13.7	13.3	14.8
Cuba		10.8	10.5	10.0	9.8	9.0	8.9	8.5	8.2	7.9
Cyprus		3.6	3.9	3.2	3.2	2.7	3.2	3.6	4.0	2.8
Czechia	13.8	12.4	12.4	12.0	11.8	10.7	11.2	11.2	11.5	11.5

Denmark		6.7	7.2	6.5	7.2	7.1	6.7	7.6	7.6	8.4
Ecuador	12.5	11.8	13.2	13.7	14.0	14.6	14.5	13.3	13.1	13.7
Estonia	24.9	22.0	17.1	16.1	14.7	15.3	15.8	14.0	13.4	12.4
Finland		13.7	12.5	11.9	11.4	12.5	12.1	13.7	14.4	14.3
Georgia	7.2	7.0	7.0	7.5	5.6	5.5	7.4	8.0	6.0	6.4
Germany		9.2	8.7	8.9	9.1	9.3	9.1	9.0	9.5	9.3
Greece		3.3	3.3	3.1	3.5	3.6	3.6	3.7	4.1	3.8
Grenada	1.4	6.3	3.7	7.1	6.4	8.4	9.9	10.1	7.2	7.0
Guatemala		29.5	29.6	29.4	28.3	32.7	31.8	31.2	29.0	29.2
Iceland	2.7	1.8	1.4	1.9	2.5	1.6	2.7	1.5	1.7	1.4
Israel		2.0	2.5	2.4	2.3	2.5	2.7	2.3	2.7	2.8
Japan		4.9	4.7	4.7	4.7	4.4	4.4	4.5	4.7	4.7
Kazakhstan	38.6	40.2	38.5	38.5	41.4	46.4	50.5	55.2	57.9	
Latvia	18.9	12.4	12.1	13.6	11.5	13.6	12.8	12.5	12.6	12.4
Lebanon	2.6	2.1	1.9							
Lithuania	20.6	19.6	15.3	15.9	16.4	19.1	18.7	19.7	22.8	21.2
Luxemburg	7.0	7.8	7.2	8.0	7.2	8.9	6.7	8.0	7.5	9.1
Malaysia		1.7	2.0	2.0	1.8	1.8	2.0	1.9	1.8	1.7
Mauritius	10.9	10.7	8.6	7.9	8.0	8.8	8.9	8.9	10.3	9.7
Mexico		25.5	24.5	24.4	25.3	26.1	24.2	24.1	25.4	26.0
Mongolia	25.9	24.5	27.2	28.9	31.0	35.6				
Netherlands		3.0	2.7	2.8	2.9	2.9	2.8	2.7	2.6	2.9
Nicaragua		15.4	15.6	19.9	19.8	23.0	20.7	23.0	22.0	19.1
North Macedonia	6.9	5.0	4.9	4.7	6.0	5.2	5.9		6.3	5.4
Oman	1.5	1.5	2.0	1.5	2.6	2.2		1.6		
Paraguay		6.4	8.1	8.4	7.4	8.2	7.5	7.1	6.3	6.6
Peru		12.1	13.2	14.2	12.6	11.1	12.4	11.9	11.4	12.8
Poland		14.5	13.5	13.3	12.3	11.6	10.7	10.4	11.2	12.0
Qatar	1.6	2.1	1.2		2.9	1.8	0.6	1.7	1.2	2.1
Republic of Korea		7.1	6.8	7.3	7.5	7.8	8.1	8.0	8.3	8.8
Saint Lucia		3.7	3.6	6.2	4.2	5.9	8.9	12.1	7.9	9.1
Saint Vincent	3.1	4.5	5.6	6.9	5.7	4.9	2.5	5.4	7.0	7.2
Serbia	6.3	5.8	5.8	5.2	5.7	5.5	5.7	5.7	5.6	5.9
Singapore		1.7	2.2	2.2	2.3	2.4	2.2	2.6	2.5	2.4
Slovenia		9.9	10.2	10.9	9.6	9.9	11.4	13.5	16.2	17.9
Spain	4.6	4.4	4.5	4.6	4.9	4.9	5.4	5.3	5.6	5.8
Switzerland		3.6	3.8	3.9	3.9	4.1	4.4	4.4	4.5	5.0
United Arab Emirates		1.8	1.9	0.0						
UK		9.4	8.1	8.2	8.5	8.4	8.2	8.1	8.1	8.0
USA		10.9	9.2	9.0	8.9	8.7	8.8	8.5	8.2	8.0
Uruguay		3.3	3.0	3.3	3.3	3.7	4.3	4.4	3.8	3.9

FOOTNOTES

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Casotto V, Grande E, Saia M, Zanetto A, and Russo FP collected the relevant bibliography and wrote the sections of the review; Barbiellini Amidei C and Casotto V contributed to "trends before the pandemic"; Zanetto A and Russo FP contributed to "COVID-19 and CLD"; Grande E and Saia M contributed to "CLD as a cause of deaths during the pandemic"; and all authors contributed to revising the first draft and approved the final version of the manuscript.

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REFERENCES

1 **GBD 2017 Cirrhosis Collaborators**. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 245-266 [PMID: 31981519 DOI: 10.1016/S2468-1253(19)30349-8]

2 **Asrani SK**, Larson JJ, Yawn B, Therneau TM, Kim WR. Underestimation of liver-related mortality in the United States. *Gastroenterology* 2013; **145**: 375-82.e1 [PMID: 23583430 DOI: 10.1053/j.gastro.2013.04.005]

3 **Fedeli U**, Avossa F, Guzzinati S, Bovo E, Saugo M. Trends in mortality from chronic liver disease. *Ann Epidemiol* 2014; **24**: 522-526 [PMID: 24861431 DOI: 10.1016/j.annepidem.2014.05.004]

4 **Sun Y**, Chang J, Liu X, Liu C. Mortality trends of liver diseases in mainland China over three decades: an age-period-cohort analysis. *BMJ Open* 2019; **9**: e029793 [PMID: 31712333 DOI: 10.1136/bmjopen-2019-029793]

5 **Ly KN**, Hughes EM, Jiles RB, Holmberg SD. Rising Mortality Associated With Hepatitis C Virus in the United States, 2003-2013. *Clin Infect Dis* 2016; **62**: 1287-1288 [PMID: 26936668 DOI: 10.1093/cid/ciw111]

6 **Fedeli U**. Increasing mortality associated with the more recent epidemic wave of hepatitis C virus infection in Northern Italy. *J Viral Hepat* 2018; **25**: 996-998 [PMID: 29532570 DOI: 10.1111/jvh.12893]

7 **Kim D**, Li AA, Gadiparthi C, Khan MA, Cholankeril G, Glenn JS, Ahmed A. Changing Trends in Etiology-Based Annual Mortality From Chronic Liver Disease, From 2007 Through 2016. *Gastroenterology* 2018; **155**: 1154-1163.e3 [PMID: 30009816 DOI: 10.1053/j.gastro.2018.07.008]

8 **Marjot T**, Moon AM, Cook JA, Abd-Elsalam S, Aloman C, Armstrong MJ, Pose E, Brenner EJ, Cargill T, Catana MA, Dhanasekaran R, Eshraghian A, García-Juárez I, Gill US, Jones PD, Kennedy J, Marshall A, Matthews C, Mells G, Mercer C, Perumalswami PV, Avitabile E, Qi X, Su F, Ufere NN, Wong YJ, Zheng MH, Barnes E, Barritt AS 4th, Webb GJ. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: An international registry study. *J Hepatol* 2021; **74**: 567-577 [PMID: 33035628 DOI: 10.1016/j.jhep.2020.09.024]

9 **Kim D**, Bonham CA, Konyn P, Cholankeril G, Ahmed A. Mortality Trends in Chronic Liver Disease and Cirrhosis in the United States, Before and During COVID-19 Pandemic. *Clin Gastroenterol Hepatol* 2021; **19**: 2664-2666.e2 [PMID: 34256143 DOI: 10.1016/j.cgh.2021.07.009]

10 **Paik JM**, Golabi P, Younossi Y, Mishra A, Younossi ZM. Changes in the Global Burden of Chronic Liver Diseases From 2012 to 2017: The Growing Impact of NAFLD. *Hepatology* 2020; **72**: 1605-1616 [PMID: 32043613 DOI: 10.1002/hep.31173]

11 **GBD 2019 Hepatitis B Collaborators**. Global, regional, and national burden of hepatitis B, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Gastroenterol Hepatol* 2022; **7**: 796-829 [PMID: 35738290 DOI: 10.1016/S2468-1253(22)00124-8]

12 **Veracruz N**, Gish RG, Cheung R, Chitnis AS, Wong RJ. Global incidence and mortality of hepatitis B and hepatitis C acute infections, cirrhosis and hepatocellular carcinoma from 2010 to 2019. *J Viral Hepat* 2022; **29**: 352-365 [PMID: 35274406 DOI: 10.1111/jvh.13663]

13 **Global Burden of Disease Liver Cancer Collaboration**, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi JY, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MS, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang YH, Kumar A, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Mądry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. *JAMA Oncol* 2017; **3**: 1683-1691 [PMID: 28983565 DOI: 10.1001/jamaoncol.2017.3055]

14 **Liu Z**, Suo C, Mao X, Jiang Y, Jin L, Zhang T, Chen X. Global incidence trends in primary liver cancer by age at diagnosis, sex, region, and etiology, 1990-2017. *Cancer* 2020; **126**: 2267-2278 [PMID: 32201944 DOI: 10.1002/cncr.32789]

15 **Liu Z**, Jiang Y, Yuan H, Fang Q, Cai N, Suo C, Jin L, Zhang T, Chen X. The trends in incidence of primary liver cancer caused by specific etiologies: Results from the Global Burden of Disease Study 2016 and implications for liver cancer prevention. *J Hepatol* 2019; **70**: 674-683 [PMID: 30543829 DOI: 10.1016/j.jhep.2018.12.001]

16 **Huang J**, Lok V, Ngai CH, Chu C, Patel HK, Thoguluva Chandraseka V, Zhang L, Chen P, Wang S, Lao XQ, Tse LA, Xu W, Zheng ZJ, Wong MCS. Disease Burden, Risk Factors, and Recent Trends of Liver Cancer: A Global Country-Level Analysis. *Liver Cancer* 2021; **10**:

330-345 [PMID: 34414121 DOI: 10.1159/000515304]
17 **Fedeli U**, Avossa F, Goldoni CA, Caranci N, Zambon F, Saugo M. Education level and chronic liver disease by aetiology: A proportional mortality study. *Dig Liver Dis* 2015; **47**: 1082-1085 [PMID: 26315625 DOI: 10.1016/j.dld.2015.07.154]
18 **Kim D**, Li AA, Perumpail BJ, Gadiparthi C, Kim W, Cholankeril G, Glenn JS, Harrison SA, Younossi ZM, Ahmed A. Changing Trends in Etiology-Based and Ethnicity-Based Annual Mortality Rates of Cirrhosis and Hepatocellular Carcinoma in the United States. *Hepatology* 2019; **69**: 1064-1074 [PMID: 30014489 DOI: 10.1002/hep.30161]
19 **Paik JM**, Henry L, De Avila L, Younossi E, Racila A, Younossi ZM. Mortality Related to Nonalcoholic Fatty Liver Disease Is Increasing in the United States. *Hepatol Commun* 2019; **3**: 1459-1471 [PMID: 31701070 DOI: 10.1002/hep4.1419]
20 **Ning Q**, Wu D, Wang X, Xi D, Chen T, Chen G, Wang H, Lu H, Wang M, Zhu L, Hu J, Liu T, Ma K, Han M, Luo X. The mechanism underlying extrapulmonary complications of the coronavirus disease 2019 and its therapeutic implication. *Signal Transduct Target Ther* 2022; **7**: 57 [PMID: 35197452 DOI: 10.1038/s41392-022-00907-1]
21 **Dufour JF**, Marjot T, Becchetti C, Tilg H. COVID-19 and liver disease. *Gut* 2022; **71**: 2350-2362 [PMID: 35701093 DOI: 10.1136/gutjnl-2021-326792]
22 **Williamson EJ**, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, Curtis HJ, Mehrkar A, Evans D, Inglesby P, Cockburn J, McDonald HI, MacKenna B, Tomlinson L, Douglas IJ, Rentsch CT, Mathur R, Wong AYS, Grieve R, Harrison D, Forbes H, Schultze A, Croker R, Parry J, Hester F, Harper S, Perera R, Evans SJW, Smeeth L, Goldacre B. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020; **584**: 430-436 [PMID: 32640463 DOI: 10.1038/s41586-020-2521-4]
23 **Pirola CJ**, Sookoian S. COVID-19 and non-alcoholic fatty liver disease: Biological insights from multi-omics data. *Liver Int* 2023; **43**: 580-587 [PMID: 36593576 DOI: 10.1111/Liv.15509]
24 **Rivera-Esteban J**, Manzano-Nuñez R, Broquetas T, Serra-Matamala I, Bassegoda O, Soriano-Varela A, Espín G, Castillo J, Bañares J, Carrión JA, Ginès P, Graupera I, Pericàs JM. Impact of the COVID-19 pandemic on the care and outcomes of people with NAFLD-related cirrhosis. *JHEP Rep* 2022; **4**: 100574 [PMID: 36061511 DOI: 10.1016/j.jhepr.2022.100574]
25 **Russo FP**, Burra P, Zanetto A. COVID-19 and liver disease: where are we now? *Nat Rev Gastroenterol Hepatol* 2022; **19**: 277-278 [PMID: 35301465 DOI: 10.1038/s41575-022-00607-9]
26 **Zanetto A**, Pelizzaro F, Campello E, Bulato C, Balcar L, Gu W, Gavasso S, Saggiorato G, Zeuzem S, Russo FP, Mandorfer M, Reiberger T, Trebicka J, Burra P, Simioni P, Senzolo M. Severity of systemic inflammation is the main predictor of ACLF and bleeding in individuals with acutely decompensated cirrhosis. *J Hepatol* 2023; **78**: 301-311 [PMID: 36150575 DOI: 10.1016/j.jhep.2022.09.005]
27 **Marjot T**, Webb GJ, Barritt AS 4th, Moon AM, Stamataki Z, Wong VW, Barnes E. COVID-19 and liver disease: mechanistic and clinical perspectives. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 348-364 [PMID: 33692570 DOI: 10.1038/s41575-021-00426-4]
28 **Andrews N**, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, Gower C, Kall M, Groves N, O'Connell AM, Simons D, Blomquist PB, Zaidi A, Nash S, Iwani Binti Abdul Aziz N, Thelwall S, Dabrera G, Myers R, Amirthalingam G, Gharbia S, Barrett JC, Elson R, Ladhani SN, Ferguson N, Zambon M, Campbell CNJ, Brown K, Hopkins S, Chand M, Ramsay M, Lopez Bernal J. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. *N Engl J Med* 2022; **386**: 1532-1546 [PMID: 35249272 DOI: 10.1056/NEJMoa2119451]
29 **Russo FP**, Izzy M, Rammohan A, Kirchner VA, Di Maira T, Belli LS, Berg T, Berenguer MC, Polak WG. Global impact of the first wave of COVID-19 on liver transplant centers: A multi-society survey (EASL-ESOT/ELITA-ILTS). *J Hepatol* 2022; **76**: 364-370 [PMID: 34653592 DOI: 10.1016/j.jhep.2021.09.041]
30 **Schmitt CA**, Tchkonia T, Niedernhofer LJ, Robbins PD, Kirkland JL, Lee S. COVID-19 and cellular senescence. *Nat Rev Immunol* 2023; **23**: 251-263 [PMID: 36198912 DOI: 10.1038/s41577-022-00785-2]
31 **Simão AL**, Palma CS, Izquierdo-Sanchez L, Putignano A, Carvalho-Gomes A, Posch A, Zanaga P, Girleanu I, Henrique MM, Araújo C, Degre D, Gustot T, Sahuco I, Spagnolo E, Carvalhana S, Moura M, Fernandes DA, Banales JM, Romero-Gomez M, Trifan A, Russo FP, Stauber R, Berenguer M, Moreno C, Gonçalves J, Cortez-Pinto H, Castro RE. Cirrhosis is associated with lower serological responses to COVID-19 vaccines in patients with chronic liver disease. *JHEP Rep* 2023; **5**: 100697 [PMID: 36844943 DOI: 10.1016/j.jhepr.2023.100697]
32 **Office for National statistics**. Alcohol-specific deaths in the UK: registered in 2021. December 8, 2022. [cited 23 May 2023]. Available from: https://www.nisra.gov.uk/news/annual-alcohol-specific-deaths-statistics-2021#:~:text=Key%20points%201%20The%20total%20number%20of%20alcohol-specific,all%20alcohol-specific%20deaths%20registered%20in%202021.%20More%20items
33 **McCoy RG**, Campbell RL, Mullan AF, Bucks CM, Clements CM, Reichard RR, Jeffery MM. Changes in all-cause and cause-specific mortality during the first year of the COVID-19 pandemic in Minnesota: population-based study. *BMC Public Health* 2022; **22**: 2291 [PMID: 36474190 DOI: 10.1186/s12889-022-14743-z]
34 **Deutsch-Link S**, Jiang Y, Peery AF, Barritt AS, Bataller R, Moon AM. Alcohol-Associated Liver Disease Mortality Increased From 2017 to 2020 and Accelerated During the COVID-19 Pandemic. *Clin Gastroenterol Hepatol* 2022; **20**: 2142-2144.e2 [PMID: 35314353 DOI: 10.1016/j.cgh.2022.03.017]
35 **Kim D**, Alshuwaykh O, Dennis BB, Cholankeril G, Ahmed A. Trends in Etiology-based Mortality From Chronic Liver Disease Before and During COVID-19 Pandemic in the United States. *Clin Gastroenterol Hepatol* 2022; **20**: 2307-2316.e3 [PMID: 35811045 DOI: 10.1016/j.cgh.2022.05.045]
36 **Gao X**, Lv F, He X, Zhao Y, Liu Y, Zu J, Henry L, Wang J, Yeo YH, Ji F, Nguyen MH. Impact of the COVID-19 pandemic on liver disease-related mortality rates in the United States. *J Hepatol* 2023; **78**: 16-27 [PMID: 35988691 DOI: 10.1016/j.jhep.2022.07.028]
37 **Zhao Y**, Yeo YH, Samaan J, Lv F, He X, Gao N, Park J, Yang JD, Ayoub W, Odden MC, Ji F, Nguyen MH. Most excess years of potential life loss among individuals with cirrhosis during the pandemic were not related to COVID-19. *Gut* 2022 [PMID: 36282906 DOI: 10.1136/gutjnl-2022-328188]
38 **Kim D**, Alshuwaykh O, Dennis BB, Cholankeril G, Knowles JW, Ahmed A. Chronic liver disease-related mortality in diabetes before and during the COVID-19 in the United States. *Dig Liver Dis* 2023; **55**: 3-10 [PMID: 36182570 DOI: 10.1016/j.dld.2022.09.006]
39 **Spijker JJA**, Trias-Llimós S. Cause-specific mortality in Spain during the pandemic: educational differences and its impact on life expectancy. *Eur J Public Health* 2023; **33**: 543-549 [PMID: 36944099 DOI: 10.1093/eurpub/ckad036]
40 **World Health Organization**. International Guidelines for Certification and Classification (Coding) of COVID-19 as Cause of Death. April 20, 2020. [cited 23 May 2023]. Available from: https://www.who.int/publications/m

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MINIREVIEWS

Progress in immunotherapy for neuroendocrine neoplasm of the digestive system

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Abstract

Neuroendocrine neoplasms (NENs) are rare heterogeneous tumors that can develop in almost any organ, with the digestive organs, including the gastrointestinal tract and pancreas being the most commonly affected sites. Despite the fact that advances in initial therapies have progressed, there is presently no recognized effective treatment for advanced NEN. Immune checkpoint inhibitors (ICIs) have shown superior efficacy in treating several types of solid tumors. Despite their successful role in the treatment of partial NENs, such as small cell lung cancer, and Merkel cell carcinoma, the role of ICIs in most of the NENs remains limited. Nevertheless, due to their specific anti-tumor mechanisms and acceptable safety profile, ICIs are a promising avenue for further study in NENs therapy. Recent clinical trials have illustrated that combination therapy with ICI is more efficient than monotherapy, and multiple clinical trials are constantly ongoing to evaluate the efficacy and safety of these combination therapies. Therefore, the purpose of this review is to provide a comprehensive summary of the clinical progress of immunotherapy in NENs affecting the digestive system, with a specific emphasis on the application of programmed cell death protein 1/programmed death receptor ligand 1 inhibitor. Furthermore, this review has an exploration of the potential beneficiary population and the inherent value of utilizing immunotherapy in the management of NENs.

Key Words: Immunotherapy; PD-1 inhibitor; Neuroendocrine neoplasm; Neuroendocrine tumor; Neuroendocrine carcinoma; Gastrointestinal; Pancreatic

Core Tip: The application of immune checkpoint inhibitor (ICI) in neuroendocrine neoplasm (NEN) is regulated by the latest clinical practice guidelines. However, immunotherapy has achieved some breakthroughs for high-grade or advanced NENs of the digestive system, for which there is currently no effective drug therapy. In this regard, we investigated the causes of the heterogeneous efficacy of ICI in NEN with different grades, differentiation, and primary organs. This review summarizes the state-of-the-art progress and trend of clinical trials for different ICI-containing regimens in NENs of the digestive system, which will aid in the conduct of subsequent clinical trials and research of related mechanisms.

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INTRODUCTION

Neuroendocrine Neoplasms (NENs) are a group of rare and heterogeneous neoplasms that may originate from cells throughout the endocrine system[1]. These tumors express neuroendocrine markers and can occur in any part of the body, with a particular prevalence in the digestive system, such as the gastrointestinal (GI) tract and hepatopancreato-biliary organs. The majority of NENs are sporadic, and the exact etiology is still unknown[2]. However, less than 5% of gastroenteropancreatic (GEP) NENs occur as hereditary neoplastic syndromes, associated with gene deletions or alterations, including multiple endocrine neoplasia type 1 associated with duodenopancreatic NENs[3,4], and the von Hippel-Lindau syndrome associated with pancreatic NENs[5].

NENs of the digestive system consist of a range of tumor types, including well-differentiated neuroendocrine tumors (NETs), poorly differentiated neuroendocrine carcinomas (NECs), and mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs), which based on the degree of NEN malignancy according to the World Health Organization (WHO) classification of tumors of the digestive system (5th edition, 2019)[6]. NETs can be further classified and graded into three categories: Neuroendocrine tumors grade 1 (NET G1; low grade), NET G2 (intermediate grade), and NET G3 (high grade). This classification is determined based on the Ki-67 proliferation index and the number of mitotic figures per 2 mm^2. NECs include large cell-type NEC (LCNEC) and small cell-type NEC (SCNEC), both of which are considered as high grades. MiNENs exhibit varying degrees of differentiation and grades because they possess both neuroendocrine and non-neuroendocrine components in a single patch of neoplastic tissue, with each component accounting for ≥ 30%.

In recent years, with widespread improvements in clinical diagnosis and treatment, including endoscopy and imaging, there has been a noticeable worldwide upward trend in the detection of early-stage and non-functioning NENs from the digestive system. NEN incidence increased 6.4 times between 1973 (1.09/100000) and 2012 (6.98/100000), according to an analysis published in 2017 and based on 64971 NEN cases from the Surveillance Epidemiology and End Results database of the National Cancer Institute[7]. According to statistics from England, the incidence of NENs was about 9/100000 in 2018, and the incidences of pancreatic and rectal NENs have increased significantly[8]. Similarly, based on Taiwanese data, the incidence of NENs in digestive organs increased from 0.15 per 100000 people in 1996 to 2.36 per 100000 people in 2015[9].

The most prevalent primary sites have also been shown to vary across different regions. In the United Kingdom, the order of frequency among all primary sites of GEP-NETs, was as follows: Small intestine (25.6%), appendix (23.6%), pancreas (17.7%), colon (9.8%), stomach (9.8%), and rectum (7.8%)[10]. In China, however, the order was different, with the pancreas (31.5%), rectum (29.6%), and stomach (27.0%) being the common sites. The small intestine (5.6%) and colon (3.0%) accounted for relatively small proportions of NENs in this region[11]. Compared with others, gallbladder NENs are more scarce and account for only 0.5% of all NENs[12]. NETs are prone to metastasis to the liver; however, the liver itself is rarely the primary site of NENs, accounting for only 0.46% of primary liver tumors[13]. Owing to the rarity of the diagnosis and a lack of valid statistics for relevant cases, the quality of published data is limited, and the epidemiology of patients with digestive MiNENs remains unknown[14].

Immune checkpoint inhibitors (ICIs) have potent, strong anti-tumor activity in the management of various cancer types. These inhibitors, which target programmed cell death protein 1 (PD-1) or programmed death receptor ligand 1 (PD-L1), are profoundly researched and widely used in tumor immunotherapy. Both PD-1 and PD-L1 targeted antibodies relieve the functional inhibition of T cells and reactivate the immune response against cancer cells. Several α-PD-1 (*e.g.*, palivizumab, nivolumab, and toripalimab) and α-PD-L1 antibodies (*e.g.*, atezolizumab and durvalumab) have been licensed by the Food and Drug Administration (FDA) for the treatment of multiple forms of tumor owing to their efficacy in clinical trials. More than 10 different cancer types have been authorized for therapy with single-agent PD-1 or PD-L1 monoclonal antibodies, with objective response rates (ORR) ranging from 15%-20%[15]. However, the PD-1 or PD-L1 axis is not the only signaling access that contributes to tumor immunosuppression, and the inhibition of this pathway alone is insufficient to effectively elicit anti-tumor immunity. Consequently, several combination immunotherapies, such as PD-1/PD-L1 antibodies combined with chemotherapy, radiotherapy, angiogenesis inhibitors, other ICIs, gut microbiota transplantation, and metabolic modulators, may improve the overall anti-tumor activity and raise the response rates (RRs) of NENs of the digestive system[16].

Herein, we comprehensively summarize the clinical application status and research progress of immunotherapy drugs, mainly PD-1 and PD-L1 inhibitors, for the treatment regimen of NENs of the digestive system, which might provide more potent and widely applicable regimens as well as direction for subsequent studies.

IMMUNOTHERAPY AND NENS OF THE DIGESTIVE SYSTEM

The use of immunotherapy to treat NENs of the digestive system is still in the clinical exploration phase and is not yet recommended as the preferred regimen. This is because available clinical trials have demonstrated low ORRs to immunotherapy using PD-1 or PD-L1 inhibitors. Currently, several immunotherapeutic strategies for NENs are undergoing clinical trials, including ICI monotherapy, dual ICI therapy, anti-angiogenesis with ICIs, and chemotherapy with ICI. Table 1 provides a summary of relevant data from the clinical trials mentioned below.

Application status of ICIs in NENs of the digestive system

According to the National Comprehensive Cancer Network Clinical Practice Guidelines of Neuroendocrine and Adrenal Tumors (version 2.2022), ICIs are not currently available as a preferred regimen for the systemic treatment of patients with unresectable locally advanced or distant metastases from NEN. In the preferred regimen, the molecularly targeted drug everolimus is more effective in advanced G1 or G2 NET. In phase III clinical research, mPFS in the everolimus group was longer than in the placebo group (11 *vs* 3.9 mo; $P < 0.00001$) and decreased recurrence rate as well as mortality by 52%[17]. Somatostatin analogs, such as octreotide and lanreotide, are mainly used in the treatment of NENs that are somatostatin receptor-positive, slow-growth as well as Ki-67 ≤ 10% and are usually combined with molecularly targeted drugs to control the symptom of functional NETs. Chemotherapy is typically the preferred treatment option for G3 NET and NEC. The ORR of cisplatin or carboplatin combined with etoposide in the treatment of advanced NECs ranged from 30.8%-63.2%, and the median overall survival (mOS) was between 8.9-12.5 mo[18,19]. Temozolomide combined with capecitabine has more efficacy in pancreatic NET (pNET) than in GI NET, and the mPFS and mOS in high-grade NET were longer than those in NEC (15.3 mo *vs* 3.3 mo; 22 mo *vs* 4.6 mo)[20].

Although most NENs have not demonstrated the greatest effectiveness, ICIs can still be used as the primary treatment for well-differentiated grade 3 NETs, extrapulmonary poorly differentiated NECs, and MiNENs. Patients with advanced tumor mutational burden-high [tumor mutational burden (TMB)-H], microsatellite instability-high (MSI-H), or mismatch repair deficiency (dMMR) tumors, as identified by an FDA-approved test performed after prior therapy, may be candidates for the PD-1 inhibitor pembrolizumab if no other appropriate options for therapy are available. For biologically favorable or unfavorable locally advanced or metastatic G3 NETs (unresectable with clinically considerable tumor burden or indication of disease progression), pembrolizumab is the primary treatment[21,22]. In the event of extrapulmonary, locoregional, unresectable, or metastatic NECs/MiNENs, pembrolizumab can also be considered for systemic therapy[21,22,23]. Nivolumab combined with ipilimumab (category 2B), which is known as a type of dual ICI therapy, is a recommended option for locally progressed or metastatic G3 NETs with unfavorable biologies[24]. Similarly, if the illness advances after chemotherapy, ipilimumab paired with nivolumab (category 2B) might also be taken into consideration for extrapulmonary poorly differentiated (LCNEC/SCNEC) or unknown primary tumors.

However, the Guidelines of the Chinese Society of Clinical Oncology for Neuroendocrine Neoplasms (version 2022) only suggest the use of ICIs as a treatment option for metastatic NECs. Specifically, pembrolizumab is recommended as a first-line treatment (category 3) or as a level-2 recommendation for the second-line treatment of metastatic NEC in patients with performance status (PS) scores of 0-2 and the presence of dMMR, MSI-H, or TMB-H (category 1A)[25]. In addition, ICIs such as ipilimumab, combined with nivolumab[24] or other ICIs[26-28], may be considered for patients with metastatic NECs who have previously received systemic therapy and continue to experience disease progression and who lack standard treatment options. For these patients, ICIs are recommended as a level-3 recommendation for second-line treatment (category 3A).

ICI monotherapy

ICI monotherapy has shown remarkably low RRs in NENs, especially in poorly differentiated ones. The Keynote-028 trial demonstrated the actual clinical outcomes of pembrolizumab monotherapy in patients who had carcinoid tumors and pNET with PD-L1 expression[29]. In the pNET cohort, 16 PD-L1-positive patients out of 106 pNET patients received pembrolizumab monotherapy with ORRs of 6.3% [95% confidence interval (CI): 0.2%-30.2%], only one patient confirmed partial response (PR), 31% developed stable disease (SD) lasting more than 6 mo, and 12-mo progression-free survival (PFS) and overall survival (OS) rates of 27% and 87%, respectively. However, studies comparing the efficiency of pembrolizumab in patients with or without PD-L1 expression reported no significant differences in the disease control rate (DCR), PFS, or OS between the two groups[30]. Among 29 patients who had experienced prior treatments, 48% and 34% had GI and pancreatic NENs, respectively. Only one patient (95%CI: 0.1-17.8%) with an esophageal LCNEC had an objective PR that persisted for 13 mo before he discontinued his participation in the study. Six patients (95%CI: 7.9-39.7%) had SDs, and DCR was 24.1%[30]. In an open-label phase II trial, pembrolizumab single-agent therapy in 107 well-differentiated NETs of the lung, appendix, small intestine, colon, rectum, or pancreas with previously failed standard treatments resulted in an ORR of only 3.7% (95%CI: 1.0-9.3). The median PFS (mPFS) was 4.1 mo (95%CI: 3.5-5.4), the estimated PFS rate at 6 mo was 39.3%, and the mOS was 24.2 mo. Among 40 patients with pancreatic NETs, 25 patients with small intestine NETs, and 18 patients with other GI NETs, only 4 patients had a PR as assessed by Response Evaluation Criteria in Advanced Solid Tumors version 1.1, including cases in the pancreas (3) and rectum (1); all of them were PD-L1 negative. It is noteworthy that the response rate in the pancreatic NETs subgroup was 7.5%[31]. In another

Table 1 Clinical trials mentioned in the review

Intervention	Study phase	Actual enrollment	NEN type of the digestive system	ORR (%)	mPFS (mo)	mOS (mo)	Ref.
Pembrolizumab	I	16	pNET	6.3	4.5	21.0	[29]
	II	29 (14 GI, 10 pancreas)	GI-NEN, pNEN	3.4	2.0	4.7	[30]
	II	107 (83 GEP)	WD NETs	3.7	4.1	24.2	[31]
Toripalimab	Ib	40 (23 GI, 9 pancreas)	GI-NEN	13.0	2.5	7.8	[32]
			pNEN	22.0			
Nivolumab	II	185 (93 GEP)	GEP-NEC	7.0	1.8	7.2	[33]
Spartalizumab	II	95 NETs (32 GI, 33 pancreas); 21 GEP-NECs	GI NET	3.1	3.8	Not estimable	[34]
			pNET	3.0			
			GEP-NEC	4.8	1.8	6.8	
Avelumab	II	27 (21 GEP)	GEP-NET	--	3.3	14.2	[35]
Nivolumab + Ipilimumab	--	34 (21 from digestive organs)	NENs	14.7	1.0	5.0 (from treatment initial); 14.0 (from diagnosis)	[36]
	II	32 (15 GI)	Non-pNETs	25.0	4.0	11.0	[37]
	II	19 (9 from digestive organs)	High-grade NENs	26.0	2.0	8.7	[38]
	II	185 (93 GEP)	GEP-NEC	14.9	1.9	5.8	[33]
	--	11	Metastatic EP-NEC (mainly of GEP origin)	--	8.5	Not reached	[39]
Durvalumab + Tremelimumab	II	123 (31 GI, 32 pancreas, 33 GEP)	G1/G2 GI-NETs	0	5.8	29.5	[40,41]
			G1/G2 pNETs	6.3	5.5	23.8	
			G3 GEP-NENs	9.1	2.4	5.9	
Atezolizumab + Bevacizumab	II	40 (20 pNET, 20 non-pNET)	pNET	20.0	19.6	30.1	[44,45]
			Non-pNET	15.0	14.9	Not reached	
Pembrolizumab + Lenvatinib	II	20 (10 GI)	GI-NEN	10.0	10.0	--	[46]
Pembrolizumab + Irinoteca/Paclitaxel	II	22 (16 GI)	GI-NEC	9.0	2.0	4.0	[47]
Nivolumab + Tezolomide	II	15 (12 evaluable and 7 GEP)	GEP-NET	25.0	--	--	[48]
Nivolumab + Cisplatin/Carboplatin	II	38 (31 GEP)	G3 NENs	54.1	5.7	13.9	[49,50]

WD: Well-differentiated; NET: Neuroendocrine tumor; NEN: Neuroendocrine neoplasm; NEC: Neuroendocrine carcinoma; GEP: Gastroenteropancreatic; SCLC: Small cell lung cancer; MCC: Merkel cell carcinoma; pNET: Pancreas NET; ORR: Objective response rate; mOS: Median overall survival; mPFS: Median progression-free survival; EP-NEC: Extrapulmonary neuroendocrine carcinoma.

multicenter phase Ib clinical trial investigating toripalimab for metastatic or recrudescent NENs where standard therapies had failed, 40 patients were enrolled. Of these, for ex-pancreatic GI-derived, pancreatic, and nondigestive NENs, the ORRs were 13.0% (3/23), 22.2% (2/9), and 37.5% (3/8), respectively[32].

The effectiveness of ICI monotherapy in treating NECs has also been proven in a number of clinical trials. In a phase II trial involving 185 patients with NECs (93 were GEP) and randomly assigned (1:1) to receive nivolumab monotherapy or nivolumab combined with ipilimumab. In the monotherapy group, the ORR at 8 wk, mPFS, and mOS were 7.2% (95%CI: 2.7-15.1), 1.8 mo (95%CI: 1.7-2.0), and 7.2 mo (95%CI: 3.7-14.1), respectively. However, these results indicated that the therapeutic effect was not ideal[33]. Spartalizumab, another anti-PD-1 agent, was evaluated in a clinical trial involving 21 patients with poorly differentiated GEP-NECs and 95 patients with metastatic G1 or G2 NETs (32 GI and 33 pancreatic), all of whom had received prior treatments for their advanced diseases. The ORR in the GI NET, pNET, and GEP-NEC groups was 3.1% (95%CI: 0.1-16.2), 3.0% (95%CI: 0.1-15.8), and 4.8% (95%CI: 0.1-23.8), respectively. In the NET cohort, the mPFS, 12-mo PFS, and OS rates were 3.8 mo, 19.5% (95%CI: 11.6-28.9), and 73.5% (95%CI: 63.0-81.4) respectively. For the GEP-NEC cohort, the respective values were 1.8 mo, 0%, and 19.1% (95%CI: 4.8-40.6)[34]. Although the efficacy of spartal-

izumab for digestive NEN treatment is limited and not worthy of further investigation, its AEs are mild and manageable. Fatigue (29.5%) and nausea (10.5%) were the most frequently reported spartalizumab-related AEs that emerged during the trial of the NET cohort; they were mainly distributed in the grade 1/2, while elevated aspartate/alanine aminotransferase levels (14.3% each) were common in the GEP NEC group[34]. Other trials, however, reported contrary conclusions regarding the use of ICI monotherapy. Two trials of avelumab monotherapy, which enrolled 27 patients in total, had 21 GEP-NET, with none achieving OR but only 33% obtaining SD. AEs related to avelumab were observed in 58% of patients, including grade 3-4 AEs in 3 cases, leading directly to trial termination[35].

The aforementioned ICI monotherapy regimens have indicated mild anti-tumor activity with neither NET nor NEN of the digestive system. Nonetheless, the AEs were manageable, which made them relatively safe and applicable under most conditions. It is speculated that the severity of adverse reactions may be correlated with the efficacy. In pembrolizumab treatment, for instance, only 21.5% of treatment-related adverse reactions occurred at grade ≥ 3, while the frequent adverse reactions were malaise (22.4%) and diarrhea (13.1%)[31]. Therefore, combination therapies to improve the efficacy of ICIs against NENs in the digestive system have emerged as a major research direction.

COMBINED IMMUNOTHERAPY FOR NENS OF THE DIGESTIVE SYSTEM

Dual ICI therapy

Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) antibody, another type of ICI, have mechanisms of action complementary to those of PD-1 and PD-L1 antibodies. The binding of CTLA-4 and CTLA-4 antibodies to the surface of activated T cells indirectly promotes the activation and proliferation of T cell development by relieving the inhibitory effect of antigen-presenting cells on T cells. One preliminary retrospective evaluation for the treatment with ipilimumab plus nivolumab reported ORR, DCR, and mPFS of 15%, 41.2%, and one month (95%CI: 0.54-1.46 mo), respectively, in 34 patients with G3 NENs (79% NEC and 21 from digestive organs) who had previously undergone at least one cytotoxic chemotherapy regimen and, on average, two prior lines of treatment[36]. These findings suggest that dual ICI therapy has therapeutic effects on aggressive NECs that progress after chemotherapy, as well as in heavily pre-treated NENs. The S1609DART trial established the combination of ipilimumab and nivolumab as an option for extrapulmonary poorly differentiated (LCNEC/SCNEC) or unknown primary tumors. Results of the trial revealed that the ORR of 32 cases was 25% (95%CI: 13%-64%), and the median overall survival (mOS) was 11 mo. The ORR was 44% for non-pancreatic (non-p) high-grade NECs (8/18, CR = 1, PR = 7), including lung primaries[37]. Furthermore, in the following research on high-grade NENs, 19 patients were involved, of which 11% (each) were from the pancreas, gastroesophageal junction, and rectum. The ORR of this cohort was 26% (95%CI: 11-45%), the DCR was 32% (95%CI: 13%-57%), and the 6-month PFS was 32% (95%CI: 16%-61%); however, durable control was observed in patients with long-term diseases[38]. Based on the existing outcomes, Mohamed *et al*[39] conducted a retrospective study of 70 metastatic EP-NEC cases, mostly derived from GEP, of which 11 received Nivolumab in combination with Ipilimumab. The PFS (56.5 d, 47 d, and 258 d) and OS (not reached, 18.7 mo and 10.5 mo) were compared among patients treated with ICI monotherapy (8 patients), cytotoxic agents (23 patients) and dual ICI therapy. Thus, dual ICI therapy did show some improvement in high-grade NENs, in line with previous findings.

However, not all dual ICI therapies elicit a significant response or treatment effect. The final results from a prospective Phase II study of durvalumab plus tremelimumab in 123 patients with NENs who experienced disease progression and failure to respond to standard therapy administration were presented at the ENETS Annual Conference 2022. The cases were divided into four cohorts based on grading and primary sites: 27 patients with typical/atypical lung carcinoids for Cohort 1 (C1), 31 patients with G1/G2 GI-NETs for C2, 32 patients with G1/G2 pNETs for C3, and 33 patients with G3 GEP-NENs (91% NECs) for C4. The ORRs for the last three cohorts were 0%, 6.3%, and 9.1%, respectively, and the mPFSs were 5.8, 5.5, and 2.4 mo, in the same order. The median OSs were 29.5 mo, 23.8 mo, and 5.9 mo, respectively[40]. The 9-month DCRs for these cases were 22.8% (95%CI: 16.0-30.8), and 35.5% (95%CI: 20.5-53.0), 25% (95%CI: 12.6-41.7), and 6.1% (95%CI: 1.3-18.1) for C2 to C4, respectively[41]. Therefore, this study showed that the therapeutic effectiveness of dual ICI therapy in well-differentiated NENs was limited after the failure of standard treatment and had a relatively lower ORR. Only G3 GEP-NEN or NEC cases showed a modest survival benefit. In the NIPI-NEC phase II trial, 185 patients with platinum-refractory disease, including 93 patients with GEP-NEC, were enrolled. They were randomly assigned to receive nivolumab monotherapy or nivolumab plus ipilimumab, with ORR at eight weeks as the primary outcome measure. The cohort of nivolumab combined with ipilimumab in the trial demonstrated a prominent ORR of 14.9% (95%CI: 8.2-24.2) when compared to the nivolumab single-agent cohort of 7.2% (95%CI: 2.7-15.1); however, PFS was 1.9 mo (95%CI: 1.6-2.1) *vs* 1.8 mo (95%CI: 1.7-2.0), OS was 5.8 (95%CI: 3.3-7.6) *vs* 7.2 mo (95%CI: 3.7-14.1)[33]. Thus, most patients with NECs are not eligible for ICIs monotherapy, and the anti-tumor activity of dual ICIs therapy for both NETs and NECs needs further validation.

ICI combined with targeted anti-angiogenesis

Patients with refractory tumors or those who are chemo-intolerant have a high-quality option to de-chemotherapy in the form of ICIs combined with anti-angiogenic medications. Basic research has demonstrated a synergistic impact between ICIs and anti-angiogenic medications, encouraging angiogenesis in healthy tissues and boosting anti-tumor immunity [42]. Besides, anti-angiogenic targeted medications not only prevent angiogenesis in tumor tissue but also support the efficacy of ICIs by relieving the negative regulatory process of vascular endothelial growth factor (VEGF) in the immune microenvironment. They also influence lymphocyte and macrophage infiltration into tumor tissue, thereby alleviating the suppression of the tumor immune system[43].

Daniel and colleagues examined atezolizumab with bevacizumab combination therapy in G1/G2 pNET and non-pNET patients who had progressed under any previous therapy. Preliminary results showed that ORR, mPFS, and 1-year PFS of the two cohorts with 20 patients each were 20% (95%CI: 6%-44%) and 15% (95%CI: 3%-38%), and 19.6 mo (95%CI: 10.6-NR), and 14.9 mo (95%CI: 6.1-NR), 75% and 52%, respectively. These findings manifested that pNET patients might benefit more from this drug application regimen than non-pNET patients[44]. More recently, the MD Anderson Cancer Center released the final trial results showing that the ORR of the two groups remained consistent with the previous data; however, the PFS of the pNET and the non-pNET group descended to 14.9 mo (95%CI: 4.4-32.0) and 14.2 mo (95%CI: 10.2-19.6), respectively[45].

Al-Toubah *et al*[46] tested an alternative regimen, involving pembrolizumab plus lenvatinib, with efficacy in 20 patients with well-differentiated GI or thoracic NETs (including 9 of the small intestine, and 1 of cecal), and the response rate of the treatment was unsatisfactory. Among them, only one NET presented with PR, and the mPFS was 10 mo (95%CI: 5.9-14.1) in the small intestine, and the adverse effect rate was also high. Potentially related or related grade 3 AEs, were experienced by 12 patients, 14 patients required dose reduction or discontinuation of one of the drugs. Consequently, further research into this regimen was not deserved since it did not demonstrate substantial effectiveness for GI-NET. Therefore, it may be more worthwhile to delve into the safety and beneficial effects of ICIs paired with anti-angiogenesis in NECs from the digestive organs.

ICI combined with chemotherapy

Immunotherapy paired with chemotherapy has been successfully applied in the treatment of non-small cell lung cancer, gastric cancer, and esophageal cancer due to their synergistic mechanisms. Moreover, to expand the scope of application, some clinical study outcomes on the application of this sort of regimen in the NEN of the digestive system have been published.

The result of a phase II trial reported by Chan *et al*[47] revealed an unsatisfactory treatment response rate. The trial included twenty-two patients with extrapulmonary poorly differentiated NECs who had experienced disease progression after prior first-line therapy. These patients participated in a clinical trial investigating the combination of pembrolizumab and chemotherapy combination (16 of these patients had a primary GI site), 17 (77%) were treated with irinotecan, and 5 (23%) were treated with paclitaxel. The ORR was 9% among the patients, PR was achieved only in 2 cases, SD in 14% of cases, and PD in 60%. As inferred from the results, after biomarker selection, combination therapy with pembrolizumab and chemotherapy may improve treatment efficacy in cases of poorly differentiated GI-NEC.

In another phaseIIclinical study, twelve of the fifteen patients with advanced NET who were enrolled in and treated with nivolumab and temozolomide could be assessed for their response to this treatment, with the major locations being six small bowels, one pancreas, and five bronchial tubes[48]. According to the interim efficacy results, 25% of patients (3/12) had the best response of PR, 67% of patients (8/12) had SD, and one patient (8%) had PD.

The NICE-NEC study, which included 38 patients with advanced or irresectable gastroenteritis or G3 NENs of unknown origin, was the first to assess the efficacy of first-line chemotherapy plus ICIs in G3 NENs. Of these patients, 81.6% were GEP-NENs, and 68% were NECs, that received nivolumab combined with cisplatin or carboplatin[49]. The latest results, presented at the ESMO Congress 2022, confirmed the combinations had relatively promising therapeutic action, with an ORR and mPFS of survivors of 54.1% and 5.7 mo (95%CI: 5.1-9 mo), respectively[50]. Preliminary findings suggest that adding nivolumab to chemotherapy for G3 NENs has conceivable effectiveness without noticeably raising the toxicity profile of preferred standard chemotherapy.

Naturally, in certain rare circumstances, the combined effects of immunotherapy and chemotherapy may be therapeutically productive for some patients with terminal NECs, or those who have failed to respond to initial therapies. Chorath *et al*[51] reported a female case with metastatic high-grade NEC of the gallbladder who received immunochemotherapy treatment. A substantial decrease in liver metastases was observed following six cycles of comprehensive treatment with etoposide, carboplatin, nivolumab, and ipilimumab with a sustained response. However, further investigation is needed to comprehend the specific mechanisms and biomarkers that predict treatment efficacy. This survival benefit occurred in the absence of known predictive biomarkers to immunotherapy (PD-L1 status, mismatch repair status, and TMB).

Contrary to conventional perception, chemotherapy drugs can induce immunogenic tumor cells to undergo apoptosis, which mobilizes antigen-presenting cells and primes tumor-specific immune responses[52]. This fundamental principle underlies the previously described synergistic impact and the advantages of chemotherapy paired with ICIs in the application of NEN from the digestive system.

Table 2 provides an overview of ongoing clinical trials, which can be tracked to obtain the latest research progress of immunotherapies in NENs of the digestive systems.

BIOMARKERS FOR PREDICTING IMMUNOTHERAPY RESPONSE

MSI/dMMR, TMB, and PD-L1 expression are key biomarkers used to judge the potential benefit of ICIs for patients. Among these, MSI-H/dMMR, and TMB-H are independent adverse prognostic indicators[53]. However, the incidence of MSI-H/dMMR in NENs is relatively low, reported to be present in only 8 out of 152 GEP-NECs (5.3%) and only 1 in 29 G3 NETs (3.4%)[54]. TMB is the ratio of non-synonymous mutations in somatic cells per megabase pair of a certain genomic region. Although TMB is linked to the prognostic status of patients after ICIs therapy for the majority of malignancies, the selection of detecting genes will directly impact the TMB calculation's outcomes[55]. It is unlikely that there is an exact universal value that defines TMB-H in a way that can predict the benefit of ICI in all types of cancer[56]. In a number of published trials, PD-L1 positive patients treated with ICIs had a higher ORR than PD-L1 negative patients,

Table 2 Ongoing clinical trials related to immune checkpoint inhibitors in neuroendocrine neoplasms of the digestive system

Indentifier of Clinical Trails	Intervention	Study phase	Primary outcome measures	Estimated or actual enrollment	Trial status	Estimated study completion date	Condition related to NENs of the digestive system
NCT04079712	Nivolumab + Ipilimumab + Cabozantinib	II	overall response rate	30	Active, not recruiting	October 2023	NECs: Excluding SCLC and MCC
NCT03980925	Nivolumab + Platinum-doublet chemotherapy	II	OS at 12 mo	38	Active, not recruiting	December 2023	G3 NENs: GEP or unknown primary site
NCT04197310	Cabozantinib + Nivolumab	II	ORR	35	Active, not recruiting	December 2023	WD-NET: Non-pancreatic (*i.e.*, carcinoid) origin
NCT03290079	Pembrolizumab + Lenvatinib	II	ORR	28	Active, not recruiting	January 2024	NETs: WD small bowel or colon origin, including unknown primary, excluding pNENs
NCT04400474	Cabozantinib + Atezolizumab	II	ORR	93	Active, not recruiting	March 2024	WD G1/2 NET: Digestive system; G3 NEN: Excluding SCLC
NCT04579757	Surufatinib + Tislelizumab	Ib/II	DLT, ORR	135	Active, not recruiting	June 2024	G1/2 NETs: Thoracic or GEP origins
NCT04525638	Nivolumab + 177Lu-DOTATATE	II	overall response rate	30	Recruiting	September 2024	G3 NET or NEC of GEP, lung, and unknown primary site
NCT05058651	Atezolizumab + Platinum drug (cisplatin or carboplatin) + Etoposide	II/III	OS	189	Recruiting	October 2024	NEC: Extrapulmonary
NCT05113355	Chidamide + Sintilimab	II	ORR	23	Recruiting	November 2024	High-grade NEN, advanced and metastatic
NCT04969887	Nivolumab + Ipilimumab	II	CBR, 6 mo PFS	240	Recruiting	December 2024	NECs and G3 NETs: Independent of primary site, excluding SCLC
NCT04701307	Dostarlimab + Niraparib	II	6 mo PFS, 3 mo ORR	48	Active, not recruiting	May 2025	NECs: Excluding prostate origin
NCT03475953	Avelumab + Regorafenib	I/II	Phase I: Recommended dose of regorafenib; Phase II (cohort G): CR or PR	747 (only cohort G consists of GEP-NETs)	Recruiting	December 2025	G2/3 GEP-NETs
NCT05746208	Lenvatinib + Pembrolizumab	II	ORR	29	Not yet recruiting	July 2027	WD G3 NET: GIsite and pancreas primary
NCT05289856	Avelumab + Cabozantinib	II	DCR, CR, PR, SD	30	Recruiting	December 2025	G3 NET: Excluding MCC and SCLC
NCT05627427	Surufatinib + Sintilimab	II	PFS	60	Recruiting	December 2024	G3 NET and NEC: Metastatic and advanced
NCT05262556	NP-101 (TQ Formula) + Nivolumab + Ipilimumab	I	CR, PR, SD	10	Recruiting	December 2024	GEP-NET, GEP-NEC: Poorly differentiated

CR: Complete response; CBR: Clinical benefit rate; DCR: Disease control rate; DLT: Dose-limiting toxicity; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; PR: Partial response; WD: Well differentiated; NET: Neuroendocrine tumor; NEN: Neuroendocrine neoplasm; NEC: Neuroendocrine carcinoma; GEP: Gastroenteropancreatic; SCLC: Small cell lung cancer; MCC: Merkel cell carcinoma; pNET: Pancreas NET; GI: gastrointestinal; G1, 2, 3: Grade 1, 2, 3.

which indicated that PD-L1 expression may be relevant to the efficacy of immunotherapy[45]. However, PD-L1 expression appears quite heterogeneous across different studies[57], even though some studies revealed no relevance between the effectiveness of ICIs and PD-L1 expression[30,31,40]. Reasons for these results may be subject to limitations due to factors such as different methods of analysis, defined cutoffs, and the freshness of biopsy tissue obtained[58]. Therefore, based on identifying the expression of PD-L1, investigate the content of other predictive biomarkers to provide patients with the best immunotherapy prediction possible.

In recent years, somatic mutation analysis of NETs has emerged as a novel approach for identification and prediction. Unlike conventional monoanalytic biomarker testing, mRNA-based liquid biopsy is a non-invasive genetic testing method. The NET transcriptome signature (NETest), a pre-spotted PCR plate that targets 51 genes, measures the amount of tumor-derived mRNA extracted from a patient's blood using PCR[59]. Compared to CGA, the diagnostic accuracy of GEP-NENs with NETest is significantly better (99% *vs* 21%-36%), and it also has good sensitivity and specificity for evaluating the course and prognosis of NENs[60]. However, despite these advancements, the gold standard for NETest has not been established in clinical practice preventing its use as a routine test for NEN and limiting its ability to completely replace biopsy procedures.

Future research should prioritize the integration of data on the circulating tumor DNA, T-cell regulatory factors, and other characteristics of NEN patients who will receive immunotherapy. By employing multidimensional and dynamic combinations of biomarkers, researchers can enhance the predictive effect and fully elucidate the potential causes of patients' poor responses to ICIs.

DISCUSSION

It is necessary for the body to trigger an appropriate anti-tumor immune response through the infiltration of immune cells into the tumor immune microenvironment (TIME). The strength of individual anti-tumor immunity depends significantly on the quantity and variety of T cells[61]. As a result, there is now broad acceptance that effector T cell plays an irreplaceable role in the anti-tumor response[62].

Malignancies that exhibit significant tumor-infiltrating lymphocyte (TIL) infiltration, high PD-L1 expression, potential genetic susceptibility, and the existence of an active anti-tumor immune response, often known as the hallmarks of "hot tumors," are more receptive to immunotherapy[63]. Although recent outcomes of clinical trials suggest that the efficacy of ICIs for NEN of the digestive system fell short of expectations, considerable TIL infiltration has been observed in various NECs, including pNECs[64]. Moreover, the likelihood that NEN will match the TIME of hot tumors increases with NEN grade. In a study of 244 patients with GEP-NEN, the G3 NEN cohort contained considerably more patients with high TILs infiltration than the G1/G2 NEN group (50% *vs* 17.1%)[65]. PD-L1-expressing GEP-NEC more frequently exhibited T cell exhaustion and an abundance of regulatory T cells than did G3-NET[66], indicating that poorly differentiated NECs are more prone to benefit from immunotherapies, particularly ICIs. Additionally, there was heterogeneity in the TIME of the primary sites of NENs. For instance, in comparison to NETs from the jejunum and ileum, the duodenum NETs had a more positive detection rate of PD-1 and immune infiltration[67]; and compared to NETs of extra-pancreatic origin, pNETs had higher expression levels of PD-1 and PD-L1, as well as more TILs[65]. At the same primary site, PD-L1 expression varies across multiple NET subtypes as well, with prior research demonstrating that the metastasis-like primary (MLP)-1 subtype expresses PD-L1 at the highest levels in pNET[68]. This illustrates the need for further research into the variations in the immune microenvironments of distinct differentiated primary sites and subtypes of NEN, which may guide the therapeutic application of ICIs and assist in selecting the best immunotherapy regimens for patients during clinical trials.In addition, probing into the processes which transform NEN with modest levels of TIL infiltration and PD-L1 expression into immunologically "hot" tumors could potentially serve as a research avenue to enhance the therapeutic effectiveness of ICIs. So far, several mechanisms have been implicated in altering the low immune state of "cold" tumors, for example, stimulating T cell priming (*e.g.*, injection of Neoepitope Cancer Vaccine), accelerating T cell expansion (*e.g.*, application of interleukin (IL)-15), and inducing T cell recruitment (*e.g.*, through the use of epigenetic modulators and chemokines), *etc*[69]. Further research is needed to evaluate the feasibility of the above treatments for NEN of the digestive system.

CONCLUSION

Due to the complexity of NENs in the digestive system, the prognosis following the failure of first-line treatment is frequently poor, and the options for subsequent treatment are constrained. ICI monotherapy for the NEN is less effective, has a shorter duration of disease control, and may only be effective in selected patients. Combination therapy may improve the efficacy of ICI treatment in certain cases. Dual immunotherapy has some effect on highly malignant NECs and may be considered after the failure of standard treatment; however, more relevant clinical studies are needed in this field. The combination of ICI and anti-angiogenic drugs has exhibited certain advantages and promising applications for advanced NETs. The combination of ICI and chemotherapy has shown some efficacy for advanced NENs and NECs; however, further studies are required to determine whether it ultimately improves patient outcomes, and how to select the best combination regimen. Additionally, several preclinical studies and clinical trials have investigated the combination of ICIs with other clinical oncology treatment modalities, including pericyte therapy (CAR-T cell therapy), cytokine therapy, oncolytic virus therapy, and cancer vaccines, but experimental data in NEN are lacking. Other strategies to modify the TME of NEN, such as enhancing T-cell homing, preventing T-cell depletion, and preserving

CD8+ T-cell immune response, are also worth investigating in conjunction with ICIs for targeted therapy[70]. TMB-H, PD-L1, and MSI-H/dMMR have been identified as biomarkers for screening patients who may benefit from immunotherapy; however, their overall predictive efficiency is limited. Further research on the immune microenvironment and the search for more predictive immunotherapy markers with higher sensitivity and specificity to guide clinical treatment are urgently needed.

Owing to the rarity of NENs of the digestive organs, most previous and ongoing clinical trials have predominantly used small samples and on small scales approaches, resulting in limited trial results on the subject. Currently, the evidence supporting the use of ICI for NENs of the digestive systems is primarily based on a small number of phase I or II studies and a few case reports. To address this limitation, it is crucial to conduct multi-center, prospective, large-sample clinical trials. Such trials can further validate the conclusions drawn from studies like those discussed here. Additionally, these trials can guide the application of ICI for the treatment of NENs from the digestive system, and provide promising ideas for future research in the field of immunotherapy.

FOOTNOTES

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REFERENCES

1 **de Mestier L**, Cros J, Neuzillet C, Hentic O, Egal A, Muller N, Bouché O, Cadiot G, Ruszniewski P, Couvelard A, Hammel P. Digestive System Mixed Neuroendocrine-Non-Neuroendocrine Neoplasms. *Neuroendocrinology* 2017; **105**: 412-425 [PMID: 28803232 DOI: 10.1159/000475527]

2 **Anlauf M**, Garbrecht N, Bauersfeld J, Schmitt A, Henopp T, Komminoth P, Heitz PU, Perren A, Klöppel G. Hereditary neuroendocrine tumors of the gastroenteropancreatic system. *Virchows Arch* 2007; **451** Suppl 1: S29-S38 [PMID: 17684762 DOI: 10.1007/s00428-007-0450-3]

3 **Marx SJ**. Recent Topics Around Multiple Endocrine Neoplasia Type 1. *J Clin Endocrinol Metab* 2018; **103**: 1296-1301 [PMID: 29897580 DOI: 10.1210/jc.2017-02340]

4 **Anlauf M**, Perren A, Klöppel G. Endocrine precursor lesions and microadenomas of the duodenum and pancreas with and without MEN1: criteria, molecular concepts and clinical significance. *Pathobiology* 2007; **74**: 279-284 [PMID: 17890894 DOI: 10.1159/000105810]

5 **Speisky D**, Duces A, Bièche I, Rebours V, Hammel P, Sauvanet A, Richard S, Bedossa P, Vidaud M, Murat A, Niccoli P, Scoazec JY, Ruszniewski P, Couvelard A; GTE Group, Groupe d'Etude des Tumeurs Endocrines. Molecular profiling of pancreatic neuroendocrine tumors in sporadic and Von Hippel-Lindau patients. *Clin Cancer Res* 2012; **18**: 2838-2849 [PMID: 22461457 DOI: 10.1158/1078-0432.CCR-11-2759]

6 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]

7 **Dasari A**, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, Shih T, Yao JC. Trends in the Incidence, Prevalence, and Survival Outcomes in Patients With Neuroendocrine Tumors in the United States. *JAMA Oncol* 2017; **3**: 1335-1342 [PMID: 28448665 DOI: 10.1001/jamaoncol.2017.0589]

8 **White BE**, Rous B, Chandrakumaran K, Wong K, Bouvier C, Van Hemelrijck M, George G, Russell B, Srirajaskanthan R, Ramage JK. Incidence and survival of neuroendocrine neoplasia in England 1995-2018: A retrospective, population-based study. *Lancet Reg Health Eur* 2022; **23**: 100510 [PMID: 36176500 DOI: 10.1016/j.lanepe.2022.100510]

9 **Chang JS**, Chen LT, Shan YS, Chu PY, Tsai CR, Tsai HJ. An updated analysis of the epidemiologic trends of neuroendocrine tumors in Taiwan. *Sci Rep* 2021; **11**: 7881 [PMID: 33846396 DOI: 10.1038/s41598-021-86839-2]

10 **Genus TSE**, Bouvier C, Wong KF, Srirajaskanthan R, Rous BA, Talbot DC, Valle JW, Khan M, Pearce N, Elshafie M, Reed NS, Morgan E, Deas A, White C, Huws D, Ramage J. Impact of neuroendocrine morphology on cancer outcomes and stage at diagnosis: a UK nationwide cohort study 2013-2015. *Br J Cancer* 2019; **121**: 966-972 [PMID: 31649320 DOI: 10.1038/s41416-019-0606-3]

11 **Fan JH**, Zhang YQ, Shi SS, Chen YJ, Yuan XH, Jiang LM, Wang SM, Ma L, He YT, Feng CY, Sun XB, Liu Q, Deloso K, Chi Y, Qiao YL. A nation-wide retrospective epidemiological study of gastroenteropancreatic neuroendocrine neoplasms in china. *Oncotarget* 2017; **8**: 71699-71708 [PMID: 29069739 DOI: 10.18632/oncotarget.17599]

12 **Niu C**, Wang S, Guan Q, Ren X, Ji B, Liu Y. Neuroendocrine tumors of the gallbladder. *Oncol Lett* 2020; **19**: 3381-3388 [PMID: 32269610 DOI: 10.3892/ol.2020.11461]

13 **Huang K**, Lan Z, Chen W, Zhang J, Wang J, Zhu H, Xu B, Zhang L, Lu T, Guo Y, Wen Z. Hepatectomy and pneumectomy combined with targeted therapy for primary hepatic neuroendocrine carcinoma: Case report and review of the literature. *Front Surg* 2022; **9**: 920276 [PMID: 35910478 DOI: 10.3389/fsurg.2022.920276]

14 **Frizziero M**, Chakrabarty B, Nagy B, Lamarca A, Hubner RA, Valle JW, McNamara MG. Mixed Neuroendocrine Non-Neuroendocrine Neoplasms: A Systematic Review of a Controversial and Underestimated Diagnosis. *J Clin Med* 2020; **9** [PMID: 31963850 DOI: 10.3390/jcm9010273]

15 **Cheng AL**, Hsu C, Chan SL, Choo SP, Kudo M. Challenges of combination therapy with immune checkpoint inhibitors for hepatocellular carcinoma. *J Hepatol* 2020; **72**: 307-319 [PMID: 31954494 DOI: 10.1016/j.jhep.2019.09.025]

16 **Yi M**, Zheng X, Niu M, Zhu S, Ge H, Wu K. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. *Mol Cancer* 2022; **21**: 28 [PMID: 35062949 DOI: 10.1186/s12943-021-01489-2]

17 **Yao JC**, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, Tomasek J, Raderer M, Lahner H, Voi M, Pacaud LB, Rouyrre N, Sachs C, Valle JW, Fave GD, Van Cutsem E, Tesselaar M, Shimada Y, Oh DY, Strosberg J, Kulke MH, Pavel ME; RAD001 in Advanced Neuroendocrine Tumours, Fourth Trial (RADIANT-4) Study Group. Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet* 2016; **387**: 968-977 [PMID: 26703889 DOI: 10.1016/S0140-6736(15)00817-X]

18 **Zhang P**, Li J, Zhang X, Zhou J, Wang X, Peng Z, Shen L, Lu M. Etoposide and cisplatin *vs* irinotecan and cisplatin as the first-line therapy for patients with advanced, poorly differentiated gastroenteropancreatic neuroendocrine carcinoma: A randomized phase 2 study. *Cancer* 2020; **126** Suppl 9: 2086-2092 [PMID: 32293725 DOI: 10.1002/cncr.32750]

19 **Frizziero M**, Spada F, Lamarca A, Kordatou Z, Barriuso J, Nuttall C, McNamara MG, Hubner RA, Mansoor W, Manoharan P, Fazio N, Valle JW. Carboplatin in Combination with Oral or Intravenous Etoposide for Extra-Pulmonary, Poorly-Differentiated Neuroendocrine Carcinomas. *Neuroendocrinology* 2019; **109**: 100-112 [PMID: 30703770 DOI: 10.1159/000497336]

20 **Rogowski W**, Wachuła E, Gorzelak A, Lebiedzińska A, Sulżyc-Bielicka V, Iżycka-Świeszewska E, Żołnierek J, Kos-Kudła B. Capecitabine and temozolomide combination for treatment of high-grade, well-differentiated neuroendocrine tumour and poorly-differentiated neuroendocrine carcinoma - retrospective analysis. *Endokrynol Pol* 2019; **70**: 313-317 [PMID: 30843182 DOI: 10.5603/EP.a2019.0010]

21 **Marabelle A**, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, Chung HC, Kindler HL, Lopez-Martin JA, Miller WH Jr, Italiano A, Kao S, Piha-Paul SA, Delord JP, McWilliams RR, Fabrizio DA, Aurora-Garg D, Xu L, Jin F, Norwood K, Bang YJ. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol* 2020; **21**: 1353-1365 [PMID: 32919526 DOI: 10.1016/S1470-2045(20)30445-9]

22 **Merino DM**, McShane LM, Fabrizio D, Funari V, Chen SJ, White JR, Wenz P, Baden J, Barrett JC, Chaudhary R, Chen L, Chen WS, Cheng JH, Cyanam D, Dickey JS, Gupta V, Hellmann M, Helman E, Li Y, Maas J, Papin A, Patidar R, Quinn KJ, Rizvi N, Tae H, Ward C, Xie M, Zehir A, Zhao C, Dietel M, Stenzinger A, Stewart M, Allen J; TMB Harmonization Consortium. Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. *J Immunother Cancer* 2020; **8** [PMID: 32217756 DOI: 10.1136/jitc-2019-000147]

23 **Marabelle A**, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, Geva R, Gottfried M, Penel N, Hansen AR, Piha-Paul SA, Doi T, Gao B, Chung HC, Lopez-Martin J, Bang YJ, Frommer RS, Shah M, Ghori R, Joe AK, Pruitt SK, Diaz LA Jr. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* 2020; **38**: 1-10 [PMID: 31682550 DOI: 10.1200/JCO.19.02105]

24 **Klein O**, Kee D, Markman B, Michael M, Underhill C, Carlino MS, Jackett L, Lum C, Scott C, Nagrial A, Behren A, So JY, Palmer J, Cebon J. Immunotherapy of Ipilimumab and Nivolumab in Patients with Advanced Neuroendocrine Tumors: A Subgroup Analysis of the CA209-538 Clinical Trial for Rare Cancers. *Clin Cancer Res* 2020; **26**: 4454-4459 [PMID: 32532787 DOI: 10.1158/1078-0432.CCR-20-0621]

25 **Le DT**, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA Jr. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; **357**: 409-413 [PMID: 28596308 DOI: 10.1126/science.aan6733]

26 **Mulvey C**, Raj NP, Chan JA, Aggarwal RR, Cinar P, Hope TA, Kolli K, Zhang L, Calabrese S, Grabowsky JA, Modarresi L, Kelly V, Stonely D, Munster PN, Reidy DL, Fong L, Bergsland EK. Phase II study of pembrolizumab-based therapy in previously treated extrapulmonary poorly differentiated neuroendocrine carcinomas: Results of Part A (pembrolizumab alone). *JCO* 2019; **37**: 363-363 [DOI: 10.1200/JCO.2019.37.4_suppl.363]

27 **Fottner C**, Apostolidis L, Ferrata M, Krug S, Michl P, Schad A, Roth W, Jaeger D, Galle PR, Weber MM. A phase II, open label, multicenter trial of avelumab in patients with advanced, metastatic high-grade neuroendocrine carcinomas NEC G3 (WHO 2010) progressive after first-line chemotherapy (AVENEC). *J Clin Oncol* 2019; **37**: 4103-4103 [DOI: 10.1200/JCO.2019.37.15_suppl.4103]

28 **Yao JC**, Strosberg J, Fazio N, Pavel ME, Ruszniewski P, Bergsland E, Li D, Tafuto S, Raj N, Campana D, Hijioka S, Raderer M, Guimbaud R, Gajate P, Pusceddu S, Reising A, Degtyarev E, Mookerjee B, Aimone P, Singh S. Activity & safety of spartalizumab (PDR001) in patients (pts) with advanced neuroendocrine tumors (NET) of pancreatic (Pan), gastrointestinal (GI), or thoracic (T) origin, & gastroenteropancreatic neuroendocrine carcinoma (GEP NEC) who have progressed on prior treatment (Tx). *Ann Oncol* 2018; **29**: viii467-viii468 [DOI: 10.1093/annonc/mdy293.001]

29 **Mehnert JM**, Bergsland E, O'Neil BH, Santoro A, Schellens JHM, Cohen RB, Doi T, Ott PA, Pishvaian MJ, Puzanov I, Aung KL, Hsu C, Le Tourneau C, Hollebecque A, Élez E, Tamura K, Gould M, Yang P, Stein K, Piha-Paul SA. Pembrolizumab for the treatment of programmed death-ligand 1-positive advanced carcinoid or pancreatic neuroendocrine tumors: Results from the KEYNOTE-028 study. *Cancer* 2020; **126**: 3021-3030 [PMID: 32320048 DOI: 10.1002/cncr.32883]

30 **Vijayvergia N**, Dasari A, Deng M, Litwin S, Al-Toubah T, Alpaugh RK, Dotan E, Hall MJ, Ross NM, Runyen MM, Denlinger CS, Halperin DM, Cohen SJ, Engstrom PF, Strosberg JR. Pembrolizumab monotherapy in patients with previously treated metastatic high-grade

neuroendocrine neoplasms: joint analysis of two prospective, non-randomised trials. *Br J Cancer* 2020; **122**: 1309-1314 [PMID: 32152503 DOI: 10.1038/s41416-020-0775-0]

31 **Strosberg J**, Mizuno N, Doi T, Grande E, Delord JP, Shapira-Frommer R, Bergsland E, Shah M, Fakih M, Takahashi S, Piha-Paul SA, O'Neil B, Thomas S, Lolkema MP, Chen M, Ibrahim N, Norwood K, Hadoux J. Efficacy and Safety of Pembrolizumab in Previously Treated Advanced Neuroendocrine Tumors: Results From the Phase II KEYNOTE-158 Study. *Clin Cancer Res* 2020; **26**: 2124-2130 [PMID: 31980466 DOI: 10.1158/1078-0432.CCR-19-3014]

32 **Lu M**, Zhang P, Zhang Y, Li Z, Gong J, Li J, Li Y, Zhang X, Lu Z, Wang X, Zhou J, Peng Z, Wang W, Feng H, Wu H, Yao S, Shen L. Efficacy, Safety, and Biomarkers of Toripalimab in Patients with Recurrent or Metastatic Neuroendocrine Neoplasms: A Multiple-Center Phase Ib Trial. *Clin Cancer Res* 2020; **26**: 2337-2345 [PMID: 32086343 DOI: 10.1158/1078-0432.CCR-19-4000]

33 **Girard N**, Mazieres J, Otto J, Lena H, Lepage C, Egenod T, Smith D, Madelaine J, Gérinière L, Hajbi FE, Ferru A, Clément-Duchêne C, Madroszyk A, Desrame J, Morin F, Langlais A, Michel P, Louvet C, Westeel V, Walter T. LBA41 Nivolumab (nivo) ± ipilimumab (ipi) in pre-treated patients with advanced, refractory pulmonary or gastroenteropancreatic poorly differentiated neuroendocrine tumors (NECs) (GCO-001 NIPINEC). *Ann Oncol* 2021; **32**: S1318 [DOI: 10.1016/j.annonc.2021.08.2119]

34 **Yao JC**, Strosberg J, Fazio N, Pavel ME, Bergsland E, Ruszniewski P, Halperin DM, Li D, Tafuto S, Raj N, Campana D, Hijioka S, Raderer M, Guimbaud R, Gajate P, Pusceddu S, Reising A, Degtyarev E, Shilkrut M, Eddy S, Singh S. Spartalizumab in metastatic, well/poorly-differentiated neuroendocrine neoplasms. *Endocr Relat Cancer* 2021 [PMID: 33480358 DOI: 10.1530/ERC-20-0382]

35 **Chan DL**, Rodriguez-Freixinos V, Doherty M, Wasson K, Iscoe N, Raskin W, Hallet J, Myrehaug S, Law C, Thawer A, Nguyen K, Singh S. Avelumab in unresectable/metastatic, progressive, grade 2-3 neuroendocrine neoplasms (NENs): Combined results from NET-001 and NET-002 trials. *Eur J Cancer* 2022; **169**: 74-81 [PMID: 35504244 DOI: 10.1016/j.ejca.2022.03.029]

36 **Al-Toubah T**, Halfdanarson T, Gile J, Morse B, Sommerer K, Strosberg J. Efficacy of ipilimumab and nivolumab in patients with high-grade neuroendocrine neoplasms. *ESMO Open* 2022; **7**: 100364 [PMID: 34973511 DOI: 10.1016/j.esmoop.2021.100364]

37 **Patel SP**, Othus M, Chae YK, Giles FJ, Hansel DE, Singh PP, Fontaine A, Shah MH, Kasi A, Baghdadi TA, Matrana M, Gatalica Z, Korn WM, Hayward J, McLeod C, Chen HX, Sharon E, Mayerson E, Ryan CW, Plets M, Blanke CD, Kurzrock R. A Phase II Basket Trial of Dual Anti-CTLA-4 and Anti-PD-1 Blockade in Rare Tumors (DART SWOG 1609) in Patients with Nonpancreatic Neuroendocrine Tumors. *Clin Cancer Res* 2020; **26**: 2290-2296 [PMID: 31969335 DOI: 10.1158/1078-0432.CCR-19-3356]

38 **Patel SP**, Mayerson E, Chae YK, Strosberg J, Wang J, Konda B, Hayward J, McLeod CM, Chen HX, Sharon E, Othus M, Ryan CW, Plets M, Blanke CD, Kurzrock R. A phase II basket trial of Dual Anti-CTLA-4 and Anti-PD-1 Blockade in Rare Tumors (DART) SWOG S1609: High-grade neuroendocrine neoplasm cohort. *Cancer* 2021; **127**: 3194-3201 [PMID: 33882143 DOI: 10.1002/cncr.33591]

39 **Mohamed A**, Vijayvergia N, Kurian M, Liu L, Fu P, Das S. Exploring Real World Outcomes with Nivolumab Plus Ipilimumab in Patients with Metastatic Extra-Pulmonary Neuroendocrine Carcinoma (EP-NEC). *Cancers (Basel)* 2022; **14** [PMID: 35681675 DOI: 10.3390/cancers14112695]

40 **Capdevila J**, Teule A, López C, García-Carbonero R, Benavent M, Custodio A, Cubillo A, Alonso V, Gordoa TA, Carmona-Bayonas A, Crespo G, Blanco-Codesido M, Jimenez-Fonseca P, Viúdez A, Muñoa ALC, Sevilla I, Llanos M, Segura A, Hernando-Cubero J, Manzano JL. 1157O A multi-cohort phase II study of durvalumab plus tremelimumab for the treatment of patients (pts) with advanced neuroendocrine neoplasms (NENs) of gastroenteropancreatic or lung origin: The DUNE trial (GETNE 1601). *Ann Oncol* 2020; **31**: S770-S771 [DOI: 10.1016/j.annonc.2020.08.1370]

41 **Capdevila J**, Hernando J, Teule A, Lopez C, Garcia-Carbonero R, Benavent M, Custodio A, Garcia-Alvarez A, Cubillo A, Alonso V, Carmona-Bayonas A, Alonso-Gordoa T, Crespo G, Jimenez-Fonseca P, Blanco M, Viudez A, Casta AL, Sevilla I, Segura A, Llanos M, Landolfi S, Nuciforo P, Manzano JL. Durvalumab plus tremelimumab for the treatment of advanced neuroendocrine neoplasms of gastroenteropancreatic and lung origin: the phase II DUNE trial (GETNE 1601). In Review [DOI: 10.21203/rs.3.rs-2174016/v1]

42 **Shigeta K**, Datta M, Hato T, Kitahara S, Chen IX, Matsui A, Kikuchi H, Mamessier E, Aoki S, Ramjiawan RR, Ochiai H, Bardeesy N, Huang P, Cobbold M, Zhu AX, Jain RK, Duda DG. Dual Programmed Death Receptor-1 and Vascular Endothelial Growth Factor Receptor-2 Blockade Promotes Vascular Normalization and Enhances Antitumor Immune Responses in Hepatocellular Carcinoma. *Hepatology* 2020; **71**: 1247-1261 [PMID: 31378984 DOI: 10.1002/hep.30889]

43 **Yang J**, Yan J, Liu B. Targeting VEGF/VEGFR to Modulate Antitumor Immunity. *Front Immunol* 2018; **9**: 978 [PMID: 29774034 DOI: 10.3389/fimmu.2018.00978]

44 **Halperin DM**, Liu S, Dasari A, Fogelman DR, Bhosale P, Mahvash A, Dervin S, Estrella J, Cortazar P, Maru DM, Mckenna EF, Wistuba II, Schulze K, Futreal PA, Darbonne WC, Yun C, Hwu P, Yao JC. A phase II trial of atezolizumab and bevacizumab in patients with advanced, progressive neuroendocrine tumors (NETs). *JCO* 2020; **38**: 619-619 [DOI: 10.1200/JCO.2020.38.4_suppl.619]

45 **Halperin DM**, Liu S, Dasari A, Fogelman D, Bhosale P, Mahvash A, Estrella JS, Rubin L, Morani AC, Knafl M, Overeem TA, Fu S-C, Solis LM, Parra Cuentas E, Verma A, Chen H-L, Gite S, Subashchandrabose P, Dervin S, Schulze K, Darbonne WC, Yun C, Wistuba II, Futreal PA, Woodman SE, Yao JC. Assessment of Clinical Response Following Atezolizumab and Bevacizumab Treatment in Patients With Neuroendocrine Tumors: A Nonrandomized Clinical Trial. *JAMA Oncology* 2022; **8**: 904-909 [DOI: 10.1001/jamaoncol.2022.0212]

46 **Al-Toubah T**, Morse B, Haider M, Valone T, Strosberg J. Phase II Study of Pembrolizumab and Lenvatinib in Advanced Well-Differentiated Neuroendocrine Tumors. *Endocrine Abstracts* 2023; **89** [DOI: 10.1530/endoabs.89.C8]

47 **Chan JA**, Raj NP, Aggarwal RR, Calabrese S, DeMore A, Dhawan MS, Fattah D, Fong L, Grabowsky J, Hope TA, Kolli KP, Munster PN, Perez K, Reidy DL, Von Fedak S, Zhang L, Bergsland EK. Phase II study of pembrolizumab-based therapy in previously treated extrapulmonary poorly differentiated neuroendocrine carcinomas: Results of Part B (pembrolizumab + chemotherapy). *JCO* 2021; **39**: 4148-4148 [DOI: 10.1200/JCO.2021.39.15_suppl.4148]

48 **Owen DH**, Wei L, Goyal A, Zhou Y, Suffren S-A, Jacob R, Pilcher C, Otterson GA, Verschraegen CF, Shah MH, Konda B. CLO20-054: A Phase 2 Trial of Nivolumab and Temozolomide in Advanced Neuroendocrine Tumors (NETs): Interim Efficacy Analysis. *J Natl Compr Canc Netw* 2020; **18**: CLO20-054 [DOI: 10.6004/jnccn.2019.7460]

49 **Riesco-Martinez MC**, Capdevila J, Alonso V, Jimenez-Fonseca P, Teule A, Grande E, Sevilla I, Viñuales MB, Alonso-Gordoa T, Custodio A, Hernando J, Polo E, Trujillo OAC, Garcia-Carbonero R. 1098O Nivolumab plus platinum-doublet chemotherapy as first-line therapy in unresectable, locally advanced or metastatic G3 neuroendocrine Neoplasms (NENs) of the gastroenteropancreatic (GEP) tract or unknown (UK) origin: Preliminary results from the phase II NICE-NEC trial (GETNE T1913). *Ann Oncol* 2021; **32**: S908-S909 [DOI: 10.1016/j.annonc.2021.08.180]

50 **Riesco Martinez MC**, Capdevila Castillon J, Alonso V, Jimenez-Fonseca P, Teule A, Grande E, Sevilla I, Benavent M, Alonso-Gordoa T, Custodio A, Antón Pascual B, Hernando J, Polo E, Castillo Trujillo OA, Garcia-Carbonero R. 496MO Final overall survival results from the

NICE-NEC trial (GETNE-T1913): A phase II study of nivolumab and platinum-doublet chemotherapy (CT) in untreated advanced G3 neuroendocrine neoplasms (NENs) of gastroenteropancreatic (GEP) or unknown (UK) origin. *Ann Oncol* 2022; **33**: S769 [DOI: 10.1016/j.annonc.2022.07.624]

51 **Chorath J**, Placencio-Hickok VR, Guan M, Nissen N, Kamrava M, Klempner SJ, Nassir Y, Hendifar A, Gong J. Durable Response to Carboplatin, Etoposide, Nivolumab, and Ipilimumab in Metastatic High-Grade Neuroendocrine Carcinoma of the Gallbladder. *Pancreas* 2020; **49**: e19-e20 [PMID: 32049953 DOI: 10.1097/MPA.0000000000001480]

52 **Galluzzi L**, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol* 2020; **17**: 725-741 [PMID: 32760014 DOI: 10.1038/s41571-020-0413-z]

53 **La Salvia A**, Barroso G, Espinosa Olarte P, Anton Pascual B, Modrego Sanchez A, Riesco-Martinez M, Garcia-Carbonero R. High TMB and MSI-high as independent predictors of survival in NENs. In: Journal of Neuroendocrinology. WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, 2021: 108-108

54 **Venizelos A**, Elvebakken H, Perren A, Nikolaienko O, Deng W, Lothe IMB, Couvelard A, Hjortland GO, Sundlöv A, Svensson J, Garresori H, Kersten C, Hofsli E, Detlefsen S, Krogh M, Sorbye H, Knappskog S. The molecular characteristics of high-grade gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer* 2021; **29**: 1-14 [PMID: 34647903 DOI: 10.1530/ERC-21-0152]

55 **Chalmers ZR**, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J, Huang F, He Y, Sun J, Tabori U, Kennedy M, Lieber DS, Roels S, White J, Otto GA, Ross JS, Garraway L, Miller VA, Stephens PJ, Frampton GM. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 2017; **9**: 34 [PMID: 28420421 DOI: 10.1186/s13073-017-0424-2]

56 **Samstein RM**, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, Kaley TJ, Kendall SM, Motzer RJ, Hakimi AA, Voss MH, Russo P, Rosenberg J, Iyer G, Bochner BH, Bajorin DF, Al-Ahmadie HA, Chaft JE, Rudin CM, Riely GJ, Baxi S, Ho AL, Wong RJ, Pfister DG, Wolchok JD, Barker CA, Gutin PH, Brennan CW, Tabar V, Mellinghoff IK, DeAngelis LM, Ariyan CE, Lee N, Tap WD, Gounder MM, D'Angelo SP, Saltz L, Stadler ZK, Scher HI, Baselga J, Razavi P, Klebanoff CA, Yaeger R, Segal NH, Ku GY, DeMatteo RP, Ladanyi M, Rizvi NA, Berger MF, Riaz N, Solit DB, Chan TA, Morris LGT. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019; **51**: 202-206 [PMID: 30643254 DOI: 10.1038/s41588-018-0312-8]

57 **Cives M**, Pelle' E, Strosberg J. Emerging Treatment Options for Gastroenteropancreatic Neuroendocrine Tumors. *J Clin Med* 2020; **9** [PMID: 33202931 DOI: 10.3390/jcm9113655]

58 **Robinson MD**, Livesey D, Hubner RA, Valle JW, McNamara MG. Future therapeutic strategies in the treatment of extrapulmonary neuroendocrine carcinoma: a review. *Ther Adv Med Oncol* 2023; **15**: 17588359231156870 [PMID: 36872945 DOI: 10.1177/17588359231156870]

59 **Oberg K**, Modlin IM, De Herder W, Pavel M, Klimstra D, Frilling A, Metz DC, Heaney A, Kwekkeboom D, Strosberg J, Meyer T, Moss SF, Washington K, Wolin E, Liu E, Goldenring J. Consensus on biomarkers for neuroendocrine tumour disease. *Lancet Oncol* 2015; **16**: e435-e446 [PMID: 26370353 DOI: 10.1016/S1470-2045(15)00186-2]

60 **Malczewska A**, Oberg K, Kos-Kudla B. NETest is superior to chromogranin A in neuroendocrine neoplasia: a prospective ENETS CoE analysis. *Endocr Connect* 2021; **10**: 110-123 [PMID: 33289691 DOI: 10.1530/EC-20-0417]

61 **Woolaver RA**, Wang X, Krinsky AL, Waschke BC, Chen SMY, Popolizio V, Nicklawsky AG, Gao D, Chen Z, Jimeno A, Wang XJ, Wang JH. Differences in TCR repertoire and T cell activation underlie the divergent outcomes of antitumor immune responses in tumor-eradicating *vs* tumor-progressing hosts. *J Immunother Cancer* 2021; **9** [PMID: 33414263 DOI: 10.1136/jitc-2020-001615]

62 **Galon J**, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov* 2019; **18**: 197-218 [PMID: 30610226 DOI: 10.1038/s41573-018-0007-y]

63 **Hegde PS**, Karanikas V, Evers S. The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. *Clin Cancer Res* 2016; **22**: 1865-1874 [PMID: 27084740 DOI: 10.1158/1078-0432.CCR-15-1507]

64 **Takahashi D**, Kojima M, Suzuki T, Sugimoto M, Kobayashi S, Takahashi S, Konishi M, Gotohda N, Ikeda M, Nakatsura T, Ochiai A, Nagino M. Profiling the Tumour Immune Microenvironment in Pancreatic Neuroendocrine Neoplasms with Multispectral Imaging Indicates Distinct Subpopulation Characteristics Concordant with WHO 2017 Classification. *Sci Rep* 2018; **8**: 13166 [PMID: 30177687 DOI: 10.1038/s41598-018-31383-9]

65 **Bösch F**, Brüwer K, Altendorf-Hofmann A, Auernhammer CJ, Spitzweg C, Westphalen CB, Boeck S, Schubert-Fritschle G, Werner J, Heinemann V, Kirchner T, Angele M, Knösel T. Immune checkpoint markers in gastroenteropancreatic neuroendocrine neoplasia. *Endocr Relat Cancer* 2019; **26**: 293-301 [PMID: 30608901 DOI: 10.1530/ERC-18-0494]

66 **Pinato DJ**, Vallipuram A, Evans JS, Wong C, Zhang H, Brown M, Dina RE, Trivedi P, Akarca AU, Marafioti T, Mauri FA, Sharma R. Programmed Cell Death Ligand Expression Drives Immune Tolerogenesis across the Diverse Subtypes of Neuroendocrine Tumours. *Neuroendocrinology* 2021; **111**: 465-474 [PMID: 32097935 DOI: 10.1159/000506745]

67 **Cives M**, Strosberg J, Al Diffalha S, Coppola D. Analysis of the immune landscape of small bowel neuroendocrine tumors. *Endocr Relat Cancer* 2019; **26**: 119-130 [PMID: 30400003 DOI: 10.1530/ERC-18-0189]

68 **Young K**, Lawlor RT, Ragulan C, Patil Y, Mafficini A, Bersani S, Antonello D, Mansfield D, Cingarlini S, Landoni L, Pea A, Luchini C, Piredda L, Kannan N, Nyamundanda G, Morganstein D, Chau I, Wiedenmann B, Milella M, Melcher A, Cunningham D, Starling N, Scarpa A, Sadanandam A. Immune landscape, evolution, hypoxia-mediated viral mimicry pathways and therapeutic potential in molecular subtypes of pancreatic neuroendocrine tumours. *Gut* 2021; **70**: 1904-1913 [PMID: 32883872 DOI: 10.1136/gutjnl-2020-321016]

69 **Gubbi S**, Vijayvergia N, Yu JQ, Klubo-Gwiezdzinska J, Koch CA. Immune Checkpoint Inhibitor Therapy in Neuroendocrine Tumors. *Horm Metab Res* 2022; **54**: 795-812 [PMID: 35878617 DOI: 10.1055/a-1908-7790]

70 **Kubli SP**, Berger T, Araujo DV, Siu LL, Mak TW. Beyond immune checkpoint blockade: emerging immunological strategies. *Nat Rev Drug Discov* 2021; **20**: 899-919 [PMID: 33686237 DOI: 10.1038/s41573-021-00155-y]

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ORIGINAL ARTICLE

Retrospective Study

Radiomics model based on contrast-enhanced computed tomography to predict early recurrence in patients with hepatocellular carcinoma after radical resection

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Abstract

BACKGROUND

Radical resection remains an effective strategy for patients with hepatocellular carcinoma (HCC). Unfortunately, the postoperative early recurrence (recurrence within 2 years) rate is still high.

AIM

To develop a radiomics model based on preoperative contrast-enhanced computed tomography (CECT) to evaluate early recurrence in HCC patients with a single tumour.

METHODS

We enrolled a total of 402 HCC patients from two centres who were diagnosed with a single tumour and underwent radical resection. First, the features from the portal venous and arterial phases of CECT were extracted based on the region of interest, and the early recurrence-related radiomics features were selected *via* the least absolute shrinkage and selection operator proportional hazards model (LASSO Cox) to determine radiomics scores for each patient. Then, the clinico-pathologic data were combined to develop a model to predict early recurrence by

Cox regression. Finally, we evaluated the prediction performance of this model by multiple methods.

RESULTS

A total of 1915 radiomics features were extracted from CECT images, and 31 of them were used to determine the radiomics scores, which showed a significant difference between the early recurrence and nonearly recurrence groups. Univariate and multivariate Cox regression analyses showed that radiomics scores and serum alpha-fetoprotein were independent indicators, and they were used to develop a combined model to predict early recurrence. The area under the receiver operating characteristic curve values for the training and validation cohorts were 0.77 and 0.74, respectively, while the C-indices were 0.712 and 0.674, respectively. The calibration curves and decision curve analysis showed satisfactory accuracy and clinical utilities. Kaplan-Meier curves based on recurrence-free survival and overall survival showed significant differences.

CONCLUSION

The preoperative radiomics model was shown to be effective for predicting early recurrence among HCC patients with a single tumour.

Key Words: Hepatocellular carcinoma; Contrast-enhanced computed tomography; Radiomics; Early recurrence

Core Tip: Hepatocellular carcinoma (HCC) is a growing health issue worldwide, ranking sixth in incidence and third in mortality among all cancers. Moreover, due to the high malignancy and suppressive immune microenvironment of HCC, there remain high recurrence and metastasis rates. Therefore, we developed a radiomics model based on preoperative contrast-enhanced computed tomography to evaluate early recurrence in HCC patients with a single tumour.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a growing health issue worldwide, ranking sixth in incidence and third in mortality among all cancers[1]. For early-stage HCC patients, liver resection and liver transplantation are considered the main strategies to prolong overall survival (OS)[2,3]. However, the majority of HCC patients are diagnosed at later disease stages, and thus, surgical care is no longer a treatment option for these individuals[4]. Moreover, due to the high malignancy and suppressive immune microenvironment of HCC[5], there remain high recurrence and metastasis rates even for patients who undergo resection. Therefore, it is of vital importance to conduct systematic surveillance of HCC recurrence, and there is an urgent need to precisely predict recurrence in patients with HCC.

Currently, the recurrence rate for postoperative HCC patients is over 50% within 5 years[6], and studies have shown that patients with early recurrence – *i.e.*, recurrence occurring within 2 years – have worse outcomes and poorer prognosis than those with recurrence over 2 years[7]. Typically, the identification of primary and recurrent tumour depends on the detection of genotype, in other words, on the molecular scale[8,9]. However, its complexities have limited the widespread adoption of this method. To evaluate the prognosis of HCC patients, many staging systems have been introduced to clinical use, such as the Barcelona Clinic Liver Cancer (BCLC) staging system and albumin-bilirubin (ALBI) grading[4, 10]. Given that the staging systems above are more suitable for use in evaluating liver function or guiding treatment, there is a lack of an efficient and accurate approach to evaluate recurrence.

Radiomics is an emerging discipline based on medical imaging. Medical imaging, which includes ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), has played a crucial role in the screening and diagnosis of HCC[11]. However, the conventional imaging process relies on the experience and judgement of radiologists and might miss or be hard to quantify some crucial details. Many studies have shown that radiomics features may contain pathophysiological characteristics, which reflect tumour heterogeneity and are associated with patient prognosis [10]. Based on artificial intelligence technology, radiomics has shown certain potential in HCC related gene analysis, microvascular invasion, postoperative survival and recurrence prediction, *etc*[12-16]. However, there are few externally validated reports that studied on the use of radiomics to assess the risk of early recurrence in HCC patients after surgery.

In this study, we aimed to establish a radiomics model to predict early recurrence for patients who underwent radical resection based on contrast-enhanced CT (CECT) and clinical variables, evaluate this model's performance, and verify its feasibility in clinical application.

MATERIALS AND METHODS

Patients

We retrospectively enrolled 537 HCC patients from two institutions (Affiliated Hospital of Guilin Medical University; Peking University People's Hospital) who underwent radical resection. According to the exclusion criteria in Figure 1, 277 patients enrolled from October 2009 to May 2017 at the Affiliated Hospital of Guilin Medical University were set as the training cohort, while 125 patients enrolled from June 2010 to December 2017 at Peking University People's Hospital were set as the validation cohort. Along with the CECT images, information regarding demographic characteristics, clinicopathological data, and laboratory data were also retrospectively collected. Patient demographic characteristics included sex and age, while clinicopathological data were collected from electronic case records, containing tumour size and vascular invasion. Furthermore, laboratory data were collected before surgery, including routine blood test, liver function, and alpha-fetoprotein (AFP) level.

Diagnostic criteria and exclusion criteria

All enrolled patients were diagnosed with HCC by postoperative pathological examination; solitary HCC was confirmed by preoperative CECT and intraoperative palpation or US. In addition to CECT, at least US and MRI supported an HCC diagnosis. Radical resection was defined as complete removal of the tumour with no residual tumour or new lesion observed in two observations at an interval of no less than 4 wk. Two independent pathologists histopathologically examined resected tumour specimens. The main exclusion criteria were as follows: (1) CECT image unavailable; (2) CECT scan over 1 mo before operation; (3) previous treatment including hepatectomy, ablation, or transarterial chemoembolization; and (4) incomplete clinical or follow-up data.

Follow-up surveillance

Postoperative surveillance at each institution was conducted according to the protocol. Patients were followed every 2 mo within the first 2 years and every 3-6 mo after 2 years postoperatively (abdominal ultrasonography, serum AFP, and other serological tests were performed). CECT or MRI examination was performed if recurrence was suspected. OS was determined as the interval between the operation date and the date of death or last follow-up. Recurrence-free survival (RFS) was determined from the operation date of radical surgery to the date of the first recurrence at any site, death, or the last follow-up. Recurrence within 2 years after operation was considered as early recurrence.

CT image acquisition

The CECT images were obtained from each institution according to routine procedures, and the usage of contrast medium is described in the Supplementary material.

Region of interest segmentation and radiomics feature extraction

Regions of interest (ROIs) were defined as the tumour areas in both arterial and portal venous phases, which were delineated *via* 3D Slicer (v4.11, https://www.slicer.org) by two experienced radiologists (Reader 1: H.Y. with 10 years of working experience; reader 2: Z.Z. with 20 years of working experience). If they could not reach a consensus, the third radiologist intervened in the evaluation.

To minimize the variability and normalize the CT images, all the images were resampled to a voxel size of 1 mm × 1 mm × 1 mm. Then, the radiomics features were extracted from the ROI segments based on the Pyradiomics package (version 3.0.1). The categories of radiomics features were as follows: First-order statistics, shape-based features (2D and 3D), grey level cooccurrence matrix (GLCM), grey level run length matrix (GLRLM), grey level size zone matrix (GLZM), neighbouring grey tone difference matrix (NGTDM), and grey level dependence matrix (GLDM). LASSO Cox regression was performed to filter and obtain the early recurrence-related radiomics features according to the minimum criteria with 10-fold cross-validation. The radiomics score was determined according to the coefficients.

Modelling and validation

A radiomics and clinical combined model was developed *via* univariate and multivariate analyses, in which the optimal cut-off value of the radiomics score was determined *via* X-tile (Version 3.6.1). The candidate clinical indicators were hepatitis B surface antigen (HBsAg) (positive *vs* negative), cirrhosis (present *vs* absent), microvascular invasion (MVI) (present *vs* absent), serum AFP (> 200 *vs* ≤ 200 ng/mL), tumour size (cm), platelets (× 10^9/L), gamma-glutamyl transpeptidase (GGT) (U/L), and ALBI. The combined model was presented as a nomogram and validated in an independent cohort.

Statistical analysis

Continuous variables that follow a normal distribution are shown as the mean ± SD and were compared by using the Student's *t* test. Otherwise, the Mann-Whitney *U* test was performed. Categorical variables were compared by chi-squared tests. RFS and OS analyses were conducted by using the Kaplan-Meier method and compared by log-rank test among different groups. Time-dependent receiver operating characteristic (ROC) curves were plotted based on the timeROC package. The concordance index (C-index) was determined to evaluate the predictive ability of the combined model. The calibration curves were plotted with the rms package, while decision curve analysis (DCA) was based on the rmda package. SPSS18.0 (SPSS Inc., Chicago, IL, United States) and R (version 4.0.3, https://www.rproject.org/) were used for statistical analyses. $P < 0.05$ was considered statistically significant.

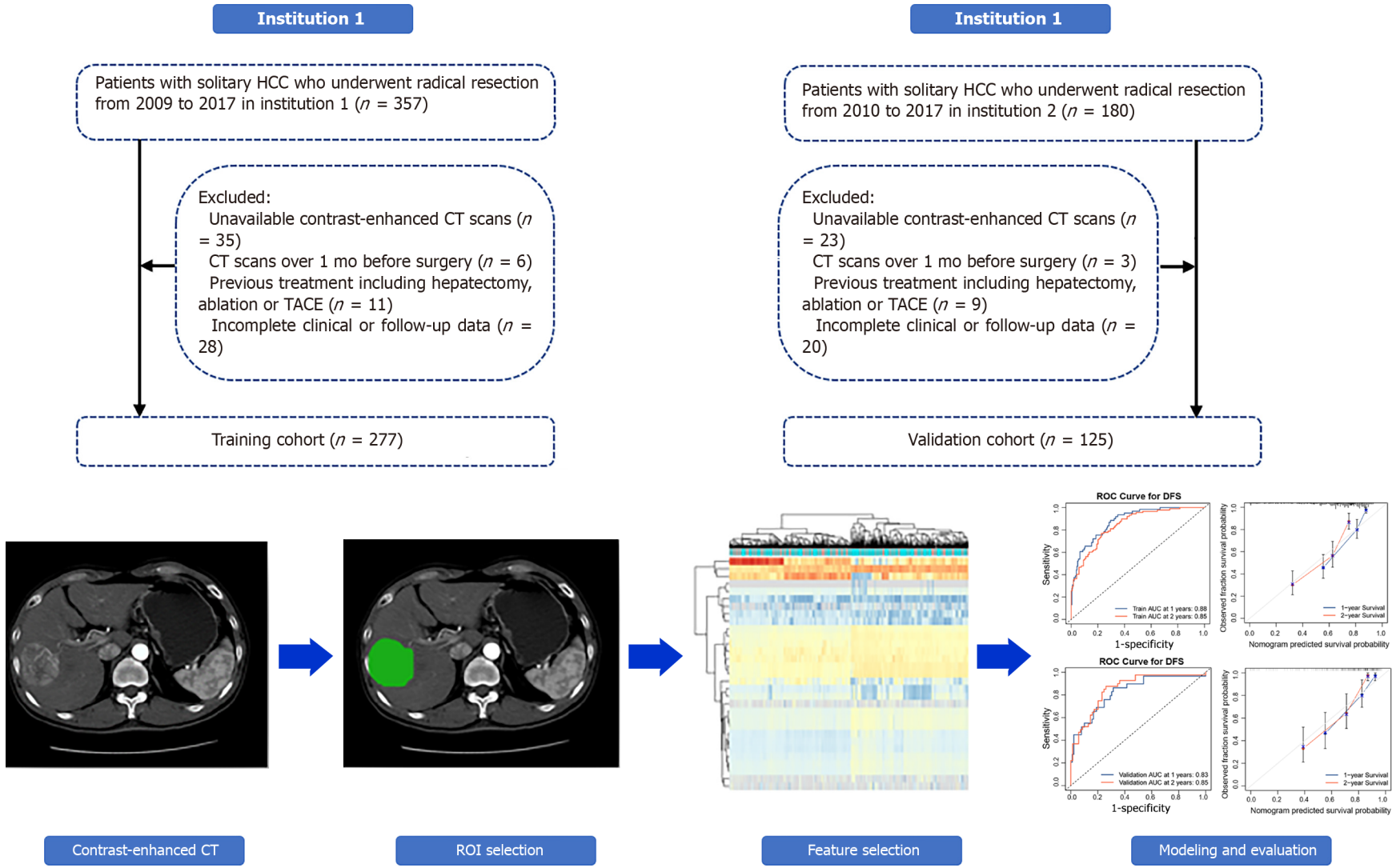

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Figure 1 Flowchart of the study cohorts. HCC: Hepatocellular carcinoma; CT: Computed tomography; TACE: Transarterial chemoembolization; ROI: Region of interest.

RESULTS

Patient characteristics

A total of 402 patients who underwent preoperative CECT were enrolled according to the exclusion criteria from two institutions, and the baseline characteristics are illustrated in Table 1. There were no significant differences in the variables evaluated between the two cohorts except aspartate aminotransferase (AST); most patients were male (both 85.60%), with liver cirrhosis (92.06% and 93.60%) and HBV-related HCC (83.75% and 82.40%, respectively). In institution 1, the median follow-up time was 54.2 mo, the median OS time was 40.0 mo, and the early recurrence rate was 36.82% (102 recurred among 277). In institution 2, the median follow-up time was 50.7 mo, the OS time was 37.0 mo, and the early recurrence rate was 32.80% (41 recurred among 125).

Determination of the radiomics score for early recurrence

A total of 1915 CECT-based radiomics features were extracted from images acquired at both the arterial and portal venous phases among 277 patients in institution 1 as the training cohort. To filter early recurrence-related features, LASSO Cox regression was performed, and 31 features were obtained (Supplementary Figure 1). Figure 2 displays the distribution and correlations among these features. Then, a radiomics score was determined according to the coefficients in Supplementary Table 1 for each patient, and the optimal cut-off value was identified as 15.93, based on which the low and high radiomics risk groups were divided accordingly. As shown in Figure 3A, the radiomics scores were significantly different between the early and nonearly recurrence groups, while the area under curve (AUC) value was 0.73 for both institutions (Figure 3B), thus demonstrating the predictive value of the radiomics score for early recurrence HCC.

Development and assessment of the radiomics and clinical combined model

To further improve the predictive performance of the model, univariate and multivariate Cox regression analyses were conducted among the radiomics model and clinicopathologic indicators. The results from univariate analysis (Table 2) illustrated that the radiomics score, MVI, serum AFP, tumour size, and GGT were statistically significant and were included in the multivariate analysis. Multivariate analysis showed that radiomics score [hazard ratio (HR), 3.86; 95%CI: 2.41-6.16; $P < 0.001$] and serum AFP (HR, 1.52; 95%CI: 1.00-2.30; $P = 0.048$) were identified as independent predictive indicators for early HCC recurrence.

Next, a predictive and visible nomogram was developed based on the radiomics and clinical combined model (Figure 4), and the AUC values of the combined model were 0.79 (95%CI: 0.73-0.84) and 0.77 (95%CI: 0.70-0.83) for 1-year and 2-year recurrence prediction in the training cohort (Figure 5A), respectively. The AUC values of the validation cohort were 0.65 (95%CI: 0.58-0.73) and 0.74 (95%CI: 0.63-0.77) for 1-year and 2-year recurrence, respectively (Figure 5B). The C-index was 0.712 and 0.674 in the training and validation cohorts, respectively. Moreover, the calibration curves illustrated good agreement between predictive and observed outcomes (Figure 5C and D), while the DCA showed that the combined model had a relative high net benefit (Figure 5E and F).

Predictive significance and subgroup analysis of the combined model

To verify the discrimination ability of the combined model, survival curves for RFS and OS were plotted based on the median risk points in the training and validation cohorts. For early recurrence, the high-risk group was more likely to present recurrence than the low-risk group in both the training and validation cohorts (log-rank, $P < 0.0001$ for both) (Figure 6A and C). Furthermore, OS was significantly different (log-rank, $P < 0.0001$ for the training cohort and $P = 0.0446$ for the validation cohort) (Figure 6B and D).

Next, we performed subgroup analysis in AFP-negative (< 20 ng/mL) and AFP-positive (≥ 20 ng/mL) groups. Regardless of the AFP level (Figure 6E-H), the model calculated points were closely related to early recurrence and OS (log-rank, $P < 0.0001$ for all). The results above illustrated the discrimination ability of the combined model.

Representative case reports

Two representative case reports were displayed to demonstrate the distinguishing ability for similar cases (Figure 7). Both of them were male with BCLC A stage HCC and similar ages, and the ROI, also known as the tumour area, was similar in tumour size and position. MVI was present in AFP-negative case 1, while MVI was absent in AFP-positive case 2. However, their outcomes were different. Case 1 was both low risk at the radiomics score and model prediction point and without recurrence until the last follow-up. Case 2 was high risk with worse outcomes, and recurrence was present at 14 mo after the operation. Thus, we concluded that the radiomics combined model could support to distinguish similar cases using CECT in clinical practice.

DISCUSSION

Due to the lack of indicators to predict early recurrence for patients who underwent radical resection, we determined a radiomics score based on preoperative CECT for patients from two independent centres, which was shown to be closely related to the occurrence of early recurrence. Next, serum AFP was included to establish a radiomics and clinical combined model, which elevated the accuracy of the model. The AUC values for the two cohorts were 0.77 and 0.74, respectively, and our findings supported that radiomics is able to be adopted to evaluate the RFS of HCC patients and might support preoperative risk stratification and postoperative surveillance management. Previous studies have

Table 1 Clinicopathologic characteristics of patients in the training and validation cohorts

Parameter		Training cohort, *n* = 277	Validation cohort, *n* = 125	*P* value
Gender	Female	40 (14.44)	18 (14.40)	0.991
	Male	237 (85.56)	107 (85.60)	
Age (yr)		50.60 ± 11.27	51.92 ± 10.50	0.265
HBsAg	Negative	45 (16.25)	22 (17.60)	0.736
	Positive	232 (83.75)	103 (82.40)	
Child-Pugh stage	A	252 (90.97)	116 (92.80)	0.543
	B	25 (9.03)	9 (7.20)	
BCLC stage	0 + A	173 (62.45)	83 (66.40)	0.447
	B + C	104 (37.55)	42 (33.60)	
Cirrhosis	Absent	22 (7.94)	8 (6.40)	0.586
	Present	255 (92.06)	117 (93.60)	
MVI	Absent	162 (58.48)	75 (60.00)	0.775
	Present	115 (41.52)	50 (40.00)	
AFP (ng/mL)	≤ 200	130 (46.93)	67 (53.60)	0.204
	> 200	147 (53.07)	58 (46.40)	
Tumor size (cm)		7.42 ± 4.51	7.07 ± 4.03	0.464
Platelets (× 10^9/L)		198.26 ± 94.67	201.52 ± 86.77	0.743
GGT (U/L, median, IQR)		73.90 (41.74, 130.23)	80.35 (46.92, 151.00)	0.198
ALT (U/L, median, IQR)		32.80 (21.11, 51.70)	29.51 (20.50, 48.82)	0.176
AST (U/L, median, IQR)		36.47 (26.75, 53.80)	35.04 (25.90, 51.60)	0.449
TB (mg/dL)		17.41 ± 26.52	17.40 ± 23.84	0.995
DB (mg/dL)		7.99 ± 17.54	8.92 ± 23.26	0.658
ALBI		-2.50 ± 0.47	-2.53 ± 0.42	0.493

HBsAg: Hepatitis B surface antigen; BCLC: Barcelona Clinic Liver Cancer stage; MVI: Microvascular invasion; AFP: Alpha-fetoprotein; GGT: Gamma-glutamyl transpeptidase; IQR: Interquartile range; ALT: Alanine transaminase; AST: Aspartate transaminase; TB: Total bilirubin; DB: Direct bilirubin; ALBI: Albumin-bilirubin.

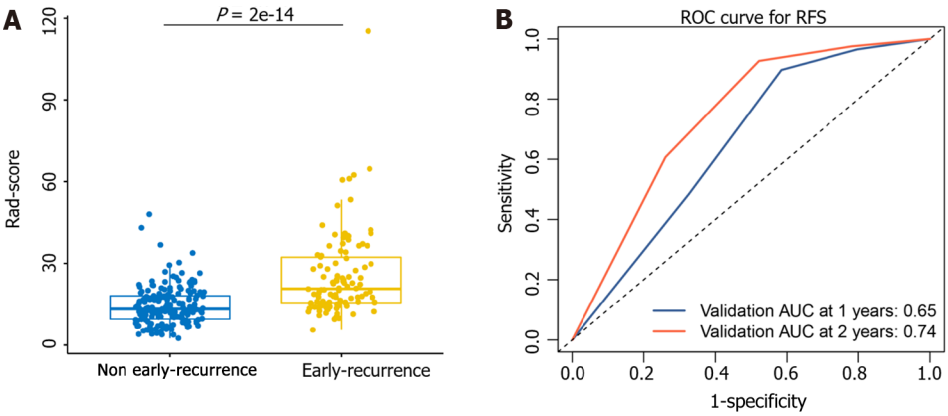

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Figure 2 Correlation and distribution of early recurrence-related radiomics features. A: Correlation of the radiomics features. The red mark represents a positive correlation, while the blue mark represents a negative correlation; B: Heatmap and clustering analysis of the radiomics features. ROC: Receiver operating characteristic; RFS: Recurrence-free survival; AUC: Area under curve.

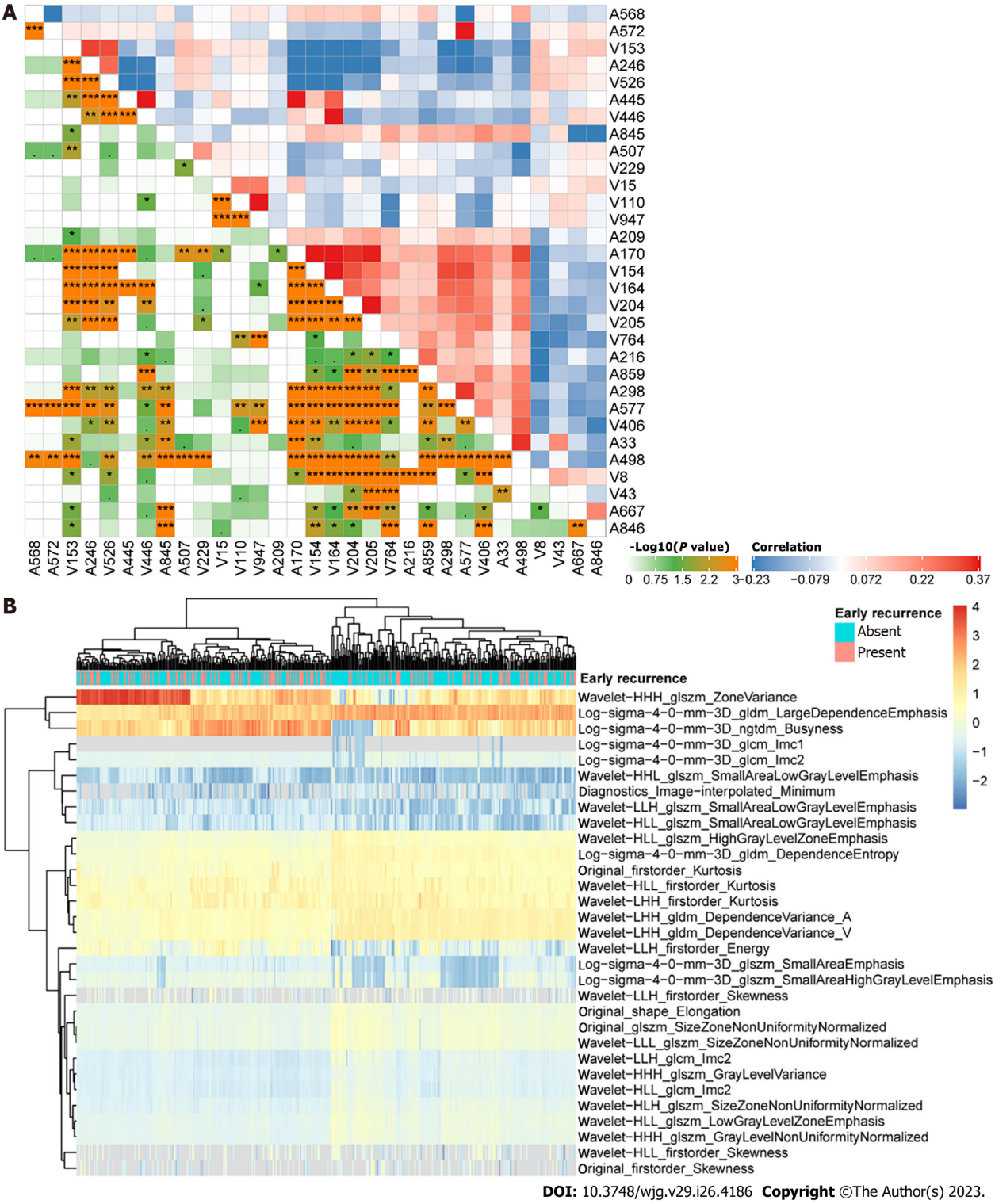

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Figure 3 Capacity of the radiomics score to distinguish early recurrence. A: Correlation of the rad-score between the nonearly recurrence and early recurrence groups; B: Receiver operating characteristic curves of the radiomics score to predict recurrence-free survival in the training and validation cohorts.

illustrated the usage of radiomics in predicting recurrence of HCC[15,16], but few studies focused on early recurrence and conducted external validation, in which early recurrence was defined as recurrence occurring within 2 years. Furthermore, the model divided the HCC patients into two risk subgroups with a favourable distinction of recurrence and a significant difference in OS. AFP-negative subgroup analysis was also performed to evaluate the reliability of the model.

The heterogeneity in primary and recurrent HCC is quite different. Typically, the gene profile and microenvironments are supposed to be able to distinguish them[8,9]. Villanueva *et al*[17] developed a composite prognostic model for HCC recurrence based on gene expression in tumour tissues. However, the majority of recurrent HCC cases occur within 2 years after surgery, which is regarded as real recurrence. Many studies have indicated that patients with early recurrence have worse outcomes than those with late recurrence[18]. Hence, it is of vital significance to monitor early recurrence in

Table 2 Univariate and multivariate Cox regression analyses of clinicopathologic characteristics for recurrence-free survival in training cohort with hepatocellular carcinoma

Variable	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Rad-score (high *vs* low) (*n*)	4.08	2.64-6.24	< 0.001	3.86	2.41-6.16	< 0.001
HBsAg (present *vs* absent) (*n*)	1.81	0.97-3.39	0.063			
Cirrhosis (present *vs* absent) (*n*)	1.84	0.75-4.52	0.183			
MVI (present *vs* absent) (*n*)	1.83	1.24-2.71	0.002	1.20	0.77-1.85	0.418
AFP (> 200 *vs* ≤ 200) (ng/mL)	1.73	1.16-2.58	0.007	1.52	1.00-2.30	0.048
Tumor size (cm)	1.05	1.01-1.09	0.005	1.00	0.94-1.04	0.621
Platelets ($\times 10^9$/L)	1.00	1.00-1.00	0.160			
GGT (U/L)	1.02	1.04-1.02	0.034	1.01	1.01-1.00	0.333
DB (mg/dL)	1.00	1.00-1.01	0.928			
ALBI	0.96	0.64-1.44	0.838			

HR: Hazard ratio; CI: Confidence interval; HBsAg: Hepatitis B surface antigen; MVI: Microvascular invasion; AFP: Alpha fetoprotein; GGT: Gamma-glutamyl transpeptidase; DB: Direct bilirubin; ALBI: Albumin-bilirubin.

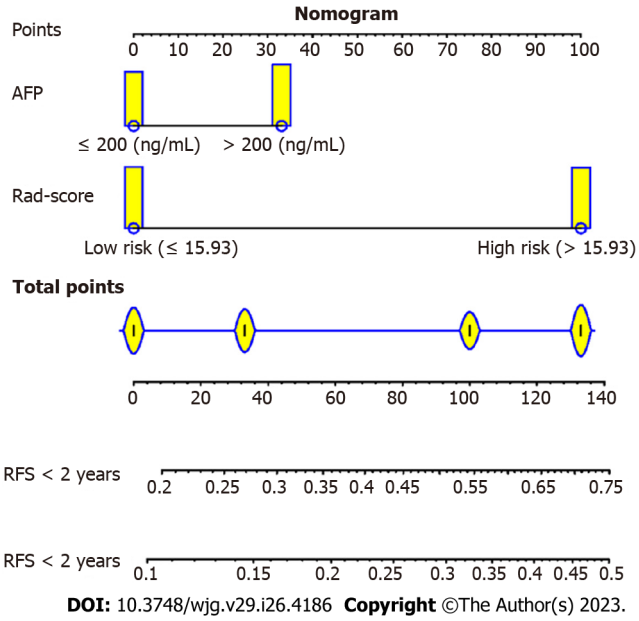

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Figure 4 A radiomics and clinical combined model is built based on the radiomics score and preoperative alpha-fetoprotein. According to the total score of each indicator, the recurrence rate corresponding to the total score is the nomogram-predicted rate. AFP: Alpha-fetoprotein; RFS: Recurrence-free survival.

clinical practice. Increasing evidence has illustrated that the immune microenvironment plays a crucial role in the early recurrence of HCC. Sun *et al*[19] revealed the unique characteristics of early relapse HCC at single-cell resolution. Additionally, metabolic microenvironments, such as lipogenesis and glycolysis, might promote oncogenic transformation [20-23]. The mechanism above reveals the complexity of recurrent HCC. Many staging systems have been applied in the diagnosis and treatment of HCC, including the AJCC TNM staging system and the BCLC and Child-Pugh staging systems. Nevertheless, none of them are capable of predicting recurrence. Serum AFP level has long been used as an indicator to predict recurrence in both hepatectomy and liver transplantation[24,25], which showed effect in our model, as well.

Preoperative imaging plays a very important role in the effective diagnosis and treatment of HCC, and CECT is part of this process. For BCLC stages A and B patients, liver resection is considered the main treatment strategy. However, the postoperative recurrence rate is extremely high. Hence, we employed radiomics to explore more details from CECT images. In this study, radiomics scores were determined based on the 31 features. There were 16 features extracted from images in the arterial phase and 15 features from images in the portal venous phase, which was consistent with the

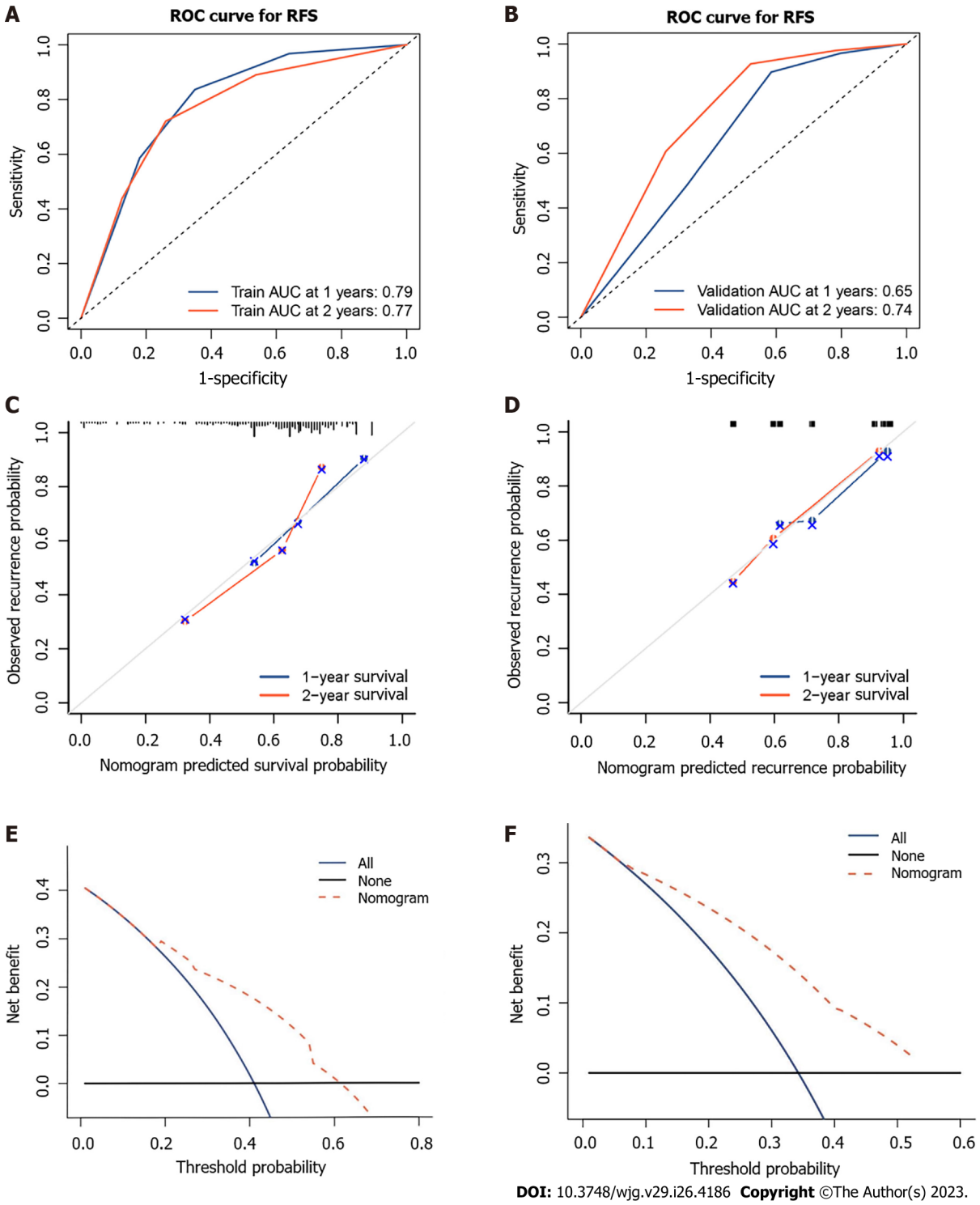

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Figure 5 Evaluation of the radiomics model to predict early recurrence. A: Receiver operating characteristic (ROC) curves to predict 1- and 2-year recurrence-free survival (RFS) in the training cohort; B: ROC curves to predict 1- and 2-year RFS in the validation cohort; C: Calibration curves of the 1- and 2-year RFS in the training cohort; D: Calibration curves of the 1- and 2-year RFS in the validation cohort; E: Decision curve analysis of the combined model in the training cohort; F: Decision curve analysis of the combined model in the validation cohort. ROC: Receiver operating characteristic; RFS: Recurrence-free survival; AUC: Area under curve.

diagnosis of HCC depending on the dynamic changes in vascular findings from the arterial phase to portal venous phase [26-29]. Consistently, among these features, most of them were based on wavelet filtered features that accounted for the greatest weight, which coincided with the previous studies[15], and might represent tumour heterogeneity of HCC and peritumoral tissues that were hard to explain and easy to be ignored by the radiologist. The potential use of radiomics has been widely reported. Hence, we combined radiomics and serum indicators to evaluate the risk of early recurrence, which was validated in an independent cohort. Our findings showed favourable value in predicting early recurrence for patients with HCC *via* non-invasive indicators and might guide clinical decisions.

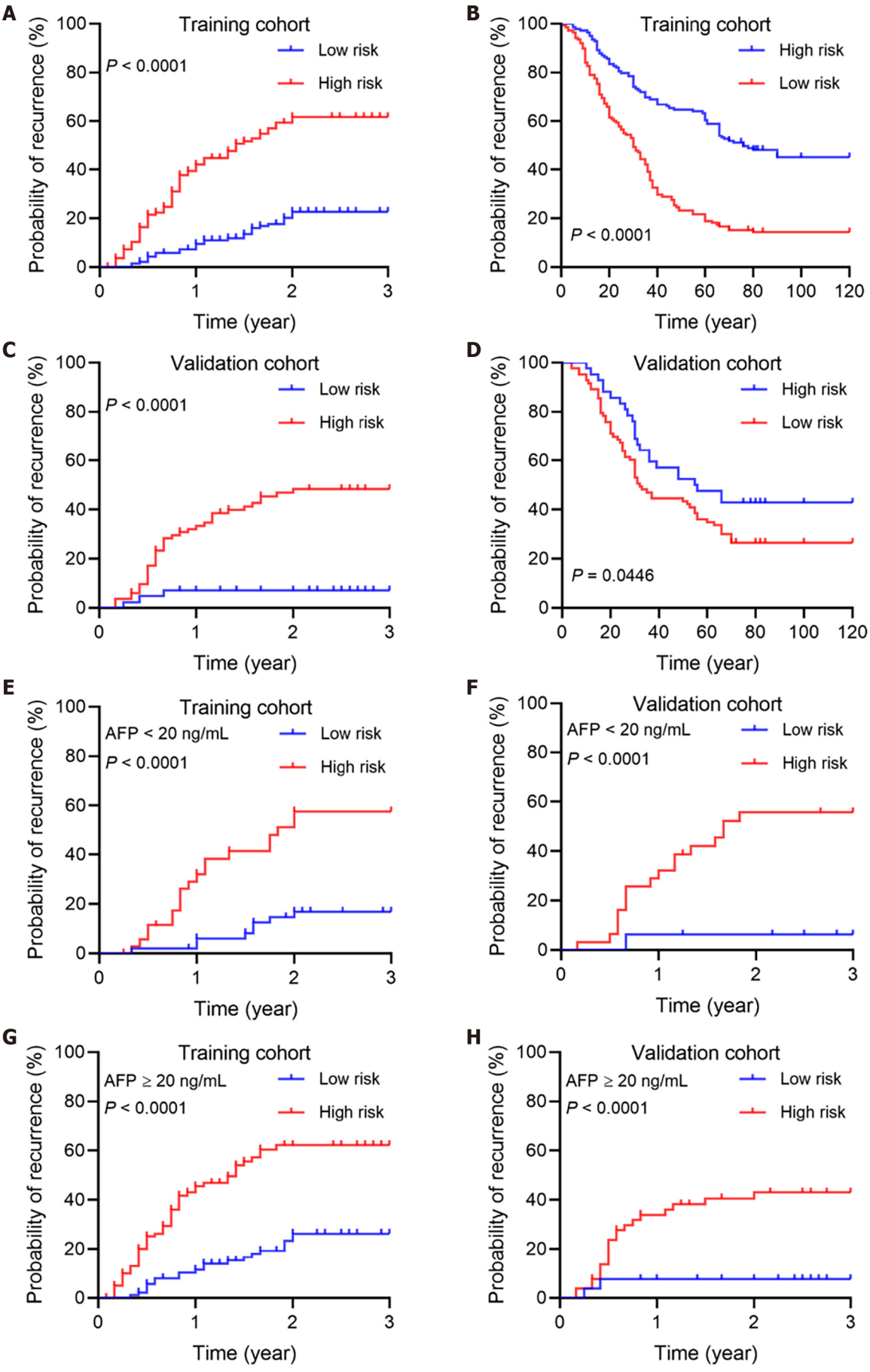

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Figure 6 Cumulative rates of early recurrence and survival rates of hepatocellular carcinoma. A: Risk accumulation curve of recurrence-free

survival (RFS) in the training cohort; B: Survival curve of overall survival (OS) in the training cohort; C: Risk accumulation curve of RFS in the validation cohort; D: Survival curve of OS in the validation cohort; E and F: Risk accumulation curve of RFS in the alpha-fetoprotein (AFP)-negative group in the training and validation cohorts; G and H: Risk accumulation curve of RFS in the AFP-positive group in the training and validation cohorts. AFP: Alpha fetoprotein.

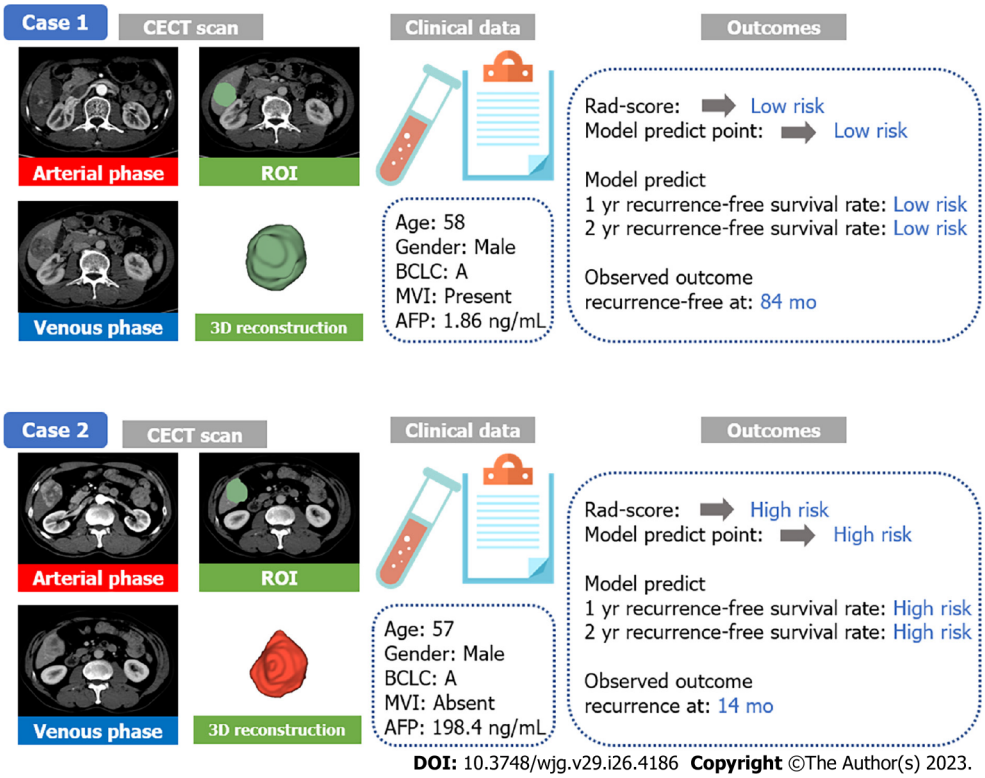

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Figure 7 Representative case reports. AFP: Alpha-fetoprotein; BCLC: Barcelona Clinic Liver Cancer; CECT: Contrast-enhanced computed tomography; MVI: Microvascular invasion; ROI: Regions of interest.

There were several limitations in our study. First, because this was a retrospective analysis, inherent biases were inevitable. Second, even though the radiomics features were extracted *via* a standard process, the features might differ and be critically dependent across CT machines and centres. Third, with the development of multimodality radiomics, Gd-EOB-DTPA-enhanced MR or 18F-FDG PET/CT has been widely used and have more favourable discrimination in adipose tissue and tumour capsules[30,31]. Therefore, we should conduct prospective trials with multimodality radiomics to confirm our results.

CONCLUSION

In summary, the radiomics scores calculated herein revealed significant differences between early and nonearly recurrence HCC patients. Then, we integrated the scores with serum AFP to develop a radiomics and clinical combined model that showed advantages and might serve as a powerful tool to predict early recurrence of HCC. We will perform further validation and translate the results into clinical application.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) seriously endangers human life and health, but there is still a lack of satisfactory treatment options. Even if it is diagnosed at early stage, the recurrence rate is still very high. The clinical monitoring strategy for HCC recurrence is limited, so there is a need to find a new and effective recurrence prediction model for HCC. And we developed a radiomics model based on preoperative contrast-enhanced computed tomography (CECT) to evaluate early recurrence in patients with a single tumour.

Research motivation

Due to the high malignancy and suppressive immune microenvironment of HCC, there are still high recurrence and metastasis rates even in HCC patients who have undergone radical resection. Therefore, it is of vital importance to conduct systematic surveillance of HCC recurrence, and there is an urgent need to precisely predict recurrence in patients with HCC. If tumour recurrence can be detected earlier, the survival and quality of life of HCC patients might be greatly improved.

Research objectives

Despite the rapid development in the treatment of HCC in recent decades, patients' outcomes remain unsatisfactory. One of the reasons is that the early diagnosis system of HCC recurrence is not yet well developed, so our research team established a recurrence prediction model for HCC based on medical imaging such as computed tomography (CT) to predict HCC recurrence earlier, so that timely treatment measures can be taken.

Research methods

We collected CT images from 537 clinical patients in two institutions and extracted valuable CT image features with 3D Slicer (v4.11, https://www.slicer.org). SPSS18.0 (SPSS Inc., Chicago, IL, United States) and R (version 4.0.3, https://www.rproject.org/) were used for statistical analyses and the prediction model of HCC recurrence was established jointly with AFP.

Research results

The radiomics scores calculated herein revealed significant differences between early and nonearly recurrence HCC patients. We combined radiomics and serum indicators to evaluate the risk of early recurrence, which was validated in an independent cohort. Our findings showed the value of predicting early recurrence in HCC patients by noninvasive indicators and might guide clinical decisions making. However, this is a retrospective analysis, and inherent biases are inevitable. We would conduct prospective trials with multimodality radiomics to confirm our results in future.

Research conclusions

The preoperative radiomics model was shown to be effective for predicting early recurrence among patients with single HCC. Compared with pathological biopsy or other tests, this model is noninvasive and more convenient for HCC patients.

Research perspectives

For HCC diagnosis, treatment, or prognosis assessment, more non-invasive methods are of great significance and needed.

FOOTNOTES

Author contributions: Li SQ, Su LL, Xu TF, and Ren LY contributed equally to this work; Liao WJ and Li SQ designed the study; Ren LY, Chen DB, Xu TF, and Su LL analyzed the data and wrote the manuscript; Yan XZ and Fan JX collected the data; Qin WY and Chen HS analyzed the images data; all authors have read and approved the final manuscript.

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Institutional review board statement: The study was reviewed and approved by the Medical Ethics Committee of the Affiliated Hospital of Guilin Medical University (Approval No. 2021WJWZC14).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: Liao WJ has received fees for serving as a speaker, a professor for the Affiliated Hospital of Guilin Medical University; Liao WJ has received research funding from the National Natural Science Foundation of China.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at liaoweijia288@163.com. Participants gave informed consent for data sharing.

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REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
2 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]
3 **Wang JH**, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. *J Hepatol* 2012; **56**: 412-418 [PMID: 21756858 DOI: 10.1016/j.jhep.2011.05.020]
4 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]
5 **Lu C**, Rong D, Zhang B, Zheng W, Wang X, Chen Z, Tang W. Current perspectives on the immunosuppressive tumor microenvironment in hepatocellular carcinoma: challenges and opportunities. *Mol Cancer* 2019; **18**: 130 [PMID: 31464625 DOI: 10.1186/s12943-019-1047-6]
6 **Cha C**, Fong Y, Jarnagin WR, Blumgart LH, DeMatteo RP. Predictors and patterns of recurrence after resection of hepatocellular carcinoma. *J Am Coll Surg* 2003; **197**: 753-758 [PMID: 14585409 DOI: 10.1016/j.jamcollsurg.2003.07.003]
7 **Tabrizian P**, Jibara G, Shrager B, Schwartz M, Roayaie S. Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis. *Ann Surg* 2015; **261**: 947-955 [PMID: 25010665 DOI: 10.1097/SLA.0000000000000710]
8 **Chen PJ**, Chen DS, Lai MY, Chang MH, Huang GT, Yang PM, Sheu JC, Lee SC, Hsu HC, Sung JL. Clonal origin of recurrent hepatocellular carcinomas. *Gastroenterology* 1989; **96**: 527-529 [PMID: 2535996 DOI: 10.1016/0016-5085(89)91581-3]
9 **Ng IO**, Guan XY, Poon RT, Fan ST, Lee JM. Determination of the molecular relationship between multiple tumour nodules in hepatocellular carcinoma differentiates multicentric origin from intrahepatic metastasis. *J Pathol* 2003; **199**: 345-353 [PMID: 12579536 DOI: 10.1002/path.1287]
10 **Johnson PJ**, Berhane S, Kagebayashi C, Satomura S, Teng M, Reeves HL, O'Beirne J, Fox R, Skowronska A, Palmer D, Yeo W, Mo F, Lai P, Iñarrairaegui M, Chan SL, Sangro B, Miksad R, Tada T, Kumada T, Toyoda H. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015; **33**: 550-558 [PMID: 25512453 DOI: 10.1200/JCO.2014.57.9151]
11 **Lambin P**, Leijenaar RTH, Deist TM, Peerlings J, de Jong EEC, van Timmeren J, Sanduleanu S, Larue RTHM, Even AJG, Jochems A, van Wijk Y, Woodruff H, van Soest J, Lustberg T, Roelofs E, van Elmpt W, Dekker A, Mottaghy FM, Wildberger JE, Walsh S. Radiomics: the bridge between medical imaging and personalized medicine. *Nat Rev Clin Oncol* 2017; **14**: 749-762 [PMID: 28975929 DOI: 10.1038/nrclinonc.2017.141]
12 **Kitao A**, Matsui O, Zhang Y, Ogi T, Nakada S, Sato Y, Harada K, Yoneda N, Kozaka K, Inoue D, Yoshida K, Koda W, Yamashita T, Kaneko S, Kobayashi S, Gabata T. Dynamic CT and Gadoxetic Acid-enhanced MRI Characteristics of P53-mutated Hepatocellular Carcinoma. *Radiology* 2023; **306**: e220531 [PMID: 36219111 DOI: 10.1148/radiol.220531]
13 **Xu X**, Zhang HL, Liu QP, Sun SW, Zhang J, Zhu FP, Yang G, Yan X, Zhang YD, Liu XS. Radiomic analysis of contrast-enhanced CT predicts microvascular invasion and outcome in hepatocellular carcinoma. *J Hepatol* 2019; **70**: 1133-1144 [PMID: 30876945 DOI: 10.1016/j.jhep.2019.02.023]
14 **Liu Q**, Li J, Liu F, Yang W, Ding J, Chen W, Wei Y, Li B, Zheng L. A radiomics nomogram for the prediction of overall survival in patients with hepatocellular carcinoma after hepatectomy. *Cancer Imaging* 2020; **20**: 82 [PMID: 33198809 DOI: 10.1186/s40644-020-00360-9]
15 **Ji GW**, Zhu FP, Xu Q, Wang K, Wu MY, Tang WW, Li XC, Wang XH. Radiomic Features at Contrast-enhanced CT Predict Recurrence in Early Stage Hepatocellular Carcinoma: A Multi-Institutional Study. *Radiology* 2020; **294**: 568-579 [PMID: 31934830 DOI: 10.1148/radiol.2020191470]
16 **Zhang X**, Wang C, Zheng D, Liao Y, Wang X, Huang Z, Zhong Q. Radiomics nomogram based on multi-parametric magnetic resonance imaging for predicting early recurrence in small hepatocellular carcinoma after radiofrequency ablation. *Front Oncol* 2022; **12**: 1013770 [PMID: 36439458 DOI: 10.3389/fonc.2022.1013770]
17 **Villanueva A**, Hoshida Y, Battiston C, Tovar V, Sia D, Alsinet C, Cornella H, Liberzon A, Kobayashi M, Kumada H, Thung SN, Bruix J, Newell P, April C, Fan JB, Roayaie S, Mazzaferro V, Schwartz ME, Llovet JM. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 1501-12.e2 [PMID: 21320499 DOI: 10.1053/j.gastro.2011.02.006]
18 **Yamamoto Y**, Ikoma H, Morimura R, Konishi H, Murayama Y, Komatsu S, Shiozaki A, Kuriu Y, Kubota T, Nakanishi M, Ichikawa D, Fujiwara H, Okamoto K, Sakakura C, Ochiai T, Otsuji E. Optimal duration of the early and late recurrence of hepatocellular carcinoma after hepatectomy. *World J Gastroenterol* 2015; **21**: 1207-1215 [PMID: 25632194 DOI: 10.3748/wjg.v21.i4.1207]
19 **Sun Y**, Wu L, Zhong Y, Zhou K, Hou Y, Wang Z, Zhang Z, Xie J, Wang C, Chen D, Huang Y, Wei X, Shi Y, Zhao Z, Li Y, Guo Z, Yu Q, Xu L, Volpe G, Qiu S, Zhou J, Ward C, Sun H, Yin Y, Xu X, Wang X, Esteban MA, Yang H, Wang J, Dean M, Zhang Y, Liu S, Yang X, Fan J. Single-cell landscape of the ecosystem in early-relapse hepatocellular carcinoma. *Cell* 2021; **184**: 404-421.e16 [PMID: 33357445 DOI: 10.1016/j.cell.2020.11.041]
20 **Zuo X**, Chen Z, Gao W, Zhang Y, Wang J, Cao M, Cai J, Wu J, Wang X. M6A-mediated upregulation of LINC00958 increases lipogenesis and acts as a nanotherapeutic target in hepatocellular carcinoma. *J Hematol Oncol* 2020; **13**: 5 [PMID: 31915027 DOI: 10.1186/s13045-019-0839-x]
21 **Calvisi DF**, Wang C, Ho C, Ladu S, Lee SA, Mattu S, Destefanis G, Delogu S, Zimmermann A, Ericsson J, Brozzetti S, Staniscia T, Chen X,

Dombrowski F, Evert M. Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 1071-1083 [PMID: 21147110 DOI: 10.1053/j.gastro.2010.12.006]

22 **Gao Q**, Zhu H, Dong L, Shi W, Chen R, Song Z, Huang C, Li J, Dong X, Zhou Y, Liu Q, Ma L, Wang X, Zhou J, Liu Y, Boja E, Robles AI, Ma W, Wang P, Li Y, Ding L, Wen B, Zhang B, Rodriguez H, Gao D, Zhou H, Fan J. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. *Cell* 2019; **179**: 561-577.e22 [PMID: 31585088 DOI: 10.1016/j.cell.2019.08.052]

23 **Li Q**, Pan X, Zhu D, Deng Z, Jiang R, Wang X. Circular RNA MAT2B Promotes Glycolysis and Malignancy of Hepatocellular Carcinoma Through the miR-338-3p/PKM2 Axis Under Hypoxic Stress. *Hepatology* 2019; **70**: 1298-1316 [PMID: 31004447 DOI: 10.1002/hep.30671]

24 **Ijichi M**, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology* 2002; **35**: 853-860 [PMID: 11915031 DOI: 10.1053/jhep.2002.32100]

25 **Notarpaolo A**, Layese R, Magistri P, Gambato M, Colledan M, Magini G, Miglioresi L, Vitale A, Vennarecci G, Ambrosio CD, Burra P, Di Benedetto F, Fagiuoli S, Colasanti M, Maria Ettorre G, Andreoli A, Cillo U, Laurent A, Katsahian S, Audureau E, Roudot-Thoraval F, Duvoux C. Validation of the AFP model as a predictor of HCC recurrence in patients with viral hepatitis-related cirrhosis who had received a liver transplant for HCC. *J Hepatol* 2017; **66**: 552-559 [PMID: 27899297 DOI: 10.1016/j.jhep.2016.10.038]

26 **Sun YF**, Xu Y, Yang XR, Guo W, Zhang X, Qiu SJ, Shi RY, Hu B, Zhou J, Fan J. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. *Hepatology* 2013; **57**: 1458-1468 [PMID: 23175471 DOI: 10.1002/hep.26151]

27 **Xu RH**, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, Yi S, Shi W, Quan Q, Li K, Zheng L, Zhang H, Caughey BA, Zhao Q, Hou J, Zhang R, Xu Y, Cai H, Li G, Hou R, Zhong Z, Lin D, Fu X, Zhu J, Duan Y, Yu M, Ying B, Zhang W, Wang J, Zhang E, Zhang C, Li O, Guo R, Carter H, Zhu JK, Hao X, Zhang K. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017; **16**: 1155-1161 [PMID: 29035356 DOI: 10.1038/nmat4997]

28 **Bruix J**, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

29 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A; OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]

30 **Vreugdenburg TD**, Ma N, Duncan JK, Riitano D, Cameron AL, Maddern GJ. Comparative diagnostic accuracy of hepatocyte-specific gadoxetic acid (Gd-EOB-DTPA) enhanced MR imaging and contrast enhanced CT for the detection of liver metastases: a systematic review and meta-analysis. *Int J Colorectal Dis* 2016; **31**: 1739-1749 [PMID: 27682648 DOI: 10.1007/s00384-016-2664-9]

31 **Tanimoto A**, Lee JM, Murakami T, Huppertz A, Kudo M, Grazioli L. Consensus report of the 2nd International Forum for Liver MRI. *Eur Radiol* 2009; **19** Suppl 5: S975-S989 [PMID: 19851766 DOI: 10.1007/s00330-009-1624-y]

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ORIGINAL ARTICLE

Clinical Trials Study

Raf kinase inhibitor protein combined with phosphorylated extracellular signal-regulated kinase offers valuable prognosis in gastrointestinal stromal tumor

Wen-Zhi Qu, Luan Wang, Juan-Juan Chen, Yang Wang

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Abstract

BACKGROUND

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Tyrosine kinase inhibitors, such as imatinib, have been used as first-line therapy for the treatment of GISTs. Although these drugs have achieved considerable efficacy in some patients, reports of resistance and recurrence have emerged. Extracellular signal-regulated kinase 1/2 (ERK1/2) protein, as a member of the mitogen-activated protein kinase (MAPK) family, is a core molecule of this signaling pathway. Nowadays, research reports on the important clinical and prognostic value of phosphorylated-ERK (P-ERK) and phosphorylated-MAPK/ERK kinase (P-MEK) proteins closely related to raf kinase inhibitor protein (RKIP) have gradually emerged in digestive tract tumors such as gastric cancer, colon cancer, and pancreatic cancer. However, literature on the expression of these downstream proteins combined with RKIP in GIST is scarce. This study will focus on this aspect and search for answers to the problem.

AIM

To detect the expression of RKIP, P-ERK, and P-MEK protein in GIST and to analyze their relationship with clinicopathological characteristics and prognosis of this disease. Try to establish a new prognosis evaluation model using RKIP and P-ERK in combination with analysis and its prognosis evaluation efficacy.

METHODS

The research object of our experiment was 66 pathologically diagnosed GIST patients with complete clinical and follow-up information. These patients received surgical treatment at China Medical University Affiliated Hospital from January 2015 to January 2020. Immunohistochemical method was used to detect the expression of RKIP, P-ERK, and P-MEK proteins in GIST tissue samples from these patients. Kaplan-Meier method was used to calculate the survival rate of 63 patients with complete follow-up data. A Nomogram was used to represent the new prognostic evaluation model. The Cox multivariate regression analysis was conducted separately for each set of risk evaluation factors, based on two risk classification systems [the new risk grade model *vs* the modified National Institutes of Health (NIH) 2008 risk classification system]. Receiver operating characteristic (ROC) curves were used for evaluating the accuracy and efficiency of the two prognostic evaluation systems.

RESULTS

In GIST tissues, RKIP protein showed positive expression in the cytoplasm and cell membrane, appearing as brownish-yellow or brown granules. The expression of RKIP was related to GIST tumor size, NIH grade, and mucosal invasion. P-ERK protein exhibited heterogeneous distribution in GIST cells, mainly in the cytoplasm, with occasional presence in the nucleus, and appeared as brownish-yellow granules, and the expression of P-ERK protein was associated with GIST tumor size, mitotic count, mucosal invasion, and NIH grade. Meanwhile, RKIP protein expression was negatively correlated with P-ERK expression. The results in COX multivariate regression analysis showed that RKIP protein expression was not an independent risk factor for tumor prognosis. However, RKIP combined with P-ERK protein expression were identified as independent risk factors for prognosis with statistical significance. Furthermore, we establish a new prognosis evaluation model using RKIP and P-ERK in combination and obtained the nomogram of the new prognosis evaluation model. ROC curve analysis also showed that the new evaluation model had better prognostic performance than the modified NIH 2008 risk classification system.

CONCLUSION

Our experimental results showed that the expression of RKIP and P-ERK proteins in GIST was associated with tumor size, NIH 2008 staging, and tumor invasion, and P-ERK expression was also related to mitotic count. The expression of the two proteins had a certain negative correlation. The combined expression of RKIP and P-ERK proteins can serve as an independent risk factor for predicting the prognosis of GIST patients. The new risk assessment model incorporating RKIP and P-ERK has superior evaluation efficacy and is worth further practical application to validate.

Key Words: Raf kinase inhibitory protein; Phosphorylated extracellular-signal-regulated kinase; Gastrointestinal stromal tumors; Immunohistochemistry; Survival analysis; Risk grade model

Core Tip: Nowadays, research reports on the expression of downstream proteins of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase pathway combined with raf kinase inhibitor protein (RKIP) in gastrointestinal stromal tumor (GIST) is scarce. This study will focus on this aspect and use immunohistochemistry methods, large sample survival analysis data, and the latest bioinformatics analysis techniques to answer the problem. Our experimental results showed that the expression of the two proteins had a certain negative correlation. The combined expression of RKIP and phosphorylated-ERK proteins can serve as an independent risk factor for predicting the prognosis of GIST patients. Furthermore, the new risk assessment model incorporating RKIP and phosphorylated-ERK has superior evaluation efficacy.

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. It is believed that GISTs originate from Cajal cells, and mutations in the kit and platelet-derived growth factor receptor-alpha (PDGFR-α) genes are considered to be the cause of most cases of GISTs. Based on this discovery, specific tyrosine kinase inhibitors, such as imatinib, have been used as first-line therapy for the treatment of GISTs. Although these drugs have achieved considerable efficacy in some patients, reports of resistance and recurrence have emerged[1]. Even second-line target therapy drugs such as sunitinib, protein kinase C (PKC) 412, or BMS-354825 are not effective for all patients[2].

To overcome tumor resistance, research on GISTs has focused on the search and validation of new targeted therapy sites and regulatory genes. The Raf-MEK-ERK pathway, which is closely related to GIST, is one of the well-studied pathways. Extracellular signal-regulated kinase 1/2 (ERK1/2) protein, as a member of the mitogen-activated protein kinase (MAPK) family, is a core molecule of this signaling pathway. It works with upstream activating molecules and downstream effector molecules to form an efficient and accurate signal transduction system. The MAPK/ERK Kinase 1/2 (MEK 1/2) protein is a MAPKK (MAPK kinase) that can activate the Thr and Tyr sites on the ERK1/2 protein, thereby phosphorylating and activating it to produce the activated form of P-ERK1/2. Raf-1 protein is an upstream MAPKKK (MAPKK Kinase) that can activate the entire pathway by phosphorylating MEK1/2 to obtain P-MEK1/2. The pathway is regulated by the cell cycle and extracellular stimuli, thus regulating downstream protein kinases, phospholipases, and transcription factors to play important biological functions[3-5].

The RKIP protein is a structurally complex protein with a "multidirectional switch" regulatory role in multiple signaling pathways. First, it can regulate multiple signaling pathways and has an inhibitory effect on the Raf-MEK-ERK pathway, affecting cell invasion and proliferation[6]. Additionally, the RKIP protein is also a phosphorylation target, such as its binding to G protein-coupled receptor kinase 2 (GRK2) in the G protein-coupled pathway[7]. Recently, the study of RKIP protein's tumor regulatory function has gradually become a hot spot, and previous studies have reported that RKIP protein is related to the size of GIST tumors, National Institutes of Health (NIH) staging, and whether the tumor invades the mucosa, but RKIP cannot be used as an independent factor for GIST prognosis evaluation[8].

Nowadays, research reports on the important clinical and prognostic value of phosphorylated (P)-ERK and P-MEK proteins closely related to RKIP have gradually emerged in digestive tract tumors such as gastric cancer, colon cancer, and pancreatic cancer. However, literature on the expression of downstream proteins of the ERK/MAPK pathway combined with RKIP in GIST is scarce. This study will focus on this aspect and use immunohistochemistry methods, large sample survival analysis data, and the latest bioinformatics analysis techniques to search for answers to the problem. We aim to find new tumor treatment targets for GIST and develop more reliable and efficient diagnostic and prognostic evaluation methods.

MATERIALS AND METHODS

Patients

The research object of the immunohistochemical experiment was 66 paraffin-embedded specimens that were surgically resected and pathologically diagnosed at China Medical University Affiliated Hospital from January 2015 to January 2020, with complete clinical and follow-up information. There were 36 male cases and 30 female cases, with an age range of 21-83 years and a mean age of 56.2 years. The tumor occurrence sites were: Stomach in 37 cases (56.1%), duodenum in 12 cases (18.2%), jejunum in 15 cases (22.7%), and colon in 2 cases (3.0%) (Table 1).

The diagnostic criteria for GISTs were histopathological features consistent with GISTs and immunohistochemical positivity for CD117, immunohistochemical negativity for CD117 but positivity for CD34, or immunohistochemical negativity for CD117 and CD34 as well as smooth muscle actin, desmin, and S-100 (to exclude smooth muscle tumors and neurogenic tumors). Among them, 59 cases (90.8%) were CD117-positive, and 50 cases (76.9%) were CD34-positive.

The risk grade criteria for GIST used the modified NIH 2008 risk classification system, which combines three assessment factors: Tumor location (gastric *vs* non-gastric: Small intestine and colon, *etc*.), tumor diameter (< 2 cm, 2-5 cm, 5-10 cm, > 10 cm), and mitotic count [< 5/50 high-power fields (HPFs), 5-10/50 HPFs, > 10/50 HPFs]. GISTs were classified as very low risk (I), low risk (II), moderate risk (III), and high risk (IV).

Among the 66 cases of GIST in this study, except for 2 cases who died due to other reasons and 1 case who was lost to follow-up, the follow-up information of the remaining 63 cases was complete. The follow-up time ranged from 5 to 61 mo, with a mean of 48 mo.

Immunohistochemical staining

The GIST specimens were fixed with formalin, embedded in paraffin, and sectioned into 4 μm-thick slices. The sections underwent routine dewaxing in water, followed by H_2O_2 treatment at room temperature to inactivate endogenous enzymes. Subsequently, they were washed three times with distilled water. Antigen retrieval was performed, followed by incubation with a blocking solution containing 5% BSA at room temperature for 20 min. Primary antibodies (RKIP/P-ERK/P-MEK antibodies, rabbit IgG) were then added at an appropriate dilution and incubated at room temperature for 12 h. Next, the sections were treated with a secondary antibody (goat anti-rabbit IgG) and incubated at 25 °C for 20 min. SABC-AP reagent was applied to the sections, followed by incubation at 25 °C for 20 min. For color development, the BCIP/NBT substrate was prepared by diluting it in TBS, thoroughly mixed, and added to the slides. The sections were incubated at 25 °C for 20 min, with the reaction time monitored under a microscope. After washing with distilled water, the sections were lightly counterstained with nuclear red, rinsed, dried, and mounted with a water-soluble mounting medium for microscopic observation.

Evaluation of immunohistochemical staining

The positive staining of RKIP, P-ERK, and P-MEK proteins was mainly located in the cytoplasm and cell membrane, while P-ERK and P-MEK were occasionally observed in the nucleus. The semi-quantitative dual scoring method was used to evaluate the protein expression of proteins. Under high-power microscopy, scoring was performed based on the staining intensity and the proportion of positive cells. Ten random high-power fields were selected on each slide, and the number of positive cells was counted among 100 cells in each field. The average value was calculated and expressed as a

Table 1 Relationship between proteins expression and clinical and pathological characteristics of gastrointestinal stromal tumors

Characteristic	*n*	RKIP			P-ERK			P-MEK		
		Negative	Positive	*P* value[a]	Negative	Positive	*P* value[a]	Negative	Positive	*P* value[a]
Sex										
Male	36	16	20	0.652	16	20	0.716	22	14	0.365
Female	30	15	15		12	18		15	15	
Age in yr										
> 56	33	17	16	0.459	13	20	0.618	15	18	0.083
≤ 56	33	14	19		15	18		22	11	
Tumor location										
Stomach	37	20	17	0.585	16	21	0.902	21	16	0.224
Duodenum	12	4	8		4	8		4	8	
Jejunoileum	15	6	9		7	8		11	4	
Colon	2	1	1		1	1		1	1	
Tumor size in cm										
< 2	12	1	11	< 0.001	8	4	0.009	7	5	0.353
2-5	20	5	15		12	8		14	6	
6-10	19	14	5		6	13		10	9	
> 10	15	11	4		2	13		6	9	
NIH risk grade										
Very low	10	2	8	< 0.001	7	3	0.004	7	3	0.319
Low	20	3	17		13	7		11	9	
Moderate	11	8	3		3	8		8	3	
High	25	18	7		5	20		11	14	
Mitotic figures as /50 HPFs										
0	11	3	8	0.074	5	6	0.015	5	6	0.604
1-4	37	15	22		21	16		22	15	
5-10	7	5	2		1	6		5	2	
> 10	11	8	3		1	10		5	6	
Mucosal invasion										
Yes	34	21	13	0.013	10	24	0.027	18	16	0.599
No	32	10	22		18	14		19	13	

[a]Raf kinase inhibitory protein (RKIP)-positive percentage were compared using the χ^2 test, $P < 0.05$ were considered statistically significant.
HPFs: High-power fields; NIH: National Institutes of Health; P-ERK: Phosphorylated extracellular-signal regulated kinase; P-MEK: Phosphorylated mitogen-activated protein kinase\ERK kinase.

percentage, representing the positivity index of proteins. The detailed scoring criteria were as follows:

(1) Staining intensity scoring criteria: no staining, 0 points; yellow, 1 point; light brown, 2 points; dark brown, 3 points;

(2) Scoring criteria for the proportion of positive cells: < 25% positive cells, 0 points; 25%-50%, 1 point; 51%-75%, 2 points; > 75%, 3 points;

And (3) The overall score was the product of the staining intensity and the proportion of positive cells, and graded as negative (0-2), mildly positive (+, 3), moderately positive (++, 4-6), or strongly positive (+++, 9). RKIP/P-ERK/P-MEK expression was judged to be either negative (0-2) or positive (3-9).

Follow-up

Telephone follow-up was the primary method, with outpatient review and correspondence as secondary measures.

Survival time was calculated as the time from the date of surgery to the date of the last follow-up for surviving patients, the date of death for deceased patients, and the date of the last follow-up for lost patients. Among them, 63 cases had complete follow-up data, 2 cases were excluded due to death from other diseases or accidents, and 1 case was excluded due to loss to follow-up. The follow-up period was from January 2020 to December 2022.

Statistical analysis

SPSS 22.0 software (IBM Corp., Armonk, NY, United States) was used for statistical analysis. Chi-square test was used for comparing percentages. Spearman analysis was used for correlation analysis of two groups of ordinal data. Kaplan-Meier method was used to calculate survival rate, and log-rank test was used. Cox univariate analysis was used for single-factor prognosis analysis, and Cox multivariate regression was used for multi-factor prognosis analysis. A Nomogram was used to represent the new prognostic evaluation model. The Cox multivariate regression analysis was conducted separately for each set of risk evaluation factors, based on two risk classification systems (the new risk grade model *vs* the modified NIH 2008 risk classification system). Receiver operating characteristic (ROC) curves were used for evaluating the accuracy and efficiency of the two prognostic evaluation systems, and statistical significance was set at $P < 0.05$.

RESULTS

Immunohistochemical results of RKIP, P-ERK, and P-MEK proteins and their correlation with clinical pathology

In GIST tissues, RKIP protein showed positive expression in the cytoplasm and cell membrane, appearing as brownish-yellow or brown granules. The immunohistochemical staining result is shown in Figure 1A and B under light microscopy. Among the 66 specimens tested, RKIP protein was expressed positively in 35 cases (53%) and negatively in 31 cases (47%). Statistical analysis revealed that the expression of RKIP was related to GIST tumor size, NIH grade, and mucosal invasion ($P < 0.05$) (Table 1).

P-ERK protein exhibited heterogeneous distribution in GIST cells, mainly in the cytoplasm, with occasional presence in the nucleus, and appeared as brownish-yellow granules, as shown in Figure 1C and D. Positive expression of P-ERK protein was detected in 38 cases (57.6%), while negative expression was detected in 28 cases (42.4%). Clinical pathology analysis indicated that the expression of P-ERK protein was associated with GIST tumor size, mitotic count, mucosal invasion, and NIH grade ($P < 0.05$) (Table 1).

P-MEK protein expression in GIST cells was observed as brownish-yellow granules, mainly distributed in the cytoplasm, with some in the nucleus, and showed a relatively uniform distribution, as shown in Figure 1E and F. Among the 66 cases, positive expression of P-MEK protein was found in 29 cases (43.9%), while negative expression was detected in 37 cases (56.1%). However, no significant correlation was observed between P-MEK protein expression and any independent clinical-pathological factors of GIST (Table 1).

RKIP, P-ERK, and P-MEK protein co-expression analysis

Spearman's correlation test was used to analyze the correlation between RKIP protein expression and P-ERK/P-MEK expression. The results showed that in GIST cases, RKIP protein expression was negatively correlated with P-ERK expression (correlation coefficient = -0.575, $P < 0.001$); RKIP protein expression also had a certain negative correlation with P-MEK protein expression (correlation coefficient = -0.323, $P < 0.001$) (Table 2).

Univariate and multivariate regression analysis

Univariate regression analysis was performed on complete follow-up data from 63 cases, which indicated that tumor location, tumor size, mitotic count, RKIP protein expression, P-ERK protein expression, RKIP combined with P-ERK protein co-expression, and NIH grading were all correlated with patient prognosis ($P < 0.05$).

These univariate factors were further included in COX multivariate regression analysis. The results showed that RKIP protein expression was not an independent risk factor for tumor prognosis ($P = 0.061$). However, tumor size ($P = 0.037$), NIH grading ($P = 0.014$), P-ERK protein expression ($P = 0.041$), and RKIP combined with P-ERK protein expression ($P = 0.044$) were identified as independent risk factors for prognosis with statistical significance. Among them, the impact of RKIP combined with P-ERK protein expression on survival time was the highest with a weight factor of Exp (B) at 11.320, followed by NIH grading, P-ERK expression, and tumor size (Table 3).

Survival analysis curve of RKIP combined with P-ERK in GIST

Based on the expression of RKIP and P-ERK proteins in GIST specimens, we divided the cases into two groups: 1. RKIP (-) and P-ERK (+) group, and 2. The control group with RKIP (+) or P-ERK (-). We drew survival curves for both groups to better represent the significance of the combination of these two proteins in predicting GIST prognosis. The results showed that the survival rate and survival time of Group 1 were significantly lower than those of Group 2 ($P = 0.0312$) (Figure 2).

Establishment of a new prognosis evaluation model using RKIP and P-ERK in combination and its prognosis evaluation efficacy analysis

We included "RKIP expression, P-ERK protein expression, tumor size, tumor location, and mitotic count" as five risk assessment factors (Table 4: Multivariate 2) and used multivariate Cox regression analysis to draw a new nomogram for this new evaluation model. We also conducted ROC analysis to evaluate its prognostic performance. As a control, we

Table 2 Correlations among proteins expression

Protein expressions	Negative	P-ERK positive	Correlation and *P* value	Negative	P-MEK positive	Correlation and *P* value
RKIP						
Negative	3	26	C[a] = -0.575	11	18	C = -0.323
Positive	25	12	*P* < 0.001	26	11	*P* = 0.008

[a]C: Correlation coefficient value is calculated using Spearman's rho test, $P < 0.05$ were considered statistically significant.
P-ERK: Phosphorylated-extracellular-signal regulated kinase; P-MEK: Phosphorylated-mitogen-activated protein kinase\ERK kinase; RKIP: Raf kinase inhibitory protein.

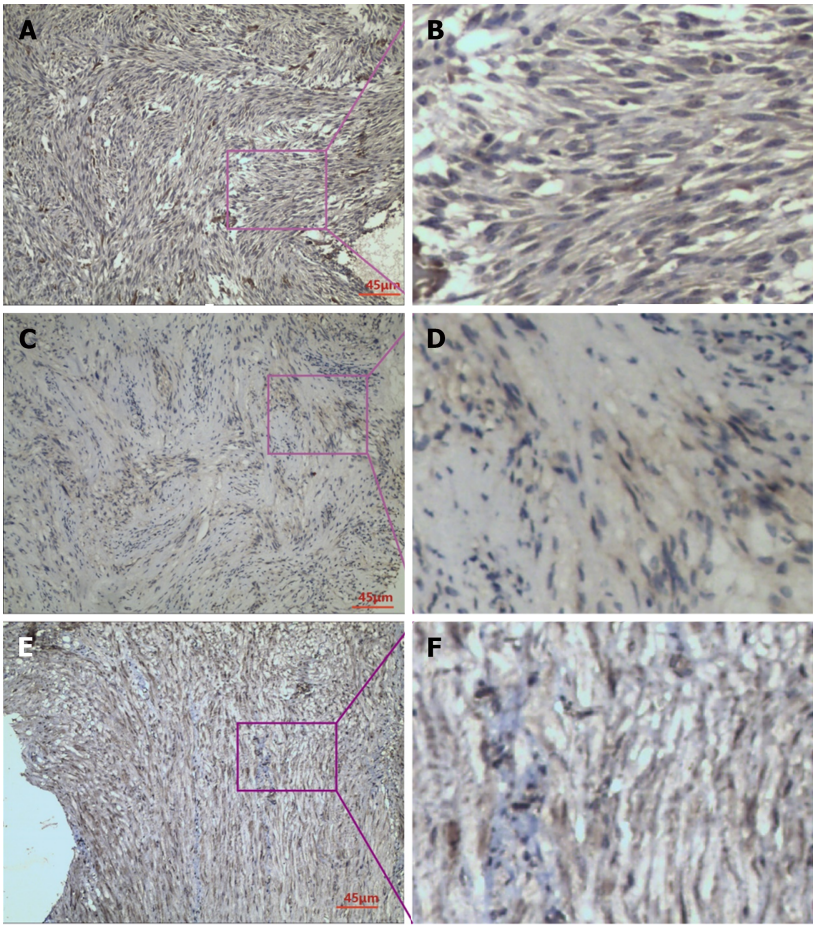

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Figure 1 Immunohistochemical staining. A: Immunohistochemical staining for raf kinase inhibitor protein (RKIP) in gastrointestinal stromal tumor (GIST) tissues; B: RKIP protein showed positive expression in the cytoplasm and cell membrane, appearing as brownish-yellow or brown granules; C: Immunohistochemical staining for phosphorylated-extracellular signal-regulated kinase (P-ERK) in GIST tissues; D: P-ERK protein exhibited heterogeneous distribution in GIST cells, mainly in the cytoplasm, with occasional presence in the nucleus, and appeared as brownish-yellow granules; E: Immunohistochemical staining for phosphorylated-mitogen-activated protein kinase/ERK Kinase (P-MEK) in GIST tissues; F: P-MEK protein expression in GIST cells was observed as brownish-yellow granules, mainly distributed in the cytoplasm, with some in the nucleus, and showed a relatively uniform distribution. A, C and E were taken under 200 × magnification.

used the three conventional risk factors (tumor location, tumor size, and mitotic count) in the modified NIH 2008 risk classification system as evaluation factors (Table 4: Multivariate 1) and performed the same statistical analysis.

After analyzing the results of the above analysis (Table 4), we obtained the nomogram of the new prognosis evaluation model (Figure 3). ROC curve analysis also showed that the new evaluation model (red curve, AUC = 0.860) had better prognostic performance than the modified NIH 2008 risk classification system (blue curve, AUC = 0.796) ($P = 0.0312$) (Figure 4).

Table 3 Analysis of factors affecting prognosis of gastrointestinal stromal tumors in Cox proportional-hazards regression models for overall survival, *n* = 63

Characteristic	*n*	Univariate[a]			Multivariate[1]		
		HR	95%CI	*P* value	B	Exp (B)	*P* value
Sex							
Male	34	0.87	0.32-2.34	0.783	0.004	1.004	0.994
Female	29						
Age in yr							
> 56	30	0.99	0.96-1.03	0.751	-0.011	0.989	0.609
≤ 56	33						
Tumor location							
Stomach	36	3.86	1.23-12.18	0.021	0.669	1.953	0.057
Duodenum	12						
Jejunoileum	15						
Tumor size in cm							
< 2	11	2.32	1.32-4.07	0.003	0.734	2.083	0.037
2-5	19						
6-10	18						
> 10	14						
NIH risk grade							
Very low	10	2.32	1.32-4.07	0.002	1.393	4.025	0.014
Low	19						
Moderate	10						
High	24						
Mitotic figures as /50 HPFs							
0	11	1.7	1.06-2.70	0.026	0.179	1.196	0.505
1-4	36						
5-10	5						
> 10	11						
RKIP expression							
Positive	35	0.14	0.04-0.50	0.002	-2.064	0.127	0.061
Negative	28						
P-ERK expression							
Positive	36	3.63	1.03-12.73	0.044	1.761	5.821	0.041
Negative	27						
P-MEK expression							
Positive	28	1.78	0.66-4.79	0.252			
Negative	35						
RKIP and P-ERK co-expression[2]							
Positive	25	0.34	012-0.94	0.037	2.427	11.32	0.044
Negative	38						

[1]Analysed factors were sex, age, tumor location, tumor size, National Institutes of Health (NIH) risk grades, mitotic figures, raf kinase inhibitory protein

(RKIP), phosphorylated-extracellular-signal regulated kinase (P-ERK) expressions, RKIP and P-ERK co-expressions.
[2]Based on the expression of RKIP and P-ERK proteins in gastrointestinal stromal tumors specimens, we defined the cases with RKIP (-) and P-ERK (+) as negative, while the cases with RKIP (+) or P-ERK (-) as positive.
[a]Log-rank test; $P < 0.05$ were considered statistically significant.
HPFs: High-power fields; HR: Hazard ratio; MAPK: Mitogen-activated protein kinase; P-MEK: Phosphorylated-MAPK\ERK kinase.

Table 4 Multivariate analysis of prognostic factors for overall survival of gastrointestinal stromal tumors with two different sets of risk factors, *n* = 63

Characteristic	*n*	Multivariate[1]			Multivariate[2]		
		HR	95%CI	*P* value[a]	HR	95%CI	*P* value[a]
Tumor location							
Stomach	36	2.59	0.69-9.69	0.156	4.13	1.07-15.98	0.04
Duodenum	12						
Jejunoileum	15						
Tumor size in cm							
< 2	11	1.92	0.93-3.96	0.079	1.17	0.52-2.62	0.71
2-5	19						
6-10	18						
> 10	14						
Mitotic figures as /50 HPFs							
0	11	1.22	0.71-2.10	0.472	1.28	0.75-2.18	0.361
1-4	36						
5-10	5						
> 10	11						
RKIP expression							
Positive	35				0.13	0.03-0.66	0.014
Negative	28						
P-ERK expression							
Positive	36				0.91	0.19-4.32	0.909
Negative	27						

[1]Analysed factors were tumor location, tumor size, mitotic figures; above are all the risk factors included in NIH 2008 Risk Grade System.
[2]Analysed factors were tumor location, tumor size, mitotic figures, raf kinase inhibitory protein (RKIP) expressions and phosphorylated-extracellular-signal regulated kinase (P-ERK) expressions.
[a]$P < 0.05$ were considered statistically significant.
HPFs: High-power fields; HR: Hazard ratio; MAPK: Mitogen-activated protein kinase; P-MEK: Phosphorylated MAPK\ERK kinase.

DISCUSSION

GIST is the most common mesenchymal tumor in the gastrointestinal tract, accounting for approximately 60%-70% of all cases. Currently, effective treatment for GIST involves surgery combined with appropriate targeted therapy[9]. However, resistance to targeted therapy often occurs, and prognosis assessment is still being explored and modified. Therefore, there is an urgent need to find better therapeutic targets and a more efficient risk grading system.

ERK and MEK proteins are two core proteins in the Raf1-MEK-ERK pathway, which are downstream targets activated by abnormal C-kit and PDGFR-α *genes* closely related to GIST. ERK protein is associated with various diseases[10] such as tumors, heart failure, developmental disorders, and autoimmune diseases[11-13]. RKIP protein is a natural inhibitor of Raf-1 protein and is widely present in living organisms. It has an inhibitory effect on the metastasis and proliferation of various tumors.

In our preliminary experiments, we found that high expression of RKIP protein was related to GIST tumor size, invasion of the mucosa, and NIH grading. However, the single expression of RKIP protein alone is not sufficient as a single risk factor for evaluating GIST prognosis. Recent reports have suggested that RKIP protein regulates the expression

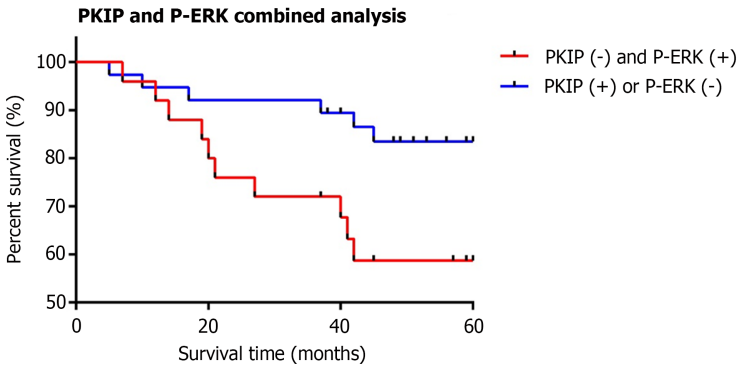

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Figure 2 Relationship between raf kinase inhibitor protein and phosphorylated-extracellular-signal regulated kinase co-expression and prognosis in gastrointestinal stromal tumors. Comparison with the raf kinase inhibitor protein (RKIP)-negative and phosphorylated-extracellular signal-regulated kinase (P-ERK)-positive groups; RKIP high expression group is correlated with a better survival rate and the difference was significant. Log-rank analysis, *P* = 0.0312.

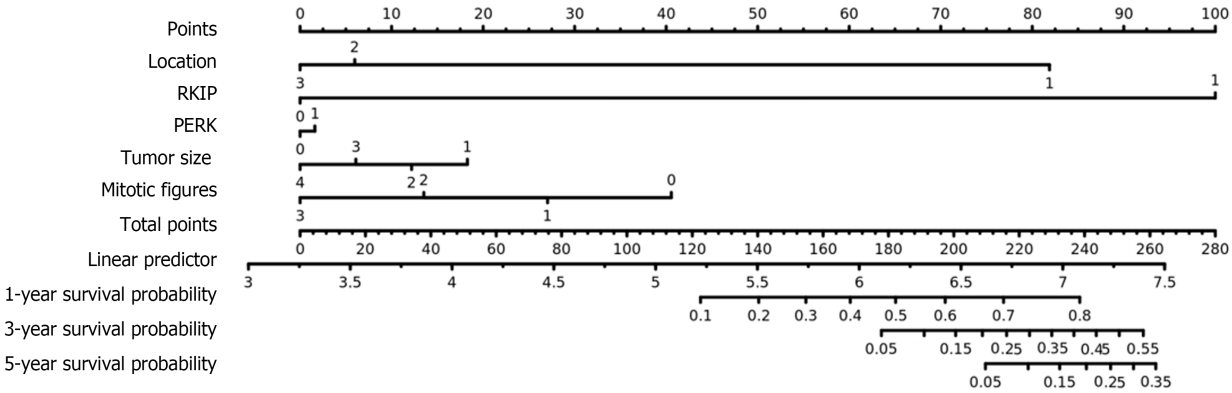

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Figure 3 Nomogram for the new risk grade model with consideration of raf kinase inhibitory protein and phosphorylated-extracellular-signal regulated kinase to predict the probabilities of 5-yr survival with gastrointestinal stromal tumors. Raf kinase inhibitory protein (RKIP) expression, phosphorylated-extracellular-signal regulated kinase (P-ERK) expression, tumor size, tumor location and mitotic count are involved as assessment factors.

of the MEK/ERK pathway in pancreatic cancer[14,15], thereby affecting tumor resistance and invasion. Additionally, it has been found that the combination of RKIP and P-ERK proteins in gastric cancer has a predictive significance for tumor prognosis[16]. These findings inspired us to focus on the combined detection of RKIP, P-ERK, and P-MEK proteins in GIST, in hopes of gaining further insights.

Clinical and pathological correlations

In this experiment, we analyzed the expression of RKIP, P-ERK, and P-MEK proteins in GIST and found that RKIP expression is related to GIST tumor size and NIH staging. This result is consistent with the research findings of Yuan *et al* [17], who found that knocking down the RKIP gene promotes cell growth in nasopharyngeal carcinoma cell lines[17].

In addition to its correlation with tumor size and NIH staging, P-ERK protein is also related to the mitotic count in GIST cells. Zhang *et al*[18] found that high expression of RKIP protein leads to low expression of P-ERK protein in the human choriocarcinoma cell line JEG-3. Furthermore, transfection with low P-ERK level tumor cells significantly reduced their growth activity compared to the control group, indicating that P-ERK protein is related to tumor cell proliferation [18].

The mechanism may be that low RKIP expression in GIST tumor cells leads to more phosphorylation of downstream ERK1/2 protein, forming P-ERK protein, which can control important structures in cell division such as centrosomes, spindle fibers, and centromeres[19,20], accelerating the tumor cell cycle and mitosis[21]. In addition, low RKIP expression causes dysregulation of its ligand Raf-1 protein, which upregulates the cell mitotic cycle[22,23], accelerating tumor growth. Moreover, ERK protein can be rapidly transported into the nucleus, further phosphorylating and activating proliferation-related transcription factors such as AP-1, ELK-1, and Serum Response Factor Accessory Protein (SAP), promoting cell proliferation and causing abnormal nuclear division[24]. Thus, low RKIP expression in GIST upregulates P-ERK, leading to tumor enlargement and increased nuclear division.

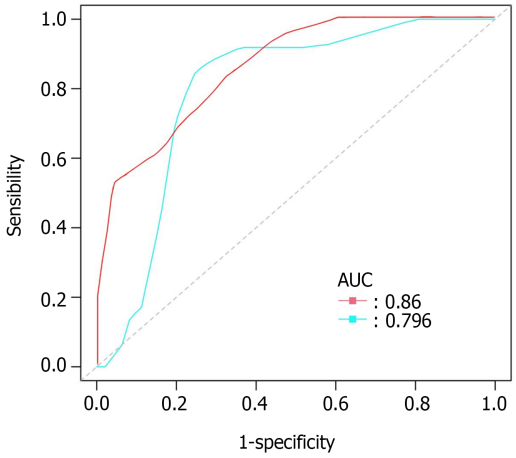

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Figure 4 Receiver operating characteristic curves for two risk grade system in predicting overall survival of gastrointestinal stromal tumors. The red curve represents for the new risk grade model and the blue one represents for National Institutes of Health (NIH) 2008 Risk Grade System. The area under the curve was 0.860 for the new risk grade model, while 0.796 for NIH 2008 Risk Grade System. Compared with the traditional NIH 2008 system, the new model which is with consideration of raf kinase inhibitory protein and phosphorylated-extracellular-signal regulated kinase is correlated with a better prediction efficiency for overall survival of gastrointestinal stromal tumors, the difference was significant. Log-rank analysis, $P = 0.0312$. AUC: Area under curve.

The NIH 2008 risk classification system is a recognized postoperative prognostic assessment system for GIST, which is based on three independent risk factors: Tumor location, tumor size, and mitotic index. RKIP and P-ERK proteins are associated with two of these factors, and it is easy to understand that protein expression is also related to NIH 2008 risk classification results. Schoppmann *et al*'s report suggests that loss of RKIP expression tends to increase the risk level of GIST cases in Fletcher's risk classification and a similar conclusion is drawn in Miettinen's risk classification[25]. Wang *et al*[26] also found that RKIP protein expression is related to TNM staging in non-small cell lung cancer[26].

We also found that both RKIP protein and P-ERK protein are related to the mucosal invasion status of GIST tumors. Martinho *et al*[27] found in experiments that low expression of RKIP protein often indicates a tendency of GIST tumors to invade and metastasize[27]. A similar correlation is found in gastric cancer between RKIP protein expression and tumor invasion depth, lymph node metastasis, and distant metastasis. The mechanism may be related to the interaction between RKIP protein and Snail protein, which regulates tumor Epithelial-Mesenchymal Transition (EMT)[28]: Snail protein can bind and downregulate E-cadherin protein, thereby regulating EMT; at the same time, it binds to the RKIP protein promoter E-box region to downregulate RKIP protein transcription. Thus, when Snail upregulates EMT, RKIP is often reduced[29].

In the analysis of clinical and pathological factors, no statistically significant results were found for P-MEK protein expression. However, there is a certain negative correlation between RKIP and P-MEK expression, which is statistically significant. This may indicate that RKIP has a certain regulatory effect on P-MEK. Schoppmann *et al*[25] found in their immunohistochemical study of GIST specimens that P-MEK1/2 expression is related to whether the tumor can be completely resected, but has no significant relationship with other factors. The conclusions of this study are mutually corroborated.

Analysis of co-expression of RKIP and P-ERK proteins

In this experiment, it was found that the expression of RKIP and P-ERK in GIST was negatively correlated ($P < 0.05$). Yang *et al*[14] found that inhibiting ERK phosphorylation would upregulate RKIP expression in pancreatic cancer cell lines; while Yuan *et al*[17] reported that high expression of RKIP in nasopharyngeal carcinoma cells can also inhibit ERK protein activation. Therefore, RKIP protein and P-ERK protein have mutual regulatory functions. Recently, the novel and revolutionary "Phospho-Theft" hypothesis about RKIP provides a perfect explanation for its possible mechanism[30]. RKIP protein has two completely different functions: When not phosphorylated, RKIP protein inhibits the activation and signal transmission of Raf-1 protein, which can reduce the activation of downstream MEK/ERK; after RKIP is phosphorylated by PKC protein at S153, P-RKIP substrate transfers to GRK2[31], which can enhance the activation of the β-AR pathway and activate downstream substrate ERK[32]. In addition, RKIP is regulated by feedback of KRAS-ERK pathway. At the same time, P-ERK can undergo self-phosphorylation, thereby activating downstream substrates such as Elk1, mitogen-and stress-activated protein Kinase, c-myc, *etc*[33].

Univariate and multivariate survival analysis and survival curve

Combining univariate and multivariate COX regression analysis, it was found that RKIP protein expression could not serve as an independent risk factor for predicting tumor prognosis ($P = 0.061$). Tumor size ($P = 0.037$), NIH grading ($P = 0.014$), P-ERK protein expression ($P = 0.041$), and RKIP combined with P-ERK protein expression ($P = 0.044$) could serve as independent prognostic factors with statistical significance. Among them, the combined expression of RKIP and P-ERK had the highest impact on survival, with a weight factor of Exp (B) at 11.320, followed by NIH grading, P-ERK expression, and tumor size. Furthermore, we plotted survival curves for GIST cases based on their combined RKIP and P-

ERK expression, revealing a significant difference in survival rates between groups. Therefore, the combined expression of RKIP and P-ERK has significant prognostic value for evaluating the prognosis of GIST patients and carries greater weight. This is consistent with findings in a study on nasopharyngeal carcinoma where RKIP was found to be an independent risk factor for predicting prognosis. In gastric cancer, the combined expression of P-ERK and RKIP was found to be associated with a 5-year relapse-free survival after surgery.

Establishment of a GIST prognostic model based on RKIP and P-ERK protein expression

Standardized treatment for GIST includes timely surgery and rational targeted therapy. The indication for targeted therapy is currently based on the NIH 2008 risk classification system applied to postoperative pathology results, with Imatinib (a representative TKI drug) for the intermediate and high-risk population[34]. Meanwhile, the Armed Forces Institute of Pathology and World Health Organization 2013 risk classifications are also used[35]. However, there are differences in the results among various risk classification systems, and there are difficulties in using and interpreting them for the public. As mentioned in this article, we found that the combination of RKIP and P-ERK protein expression is of great significance for the prognostic evaluation of GIST patients. Therefore, we incorporated RKIP and P-ERK protein expression, as well as tumor size, tumor location, and mitotic count, into a new GIST risk assessment model and plotted a survival curve. We analyzed its evaluation efficiency using the ROC curve, and the results showed that it is superior to the NIH 2008 risk classification system (Area Under Curve 0.860 *vs* 0.796).

Perspectives and limitations

In recent years, GIST-related research has been devoted to identifying new biomarkers and finding new therapeutic targets[36]. As one of the hotspots, our study found that the combination of RKIP and P-ERK proteins can effectively indicate the prognosis of GIST and can be used to guide GIST targeted therapy. More researchers have focused on the application and exploration of RKIP and P-ERK proteins in tumor diagnosis and treatment, such as the recent successful case of using urine RKIP monitoring to evaluate the prognosis of clear cell renal cell carcinoma (ccRCC)[37]. Serum RKIP levels have also been used to monitor the efficacy of treatment for multiple sclerosis[38]. In terms of treatment, ERK inhibitors combined with chloroquine can improve the adjuvant therapy for pancreatic cancer with K-Ras mutations[39], and imatinib combined with chloroquine (to inhibit autophagy) has also been attempted in GIST drug-resistant experimental studies[40].

Our experiment fills the gap in the research on the relevance of RKIP and P-ERK in GIST, further confirming the significance of these proteins in tumor physiology and clinical treatment. However, the experiment still has some limitations. Firstly, due to the lower incidence of GIST comparing to other gastrointestinal tumors, the sample size was limited. We will continue to recruit more samples and track their follow-up, using more abundant data for further validation and research. Secondly, this experiment used immunohistochemical methods for protein level research. In the future, we will supplement molecular level experiments to further elucidate the mechanism of action of relevant sites on the RKIP and ERK/MAPK pathways, and better guide targeted therapy. The above is also the focus of our project and ongoing work. We are confident in making improvements and will strive to promote a new chapter in the RKIP-related diagnosis and treatment of GIST.

CONCLUSION

Our experimental results showed that the expression of RKIP and p-ERK proteins in GIST was associated with tumor size, NIH 2008 staging, tumor invasion, and p-ERK expression was also related to mitotic count. The expression of the two proteins had a certain negative correlation. The combined expression of RKIP and p-ERK proteins can serve as an independent risk factor for predicting the prognosis of GIST patients. The new risk assessment model incorporating RKIP and p-ERK has superior evaluation efficacy and is worth further practical application to help validate results.

ARTICLE HIGHLIGHTS

Research background

Nowadays, research reports on the important clinical and prognostic value of phosphorylated-extracellular signal-regulated kinase (P-ERK) and phosphorylated-mitogen-activated protein kinase (MAPK/ERK) kinase (P-MEK) proteins closely related to raf kinase inhibitor protein (RKIP) have gradually emerged in digestive tract tumors.

Research motivation

The expression of downstream proteins of the ERK/MAPK pathway combined with RKIP in gastrointestinal stromal tumor (GIST) is scarce.

Research objectives

To detect the expression of RKIP, P-ERK, and P-MEK proteins in GIST and to analyze their relationship with clinicopathological characteristics and prognosis of this disease. Try to establish a new prognosis evaluation model using RKIP and P-ERK in combination and analyze its prognosis evaluation efficacy.

Research methods

This study will focus on this aspect and use immunohistochemistry methods, large sample survival analysis data, and the latest bioinformatics analysis techniques to search for answers to the problem.

Research results

Our experimental results showed that the expression of RKIP and P-ERK proteins in GIST was associated with tumor size, NIH 2008 staging, tumor invasion, and P-ERK expression was also related to mitotic count. The expression of the two proteins had a certain negative correlation.

Research conclusions

The combined expression of RKIP and P-ERK proteins can serve as an independent risk factor for predicting the prognosis of GIST patients. The new risk assessment model incorporating RKIP and P-ERK has superior evaluation efficacy and is worth further practical application to validate.

Research perspectives

As one of the hotspots, our study found that the combination of RKIP and P-ERK proteins can effectively indicate the prognosis of GIST and can be used to guide GIST targeted therapy. More researches are needed to focus on the application and exploration of RKIP and P-ERK proteins in tumor diagnosis and treatment.

FOOTNOTES

Author contributions: Wang Y designed research; Wang Y, Wang L and Qu WZ performed research; Wang Y and Qu WZ analyzed data; Wang Y, Chen JJ and Wang L wrote the paper; Wang Y edited the paper and provided primary revised opinion; All authors have read and approve the final manuscript.

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Informed consent statement: Consent was not obtained but the presented data are anonymized, and risk of identification is low. With the approval of the ethics committee, informed consent was waived.

Conflict-of-interest statement: The authors report grants from Natural Science Foundation of Liaoning Province, China, during the conduct of the study. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted and entitled 'Raf kinase inhibitor protein combined with phosphorylated extracellular signal-regulated kinase offers valuable prognosis in gastrointestinal stromal tumor'.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at wwyy840731@126.com. Consent was not obtained but the presented data are anonymized, and risk of identification is low. No additional data are available.

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REFERENCES

1 **Heinrich MC**, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, Eisenberg BL, von Mehren M, Fletcher CD, Sandau K, McDougall K, Ou WB, Chen CJ, Fletcher JA. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol* 2006; **24**: 4764-4774 [PMID: 16954519 DOI: 10.1200/JCO.2006.06.2265]

2 **Demetri GD**, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; **368**: 1329-1338 [PMID: 17046465 DOI: 10.1016/S0140-6736(06)69446-4]

3 **Riller Q**, Rieux-Laucat F. RASopathies: From germline mutations to somatic and multigenic diseases. *Biomed J* 2021; **44**: 422-432 [PMID: 34175492 DOI: 10.1016/j.bj.2021.06.004]

4 **Yan ZP**, Li JT, Zeng N, Ni GX. Role of extracellular signal-regulated kinase 1/2 signaling underlying cardiac hypertrophy. *Cardiol J* 2021; **28**: 473-482 [PMID: 32329039 DOI: 10.5603/CJ.a2020.0061]

5 **Fan HY**, Tong C, Sun QY. [Mitogen-activated protein kinase (MAPK) Signaling Pathways: State-of-the-art]. *Donwuxue Zazhi* 2002; **37**: 98-102 [DOI: 10.3969/j.issn.0250-3263.2002.05.026]

6 **Yeung K**, Janosch P, McFerran B, Rose DW, Mischak H, Sedivy JM, Kolch W. Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein. *Mol Cell Biol* 2000; **20**: 3079-3085 [PMID: 10757792 DOI: 10.1128/MCB.20.9.3079-3085.2000]

7 **Granovsky AE**, Clark MC, McElheny D, Heil G, Hong J, Liu X, Kim Y, Joachimiak G, Joachimiak A, Koide S, Rosner MR. Raf kinase inhibitory protein function is regulated *via* a flexible pocket and novel phosphorylation-dependent mechanism. *Mol Cell Biol* 2009; **29**: 1306-1320 [PMID: 19103740 DOI: 10.1128/MCB.01271-08]

8 **Wang Y**, Chen JJ, Wang XF, Wang Q. Clinical and prognostic significance of Raf kinase inhibitory protein expression in gastrointestinal stromal tumors. *World J Gastroenterol* 2018; **24**: 2508-2517 [PMID: 29930472 DOI: 10.3748/wjg.v24.i23.2508]

9 **Sun K**, Lv H, Chen B, Nie C, Zhao J, Wang S, Wang J, Xu W, Chen X. Dawning precision treatment for gastric cancer: The latest biomarkers. *J Transl Int Med* 2021; **9**: 228-230 [PMID: 35136720 DOI: 10.2478/jtim-2021-0023]

10 **Lorenz K**, Rosner MR. Harnessing RKIP to Combat Heart Disease and Cancer. *Cancers (Basel)* 2022; **14** [PMID: 35205615 DOI: 10.3390/cancers14040867]

11 **Sugiura R**, Satoh R, Takasaki T. ERK: A Double-Edged Sword in Cancer. ERK-Dependent Apoptosis as a Potential Therapeutic Strategy for Cancer. *Cells* 2021; **10** [PMID: 34685488 DOI: 10.3390/cells10102509]

12 **Gilbert CJ**, Longenecker JZ, Accornero F. ERK1/2: An Integrator of Signals That Alters Cardiac Homeostasis and Growth. *Biology (Basel)* 2021; **10** [PMID: 33923899 DOI: 10.3390/biology10040346]

13 **Gallo S**, Vitacolonna A, Bonzano A, Comoglio P, Crepaldi T. ERK: A Key Player in the Pathophysiology of Cardiac Hypertrophy. *Int J Mol Sci* 2019; **20** [PMID: 31052420 DOI: 10.3390/ijms20092164]

14 **Yang K**, Li Y, Lian G, Lin H, Shang C, Zeng L, Chen S, Li J, Huang C, Huang K, Chen Y. KRAS promotes tumor metastasis and chemoresistance by repressing RKIP *via* the MAPK-ERK pathway in pancreatic cancer. *Int J Cancer* 2018; **142**: 2323-2334 [PMID: 29315556 DOI: 10.1002/ijc.31248]

15 **Bian Y**, Jiang H, Zheng J, Shao C, Lu J. Basic Pancreatic Lesions: Radiologic-pathologic Correlation. *J Transl Int Med* 2022; **10**: 18-27 [PMID: 35702187 DOI: 10.2478/jtim-2022-0003]

16 **Fujimori Y**, Inokuchi M, Takagi Y, Kato K, Kojima K, Sugihara K. Prognostic value of RKIP and p-ERK in gastric cancer. *J Exp Clin Cancer Res* 2012; **31**: 30 [PMID: 22463874 DOI: 10.1186/1756-9966-31-30]

17 **Yuan L**, Yi HM, Yi H, Qu JQ, Zhu JF, Li LN, Xiao T, Zheng Z, Lu SS, Xiao ZQ. Reduced RKIP enhances nasopharyngeal carcinoma radioresistance by increasing ERK and AKT activity. *Oncotarget* 2016; **7**: 11463-11477 [PMID: 26862850 DOI: 10.18632/oncotarget.7201]

18 **Zhang M**, Zhang D, Fan Q. RKIP suppresses the proliferation and invasion ofchoriocarcinoma cells through inhibiting the MAPK signaling pathway. *Int J Clin Exp Med* 2015; **8**: 22183-22190 [PMID: 26885193]

19 **Shapiro PS**, Vaisberg E, Hunt AJ, Tolwinski NS, Whalen AM, McIntosh JR, Ahn NG. Activation of the MKK/ERK pathway during somatic cell mitosis: direct interactions of active ERK with kinetochores and regulation of the mitotic 3F3/2 phosphoantigen. *J Cell Biol* 1998; **142**: 1533-1545 [PMID: 9744882 DOI: 10.1083/jcb.142.6.1533]

20 **Zecevic M**, Catling AD, Eblen ST, Renzi L, Hittle JC, Yen TJ, Gorbsky GJ, Weber MJ. Active MAP kinase in mitosis: localization at kinetochores and association with the motor protein CENP-E. *J Cell Biol* 1998; **142**: 1547-1558 [PMID: 9744883 DOI: 10.1083/jcb.142.6.1547]

21 **Yesilkanal AE**, Rosner MR. Raf kinase inhibitory protein (RKIP) as a metastasis suppressor: regulation of signaling networks in cancer. *Crit Rev Oncog* 2014; **19**: 447-454 [PMID: 25597354 DOI: 10.1615/CritRevOncog.2014012000]

22 **Ziogas A**, Lorenz IC, Moelling K, Radziwill G. Mitotic Raf-1 is stimulated independently of Ras and is active in the cytoplasm. *J Biol Chem* 1998; **273**: 24108-24114 [PMID: 9727031 DOI: 10.1074/jbc.273.37.24108]

23 **Hayne C**, Tzivion G, Luo Z. Raf-1/MEK/MAPK pathway is necessary for the G2/M transition induced by nocodazole. *J Biol Chem* 2000; **275**: 31876-31882 [PMID: 10884385 DOI: 10.1074/jbc.M002766200]

24 **Boilly B**, Vercoutter-Edouart AS, Hondermarck H, Nurcombe V, Le Bourhis X. FGF signals for cell proliferation and migration through different pathways. *Cytokine Growth Factor Rev* 2000; **11**: 295-302 [PMID: 10959077 DOI: 10.1016/S1359-6101(00)00014-9]

25 **Schoppmann SF**, Beer A, Nirtl N, Ba-Ssalamah A, Brodowicz T, Streubel B, Birner P. Downregulation of phosphatidylethanolamine binding protein 1 associates with clinical risk factors in gastrointestinal stromal tumors, but not with activation of the RAF-1-MEK-ETV1 pathway. *Cancer Lett* 2013; **335**: 26-30 [PMID: 23376254 DOI: 10.1016/j.canlet.2013.01.044]

26 **Wang A**, Duan G, Zhao C, Gao Y, Liu X, Wang Z, Li W, Wang K, Wang W. Reduced RKIP expression levels are associated with frequent non-small cell lung cancer metastasis and STAT3 phosphorylation and activation. *Oncol Lett* 2017; **13**: 3039-3045 [PMID: 28521411 DOI: 10.3892/ol.2017.5846]

27 **Martinho O**, Gouveia A, Silva P, Pimenta A, Reis RM, Lopes JM. Loss of RKIP expression is associated with poor survival in GISTs. *Virchows Arch* 2009; **455**: 277-284 [PMID: 19705153 DOI: 10.1007/s00428-009-0821-z]

28 **Cessna H**, Baritaki S, Zaravinos A, Bonavida B. The Role of RKIP in the Regulation of EMT in the Tumor Microenvironment. *Cancers (Basel)* 2022; **14** [PMID: 36230521 DOI: 10.3390/cancers14194596]

29 **Beach S**, Tang H, Park S, Dhillon AS, Keller ET, Kolch W, Yeung KC. Snail is a repressor of RKIP transcription in metastatic prostate cancer cells. *Oncogene* 2008; **27**: 2243-2248 [PMID: 17952120 DOI: 10.1038/sj.onc.1210860]

30 **Skinner JJ**, Wang S, Lee J, Ong C, Sommese R, Sivaramakrishnan S, Koelmel W, Hirschbeck M, Schindelin H, Kisker C, Lorenz K, Sosnick TR, Rosner MR. Conserved salt-bridge competition triggered by phosphorylation regulates the protein interactome. *Proc Natl Acad Sci U S A* 2017; **114**: 13453-13458 [PMID: 29208709 DOI: 10.1073/pnas.1711543114]

31 **Lorenz K**, Lohse MJ, Quitterer U. Protein kinase C switches the Raf kinase inhibitor from Raf-1 to GRK-2. *Nature* 2003; **426**: 574-579 [PMID: 14654844 DOI: 10.1038/nature02158]

32 **Maimari T**, Krasel C, Bünemann M, Lorenz K. The N-termini of GRK2 and GRK3 simulate the stimulating effects of RKIP on β-adrenoceptors. *Biochem Biophys Res Commun* 2019; **520**: 327-332 [PMID: 31604529 DOI: 10.1016/j.bbrc.2019.09.135]

33 **Robinson EL**, Drawnel FM, Mehdi S, Archer CR, Liu W, Okkenhaug H, Alkass K, Aronsen JM, Nagaraju CK, Sjaastad I, Sipido KR, Bergmann O, Arthur JSC, Wang X, Roderick HL. MSK-Mediated Phosphorylation of Histone H3 Ser28 Couples MAPK Signalling with Early Gene Induction and Cardiac Hypertrophy. *Cells* 2022; **11** [PMID: 35203255 DOI: 10.3390/cells11040604]

34 **Blay JY**, Hindi N, Bollard J, Aguiar S Jr, Angel M, Araya B, Badilla R, Bernabeu D, Campos F, Caro-Sánchez CHS, Carvajal B, Carvajal Montoya A, Casavilca-Zambrano S, Castro-Oliden V, Chacón M, Clara M, Collini P, Correa Genoroso R, Costa FD, Cuellar M, Dei Tos AP, Dominguez Malagon HR, Donati D, Dufresne A, Eriksson M, Farias-Loza M, Fernandez P, Frezza AM, Frisoni T, Garcia-Ortega DY, Gelderblom H, Gouin F, Gómez-Mateo MC, Gronchi A, Haro J, Huanca L, Jimenez N, Karanian M, Kasper B, Lopes David BB, Lopez-Pousa A, Lutter G, Martinez-Said H, Martinez-Tlahuel J, Mello CA, Morales Pérez JM, Moura David S, Nascimento AG, Ortiz-Cruz EJ, Palmerini E, Patel S, Pfluger Y, Provenzano S, Righi A, Rodriguez A, Salas R, Santos TTG, Scotlandi K, Soule T, Stacchiotti S, Valverde C, Waisberg F, Zamora Estrada E, Martin-Broto J. SELNET clinical practice guidelines for soft tissue sarcoma and GIST. *Cancer Treat Rev* 2022; **102**: 102312 [PMID: 34798363 DOI: 10.1016/j.ctrv.2021.102312]

35 **Chen T**, Xu L, Ye L, Qiu H, Hu Y, Liu H, Zhou Z, Li G, Yu J. A new nomogram for recurrence-free survival prediction of gastrointestinal stromal tumors: Comparison with current risk classification methods. *Eur J Surg Oncol* 2019; **45**: 1109-1114 [PMID: 30594406 DOI: 10.1016/j.ejso.2018.12.014]

36 **Dz Chen J**, Zhu Y, Wang Y. Emerging Noninvasive Neuromodulation Methods for Functional Gastrointestinal Diseases. *J Transl Int Med* 2022; **10**: 281-285 [PMID: 36860625 DOI: 10.2478/jtim-2022-0060]

37 **Papale M**, Vocino G, Lucarelli G, Rutigliano M, Gigante M, Rocchetti MT, Pesce F, Sanguedolce F, Bufo P, Battaglia M, Stallone G, Grandaliano G, Carrieri G, Gesualdo L, Ranieri E. Urinary RKIP/p-RKIP is a potential diagnostic and prognostic marker of clear cell renal cell carcinoma. *Oncotarget* 2017; **8**: 40412-40424 [PMID: 28418894 DOI: 10.18632/oncotarget.16341]

38 **Bedri SK**, Nilsson OB, Fink K, Månberg A, Hamsten C, Ayoglu B, Manouchehrinia A, Nilsson P, Olsson T, Hillert J, Grönlund H, Glaser A. Plasma protein profiling reveals candidate biomarkers for multiple sclerosis treatment. *PLoS One* 2019; **14**: e0217208 [PMID: 31141529 DOI: 10.1371/journal.pone.0217208]

39 **Bryant KL**, Stalnecker CA, Zeitouni D, Klomp JE, Peng S, Tikunov AP, Gunda V, Pierobon M, Waters AM, George SD, Tomar G, Papke B, Hobbs GA, Yan L, Hayes TK, Diehl JN, Goode GD, Chaika NV, Wang Y, Zhang GF, Witkiewicz AK, Knudsen ES, Petricoin EF 3rd, Singh PK, Macdonald JM, Tran NL, Lyssiotis CA, Ying H, Kimmelman AC, Cox AD, Der CJ. Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer. *Nat Med* 2019; **25**: 628-640 [PMID: 30833752 DOI: 10.1038/s41591-019-0368-8]

40 **Zheng S**, Shu Y, Lu Y, Sun Y. Chloroquine Combined with Imatinib Overcomes Imatinib Resistance in Gastrointestinal Stromal Tumors by Inhibiting Autophagy *via* the MAPK/ERK Pathway. *Onco Targets Ther* 2020; **13**: 6433-6441 [PMID: 32753885 DOI: 10.2147/OTT.S256935]

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CASE REPORT

Value of contrast-enhanced ultrasound in deep angiomyxoma using a biplane transrectal probe: A case report

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Abstract

BACKGROUND

Deep angiomyxoma (DAM) is a very rare tumor type. Magnetic resonance imaging (MRI) is considered the best imaging modality for diagnosing DAM. Computed tomography (CT) is used mainly to assess the invasion range of DAM. The value of ultrasonography in the diagnosis of DAM is still controversial. Through a literature review, we summarized the current state of ultrasonic examination for DAM and reported for the first time the contrast-enhanced ultrasound (CEUS) features of DAM seen using a biplane transrectal probe.

CASE SUMMARY

A 37-year-old woman presented with a sacrococcygeal mass that had gradually increased in size over the previous 6 mo. MRI and CT examinations failed to allow a definite diagnosis to be made. Transperineal core needle biopsy (CNB) guided by transrectal ultrasound and CEUS was suggested after a multidisciplinary discussion. Grayscale ultrasound of the lesion showed a layered appearance with alternating hyperechoic and hypoechoic patterns. Transrectal CEUS showed a laminated distribution of the contrast agent that was consistent with the layered appearance of the tumor on grayscale ultrasound. We performed transperineal CNB of the enhanced area inside the tumor under transrectal CEUS guidance and finally made a definitive diagnosis of DAM through histopathology. The patient underwent laparoscopic-assisted transabdominal surgery combined with transperineal surgery for large pelvic tumor resection and pelvic floor peritoneal reconstruction. No recurrence or metastasis was found at the nine-month follow-up.

CONCLUSION

Transrectal CEUS can show the layered perfusion characteristics of the contrast agent, guiding subsequent transperineal CNB of the enhanced area within the DAM.

Key Words: Contrast-enhanced ultrasound; Transrectal ultrasound; Transperineal core needle biopsy; Deep angiomyxoma; Pelvic tumor; Case report

Core Tip: Deep angiomyxoma (DAM) is a very rare tumor. Imaging examinations play an important role in the diagnosis of DAM. Magnetic resonance imaging is considered the best imaging modality for diagnosing DAM. Computed tomography is used mainly to assess the invasion range of DAM. The value of ultrasonography in the diagnosis of DAM is still controversial. We reported for the first time the contrast-enhanced ultrasound (CEUS) features of DAM seen using a biplane transrectal probe. Transrectal CEUS can provide more abundant diagnostic information in terms of the blood perfusion characteristics of DAM, guiding subsequent transperineal puncture of the enhanced area within the tumor.

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INTRODUCTION

Deep angiomyxoma (DAM) is a very rare tumor that mainly occurs in the pelvis and perineum of women of reproductive age[1]. DAM is classified as a "tumor of uncertain differentiation" in the World Health Organization classification. Since it was first described in 1983, DAM has been described in multiple single case reports and some case series reports[1-3]. Imaging examinations play an important role in the diagnosis of DAM and in evaluating the scope of lesion invasion. Magnetic resonance imaging (MRI) is considered the best imaging modality for the diagnosis of DAM; computed tomography (CT) is mainly used to evaluate the invasion range of DAM, and the value of ultrasound in the diagnosis of DAM is still controversial. This article reports for the first time the contrast-enhanced ultrasound (CEUS) features of DAM seen using a biplane transrectal probe and reviews the imaging features of DAM in the literature.

CASE PRESENTATION

Chief complaints

A 37-year-old woman presented with a sacrococcygeal mass that had been increasing in size over the previous 6 mo.

History of present illness

The patient inadvertently found a sacrococcygeal mass 6 mo prior, which was approximately the size of a pigeon egg at first, without pain or other discomfort. The mass gradually increased in size, accompanied by lumbosacral distension. No definite diagnosis was made after an examination at the local hospital; thus, the patient was transferred to our hospital for further diagnosis and treatment.

History of past illness

The patient had no previous medical history.

Personal and family history

The patient and family histories were negative.

Physical examination

A digital rectal examination revealed a large solid mass in the anterior rectal wall, with a hard texture and fixed position. The mass boundary could not be palpated.

Laboratory examinations

The results of laboratory examinations were normal, including routine blood analysis, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), carbohydrate antigen (CA) 19-9, and CA125 results.

Imaging examinations

Preoperative MRI showed a paracervical mass in the pelvis, measuring approximately 9.5 cm × 8.1 cm in size (Figure 1A). T2-weighted imaging (T2WI) presented stratification changes with alternating high and low signals that were clearly demarcated from the surrounding tissue, and the uterus was pushed and displaced cephalad. Preoperative contrast-

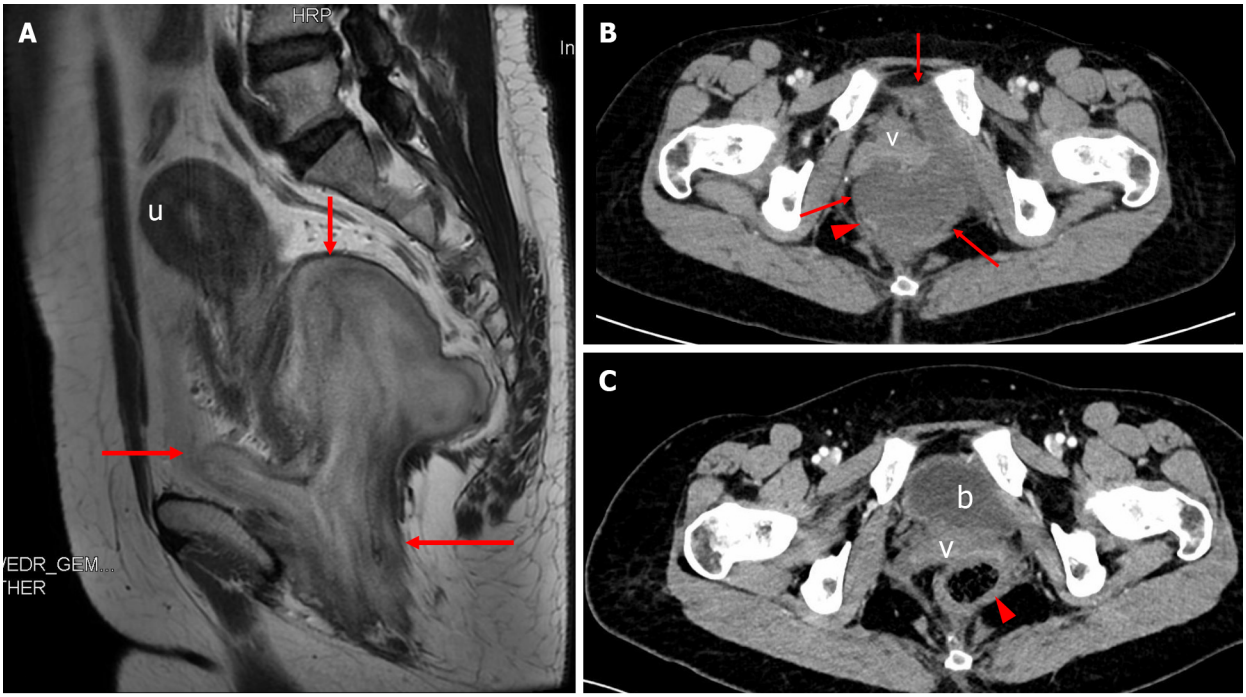

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Figure 1 Magnetic resonance imaging and contrast-enhanced computed tomography images in our case. A: Preoperative sagittal T2-weighted imaging. The mass (arrows) was 9.5 cm × 8.1 cm in size, showing a layered appearance with alternating high and low signals and a clearly demarcated boundary with respect to surrounding tissue, and the uterus was pushed and displaced cephalad; B: Preoperative transverse contrast-enhanced computed tomography (CECT) showed that the anterior vagina and the right posterior rectum (arrowhead) were pushed by the mass (arrows); C: Six months after surgery, transverse CECT showed that the filled bladder, vagina, and rectum (arrowhead) were in normal positions, with no evidence of tumor recurrence. u: Uterus; v: Vagina; b: Bladder.

enhanced CT (CECT) demonstrated a pelvic mass between the cervix and rectum with nonenhanced areas (Figure 1B). The mass pushed against and displaced the anterior vagina and the right posterior rectum.

Preoperative transrectal ultrasound (TRUS) was performed with the MyLab Twice ultrasound system (Esaote, Genoa, Italy) equipped with a biplane endoscopic probe (TRT33, linear frequency of 4-13 MHz, convex frequency of 3-9 MHz). Grayscale ultrasound showed that the left pelvic mass had an irregular shape (Figure 2A). The internal echo of the mass presented a layered appearance with alternating hyperechoic and hypoechoic patterns, and the boundary between the mass and rectum wall was clear. Color Doppler flow imaging (CDFI) showed some blood vessels scattered in the tumor (Figure 2B), and the dispersed intratumoral blood vessels were consistent with the layered appearance of the tumor on grayscale ultrasound. Then, transrectal CEUS was performed with a bolus injection of 2.4 mL of SonoVue (Bracco, Milan, Italy) through the elbow vein (Figure 2C). A laminated distribution of the contrast agent was observed within the mass, which was also consistent with the layered appearance of the tumor on grayscale ultrasound.

Further diagnostic work-up

Transperineal core needle (16G) biopsy (CNB) was performed under the guidance of TRUS (Figure 2D). The probe was switched to linear mode, and puncture sampling was performed under real-time monitoring by TRUS, avoiding the nonenhanced areas of the tumor. No complications occurred during the biopsy.

FINAL DIAGNOSIS

Postoperative immunohistochemical staining showed the following results: CD34 (vascular +), CK (pan) (-), ER (+), PR (+), Desmin (+), S-100 (-), SMA (+), CDK4 (-), and MDM2 (±). FISH revealed an unbalanced translocation of the HMGA2 gene. The final diagnosis was a DAM in the deep pelvis (Figure 3).

TREATMENT

A preoperative examination was completed. The patient underwent laparoscopic-assisted transabdominal surgery combined with transperineal surgery for large pelvic tumor resection and pelvic floor peritoneal reconstruction on February 10, 2022.

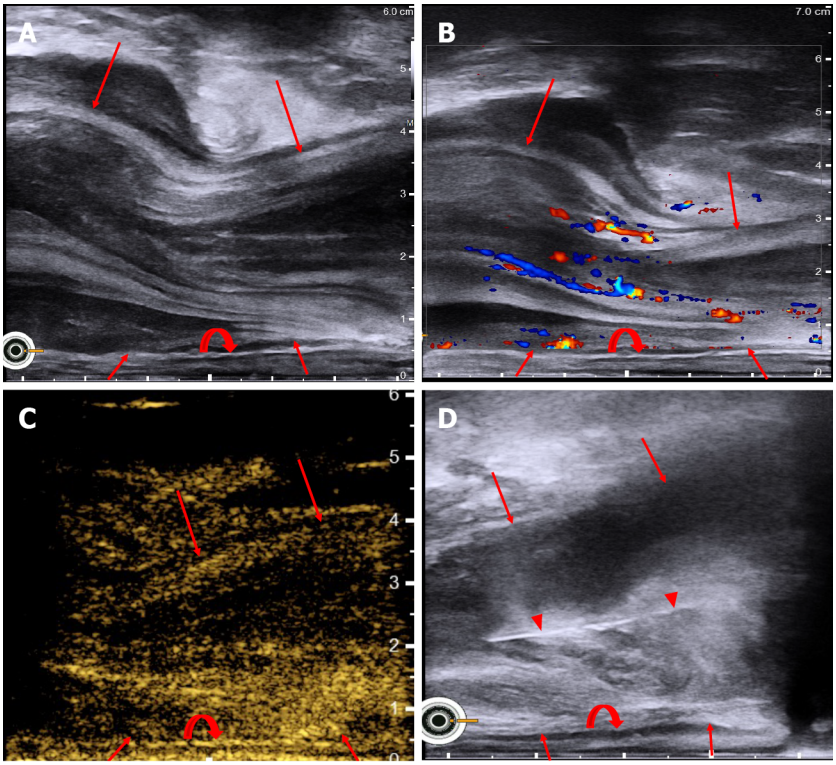

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Figure 2 Scanning with a TRT33 biplane transrectal probe linear array. A: Grayscale ultrasound showed that the mass (arrows) was irregular in shape, with a layered appearance of alternating hyperechoic and hypoechoic areas inside and a clearly demarcated boundary with respect to the rectal wall (curved arrow); B: Color Doppler flow imaging showed that the dispersed intratumoral blood vessels were consistent with the layered appearance of the mass (arrows) on grayscale ultrasound; C: Transrectal contrast-enhanced ultrasound showed a laminated distribution of the contrast agent in the mass (arrows), also consistent with the layered appearance of the tumor on grayscale ultrasound; D: Transperineal core needle (arrowheads) biopsy was performed under the guidance of transrectal ultrasound.

During surgery, the tumor was found to be located below the pelvic peritoneal reflex plane, behind the cervix, and in front of the left side of the rectum. It was irregular in shape, with a smooth capsule, and contained both cystic and solid components. The postoperative pathological report was consistent with DAM.

OUTCOME AND FOLLOW-UP

The patient recovered well after surgery and underwent regular outpatient follow-up examinations. CECT of the pelvic cavity was performed on August 12, 2022 (Figure 1C), six months after resection, and indicated no obvious signs of tumor recurrence. No recurrence or metastasis was found by the last follow-up on November 9, 2022.

DISCUSSION

DAM is a very rare type of tumor that most commonly occurs in the pelvis and perineum of women of reproductive age [1]. Histologically, the typical morphology of DAM is an infiltrating, uniformly hypocellular tumor, consisting of small-sized spindled or stellate cells scattered randomly in the myxoid stroma and containing many small to medium/Large blood vessels, without nuclear atypia[1] (Figure 3). Clinically, DAM presents as a slowly growing painless mass. DAM is characterized by locally aggressive growth, with recurrence and metastasis occurring in some cases[2,3]. Accurate preoperative diagnosis and complete resection of the lesion are key to the treatment and prevention of recurrence[4]. Imaging examinations play an important role in the diagnosis of DAM and in assessing the extent of lesion invasion[5]. Commonly used imaging examinations include MRI, CT, and ultrasound[6].

CT is mainly used to evaluate the invasion range of DAM and perform follow-up observations, but its value in displaying typical signs such as a laminated or swirled appearance of the DAM tissue is limited[5-7]. MRI is considered the best imaging modality for the diagnosis of DAM[6,8-10]. T2WI can reveal the swirled or layered appearance of the mass, which has been reported in at least 83% of cases[6]. MRI is also critical to determining the range of tumor extension into the surrounding space and the mode of tumor invasion into surrounding organs[6,9]. Retrospective analysis of the T2WI MRI data in our case showed a typical laminated and swirled appearance consistent with the reports in the literature[8-10] (Figure 1A). However, we failed to make a definitive diagnosis, mainly because DAM is rare; we had insufficient experience in diagnosing this tumor. Some experts believe that it is difficult to accurately diagnose DAM

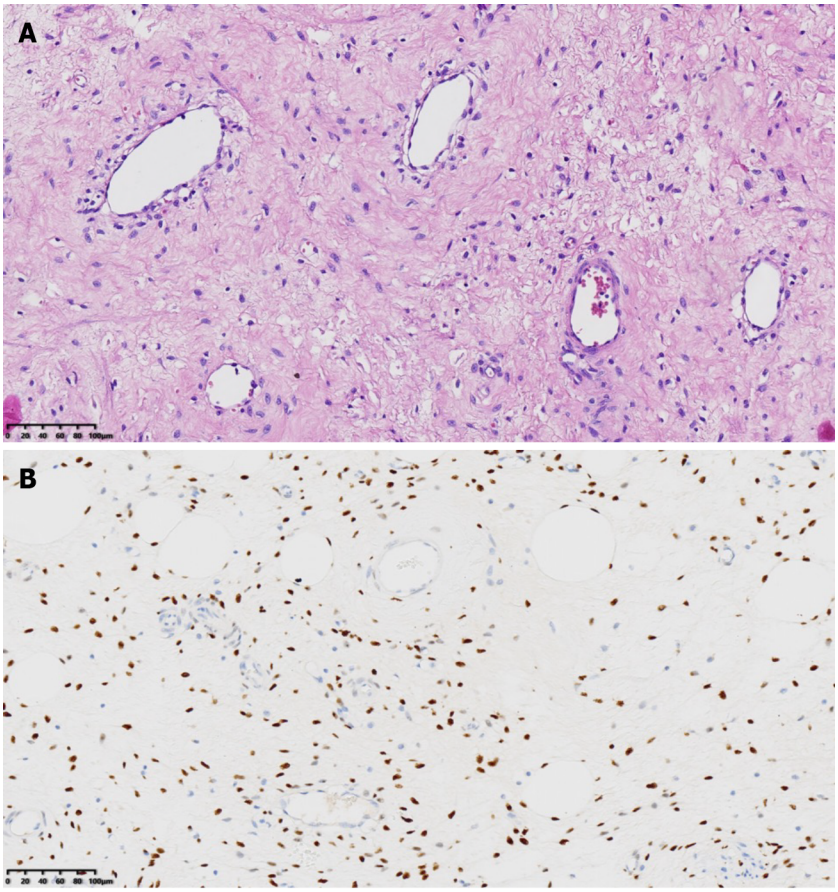

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Figure 3 Pathological results of the deep angiomyxoma puncture sample. A: Small-sized spindled or stellate cells scattered in the myxoid stroma containing many blood vessels of varying caliber, without cellular atypia or mitotic activity (HE × 200); B: Immunohistochemistry showed positive expression of estrogen receptors in the tumor cells (× 200).

before surgery, and these tumors are often misdiagnosed as other soft tissue tumors[11-13], such as Bartholin cyst, lipoma, and cellular angiofibroma. The misdiagnosis rate is as high as 82%[11].

Although MRI has many advantages in the diagnosis of DAM, the cost of MRI is relatively high, and patients with metal in the body cannot be examined by MRI. Similar to MRI, ultrasound can also be applied to avoid radiation exposure. Because of its low cost, convenient operation, and noninvasiveness, ultrasound can be used for multiple examinations in cases of DAM and follow-up examinations after surgery. In addition, ultrasound is not limited by the presence of metal objects such as heart stents and is also the preferred choice for patients with claustrophobia.

However, the literature related to ultrasound for DAM is scarce[14], and the value and role of ultrasound have not been systematically summarized. Through a literature review, we found that the previous ultrasonography methods related to DAM mainly consisted of examinations with transabdominal convex array probes[10,15], intracavity convex array probes[9,14,16,17], and transperineal linear array probes[18,19]. Among the 53 reports related to DAM, only 2[9,15] reported a typical laminated or swirled appearance on grayscale ultrasound. In all reports, CDFI did not show swirled or layered blood vessel distribution characteristics corresponding to the grayscale ultrasound findings, and CEUS examination was not performed in any of the previous reports, as shown in Table 1.

We report for the first time the CEUS features of DAM in the pelvic cavity seen using a biplane transrectal probe. In this case, transrectal grayscale ultrasound showed a laminated appearance with alternating hyperechoic and hypoechoic patterns inside the mass (Figure 2A), demonstrating the same details as MRI and excellent resolution, which confirms the high sensitivity of transrectal high-frequency ultrasound in detecting the characteristic histological structure of DAM. We also observed that the distribution of scattered blood vessels within the tumor on CDFI was consistent with the layered appearance of the tumor on grayscale ultrasound (Figure 2B). This layered distribution of vascular features has not been previously reported in the literature[9-11,15,18,19], which may be related to the fact that more anatomical details of the tumor can be displayed by high-frequency ultrasound with a biplane transrectal probe[9,20,21]; however, this is yet to be verified by subsequent cases. Reportedly, CEUS may be of great value in the diagnosis of DAM[15], as this modality can better show the characteristics of DAM and help to determine the role of ultrasound in tumor treatment. However, to our knowledge, the diagnosis of DAM by CEUS has not been reported. In our case, the mass was examined by transrectal CEUS (Figure 2C). The characteristic laminated perfusion of the contrast agent was observed within the mass, which was also consistent with the layered appearance of the tumor on grayscale ultrasound.

Table 1 Summary of sonographic features of deep angiomyxoma from the literature

No.	Ultrasonographic approach and probe	No. of tumors	Sex	Age (yr)	Location	Maximal diameter (cm)	Internal echogenicity	No. of cystic component	Laminated/swirled pattern in 2D
1	Transabdominal convex array probe	4	F	18-50	Pelvis	3-38.8 (mean: 16.2 ± 9.4)	Hypoechoic or isoechoic internal components	3	No
2	Transabdominal convex array probe	8	7F; 1M	35-64 (median 39)	Vulva, pelvis, perineal region, spermatic cord, and scrotum	7-21	Heterogeneous, isoechoic internal components	2	Yes
3	Transvaginal end-fire probe	1	F	40	Pelvis extending to the perineum	11.8	Mixed echogenicity	0	Yes
4	Transvaginal end-fire probe	1	F	54	Behind the uterus	18	Heterogeneous hypoechoic internal components	1	No
5	Transvaginal end-fire probe	1	F	44	Left pelvis	12	Hypoechoic to isoechoic internal components	0	No
6	Transrectal radial probe	1	F	61	Left perirectal mass	10.7	Hypoechoic to isoechoic internal components	NA	No
7	Transperineal linear probe	36	M	1-81 (mean 48.3 ± 20.6)	Epididymis, testes, spermatic cord, scrotum	1.6-25 (mean: 8.36)	Heterogeneous hypoechoic internal components	NA	No
8	Transperineal linear probe	1	F	28	Vulva	4.8	Heterogeneous hypoechoic internal components	0	No

F: Female; M: Male; NA: Not available.

We believe that the advantages of ultrasound with a biplane transrectal probe and CEUS in the diagnosis of DAM in the deep pelvic cavity are as follows:

First, the biplane transrectal high-frequency probe can enter the pelvic cavity along the anal canal and rectum and clearly display the laminated or swirled appearance of DAM, as well as the boundary of the mass and its relationships with adjacent structures, allowing this modality to be comparable to MRI. Typically, transperineal ultrasound and transabdominal ultrasound cannot simultaneously meet the requirements of satisfactory detection depth and high resolution of images when examining deep pelvic lesions. Additionally, the biplane transrectal probe effectively overcomes the obvious limitation of the observation angle of the convex end-fire probe.

Second, the laminated perfusion characteristics inside the mass can be displayed in real time by CEUS, which is helpful for the diagnosis and differential diagnosis of DAM.

Third, the gold standard for the diagnosis of DAM depends on histopathology and immunohistochemistry[18]. However, the proportion of cystic components in DAM is as high as 75%[10], and these components appeared as unenhanced areas on transrectal CEUS, which were easy to distinguish from enhanced areas of the mass, guiding subsequent precise and effective puncture of the enhanced area (Figure 2D). After transrectal CEUS, our patient immediately underwent transperineal CNB[22,23] guided by TRUS at the examination bed, and a clear pathological diagnosis was obtained, which provided a basis for formulating the surgical plan and reducing the patient's waiting time before surgery.

Fourth, compared with MRI and CT, TRUS is convenient, rapid, radiation-free, and economical. TRUS can dynamically display the internal and adjacent structures of the lesion in real time and can be used for repeated monitoring of the lesion before surgery.

Fifth, for patients with cardiac pacemakers, internal metal stents, or claustrophobia, ultrasound with a biplane transrectal probe and CEUS can be used instead of MRI.

Sixth, long-term follow-up is necessary due to the high recurrence rate of DAM[24]. Due to the above advantages, ultrasound with a biplane transrectal probe and CEUS are ideal tools for the postoperative follow-up of pelvic DAM patients. In summary, ultrasound with a biplane transrectal probe and CEUS can be one of the first choices for the diagnosis and follow-up of DAM patients.

CONCLUSION

In conclusion, pelvic DAM is a rare tumor. In our case, the typical laminated appearance of DAM could be observed by ultrasound with a biplane transrectal probe and CEUS. Moreover, CNB of the enhanced area inside the tumor could be accurately guided by transrectal CEUS, enabling the pathological gold standard for the diagnosis of DAM; thus, this modality is expected to be one of the preferred methods for the diagnosis of DAM in the pelvic cavity.

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FOOTNOTES

Author contributions: Zhang Q and Luo Y contributed to the study conceptualization; Luo Y and Lu Q supervised the study; Zhang Q and Yan HL contributed to data collection and manuscript drafting; Yan HL and Lu Q contributed to manuscript revision; and all authors have approved the final manuscript.

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REFERENCES

1 **Steeper TA**, Rosai J. Aggressive angiomyxoma of the female pelvis and perineum. Report of nine cases of a distinctive type of gynecologic soft-tissue neoplasm. *Am J Surg Pathol* 1983; **7**: 463-475 [PMID: 6684403 DOI: 10.1097/00000478-198307000-00009]
2 **Gay F**, Champigneulle J, Tortuyaux JM, Cuny T, Régent D, Laurent-Croisé V. Aggressive angiomyxoma. *Diagn Interv Imaging* 2013; **94**: 657-661 [PMID: 23453353 DOI: 10.1016/j.diii.2013.01.009]
3 **Giraudmaillet T**, Mokrane FZ, Delchier-Bellec MC, Motton S, Cron C, Rousseau H. Aggressive angiomyxoma of the pelvis with inferior vena cava involvement: MR imaging features. *Diagn Interv Imaging* 2015; **96**: 111-114 [PMID: 25533499 DOI: 10.1016/j.diii.2014.03.006]
4 **Song M**, Glasgow M, Murugan P, Rivard C. Aggressive Angiomyxoma of the Vulva and Bladder. *Obstet Gynecol* 2017; **130**: 885-888 [PMID: 28885430 DOI: 10.1097/AOG.0000000000002254]
5 **Outwater EK**, Marchetto BE, Wagner BJ, Siegelman ES. Aggressive angiomyxoma: findings on CT and MR imaging. *AJR Am J Roentgenol* 1999; **172**: 435-438 [PMID: 9930798 DOI: 10.2214/ajr.172.2.9930798]
6 **Surabhi VR**, Garg N, Frumovitz M, Bhosale P, Prasad SR, Meis JM. Aggressive angiomyxomas: a comprehensive imaging review with clinical and histopathologic correlation. *AJR Am J Roentgenol* 2014; **202**: 1171-1178 [PMID: 24848813 DOI: 10.2214/AJR.13.11668]
7 **Jeyadevan NN**, Sohaib SA, Thomas JM, Jeyarajah A, Shepherd JH, Fisher C. Imaging features of aggressive angiomyxoma. *Clin Radiol* 2003; **58**: 157-162 [PMID: 12623047 DOI: 10.1053/crad.2002.1127]
8 **Srinivasan S**, Krishnan V, Ali SZ, Chidambaranathan N. "Swirl sign" of aggressive angiomyxoma-a lesser known diagnostic sign. *Clin Imaging* 2014; **38**: 751-754 [PMID: 24852676 DOI: 10.1016/j.clinimag.2014.04.001]
9 **Tariq R**, Hasnain S, Siddiqui MT, Ahmed R. Aggressive angiomyxoma: swirled configuration on ultrasound and MR imaging. *J Pak Med Assoc* 2014; **64**: 345-348 [PMID: 24864615]
10 **Kumar N**, Goyal A, Manchanda S, Sharma R, Kumar A, Bansal VK. Aggressive pelvic angiomyxoma and its mimics: can imaging be the guiding light? *Br J Radiol* 2020; **93**: 20200255 [PMID: 32401545 DOI: 10.1259/bjr.20200255]

11 **Bai HM**, Yang JX, Huang HF, Cao DY, Chen J, Yang N, Lang JH, Shen K. Individualized managing strategies of aggressive angiomyxoma of female genital tract and pelvis. *Eur J Surg Oncol* 2013; **39**: 1101-1108 [PMID: 23899874 DOI: 10.1016/j.ejso.2013.06.013]

12 **Smith HO**, Worrell RV, Smith AY, Dorin MH, Rosenberg RD, Bartow SA. Aggressive angiomyxoma of the female pelvis and perineum: review of the literature. *Gynecol Oncol* 1991; **42**: 79-85 [PMID: 1916515 DOI: 10.1016/0090-8258(91)90235-w]

13 **Zou R**, Xu H, Shi Y, Wang J, Wang S, Zhu L. Retrospective analysis of clinicopathological features and prognosis for aggressive angiomyxoma of 27 cases in a tertiary center: a 14-year survey and related literature review. *Arch Gynecol Obstet* 2020; **302**: 219-229 [PMID: 32430760 DOI: 10.1007/s00404-020-05592-5]

14 **Yang X**, Zhang L, Zhao W, Zhang Y, Yu J. Invasive angiomyxoma diagnosed by transvaginal ultrasound: a case report. *Ann Palliat Med* 2021; **10**: 5870-5874 [PMID: 33040540 DOI: 10.21037/apm-20-321]

15 **Zhao CY**, Su N, Jiang YX, Yang M. Application of ultrasound in aggressive angiomyxoma: Eight case reports and review of literature. *World J Clin Cases* 2018; **6**: 811-819 [PMID: 30510948 DOI: 10.12998/wjcc.v6.i14.811]

16 **Hajjar R**, Alharthi M, Richard C, Gougeon F, Loungnarath R. Pelvic Aggressive Angiomyxoma: Major Challenges in Diagnosis and Treatment. *Cureus* 2019; **11**: e4419 [PMID: 31245206 DOI: 10.7759/cureus.4419]

17 **Huang CC**, Sheu CY, Chen TY, Yang YC. Aggressive angiomyxoma: a small palpable vulvar lesion with a huge mass in the pelvis. *J Low Genit Tract Dis* 2013; **17**: 75-78 [PMID: 23222052 DOI: 10.1097/LGT.0b013e3182507df8]

18 **Sun J**, Lian PH, Ye ZX, Dong X, Ji ZG, Wen J, Li HZ. Aggressive Angiomyxoma in the Scrotum: A Case Series and Literature Review. *Front Surg* 2022; **9**: 762212 [PMID: 35310439 DOI: 10.3389/fsurg.2022.762212]

19 **Ota H**, Otsuki K, Ichihara M, Ishikawa T, Okai T. A case of aggressive angiomyxoma of the vulva. *J Med Ultrason (2001)* 2013; **40**: 283-287 [PMID: 27277251 DOI: 10.1007/s10396-012-0427-y]

20 **Wenig BM**, Vinh TN, Smirniotopoulos JG, Fowler CB, Houston GD, Heffner DK. Aggressive psammomatoid ossifying fibromas of the sinonasal region: a clinicopathologic study of a distinct group of fibro-osseous lesions. *Cancer* 1995; **76**: 1155-1165 [PMID: 8630892 DOI: 10.1002/1097-0142(19951001)76:7<1155::aid-cncr2820760710>3.0.co;2-p]

21 **Granter SR**, Nucci MR, Fletcher CD. Aggressive angiomyxoma: reappraisal of its relationship to angiomyofibroblastoma in a series of 16 cases. *Histopathology* 1997; **30**: 3-10 [PMID: 9023551 DOI: 10.1046/j.1365-2559.1997.d01-556.x]

22 **Zhang Q**, Zhao JY, Zhuang H, Lu CY, Yao J, Luo Y, Yu YY. Transperineal core-needle biopsy of a rectal subepithelial lesion guided by endorectal ultrasound after contrast-enhanced ultrasound: A case report. *World J Gastroenterol* 2021; **27**: 1354-1361 [PMID: 33833488 DOI: 10.3748/wjg.v27.i13.1354]

23 **Nuernberg D**, Saftoiu A, Barreiros AP, Burmester E, Ivan ET, Clevert DA, Dietrich CF, Gilja OH, Lorentzen T, Maconi G, Mihmanli I, Nolsoe CP, Pfeffer F, Rafaelsen SR, Sparchez Z, Vilmann P, Waage JER. EFSUMB Recommendations for Gastrointestinal Ultrasound Part 3: Endorectal, Endoanal and Perineal Ultrasound. *Ultrasound Int Open* 2019; **5**: E34-E51 [PMID: 30729231 DOI: 10.1055/a-0825-6708]

24 **Draeger DL**, Protzel C, Hakenberg OW. Aggressive Angiomyxoma as a Rare Differential Diagnosis of Enlargement of the Scrotum. *Clin Genitourin Cancer* 2016; **14**: e237-e239 [PMID: 26774350 DOI: 10.1016/j.clgc.2015.12.022]

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