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*REVIEW*

# Salvage locoregional therapies for recurrent hepatocellular carcinoma

Cody R Criss, Mina S Makary

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## Abstract

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death worldwide. Despite the advent of screening efforts and algorithms to stratify patients into appropriate treatment strategies, recurrence rates remain high. In contrast to first-line treatment for HCC, which relies on several factors, including clinical staging, tumor burden, and liver function, there is no consensus or general treatment recommendations for recurrent HCC (R-HCC). Locoregional therapies include a spectrum of minimally invasive liver-directed treatments which can be used as either curative or neoadjuvant therapy for HCC. Herein, we provide a comprehensive review of recent evidence using salvage loco-regional therapies for R-HCC after failed curative-intent.

**Key Words:** Recurrent hepatocellular carcinoma; Locoregional therapy; Transarterial chemoembolization; Transarterial embolization; Transarterial radioembolization; Ablation; Salvage therapy



**Core Tip:** Management of recurrent hepatocellular carcinoma (R-HCC) includes surgical resection, systemic treatment, or locoregional therapies including ablation, transarterial chemoembolization, or radioembolization, and stereotactic body radiation therapy. In the setting of recurrence, locoregional therapies offer unique advantages over surgery for select patients. Recent investigations have also highlighted the potential of combining locoregional therapies or adding systemic retreatments for R-HCC.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 75%-90% of liver malignancies and is the second most common cause of cancer death worldwide[1-3]. While advancements in surveillance efforts have improved prevention and screening, incidence and mortality of HCC in recent decades have gradually increased in the United States[4,5]. Prevalence is increased in East Asia and Africa, and at-risk populations include those with cirrhosis and hepatitis B or C[4,5].

Treatment strategies for patients with HCC are tailored to tumor burden, invasiveness, and liver function, stratified using the Barcelona Clinic Liver Cancer staging (BCLC)[6]. First-line and curative treatment for HCC includes surgical resection or orthotopic liver transplantation with eligibility determined *via* the Milan criteria[5,7]. In patients with early-stage HCC who are not eligible for liver transplantation, surgical resection may be performed[6]. In patients who do not qualify as surgical candidates, the use of locoregional therapies using image-guided techniques has grown in popularity over the last several decades, providing a minimally invasive treatment approach to HCC[8,9]. Locoregional therapy is comprised of radiofrequency ablation (RFA)/ thermal microwave ablation (MVA), transarterial chemoembolization (TACE), or radioembolization (TARE), which have been commonly used neo-adjunctively to bridge or downstage patients with HCC in order to meet surgical eligibility (Figure 1)[10]. Ablation, in particular, offers a curative-intent option for nonsurgical candidates with early-stage HCC (BCLC 0/A) with a corresponding 5-year survival rate of 50%-80%[11]. Locoregional therapies provide an alternative strategy with the benefit of reduced comorbidity[12], and avoidance of complications that may worsen clinical outcomes associated with traditional surgery[13,14].

Long-term prognosis for the treatment of HCC remains poor, with a recurrence rate of 41%-70% within 5 years following resection[15-18]. Depending on tumor size, severity, liver function, and clinical indices, repeat hepatectomy may not be suitable for some patients. Therefore, alternative treatment options should be explored after initial curative attempts. No definitive consensus on standard salvage treatment approaches exist for recurrent HCC, but common therapies include repeat resection, liver transplantation, tyrosine kinase inhibitors, locoregional therapies, or a combination of multiple modalities[19]. This manuscript provides a comprehensive review of the current state of the literature for the use of salvage loco-regional therapies for recurrent HCC (R-HCC).

### *Risk Factors for Recurrent HCC*

Prognostic factors associated with the increased risk of recurrence can vary from morphologic and surgical factors to molecular factors[20,21]. Larger tumors, or nodules with diameters ≥ 5 cm, are associated with increased rates of recurrence. Other morphological risk factors include the presence of multiple tumor nodules and satellite lesions[21-23]. The association between tumor size and recurrence is due to its correlation with invasiveness and propensity for portal vein-mediated intrahepatic metastasis and vascular invasion[21,24-26]. Microvascular invasion is a poor prognostic factor for R-HCC[27-29], defined as the histopathological observance of malignant cells within hepatic tissue and vascular cavities of the surrounding portal or hepatic vessels[30]. Other tumor-related factors associated with risk of recurrence after resection or liver transplantation, such as alpha-fetoprotein levels > 400 ug/L[31,32]. Overexpression of other histological and circulating biomarkers are also associated with negative prognostic factors related to recurrence[33].

## SALVAGE LOCOREGIONAL THERAPY FOR RECURRENT HCC

Salvage locoregional therapy for R-HCC is frequently used after resection or in the setting of advanced, unresectable disease[8,9,33]. Compared to locoregional therapy or resection, liver transplantation carries a superior survival benefit for R-HCC[34-37]. However, the utility of transplantation is limited due to strict inclusion criteria, donor availability, high treatment costs, and surgical candidacy[9]. In patients who do not meet Milan criteria or not eligible for transplantation, the decision between locoregional therapies such as ablation, or repeated resection remains controversial. While resection is recognized as a primary treatment for HCC[6], portal hypertension, poor functional reserve from the future liver remnant, and technical difficulties (*e.g.*, adhesions, anatomy modifications) can make repeat resection challenging and risky[38,39]. Therefore, the efficacy of alternative methods may be uniquely promising for R-HCC. The following section includes an overview of specific locoregional therapy modalities and their efficacy for R-HCC.

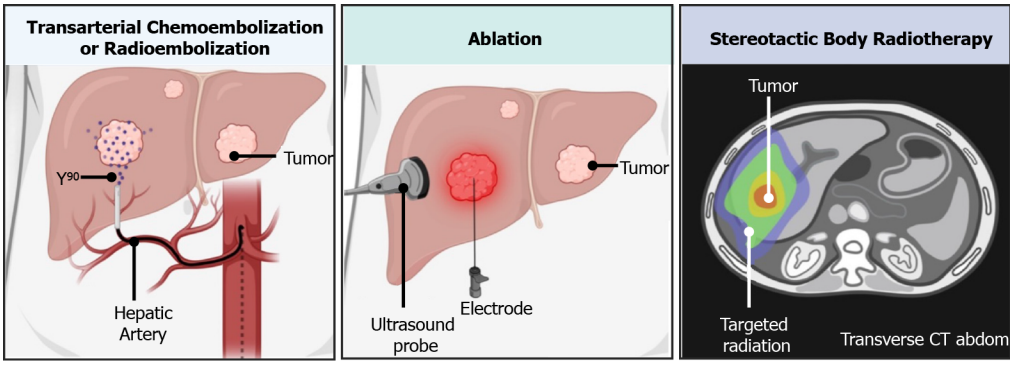


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**Figure 1 Schematic depiction of locoregional therapies.** CT: Computed tomography.

### *TACE*

The liver parenchyma utilizes a dual blood supply with approximately two-thirds of originating from the portal vein and the remaining third from the hepatic artery. Transarterial embolization (TAE) involves selective angiographic occlusion of tumor-supplying vessels from the hepatic artery resulting in tumor ischemia and necrosis[9,40]. Similarly, TACE involves the use of embolizing microparticles combined with regional chemotherapy[9]. Several variations of TACE exist, but embolization is commonly completed using gelatin sponge particles, polyvinyl alcohol particles, or spherical embolic agents[41]. Of note, conventional TACE utilizes a chemotherapeutic agent emulsed with lipiodol, whereas the use of drug-eluting beads carry the added benefit of increased concentration to the target[9,42,43]. Damage to healthy liver parenchyma is spared *via* arterial supply from the unobstructed portal vein[9,44].

TACE can be used as a bridge to transplantation and is currently a first-line multinodular HCC and intermediate-stage disease (BCLC B)[6,9]. It is also reserved for early-stage disease (BCLC A) who do not meet surgical criteria[9]. TACE after resection is particularly beneficial to patients with poor prognostic factors such as microvascular invasion[45-48]. Similar to primary HCC, TACE for R-HCC is tolerable and an optimal therapeutic modality for patients with poor liver function or multifocal HCC [49-51]. Two recent meta-analyses found adjuvant TACE improved overall survival (hazard ratio: 0.64-0.71)[46,52] and disease free survival (hazard ratio: 0.73)[52]. Overall 1- and 3-year survival rates for TACE for R-HCC are reportedly 28%-82% and 32%-43.9%, respectively[50,53]. Meta-analysis has reported 5-year survival rates for TACE to be 15.5%[54]. Poorer outcomes and prognosis in patients treated with TACE for R-HCC are multiple sessions, tumor size > 5 cm and ≥ 2 lesions[50]. TACE offers a unique benefit in the presence of microvascular invasion or multifocal disease but studies to date have been largely retrospective and a need for randomized control trials is required before clinical considerations are definitive. A prospective investigation of 629 patients found worse outcomes in patients treated with TACE ($n$ = 339), compared to radiofrequency ablation ($n$ = 162), and re-hepatectomy ($n$ = 128)[49]. Yet, a meta-analysis of seven studies including patients with R-HCC reported no overall survival differences between TACE ($n$ = 807) and repeated resection ($n$ = 267). Therefore, TACE appears to be an effective treatment option for R-HCC, with a preferential advantage to patients with morphological factors such as multiple tumors or disease complicated by microvascular invasion[33].

### *TARE*

TARE is a local radiation therapy also referred to as selective internal radiotherapy, whereby Yttrium-90 Labeled microspheres are delivered through the hepatic arteries to the tumor[55,56]. Yttrium-90 is a β-emitter, and has a tumoricidal effect at a sufficient dosage of 400Gy or greater[11]. Similar to TACE, radioembolization is used as a neoadjuvant treatment for downstaging and bridging patients for transplantation or resection[57] and considered a curative approach for early-HCC or BCLC 0/A[58]. TARE has become increasingly popular over the last decade as a safe and tolerable procedure for HCC [59], with shorter hospital length of stay and decreased risk of post-embolization syndrome when compared to TACE[60-62]. Additionally, TARE carries less risk for portal vein tumor thrombosis[63]. Recently, TARE has been adopted within the BCLC algorithm as a second-line treatment for early-stage HCC[11,64]. This change is primarily driven by the LEGACY (Local radioembolization using Glass Microspheres for the Assessment of Tumor Control with Y-90) study, which found radioembolization > 400 Gy to be safe and an effective curative approach for patients with nodules less than 8 cm[65].

For R-HCC, there is a scarcity of investigations determining the utility of TARE after failed curative-intent. Meta-analyses have shown similar outcomes between TACE and TARE for unresectable HCC [66]. It is also important to note that a randomized control trial by Salem *et al*[65] found better tumor control outcomes in patients with HCC BCLC stages A/B treated with TARE as opposed to TACE (time to progression: > 26 mo; 6.8 mo, respectively). Sangro *et al*[67] reported no differences in adverse events

in patients receiving TARE with prior failed curative-intent treatments (surgical or non-surgical) compared to treatment naïve patients receiving TARE. A retrospective investigation of 41 patients reported a time to progression of 11.3 mo and overall survival of 22.1 mo patients receiving TARE after prior resection[68]. Due to the advantages of TARE listed above, it has been advocated for advanced, unresectable disease[33,69]. More data is needed to determine the efficacy and optimal patient-selection strategies of radioembolization in the context of R-HCC.

### Ablation

Ablation involves using a probe placed percutaneously under image guidance into the tumor to induce necrosis *via* thermal energy[11,70]. Ablation consists of either RFA or MVA. RFA is moderated by the "heat sink effect" which can negatively impact tumor response. Blood flow from nearby tissue can dissipate heat transfer and result in a cooling effect[71]. MVA is less impacted by heat sink due to the use of higher temperatures and larger, homogenous ablation zone, but at a cost of increased risk of injury to adjacent structures[72-75]. For both types of ablation, tumor location efficacy can be impacted by location, where tumors abutting nearby structures like the gallbladder, bowel, and diaphragm can be injured or result in insufficient safety margins that leave residual tumor[76]. Ablation is considered a curative treatment for early-stage HCC (BCLC 0/A)[6,11]. A major advantage of ablation is it can be performed quicker and may be more feasible than surgery with the added benefit of fewer complications and faster recovery[77].

A retrospective review of 211 patients with R-HCC found the 1-year survival rate for locoregional therapy (RFA, TAE, and/or percutaneous ethanol injection; $n$ = 170, 91.6%) to be greater than salvage liver transplantation ($n$ = 41, 90.2%)[37]. However, survival rates became superior in salvage liver transplantations at 3- and 5-years (80.4, and 80.4%, respectively) relative to the locoregional therapy group (71.7, and 51.1%, respectively)[37]. A meta-analysis of retrospective investigations by Chen *et al* [78] found improved clinical outcomes for 3- and 5-year survival rates in repeated hepatectomy compared to RFA for R-HCC. Therefore, repeated hepatectomy carries improved long-term efficacy, although the authors acknowledge selection bias may confound these results since a higher proportion of patients with improved liver function and limited tumor spread may be candidates for surgery. A meta-analysis of randomized control trials and observational studies by Yuan *et al*[79] found similar survival rates between ablation (MVI or RFA) compared to re-resection, but lower perioperative morbidity rates were observed in patients undergoing ablation (3.3%) relative to re-resection (17%). The majority of these studies included tumors ≤ 3 cm, and therefore the decision to utilize ablation over surgery for R-HCC may be appropriate for smaller tumors[80]. In tumors ≤ 3 cm, disease-free survival rates are similar to resection, but hospital length of stay and perioperative morbidity is lower in RFA (5 d, 7%, respectively) compared to repeated resection (13 d, 16%, respectively)[81]. Yang *et al*[82] echoed these findings, illustrating repeat resection for R-HCC has superior overall survival rates, but sub-group analyses of outcomes for smaller tumors diminish survival differences between these two methods. Larger, more homogenous ablation volumes associated with MVA may broaden ablation applicability to larger tumors[83]; however, studies to date evaluating MVA for R-HCC are limited.

### Stereotactic body radiotherapy

Stereotactic body radiotherapy (SBRT) is a localized therapy whereby fractionated high-dose radiation is used to ablate liver parenchymal tumors (Figure 1)[84]. Conventionally, SBRT is dedicated to salvage therapy for R-HCC or advanced disease when ablation or embolization has failed or is contraindicated [85]. SBRT is currently not included in the BCLC but is included in the National Comprehensive Cancer Guidelines[84]. Kimura *et al*[86] reviewed patients with HCC who either failed or were not eligible for resection or other locoregional therapies, reporting safe and satisfactory overall survival rates for first and second SBRT ($n$ = 81, 60.4%, and 61%, respectively). In patients receiving salvage SBRT after TACE, overall survival rates at 3 years were 72.7% ($n$ = 302), with 95.4% tumors reaching complete response [87]. Therefore, in patients who fail TACE and curative modalities are not suitable, salvage SBRT could be offered as a potential subsequent treatment option.

### Multimodal Locoregional Therapy Approaches

Approaches that combine locoregional therapies (*e.g.*, TACE and RFA/MVA) have been proposed. Several mechanisms have been suggested to explain the synergistic or additive effects of combining modalities. Multimodality therapies may overcome individual limitations of monotherapy, such as providing adequate control for intermediate to larger tumors[72,88-90]. TACE is suggested to mitigate the heat sink effect and therefore, positively impact the efficacy of RFA[71]. Chemoembolization may also reduce tumor burden, which can aid RFA by extending the safety margin and the resultant coagulation zone[90,91]. A meta-analysis of 8 randomized control trials using RFA-TACE for primary HCC found improved overall survival [hazard ratio (HR) = 0.58, confidence interval (CI) 0.41 - 0.80] and recurrence free survival (HR = 0.65 CI =0.47 - 0.76) compared to RFA alone.

To date, few investigations have sought to determine the efficacy of multimodal therapy as a salvage treatment approach in unresectable disease or instances of R-HCC. For the treatment of larger R-HCC tumors (≤ 7 cm), TACE followed by RFA can reveal additional satellite lesions and have greater 1-, 3-, 4-

year survival rates (92.6%, 66.6%, 61.8%) than RFA alone (85.3%, 59%, 45%)[92,93]. Studies comparing the efficacy of TACE-RFA have indicated comparable 1-, 3-, and 5-year survival outcomes between the two salvage treatment approaches for both smaller tumors (≤ 5 cm) [94,95] and larger ones (> 5 cm)[96]. Interestingly, TACE-RFA achieved satisfactory outcomes with a lower rate of complications (*e.g.*, bleeding, liver failure) and shorter hospital stays[94-96]. Yang *et al*[97] published a retrospective investigation of 103 patients with R-HCC treated with either RFA, TACE, or combination therapy of RFA and TACE. Intrahepatic rates of recurrence were lower in the combination group (20.7%) compared to TACE (57.1%) and the RFA group (43.2%). 1-, 3- and 5-year survival rates were also greater in the combination group (88.5%, 64.6%, 44.3%) compared to the TACE alone group (65.8%, 38.9%, 19.5%). Other multimodal regimens for R-HCC have been explored, including TACE and MVA, of which when combined, improve tumor response and prolong progression-free survival compared to TACE monotherapy for small R-HCC tumors (≤ 3 cm)[98]. Although prospective investigations are required prior to establishing recommendations, in general, current evidence indicates a potential survival benefit to multimodality approaches with some investigators advocating for the adoption of multimodal therapy in future BCLC treatment guidelines[99].

### *Combining Locoregional Therapy and Systemic Therapy*

Sorafenib, an oral tyrosine kinase inhibitor, is reserved for advanced-stage disease (BCLC class C) based on the results of the SHARP trial[6]. Overall, Sorafenib can offer survival benefit for unresectable HCC, but worse tumor response and greater adverse events when compared to locoregional therapies[100, 101]. Challenges of using sorafenib are further compounded by heterogenous response rates and acquired resistance[102-104]. However, investigations have explored the utility of combining oral systemic agents with locoregional therapy (Table 1). A retrospective study reviewed 1126 patients with R-HCC in patients who received sorafenib and concurrent TACE or TACE monotherapy. The addition of sorafenib to TACE offered significantly improved survival time compared to TACE alone (20.23 *vs* 13.87 mo, respectively)[105]. Peng *et al*[106] retrospectively reviewed patients with advanced R-HCC receiving either sorafenib monotherapy ($n$ = 101), or a combination of sorafenib and TACE-RFA ($n$ = 106). While the toxicity profile was similar between both groups, median overall survival and time to progression in TACE-RFA + sorafenib (14 mo; 7 mo, respectively) was superior to sorafenib monotherapy (9 mo; 4 mo, respectively)[106]. A randomized, multicenter control trial comparing TACE ($n$ = 76 and TACE with sorafenib ($n$ = 80) for unresectable HCC, resection, found median progress-free survival to be greater in the combined treatment group (25.2 *vs* 13 mo)[107]. Although this trial included treatment naiive patients, a large portion of patients received prior locoregional therapy treatments Multicenter phase III randomized control trials comparing TACE alone and TACE with sorafenib for recurrent, unresectable HCC are currently underway.

## FUTURE DIRECTIONS

### *Immuno-locoregional combination therapy*

Immunological properties associated with HCC have driven a growing use of immune checkpoint modulators such as anti-PD-1 antibodies (*e.g.*, nivolumab, pembrolizumab, camrelizumab) or CTL-A-4 inhibitors (*e.g.*, ipilimumab, tremelimumab)[108-111] over the last decade. Thus far, phase 2 and 3 trials have found promising tumor response rates and safety profiles compared to previous standard systemic therapies[112]. In addition to tumor necrosis, there has been some evidence that locoregional therapy can activate T-cell responses and augment the expression of multiple immune-mediated processes within the tumor microenvironment[113]. Development of treatment strategies for HCC that combine locoregional therapies and immunomodulators have thus emerged. Despite this rise in utilization, Guo *et al*[109] found no difference in clinical outcomes or tumor response for combined TACE and camrelizumab compared to TACE monotherapy. Studies determining the efficacy of immunotherapy combined with locoregional therapy are scarce, but multiple trials combining immunomodulators and locoregional therapies are currently underway[114]. It should be noted, adverse events with immunocheckpoint blockers, such as hyperprogressive disease, have been reported and pose a unique challenge influencing clinical judgment to utilize these agents. Hyperprogressive disease is characterized by a rapid increase in tumor burden and subsequent clinical deterioration in patients treated with immunotherapy agents. Other immunotherapies benefits (*e.g.*, vaccines, oncolytic viruses and adoptive cellular therapies) have also been speculated to be therapeutic but remain under clinical investigation[111].

### *Determining treatment algorithms for recurrent HCC*

After the failure of curative-intent or tumor recurrence, the use of locoregional therapies is warranted, especially in patients no longer eligible for surgery. Ablation, however, should be considered as a comparable alternative to repeat-resection in patients with recurrent small solitary tumors, notably ≤ 3 cm. Similar to prior reviews, in patients with early recurrence (< 1 year), multifocal disease (> 2 - 3 nodules) or in the presence of microvascular invasion, TACE should be considered[33]. Moreover, due to lower toxicity and longer time-to-progression for advanced disease[62], the use of radioembolization

**Table 1 Outcomes of multimodal locoregional therapy for recurrent hepatocellular carcinoma**

| Ref. | Study design | Treatment | Number of patients | Outcomes |
|---|---|---|---|---|
| Song *et al* [95] | Retrospective | Recurrent HCC ≤ 5 cm | 63 TACE; 96 TACE-RFA | TACE-RFA lower disease progression than TACE monotherapy; No difference in overall survival |
| Zhang *et al* [115] | Retrospective | Treatment Naïve HCC, DEB-TACE-RFA for Recurrent HCC (Group B), and hepatectomy | 40 DEB-TACE as primary treatment; 36 DEB-TACE Recurrent HCC; 40 hepatectomy as primary | DEB-TACE-RFA can prolong survival time for recurrent HCC |
| Zheng *et al* [96] | Retrospective | TACE-RFA or repeat hepatectomy | 63 TACE-RFA; 38 repeat hepatectomy | Similar overall survival for TACE-RFA (38 months) compared to repeat hepatectomy (42 months); No difference in progression free survival |
| Peng *et al* [94] | Retrospective | Recurrent HCC ≤ 5 cmTACE-RFA or repeat hepatectomy | 107 TACE-RFA; 79 repeat hepatectomy | No difference in overall survival or disease-free survival; TACE-RFA has lower complications and shorter hospital stays |
| Ji *et al*[98] | Retrospective | Recurrent HCC with three or fewer tumors < 3 cm | 17 TACE-MWA; 28 TACE | TACE-MWA showed better 1-,3-, 6- month tumor response; TACE-MWA showed prolonged 1-,3-, 5-year progression free survival; No difference in overall survival |

HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; DEB-TACE: Drug-eluting bead transarterial chemoembolization; MWA: Microwave ablation. TACE-RFA: Transarterial chemoembolization and radiofrequency ablation; DEB-TACE-RFA: Drug-eluting bead transarterial chemoembolization and radiofrequency ablation; TACE-MWA: Transarterial chemoembolization and Microwave ablation.

offers a favorable alternative to TACE. Evidence supports that multimodal therapy provides superior clinical benefit to monotherapy as well as repeat-resection for smaller tumors (Table 1) for R-HCC. To date, it is unclear which additional patient populations (*e.g.*, those not currently suitable for locoregional monotherapy) may benefit from multimodal or strategies that combine locoregional and systemic therapy (Table 2).

## CONCLUSION

Treatment strategies for R-HCC remain a challenge, and there is no consensus on how to manage patients who fail curative-intent therapies. The use of targeted locoregional therapies can improve clinical outcomes after recurrence in patients not eligible for or awaiting transplantation, or in cases of advanced disease. The emerging use of multimodal and additive systemic agents exhibit promise as a novel treatment approach in the setting of recurrence; however, prospective studies are necessary before definitive recommendations can be made.

**Table 2 Locoregional therapy and oral agents for recurrent hepatocellular carcinoma**

| Ref. | Study Design | Treatment | Number of Patients | Outcomes |
|---|---|---|---|---|
| Wan *et al*[105] | Retrospective | Recurrent HCC ≤ 5 cm | 127 TACE; 127 Sorafenib + TACE | Sorafenib + TACE increased survival time compared to TACE alone (30.7 *vs* 18.22 mo); Longer duration of Sorafenib when treated with Sorafenib + TACE associated with survival |
| Peng *et al*[106] | Retrospective | Recurrent HCC ≤ 7 or five nodules ≤ 3 cm | 106 TACE-RFA + Sorafenib; 101 Sorafenib | Longer median overall survival and time to progression for combination therapy |
| Guo *et al*[109] | Retrospective | Recurrent HCC | 20 TACE+ camrel-izumab; 51 TACE | No difference in tumor response, progression-free survival, or overall survival |

HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; TACE-RFA: Transarterial chemoembolization and radiofrequency ablatio.

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*REVIEW*

# COVID-19 and hepatic injury: cellular and molecular mechanisms in diverse liver cells

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## Abstract

The coronavirus disease 2019 (COVID-19) represents a global health and economic challenge. Hepatic injuries have been approved to be associated with severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection. The viral tropism pattern of SARS-CoV-2 can induce hepatic injuries either by itself or by worsening the conditions of patients with hepatic diseases. Besides, other factors have been reported to play a crucial role in the pathological forms of hepatic injuries induced by SARS-CoV-2, including cytokine storm, hypoxia, endothelial cells, and even some treatments for COVID-19. On the other hand, several groups of people could be at risk of hepatic COVID-19 complications, such as pregnant women and neonates. The present review outlines and discusses the interplay between SARS-CoV-2 infection and hepatic injury, hepatic illness comorbidity, and risk factors. Besides, it is focused on the vaccination process and the role of developed vac-cines in preventing hepatic injuries due to SARS-CoV-2 infection.

**Key Words:** COVID-19; Hepatic injury; Viral tropism; COVID-19 comorbidity; Vaccination

**Core Tip:** The association between coronavirus disease-19 and hepatic injury is demonstrated by determining the viral tropism and its different pathological implications. A better understanding of the diversity and risk factors of severe acute respiratory syndrome coronavirus-2-induced hepatic injury provides a fundamental approach to overcoming adverse effects. Moreover, vaccination can influence assessment and evaluation.

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## INTRODUCTION

Coronavirus (CoV) is derived from the Latin word "corona," which means "crown"[1]. It can cause various human respiratory tract diseases, ranging from mild cold to severe respiratory distress syndrome (RDS)[2]. CoV has presented several challenges throughout its history, including viral isolation, detection, prevention, and vaccine development[3]. CoV is a member of the order *Nidovirales* and has the largest RNA genome[4]. Furthermore, it is recognized as arising from a zoonotic origin and frequently spreads by contact or respiratory droplets. The affected individual has non-specific clinical characteristics requiring virological diagnosis and molecular confirmation[5]. Seven coronaviruses have been recognized to infect humans, with SARS-CoV-2 being the most recent, and this might be due to frequent infections across different species and sporadic spillover episodes[4]. Two of these previously recognized coronaviruses are the Middle East Respiratory Syndrome CoV, which originated in the Middle East in 2012, and the Severe Acute Respiratory Syndrome CoV (SARS-CoV), which originated in China from 2002 to 2003 and was responsible for significant epidemics in the previous two decades[6]. The recent CoV illness, also known as coronavirus disease of 2019 (COVID-19), poses a risk to global health[7]. The COVID-19 pandemic began in the Chinese city of Wuhan near the end of December 2019 and spread rapidly in the following months to Thailand, Japan, South Korea, Singapore, and Iran[8]. This was followed by a viral outbreak worldwide, particularly in Spain, Italy, the United States of America, the United Arab Emirates (UAE), and the United Kingdom (UK). The COVID-19 disease is classified as a pandemic by the World Health Organization (WHO)[9]. The three types of coronaviruses are zoonotic, can infect people, and cause severe and fatal diseases[10]. New coronaviruses are expected to emerge and cause sporadic seasonal outbreaks due to their great genetic diversity, frequent genome recombination, and rise in human-animal interface activities brought on by contemporary agricultural methods[11].

## VIRAL TROPISM

In COVID-19, viral tropism is responsible for spreading infection outside the respiratory tract and predisposing it to systemic symptoms, aggravating pre-existing disorders, and multiorgan damage in the kidney, heart, nervous system, liver, and gastrointestinal tract[12,13]. However, the available data indicate the second multiorgan dysfunction inherent to the immune discrepancy or cytokine storm, developing hypoxic or ischemic injury and drug-induced injury[14,15]. Although viral tropism should be considered to understand the SARS-CoV-2 infection, the S protein of the virus mediates SARS-CoV-2 cell entrance, which represents a high affinity for cells expressing angiotensin-converting enzyme 2 receptors (ACE2)[16]. Furthermore, the affinity of the S protein to ACE2 receptors increases when SARS-CoV-2 is proteolytically activated[17]. In an *in vitro* study by Letko *et al*[18], the S protein of lineage B beta-coronaviruses such as SARS-CoV and the recent SARS-CoV-2 significantly improved its affinity for its receptor when it was pre-incubated with proteolytically activated trypsin. Trypsin is expressed by liver epithelial cells[19]. Additionally, the protein of the SARS-CoV-2 contains a furin-like proteolytic site that has never been observed in other coronaviruses[20]. It is worth mentioning that furin is expressed in organs such as the salivary glands, liver, kidney, and pancreas involved in SARS-CoV-2 infection[21]. As a result, to determine tropism for a particular tissue, ACE2 should be present at the host cell surface[22]. Consequently, ACE2 expression is considered a mirror of viral load[23]. Controversially, the highest levels of ACE2 are detected in the small intestine, testis, heart, colon, and thyroid gland[24]. Nevertheless, respiratory system symptoms are dominant in COVID-19 because the nasal ciliated cells are the primary targets for SARS-CoV-2 replication in the early stages of infection[25]. Besides, ACE2 is abundantly expressed in more than 80% of alveolar lung cells, consequently affecting all respiratory functions[23].

## DIAGNOSIS

With increasing COVID-19 prevalence and mortality rates, as of 14 August 2022, the WHO reported that over 587 million people were infected with SARS-COV-2, including over 6 million deaths[26]. Therefore, the nation's healthcare systems face overwhelming psychological and economic burdens. Consequently, the most efficient method to prevent infection is to separate symptomatic persons, quarantine others, and manage concomitants while increasing immunization rates.

The molecular test is the most practical method to confirm the diagnosis of COVID-19, using the reverse transcription polymerase chain reaction (RT-PCR) to detect viral genetic materials in different sample swabs from the nasal cavity, mouth, sputum, and feces[27,28]. This molecular test provides high sensitivity and specificity; however, it has several drawbacks, such as requiring trained technicians, being time-consuming, high cost, shortages in test kit supplies, and false negative thresholds[29]. Therefore, it is critical to develop new quick, reliable, and affordable diagnostic techniques.

Patients with fever, cough, and chest pain with breathing problems or pneumonia are usually diagnosed by imaging tests, such as chest X-ray or computed tomography (CT)[30]. Imaging tests are predominantly available worldwide, and the scanning process is relatively simple and rapid, enabling a large population's screening[31]. In a study based on chest X-ray findings and severity scores, a chest X-ray is a limited tool because it has an abnormality observed at a specific point[32]. In the same context, Borghesi A. and R. Maroldi mentioned that chest X-ray is an insensitive diagnostic tool for the early detection of lung abnormalities. In contrast, it is a valuable tool for monitoring (day after day) the rapid progression of lung abnormalities in infected patients, particularly in intensive care units[33]. Despite its limited sensitivity, the appearance of a local or bilateral patchy shadow infiltrating a chest X-ray is the most typical radiological presentation[34].

Currently, CT plays a pivotal role and is the main technique for diagnosing and following patients with COVID-19[35]. The CT finding is more sensitive than the chest x-ray, particularly in the initial assessment[32,36]. CT findings may be present early, even before the onset of the symptoms[36]. Additionally, Li Y. and L. Xia's comparative study reflected the low misdiagnosed rate of CT scans and detected positivity earlier than RT-PCR[37]. The most common chest CT findings included ground-glass opacity, ill-defined boundaries, smooth or uneven interlobular septal thickening, an air bronchogram, a crazy-paving pattern, and thickening of the nearby pleura[38]. Due to numerous drawbacks, chest CT has some restrictions; for instance, radiation exposure, overuse of health care resources, hygiene, or inability to get a CT scan, as in critically ill patients, or clinically unstable, as in the case of intensive care unit (ICU) admission[39]. As a result, other methods are required to define and monitor patients rapidly.

Moreover, clinical pathologists have a significant role in monitoring inflammatory markers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cells (WBCs). The most significant markers during SARS-COV-2 infection and highly associated with COVID-19 progression were lymphocytopenia, elevated CRP, and alternation in the ESR levels[40-42]. Data obtained from 452 patients with COVID-19 revealed that lymphocytopenia, high WBCs, a high neutrophil-lymphocyte ratio, and lower percentages of monocytes, eosinophils, and basophils were mainly observed in severe cases[43]. Similar findings were demonstrated by Henry *et al*[44] (2020) in their meta-analysis of 21 studies that included 3377 individuals who tested positive for COVID-19. They found that patients with severe and fatal diseases had more dramatic leukocytosis and lymphocytopenia and thrombocytopenia than mild to moderate diseased and survivor patients. The study by Mardani *et al*[41] (2022) attempted to explain the association between the inflammatory markers and COVID-19 progression and found that elevated CRP was correlated with the severity of COVID-19; furthermore, high ESR levels were observed in the severe cases only. Additionally, interleukin (IL)-7, IL-8, IL-9, IL-10, granulocyte-colony stimulating factor, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor A (VEGFA) were all found at high blood levels in COVID-19 patients[45].

In contrast, children show inconsistency and require further investigation. According to Del Valle *et al*[46] (2022) children with SARS-CoV-2-associated community-acquired pneumonia have low CRP levels. Additionally, a systematic review by Patel NA (2020) describes 2914 pediatric patients with COVID-19, the lab results for these children indicate stable WBC, lymphocyte count, and CRP levels[47]. Even though pneumonia causes an elevated CRP level, pneumonia with COVID-19 causes a drastic increase in CRP. This was revealed in a retrospective comparative study by analysis of the laboratory markers among children affected with pneumonia in the presence or absence of SARS-CoV-2 infection[48]. A meta-analysis study covers 20 eligible studies to identify the laboratory abnormalities among 1810 pediatric patients including Leukopenia, lymphopenia and elevated CRP[49]. Furthermore, the major conclusion of a retrospective cohort study by Graff *et al*[50] (2021), which included 454 patients, was that elevated CRP is a predictor of severe COVID-19 in children. All the previous studies show defects in the number of people involved in the studies. Hence, we recommend further investigation into many children.

Numerous investigations have demonstrated that liver damage occurred in SARS patients. This damage primarily took the form of mild to moderate elevations of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) in the early stages of the illness. Some individuals' blood albumin

levels dropped as their bilirubin levels increased[51]. Compared to moderate cases, patients were more likely to have severe hepatic damage[52]. According to recent investigations into COVID-19, liver damage can occur in between 14.8% and 53% of cases, with aberrant ALT/AST values and slightly increased bilirubin levels serving as the significant indicators[53]. Severe cases reduced albumin (26.3-30.9 g/L)[54]. In recent research, including 1100 Chinese patients, Guan *et al*[34] found that 56% of patients with a severe COVID-19 infection and about 18% of patients with a non-severe COVID-19 disease had increased blood AST levels. Additionally, it was shown that patients with a non-severe COVID-19 illness accounted for 20% of patients with increased blood levels of ALT. In contrast, patients with severe COVID disease constituted 28% of patients. In COVID-19 fatality cases, liver damage occurred between 58.06% and 78% of the time[55]. A study showed that a patient with severe COVID-19 had blood ALT and AST values of 7590 and 1445 U/L, respectively[54].

## RISK FACTORS

Intriguingly, lifestyle characteristics such as smoking, a high body mass index (BMI), male gender, postmenopausal status, and higher age in females were cited as the most significant risk factors for SARS-CoV-2 infection, regardless of comorbidities[56-58]. According to some studies, the age for an elevated risk is > 64 or > 65 years old. With six records, hypertension[59] and diabetes[60] are the most prevalent pre-existing comorbidities, followed by cardiovascular disease with three records. On rare occasions, associations were found between severity and TB, chronic renal illness, chronic obstructive pulmonary disease, or cerebrovascular disease. Significant effects on disease severity were reported for eight comorbidities that emerged because of COVID-19 infection[61]. Among them are organ failure, immune dysfunction, acute liver damage, hypoproteinemia, acute RDS, severe pneumonia, an uncontrolled inflammatory response, and hypercoagulable conditions[58,61].

Because their host defenses are compromised, patients with pre-existing liver diseases, such as cirrhosis of the liver and hepatocellular carcinoma (HCC), are more susceptible to infections and sepsis in general. Chronic liver diseases (CLDs) were present in 0.6% to 1.4% of hospitalized COVID-19 patients[62,63]. These individuals were more likely to experience severe illness (up to 60%) and increased death (up to 18%)[64]. Additionally, SARS-CoV-2 infection worsened the clinical prognosis and exacerbated liver damage in persons with CLDs resulting in decompensation in 20% of cirrhotic patients and worsening the clinical outcomes of people who were unstable[65].

The relationship between metabolically associated fatty liver disease (NAFLD) and COVID-19, among instances of chronic liver disorders and COVID-19, has received full attention. According to two investigations by Qian *et al*[66] and Ji *et al*[67], Patients with NAFLD have a longer viral shedding period and are more likely to have abnormal liver functions from the time of admission until discharge. Moreover, other investigations reported the same findings, with more significant mortality in patients with NAFLD, obesity, and those over 60 years old[68].

Additionally, the chance of rapid SARS-CoV-2 infection and developing COVID-19 complications appear with immunomodulatory and immunosuppressive drugs mainly used in autoimmune liver diseases. Therefore, patients with autoimmune hepatitis receiving immunosuppressive therapy should be viewed as having a high risk of developing severe COVID-19[69]. In contrast, the incidence of SARS-CoV-2 infection in patients with autoimmune hepatitis was like the general population, and the prevalence of severe COVID-19 was low[70]. Hence, we recommended further studies on patients with autoimmune hepatitis receiving immunosuppressive therapy.

Finally, according to preliminary findings on coinfection with SARS-CoV-2 and other viruses, it seems to cause severe progression, poor outcomes, or vial reactivation as in the case of the hepatitis C virus (HCV) and Hepatitis B virus (HBV) coinfection[71-73].

## CLINICAL CHARACTERISTICS OF LIVER INJURY IN COVID-19

Recent research shows that the frequent symptoms of fever and cough coincide with the beginning of COVID-19 infection. Other clinical characteristics, such as diarrhea, nausea, vomiting, and lack of appetite, represent at least a digestive system symptom[34]. CoV infection has been linked to liver damage in SARS and Middle East respiratory disease patients[74]. In cases with COVID-19, abnormal liver function was observed, shown as isolated elevations in blood transaminase and lactate dehydrogenase (LDH) levels[75]. Alkaline phosphatase (ALP), LDH, ALT, AST, and prothrombin time levels gradually increased during the hospitalization of the first COVID-19 case in the United States[76]. According to a study from Jin Yin-tan Hospital, out of the 99 patients with COVID-19, 43 had ALT or AST levels above the normal range, 75 had elevated LDH levels, and one had a severe disruption in liver function[54]. With 3.75% of all cases in Jiangsu province being imported and cases outside Wuhan, liver damage was said to be less common in these patients[77]. In an analysis of liver function among patients outside intensive care units, males were more likely to experience liver impairment than females[78]. In pediatric instances, liver damage was discovered in 22% of kids, most often between 2

and 18 d after admission[79]. In Wuhan, liver injury is a common factor among patients who are admitted to the ICU and non-survivors hospitalized patients. This reflects the relationship between liver injury and the severity of COVID-19[80]. Fifty-two patients who required mechanical breathing or had at least 60% inspired oxygen been included in a study of critically ill individuals. Twenty-nine percent of patients with critical conditions had liver damage. Fifteen percent had acute renal disease, and fifteen percent had cardiac injury[81]. In a multicenter study involving 1099 patients and 552 hospitals, abnormal liver function was generally detected in critically ill participants, whereas jaundice was less frequently observed in COVID-19 patients. In harmony with the elevation of total bilirubin levels in 10% of patients, the percentage was increased in severe cases up to 20.5%[34]. Furthermore, a multicenter retrospective cohort study including 5771 patients in Hubei province suggested that upregulation in liver injury markers, particularly AST, is closely correlated with the probability of death during COVID-19[75]. Therefore, the dynamic patterns of liver injury markers and their putative risk variables may provide a significant explanation for the liver damage linked to COVID-19. Additionally, all studies indicated that liver injury parameters should be monitored during hospitalization.

## POSSIBLE MECHANISMS OF COVID-19-ASSOCIATED LIVER INJURY

### *SARS-CoV-2 tropism and liver injury*

ACE2 expression aroused the curiosity of researchers and scientists due to unusual ACE2 hepatic distribution and unexpected outcomes. Chai and colleagues assumed the hepatic abnormalities during COVID-19 were ascribed to cholangiocytes dysfunction, not due to hepatocytes damage (Figure 1). Their investigation using single-cell RNA-seq revealed that the primary target for SARS-CoV-2 in the liver was cholangiocytes. The ACE2 expression in hepatocytes is 20 times less than observed in cholangiocytes. Despite this, clinical data from COVID-19 patients showed rising ALT, AST, and LDH levels, while ALP and gamma-glutamyl transferase, which describe bile duct injury, did not significantly increase[82]. At the same time, histological and immunohistochemistry assessments of Kupffer cells and T and B lymphocytes did not express ACE2[83], even though COVID-19-infected patients' livers frequently showed Kupffer cell activation and proliferation[84,85]. Additionally, systemic inflammation typically results in Kupffer cell activation and proliferation[84]. Although Kupffer cells do not express, ACE2 may have a crucial role in the propagation of inflammation that results in SARS-CoV-2-mediated liver damage. It is noteworthy that prediction of SARS-CoV-2 consecutive signaling, and outcome is challenging because the expression of ACE2 level is regulated by many factors and conditions, for example, liver fibrosis, liver cirrhosis, hypertension, diabetes, chronic pulmonary diseases, hypoxia, old age, and smoking, which represent factors for COVID-19[17,86,87].

SARS-CoV-2 uses ACE2 receptors to invade host cells and utilizes other molecules to facilitate infection, such as furin, transmembrane serine protease 11A (TMPRSS11a), and neuropilin-1[88,89].

Neuropilin-1 is embedded in the liver, causing physiological and pathological conditions. Activation of the neuropilin-1 cascade triggers angiogenesis process *via* controlling cell proliferation, cell survival, and cell migration[90]. Regardless of the cause of hepatic injury and conditions resulting from a viral infection, the elevation of neuropilin-1 is the defense mechanism. Consequently, neuropilin-1 may influence liver damage induced by SARS-CoV-2[89]. Neuropilin-1 has been reported to be found and expressed in liver sinusoidal endothelial cells and hepatic stellate cells[91]. Meanwhile, hepatic stellate cells' activation is postulated to be the primary cause of liver disease and fibrosis[92,93]. In different conditions, the hepatic stellate increases proinflammatory and profibrotic cytokines[94]. One of those cytokines is IL-6, produced when SARS-CoV-2 activates the immune system in COVID-19 patients and is associated with altered liver enzyme levels[95]. Therefore, propagation of neuropilin-1 expression with activation of hepatic stellate cells promotes signaling transcription and stimulates the release of growth factors such as transforming growth factor -β and VEGF, elucidating their role in the progression of liver damage during SARA-CoV-2 infection[96].

All the data mentioned above are consistent with a detailed histological examination clarifying the possible mechanisms of hepatic injury. Wang *et al*[84] uncovered the presence of intact SARS-CoV-2 viral particles in the cytoplasm of hepatocyte samples obtained from 156 dead COVID-19 patients. Further observations revealed conspicuous mitochondrial swelling, endoplasmic reticulum dilatation, glycogen granule decrease, fibrin deposition, granulomas, massive central necrosis, and apoptosis. Another study by Fiel *et al*[97], using in situ hybridization and electron microscopy, reported that SARS-CoV-2 directly invades liver cells and induces histological changes such as apoptosis, especially in cholangiocytes, abundant mitoses, mixed inflammatory infiltrates in portal tracts, endothelins, and severe bile duct damage. In a case study by Melquist *et al*[98], the direct SARS-CoV-2 cytopathic effect caused a rapid progression of acute hepatitis to fulminant liver failure with a mild increase in transaminase levels without developing respiratory symptoms. Data from the international study involving 130 centers in 29 countries revealed that the stage of liver disease is closely correlated with COVID-19 mortality. The highest rates of hepatic decompensation and mortality were observed in patients with advanced liver cirrhosis and those with alcoholic liver disease[99].

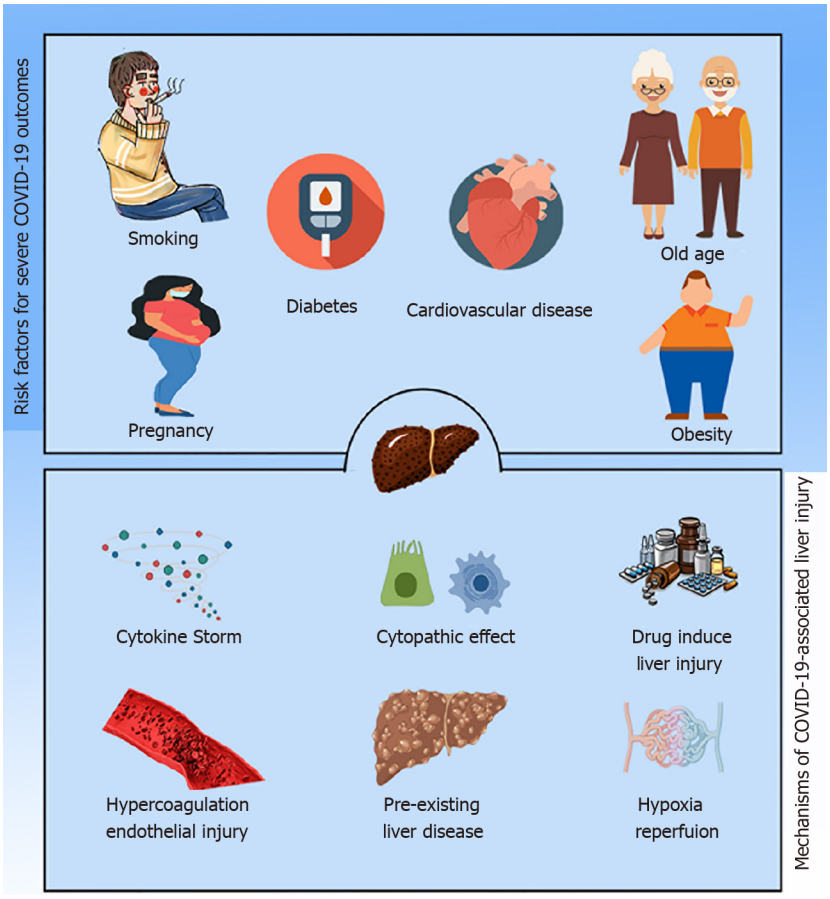


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**Figure 1 Risk factors and possible mechanisms of COVID-19-associated liver injury.** COVID-19: Coronavirus disease 2019.

### *Cytokine storm and liver injury*

SARS-CoV-2 induces immune dysregulation associated with the unspecified release of proinflammatory cytokines and coagulation enzymes. The massive release of cytokines is known as a cytokine storm or cytokine release syndrome and is characterized by the magnitude of the release of interferons, TNFs, ILs, and chemokines[100]. Hence, uncontrolled systemic proinflammatory cytokine release represents unfavorable clinicopathological conditions in COVID-19 patients, for instance, progressive liver damage and liver failure.

IL-6 is the most significant cytokine in liver hepatocytes and is a crucial inducer of the acute phase response and infection defense[101]. IL-6 stimulates hepatocytes during the initial phase of inflammation to upregulate CRP, fibrinogen, haptoglobin, alpha-antitrypsin, and serum amyloid-A which induce acute inflammatory phase[101]. Additionally, prolonged inflammation stimulates IL-6, targeting monocyte chemotaxis toward tissue-destructive injury[102]. Furthermore, IL-6 induces multiple effects during the storm *via* the activation of different transduction signaling pathways, *e.g.*, nuclear factor *kappa*-light-chain-enhancer of activated B cells (NF-κB), janus kinase (JAK)/ signal transducer and activator of transcription (STAT), and the Akt/Phosphatidylinositol-3-kinase pathway[71,103].

Similarly, attention must be paid to the crucial roles of the ACE/Ang II/ angiotensin II receptor type 1 pathways. Ang II can directly activate the NF-κB pathway, increasing the secretion of IL-6, IL-1β, TNF-α, and IL-10[104]. Moreover, Ang II has been reported to induce mitogen-activated protein kinases activation, which in turn induce pro-inflammatory cytokines' release[105].

A case series study by Li *et al*[106], revealed elevated serum transaminase levels attributed to systemic inflammation, cytokine storm syndrome, and hepatocyte damage. Darif *et al*[95] reported that hepatic injury in patients with COVID-19 was attributed to systemic inflammation. Therefore, significant elevations in CRP, TNFα, and IL-6, concomitant with a significant elevation in aminotransaminase, describe hepatic injury associated with SARS-CoV-2 infection[107,108]. All these data confirm the relationship between inflammation during COVID-19 and hepatic injury.

### *Hypoxia and liver injury*

One of the most common complications of COVID-19 is acute respiratory distress syndrome requiring a high level of management[81,109,110]. COVID-19 is associated with impaired respiration, an insult to blood flow, and hypotension, which are clues to hypoxic hepatitis, and might exacerbate liver damage

or even lead to liver failure[11,106]. Ischemia induces profoundly detrimental cellular effects and results in metabolic abnormalities, for example, disturbances in lipid metabolism as well as lack of oxygen supply initiate hepatocellular death[112]. Furthermore, rapid recovery of blood flow with reoxygenation of hepatocytes results in metabolic abnormalities, the generation of reactive oxygen species, an inflammatory response, and cellular death[113]. Hence, hepatic ischemia deteriorates hepatic status *via* destructive cellular reactions concomitant with immune stimulation[112-114]. Hypoxia has been determined as the primary pathway to regulating ACE2 expression in hepatic cells[115]. These phenomena rapidly progress with a conspicuous elevation of transaminase levels, accompanied by LDH elevation[116]. A retrospective study by Huang *et al*[117] revealed that hypoxic hepatitis is apparent in intensive care units and is often associated with a drastic elevation in ALT levels, multiorgan damage, and high mortality risk. Additionally, patients with COVID-19 and hypoxic hepatitis are sometimes comorbid with respiratory failure, septic shock, or heart failure[53,80,118]. All these findings suggest an association between hepatic ischemia/hypoxia-reperfusion injury and liver injury during the SARS-CoV-2 infection.

### Endothelial cells and liver injury

SARS-CoV-2 induces hypercoagulation, with the incidence of pulmonary embolism associated with complications aggravating heart failure and liver congestion[119]. Hypercoagulation and clotting disorders might occur through direct infection of platelets or a cytokine storm[120]. As mentioned above, patients with COVID-19 reported a change in platelet count and prothrombin time with an elevation in D-dimer and fibrinogen concentrations[80,121-123]. A multicenter, retrospective cohort study found that patients who died from COVID-19 were more likely to have severe hematological (lymphopenia, ferritin, and elevated D-dimer) and cardiogenic factors (troponin and lactate dehydrogenase), providing support for this hypothesis[80]. Goshua *et al*[123] reported that patients with COVID-19 showed a disturbance in epitheliopathy and platelet activation markers, particularly von Willebrand factor (vWF) antigen, P-selectin, and soluble thrombomodulin, anticipating a poor outcome or death. Furthermore, a case report study by Antunes de Brito *et al*[124] observed hepatic artery thrombosis in a patient with COVID-19 who experienced acute abdominal pain in harmony with elevations in protein C and D-dimer. Histological examination implied a severe disruption of the intrahepatic blood vessel network secondary to systemic changes induced by the virus that might also affect the cardiovascular system, coagulation cascade, and endothelial layer of blood vessels[125]. Additionally, a series of pathological examinations of liver autopsies obtained from deceased COVID-19 patients elucidated platelet aggregation in some portal veins as well as hepatic sinusoidal injury due to platelet-fibrin microthrombi[126]. However, ischemic-type damage in the liver has been observed in some cases [126]. Massive data indicate a relationship between hypercoagulation and liver injury in COVID-19 patients.

SARS-CoV-2 infection induces a pathological thromboinflammation response, including platelet hyperreactivity, hypercoagulability, and hypofibrinolysis[127]. SARS-CoV-2 binds to ACE2 receptors on the surface of endothelial cells and subsequently induces endothelial injury[127]. Additionally, SARS-CoV-2 invades megakaryocytes and platelets[128]. Endothelial cell activation and injury were confirmed by elevation of several blood hemostatic factors including vWF, thrombomodulin, and factor VIII[122, 123]. Collectively, they trigger a platelet plug activation[129,130]. A procoagulant molecule and platelet tissue factor, produced by hepatocytes and endothelial cells, attach, and activate factor VII, a procoagulant molecule that circulates in the blood. Activated factor VII activates factor X, which subsequently resulted in thrombin formation. Thrombin promotes a series of coagulation processes to produce fibrin which build a substantial fibrin mesh, in addition to platelet activation and aggregation [131,132]. Furthermore, Ang II increases plasminogen activator inhibitor-1 (PAI-1) expression in endothelial cells, which inhibits fibrinolysis and induces a hypercoagulable state[133].

Furthermore, hypoxia promotes coagulation through multiple pathways, such as hypercoagulation and inflammation. Hypoxia attenuates endothelial cells' anticoagulant function by suppressing thrombomodulin with increased PAI-1 upregulation. It promotes NF-κB and toll-like receptor 4 signaling pathways in macrophages and neutrophils, stimulating the release of IL-6 and TNFα[134-136].

Excessive inflammatory cytokines, particularly IL-6, facilitate SARS-CoV-2 and mediate coagulopathy[137]. IL-6 stimulates platelet formation and megakaryocytopoiesis generation, which could generate a hypercoagulability state[138]. A retrospective study by McConnell *et al*[139] revealed that the IL-6/JAK/STAT pathway is responsible for coagulopathy and hepatic epitheliopathy associated with COVID-19 and could be the potential mechanism of liver injury in these patients.

### Drug-induced liver injury

Several medications can induce liver dysfunction and hepatocellular damage. Some are used as over-the-counter medications, for example, paracetamol, and others are used with precautions such as antibiotics, including azithromycin[140]. Although drug-induced liver damage is rare, it can immediately result in acute liver failure and require a liver transplant[141]. Drug metabolism is a possible cause of drug-induced liver injury (DILI) development by generating chemically reactive drug metabolites. The failure to metabolize reactive drugs can result in mitochondrial damage and oxidative stress, activating different signaling pathways[142]. Furthermore, reactive metabolites can act as haptens

and create neoantigens, which, when presented on human leukocyte antigen (HLA) molecules or attached to HLA molecules, can activate T cells, and trigger an adaptive immune response[143].

Several antiviral medications, supportive care, and trials of complementary therapies are among the therapeutic options being investigated against SARS-CoV-2. Hepatotoxicity from nucleoside analogs and protease inhibitors, which are used to manage COVID-19, can occur because the liver is involved in the metabolism of many medications. In a case study from Wuhan, after receiving lopinavir and ritonavir, the patient developed liver damage[143]. A recent randomized controlled study compared the elevation of AST, ALT, and total bilirubin in COVID-19 patients associated with lopinavir and ritonavir [144]. In a retrospective analysis of COVID-19, Fan *et al*[145] found that significantly elevated liver enzymes and liver abnormalities were in harmony with receiving combination therapy. In this study, 47.3% of the released patients had increased liver function tests (LFTs) at baseline, and 23.7% experienced abnormalities during hospitalization, which might be due to treatments or the disease.

It was discovered that many COVID-19 patients had previously used antipyretics and analgesics, most frequently paracetamol, whose overdose is recognized as a cause of liver injury with a significant elevation of serum aminotransferases[146]. Additionally, hepatic injury worsens in critical illnesses and patients with preexisting CLDs[147]. Therefore, healthcare providers should be aware of over-the-counter medications used to control common COVID-19 symptoms such as fever and pain. Physicians play a role in monitoring abnormalities in LFTs as they can indicate unknown drug hepatotoxicity.

Hydroxychloroquine is one of the drugs suggested for COVID-19 therapy regimens, an anti-malarial medicine that relies on scant data in limited clinical settings[148]. Based on clinical data, hydroxychloroquine hepatotoxicity during COVID-19 is rare[149]. A few incidences of significant increases in aminotransferases brought on by hydroxychloroquine have never been documented[150]. Therefore, patients with liver disorders should use hydroxychloroquine cautiously since it can accumulate[151].

Azithromycin is an antibacterial drug belongs to macrolide antibiotic. It was used to treat bacterial infections before treating COVID-19 alone or in combination with hydroxychloroquine[152]. Hence, the hepatic injury should be considered due to the high use of these medications. Liver damage may rarely occur within the first two to three weeks of starting azithromycin. Most patients fully recover from it, and it is predominately a hepatocellular pattern[153].

Remdesivir belongs to an adenosine analog with antiviral action[154]. A multicenter, randomized, double-blind, placebo-controlled trial by Wang *et al*[155] revealed that 10% of the remdesivir group had high blood bilirubin and 5% had increased aminotransferases. Additionally, Remdesivir was used to treat COVID-19 in a case series ($n$ = 53), and 23% of the patients experienced elevated liver enzyme levels that required early treatment termination[156]. However, clinical data implied that the relation between remdesivir and hepatic injury during COVID-19 treatment needs more explanation[154].

To conclude, medications that reduce inflammation and preserve the liver should be given to individuals who are expected to experience liver damage, regardless of the drug, dosage, or dose[140].

All studies regarding possible mechanisms of COVID-19-associated liver injury were summarized in Table 1.

## COVID-19 COMORBIDITY WITH DIFFERENT HEPATIC ILLNESSES

### *COVID-19 and viral hepatitis*

HBV is a double-stranded DNA virus, a member of the *Hepadnaviridae* family. In contrast, HCV is a single-stranded RNA virus belonging to the Flaviviridae family[100]. Recently, several studies have indicated that the coinfection of COVID-19 and HCV is a predictor of acute-on-chronic liver failure and a high potential for ICU admission. A cohort study indicated that HCV patients with SARS-CoV-2 coinfection were more likely to be hospitalized. However, the mortality rate did not change[157].

In a retrospective cohort study that included 242 patients with hepatitis C cirrhosis, 46 patients were coinfected with SARS-CoV-2 and HCV and had high levels of ferritin, creatinine, blood urea nitrogen, prothrombin time, and HCV viral load, anticipating the development of acute-on-chronic liver failure and the potential for ICU admission[158]. An observational study by Toma *et al*[159] among patients with SARS-CoV-2, active HCV, and cure HCV in a control group showed the highest serum concentrations of ALT, AST, CRP, and ferritin. Moreover, serum and fecal calprotectin were detected in a patient with SARS-CoV-2 infection. In a serological study by León *et al*[160], patients with both HCV coinfection demonstrated a considerable elevation in IL-6 and IL-17, with lower TNF-α levels when compared with patients infected with HCV or SARS-CoV-2.

On the other hand, a nationwide population-based study has reported that patients infected with HBV were predisposed to have severe symptoms of SARS-CoV-2, a high probability of ICU admission, and more organ failures than patients without HBV infections, especially in older patients[161].

In addition, severe monocytopenia, lymphopenia, hypoalbuminemia, and lipid metabolism deficiency were observed in the liver of coinfected with SARS-CoV-2 and HBV[162,163]. Besides the elevation of liver impairment markers, including ALT, AST, ALP, and total bilirubin, several novel risk factors have been identify ed, including elevated LDH, D-dimer, decreased albumin, and albumin/globulin ratio[164]. However, other studies have found that HBV is not related to the poor outcomes of

**Table 1 Explore the main causes of liver injuries during COVID-19**

| Cause of liver injury | The main finding of the study | Ref. |
|---|---|---|
| | SARS-CoV-2 directly invades the liver and displays hepatic impairment characterized by liver enzyme abnormalities | Wang *et al*[84], 2020 |
| SARS-CoV-2 tropism | Intrahepatic SARS-CoV-2 contributes to liver inflammation, endothelium, and bile duct damage | Fiel *et al*[97], 2021 |
| | SARS-CoV-2 cytopathic effect involved in the rapid progression of acute liver injury to acute liver failure | Melquist *et al*[98], 2020 |
| Cytokine storm | Elevation of liver enzymes in COVID-19 is mainly related to immune dysregulation caused by cytokine storm and hepatic damage | Li *et al*[106], 2020 |
| | Systemic inflammation is the fuel for hepatic injury in COVID-19 patients | Effenberger *et al*[244], 2021 |
| Hypoxic liver injury | Hypoxic hepatitis is not a rare condition in COVID-19 patients admitted to the intensive care unit and is dramatically associated with elevated liver enzymes | Huang *et al*[117], 2020 |
| | Hepatic artery thrombosis is highly associated with hepatic injury and abdominal pain during COVID-19 | Antunes de Brito *et al*[124],2021 |
| | SARS-CoV-2 induces severe disruption of the intrahepatic blood vessel and also affects the endothelial layer of blood vessels | Sonzogni *et al*[125], 2020 |
| Endothelial cells and liver injury | Hepatic injury is attributed to platelet-fibrin microthrombi in the hepatic sinusoids along with some portal vein platelet aggregates | Rapkiewicz *et al*[126], 2020 |
| | SARS-CoV-2 activates IL-6/JAK/STAT pathway consequently, stimulating coagulopathy and hepatic epitheliopathy | McConnell *et al*[139], 2021 |

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; IL-6: Interleukin-6; JAK: Janus kinases; STAT: Signal transducer and activator of transcription proteins.

COVID-19[165]. Furthermore, the reactivation of HBV may occur due to the COVID-19 vaccine, as observed in some cases[166-168].

### *COVID-19 and viral hepatitis during pregnancy and its impacts on neonates*

Acute HBV infection during pregnancy is not a risk factor for fetal death or teratogenicity. However, many complications in HBV-infected pregnant women may be associated with an increased risk of gestational diabetes, postpartum hemorrhage, premature birth, and low birth weight[169]. Furthermore, in a prospective cohort study, Rajan *et al*[170] indicated that pregnant women with both HBV and SARS-CoV-2 coinfection had a high proportion of preterm deliveries and a low mean birth weight. In rare cases, the coinfection of both viruses has led to intrahepatic cholestasis in pregnancy and acute fatty liver disease of pregnancy (AFLP)[171]. Nevertheless, there is some indication that HBV and COVID-19 coinfection does not lead to worse results[170,172]. On the other hand, some studies have provided evidence that treatment regimens including antivirals, hepatoprotective, and low-dose dexamethasone drugs might be recommended in cases of pregnant women with HBV and COVID-19 coinfection, besides coagulation function monitoring as part of the management process[171,173].

Similarly, pregnant women with HCV infection are more likely to have infants born prematurely, stillborn infants, newborns with low birth weight, or infants with birth abnormalities[174,175]. Furthermore, from an epidemiological point of view, the worldwide hepatitis elimination program has been affected due to COVID-19 spreading, and this may require new policies and strategies for hepatitis elimination[176-178].

Ahmed *et al*[179] reported a case-report study in which a 26-year-old Asian female pregnant patient was affected by a sudden onset of severe preeclampsia complicated by AFLP and acute kidney injury (AKI) following SARS-CoV-2 infection. Besides, the comorbidities of SARS-CoV-2 and preeclampsia in pregnancy can lead to AFLP and AKI. This comorbidity can cause calcifications of the bowel and gallbladder of the fetus[180,181], besides a liver parenchymal disease associated with liver rupture[182], liver coagulation, liver impairment, and preterm delivery[183]. Furthermore, a pregnant woman with SARS-CoV-2 infection at 28 wk with a low-lying placenta was complicated by obstetric cholestasis and several episodes of minor antepartum hemorrhage[184]. Moreover, placental insufficiency and subsequent fetal hypoxia may occur[185].

### *COVID-19 and pregnancy: Several mechanisms for complications*

Recently, pregnant patients who were coinfected with SARS-CoV-2 showed a higher risk of developing complications than those who were not pregnant. Studies have shown that pregnant women with SARS-CoV-2 infection increased the probability of developing preeclampsia compared to individuals who did not have SARS-CoV-2 infection during pregnancy[186]. Nevertheless, symptomatic patients were more

likely to have preeclampsia than asymptomatic ones[186,187].

On the other hand, several hypotheses may illustrate the high rate of preeclampsia associated with SARS-CoV-2 infection. A direct cytopathic effect with dysregulation of the RAAS system induces a change in the placenta's function[188-191] because it controls the proliferation of trophoblasts, angiogenesis, and placental blood supply. Thus, the interaction between SARS-CoV-2 and ACE2 receptors described in RAS system down-regulation and reduction of vasodilatory angiotensin 1 to 7 results in continuous vasoconstriction and pro-inflammatory effects of angiotensin II, which finally lead to a pathophysiological mechanism of preeclampsia[192-196]. A study conducted by Verma *et al*[197] suggested that the infected placenta had a reduction in ACE2 receptor expression, proangiogenic factors, and an increase in the production of soluble FMS-like tyrosine kinase-1 (sFlt-1), which are biomarkers for preeclampsia. An in-silico study by Seethy *et al*[198] concluded that interactions between SARS-CoV-2 and the placenta are regulated through trophoblast invasion, migration, proliferation, and differentiation processes by the milk fat globule-EGF factor 8 protein, plasminogen activator, and protease-activated receptor 2 proteins.

In parallel, pregnant women might be able to develop a pre-eclampsia-like syndrome characterized by proteinuria, hypertension, thrombocytopenia, the elevation of liver enzymes, an abnormal uterine artery pulsatility index, and increased sFlt-1/placental growth factor[199], besides preeclampsia, coagulopathy, and the HELLP (hemolysis, elevated liver enzymes, low platelet count)[200].

### COVID-19 and liver fibrosis/cirrhosis

Recently, it has been hypothesized that patients with a hepatic illness have a higher mortality rate after SARS-CoV-2 infection. Non-invasive indices, including the Fibrosis-4 index (FIB-4), the NAFLD fibrosis score, and the AST to platelet ratio index, have been developed to determine the severity of fibrosis, which plays a crucial role in assessing liver fibrosis[201]. In a multicenter observational study, Kim *et al*[202] reported that patients with diabetes mellitus (DM) showed a higher FIB-4 index, serious complications such as severe respiratory failure, venous thromboembolism, hepatic injury, and a high mortality rate compared to patients without DM. Meanwhile, the FIB-4 index might be used to assess the risk of progression to hepatic illness in middle-aged patients with COVID-19[203]. An association was observed between liver fibrosis scores and poor outcomes, and these findings were consistent with previous research that found worse outcomes in COVID-19 individuals with pre-existing chronic liver disorders, including a high proportion of ICU admission and the need for mechanical ventilation[204, 205]. An explanation for liver injury could be the presence of high levels of lymphocytes and natural killer cells inside the hepatic tissue[206].

On the other hand, An *et al*[207] conducted a STROBE observational study and reported that patients with liver cirrhosis and COVID-19 were frequently admitted to the hospital more than those with liver cirrhosis only. Unlikely, in the same study, cirrhotic patients who lacked COVID-19 experienced more severe liver cirrhosis-related consequences and needed immediate treatment. In a multicenter cohort study, Bajaj *et al*[208] illustrated that those with cirrhosis alone or with COVID-19 had equal death rates, while patients with COVID-19 alone had a greater mortality rate.

### COVID-19 and liver fibrosis/cirrhosis during pregnancy

As discussed above, having an infection makes pregnant women more susceptible to developing more severe symptoms. Biomarkers such as ALT, AST, ALP, elevated D-dimer levels, fibrin degradation, and prolonged prothrombin time lead to liver injury, liver fibrosis, and liver cirrhosis; hence, increasing the possibility of preeclampsia with HELLP syndrome[179].

### COVID-19 and HCC

HCC is the third most important cause of cancer-related mortality and the sixth most frequent cancer in the world. SARS-CoV-2 virus infection has recently been considered a risk factor for cancer patients because SARS-CoV-2 might aggravate liver damage in HCC patients[209]. Furthermore, a US multicenter study by Kim *et al*[210] reported that having HCC indicates a greater mortality rate in individuals with HCC infected by SARS-CoV-2 than COVID-19 alone, especially in patients with obesity, DM, hypertension, hyperlipidemia, older patients (≥ 65 years), and Hispanic ethnicity. Also, in China, patients with HCC and COVID-19 were shown to be more susceptible to a higher risk of death and admission to the ICU[211]. In parallel, Leo *et al*[212] retrospectively analyzed 119 patients with HCC and COVID-19 infection. They found that about one-third of patients required hospital admission. Two-thirds had an elevation of transaminases, particularly ALP, which was independently linked to a high mortality rate, higher CRP levels, and more severe respiratory failure upon admission to the hospital.

### Liver transplantation and COVID-19

According to the American Society of Transplantation, there has yet to be an agreement on the ideal timing of liver transplantation (LT) in patients infected with SARS-CoV-2. However, it is recommended that before transplantation, recipients should have a negative SARS-CoV-2 test[213]. Nevertheless, Martinez-Reviejo *et al*[214] determined that, regardless of symptoms at the time of infection, using LT from SARS-CoV-2 positive donors appears to be a safe technique with a low risk of transmission.

Furthermore, a multicenter network study by Mansoor *et al*[215] found that LT patients with COVID-19 had a substantially larger possibility of hospitalization but not mortality, thrombosis, or ICU admission when compared to those without LT and COVID-19. In contrast, a case-control study by Shafiq *et al*[216] stated that regarding death and hospitalization rates, there was no significant difference between the case and control groups in liver enzyme ratios, and both had a normalized value at the time of discharge. In addition, the only difference in the patient's pathological characteristics is the type of liver graft, alkaline phosphatase levels, and lymphovascular invasion[217]. A case-report study indicated that some LT could be successful in active SARS-CoV-2 patients without developing post-operative COVID-19 symptoms[213]. Furthermore, an Italian multicenter series by Romagnoli *et al*[218] found that liver transplantation from COVID-19-positive donors to informed recipients with SARS-CoV-2 immunity might help increase the safety of the donor pool. Rela *et al*[219] reported a successful LT in patients with severe liver failure due to cholestasis with good graft function and recovering function in the native liver remnant.

Collectively, the effect of comorbid hepatic disorders with SARS-CoV-2 infection was summarized in Table 2.

## VACCINES USED FOR COVID-19 PREVENTION

Recombinant DNA, mRNA, and adenovirus vector-based technologies were the three main methods of vaccine development that demonstrated immediate success. All have been shown to help prevent infections, especially in severe diseases, because breakthrough infections are typically asymptomatic or mild-to-moderate. BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) were emergently approved in the United States as the first mRNA vaccines[220]. Following that, an Emergency Use Authorization (EUA) license was granted for the two most effective adenovirus-based vaccinations in the United States (Ad26.COV2.S) (Janssen-Johnson & Johnson) and Europe (ChAdOx1.nCoV-19; Oxford-Astra Zeneca). Adenovirus-vectored vaccines have demonstrated effectiveness in China (Ad5-vectored COVID-19 vaccine) and countries that produce traditional, inactivated viral vaccines. The most frequently used COVID-19 vaccinations are intramuscular injections, and a first dose is recommended to be followed by a second dose within three to four weeks. Currently, a booster dose is recommended administrated after six months of the initial immunization. Individuals 18 years of age and older may get a booster dose of the Johnson & Johnson COVID-19 vaccination 2 mo following the initial single dose[221,222].

### *Pfizer-BioNTech vaccine (BNT 162b2)*

An intramuscular mRNA vaccine called BNT 162b2 is administered in two doses (30 μg *per* dose) at 21-d intervals. The vaccine is accessible in multidose vials and must be refrigerated at a temperature between 60 °C and 80 °C[223], which might present a logistical challenge in developing nations. According to phase I/II/III, randomized, placebo-controlled trials published in December 2020, the Food and Drug Administration (FDA) approved it for emergency use[224]. In the study, 43448 volunteers were randomly assigned in a 1:1 ratio to the vaccination arm and the placebo arm. Compared to the placebo, the vaccination showed a 95% efficiency in preventing COVID-19, and this efficacy was maintained for subgroups based on age, sex, BMI, ethnicity, and comorbidities. Local site responses were the most prevalent adverse effects. Young patients were more likely to experience systemic symptoms such as fever, joint discomfort, and chills, which increased following the second dosage[225]. Just three individuals with moderate or severe liver disease were included in the trials, with 214 participants having mild liver disease. The virological status and disease severity of patients with HBV and HCV infections were included; however, it was unknown how severe their conditions were. Furthermore, immunosuppressive drug users were excluded. Hence, more information is required concerning people with liver illnesses[226,227].

### *Moderna vaccine (mRNA-1273)*

The mRNA-1273 is another mRNA vaccination given in two doses of 100 μg each, separated by 28 d. Based on phase III randomized placebo-controlled trial published in December 2020, in which 30420 participants were randomly allocated to the immunization and placebo groups in a 1:1 ratio, the FDA approved the vaccine. The effectiveness of the vaccination in preventing COVID-19 was 94.1%. Only the placebo group experienced severe COVID-19, resulting in one participant's death. Serious, unanticipated adverse reactions to vaccinations were more frequent in the vaccine group, but none were fatal or forced to be completed until the research's end. After the second dose and in younger people, the unwanted local and systemic responses were more prevalent[228,229]. Although the liver condition was not specified, the study included 196 individuals with liver disease (divided equally between the vaccination and placebo groups). Participants in the experiment who were on systemic immunosuppressive medication were not allowed. For individuals with hepatic illness, no independent efficacy and safety data were available[228].

**Table 2 Summarizing the effect of comorbid hepatic disorders with SARS-CoV-2 infection**

| Hepatic disorders | Main finding | Ref. |
|---|---|---|
| | SARS-CoV-2 comorbidity with HCV shows a high percentage of ferritin, white blood cell count, prothrombin time, lymphocyte count, and hypoglycemia | Cerbu *et al* [159], 2022 |
| | SARS-CoV-2 and HCV coinfection reported higher levels of IL-6 and IL-17, and TNF-α when compared with HCV and COVID-19 alone | León *et al*[161], 2022 |
| HCV | The Serum levels of ALT, AST, CRP and ferritin, and calprotectin were significantly elevated in patients with COVID-19 infection than in patients with active HCV and patients with cured HCV infection | Toma *et al* [160], 2022 |
| | HCV patients with SARS-CoV-2 infection are more likely to be hospitalized with a high possibility of liver fibrosis and mortality | Butt *et al*[158], 2021 |
| | Individuals with HCV and SARS-CoV-2 co-infection are more vulnerable to developing liver cirrhosis | Afifyet al[245], 2021 |
| | Patients with a history of HBV are anticipated to have a worse outcome with a high probability of ICU admission, and more organ failures | Choe *et al*[162], 2022 |
| | S SARS-CoV-2 and chronic HBV showed severe monocytopenia, lymphopenia, thrombocytopenia, hypoalbuminemia, and lipid metabolism deficiency in the liver | Zou *et al*[164], 2021 |
| HBV | Patients with HBV and SARS-CoV-2 coinfection died from severe liver disease and haptic sclerosis | Chen *et al* [163], 2020 |
| | Patients with HBV who have COVID-19 were more likely to develop devastating illnesses and/or death. Additionally, the elevation of LDH, and D-dimer, with decreased albumin, and albumin/globulin ratio is helpful for early clinical surveillance | Wang *et al* [165], 2022 |
| | Patients with DM with advanced liver fibrosis infected by SARS-CoV-2 are assumed to have a 10-time risk of mortality when compared with patients without comorbidities | Kim *et al*[203], 2021 |
| | The high proportion of ICU admission, and the need for mechanical ventilation | Hassnine *et al* [206], 2022 |
| Liver cirrhosis | Patients with liver cirrhosis and COVID-19 were admitted to the hospital than liver cirrhosis alone | An *et al*[208], 2021 |
| | Those with cirrhosis alone or cirrhosis with COVID-19 had equal death rates, while patients with COVID-19 alone had a greater mortality rate | Bajaj *et al*[209], 2021 |
| | HCC predicts a greater mortality rate in individuals with HCC infected by SARS-CoV-2 than COVID-19 alone, especially in patients with obesity, diabetes mellitus, hypertension, and hyperlipidemia, older patients ≥65 years, and Hispanic ethnicity | Kim *et al*[211], 2021 |
| HCC | HCC and COVID-19 were shown to be more susceptible to have a higher risk of death and admitted to the ICU | Liang *et al* [212], 2020 |
| | Patients with HCC-COVID-19 coinfection found that about one-third of patients need hospital admission, and two-thirds of patients have an elevation of transaminases. Alkaline phosphatase which independently linked to a high mortality rate, higher C reactive protein levels, and more severe respiratory failure upon admission to the hospital | Leo *et al*[213], 2022 |
| | LT patients with COVID-19 had a considerably increased risk of hospitalization but not a significantly higher risk of mortality, thrombosis, or need for ICU admission | Mansoor *et al* [216], 2021 |
| | High alkaline phosphatase levels, and lymphovascular invasion | Shafiq *et al* [217], 2022 |
| LT | LT cases could be successful in active SARS-CoV-2 patients without developing post-operative COVID-19 symptoms | Mouch *et al* [214], 2022 |
| | Found that liver transplantation from COVID-19-positive donors to informed recipients who have SARS-CoV-2 immunity may help to increase the donor pool safely | Romagnoli *et al* [219], 2021 |
| | Successful LT In patients with severe liver failure due to cholestasis with a good graft function and recovering function in the native liver remnant | Rela *et al*[220], 2022 |

HCV: Hepatitis C virus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; IL-6: Interleukin-6; IL-17: Interleukin-17; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C-reactive protein; HBV: Hepatitis B virus; ICU: Intensive care unit; HCC: Hepatocellular carcinoma; LT: Liver transplantation.

### *ChAdOx1nCoV-19 vaccine (AZD1222)*

ChAdOx1 nCoV-19 vaccine (AZD1222) was created by the University of Oxford, which uses a replication-deficient chimpanzee adenovirus as a vector containing the gene encoding for the SARS-CoV-2 spike glycoprotein. Storage conditions may be kept between 2 and 8 °C and are less strict than mRNA vaccines. AstraZeneca and Serum Institute of India produce it (SII). In December 2020, the UK

granted emergency use authorization for the vaccine produced by AstraZeneca. The vaccine, produced by SII under the brand name COVISHIELDTM®, was approved for use in India by the Drug Controller General of India[229]. Two intramuscular vaccine doses, each containing 0.5 mL, were given over a 4–6 wk interval. In patients who got a single dose, antibody responses peaked on day 28, and in individuals who received a booster dose four weeks later, they peaked on day 56[228,229]. A pooled intermediate analysis of four randomized controlled trials by Voysey *et al*[230] conducted in Brazil, South Africa, and the UK, which included 23848 people, was used to support the authorization. Of these, 11636 patients were included in the interim study. The experiment showed total vaccination effectiveness of 70.1%. After 21 d following immunization, 10 COVID cases were recorded; all were in the control group and included two cases of severe COVID and one case of death. In addition, only three of the 175 cases with adverse effects might have been caused by vaccination. Individuals with hepatic disorders were mostly excluded from the 4 studies described above. Patients with severe liver diseases were not included in the trials in the UK and Brazil, although the severity standards were unclear. Furthermore, individuals using immunosuppressive drugs and those with alcohol dependence were excluded. Abnormal LFTs, Australian antigen-positive status, CLDs, and alcohol misuse were listed as exclusion criteria in a South African study. Only two individuals (one from each vaccination and control group) had abnormal liver function[231].

### *Janssen vaccine/Ad26.COV2.S*

This full-length SARS-CoV-2 S protein-containing non-replicating human adenovirus type 26 triggers an immune response to the SARS-CoV-2 infection. The SARS-CoV-2 virus is prevented from invading type 2 alveolar cells in the lungs by an antibody directed against the S protein, lessening the severity and morbidity of the infection[232]. Adjuvant properties, scalability, and broad tissue tropism are benefits of adenoviral vectors[233,234]. Since these labs need biosafety level 2 certification, vaccine production will likely go more slowly during this pandemic. Additionally, a person with immunity to viral vectors would reduce the vaccine's efficacy. Employing the chimpanzee adenovirus (ChAdOx1), which serves as an alternative to the human Ad vector and does not confer any immunity on humans, Oxford/AstraZeneca could overcome this drawback[235,236].

Moreover, Sadoff *et al*[231] revealed that a single-shot Janssen vaccination prevents severe SARS-CoV-2 infections. A total of 43783 seronegative volunteers participated in this study, and they were separated into two age groups: Group 1 (18-59 years old) and group 2 (≥ 60 years old). These participants were randomly divided into two groups of like-minded individuals in a 1:1 ratio, one receiving the placebo and the other the vaccination. The study group collected 468 confirmed cases after receiving the vaccination for 14 d. A total of 464 cases, including 116 from the vaccination group and 348 from the placebo group, were mild to moderate in severity, indicating an effectiveness of 66.9%. More than 66 moderates to severe-critical cases were confirmed to belong to the vaccine group after 28 d of follow-up, compared to 193 cases that belonged to the placebo group. Moreover, less severe-critical cases were observed among older patients than younger patients, suggesting possible early protection from the vaccine, especially in the elderly. The effectiveness of the immunization was equal across all age groups after 28 d[233].

### *Sinopharm COVID-19 vaccine (Sinovac)*

At least five distinct COVID-19 vaccines, including conventional inactivated viral vaccines and vaccines based on an adenovirus vector, have been created and given the go-ahead for use in China. The safety and efficacy of the majority have not been extensively reported. As part of its international COVID-19 immunization global project known as COVAX®, the WHO has authorized two vaccines, the Sinopharm, Beijing, and Sinovac Corona Vac vaccines, both traditional inactivated viral vaccines, are essential to China's ambition to immunize most of its inhabitants by 2022[237]. After two dosages, the efficacy rates in clinical trials examining their safety and effectiveness from various regions of the world range from 50% to 91%. Other nations use these vaccinations, including Russia, Turkey, Brazil, Chile, Argentina, Peru, Mexico, Egypt, the UAE, Jordan, Morocco, Indonesia, and Pakistan. Although the range and incidence of adverse effects following the Sinopharm and Sinovac COVID-19 vaccinations are not documented, the methods of manufacture would imply that these vaccines are generally safe and unlikely to cause hepatocellular damage[222,238,239].

Patients with CLDs are particularly susceptible groups to increase the risk of death and more severe types of COVID-19. Many procedures or treatments for this demographic were postponed due to hospital overcrowding or to avoid putting patients at further risk. This population requires specific attention due to their underlying condition. Therefore, for these patients, immunization should also be a top priority. Interestingly, vaccination appears to be safe in stable CLDs[224]. Additionally, immunization priority was given to the high-risk liver disease such decompensated cirrhosis, liver cancer, and liver transplant recipients. They should receive the vaccination faster when their scores are higher. Indeed, the severity of the immune response induced by vaccine in these participants is unknown, and it is anticipated that it will be insufficient given their underlying illnesses and treatments. The mRNA COVID-19 vaccines are especially remarkable since they are expected to have favorable, safe, and effective characteristics in these individuals[240]. Accordingly, to get COVID-19 vaccinations, patients with CLDs receiving medical care do not need to cease their medication. Besides, patients with

HCC receiving systemic or locoregional therapy can get the vaccine without interrupting their medical care. Nevertheless, immunization should be postponed until the situation is stabilized in recent disease or fever cases. Intriguingly, immune-related adverse events are a potential outcome of vaccination interactions with immune checkpoint inhibitors, which raises concerns about their usage in patients with certain liver disorders (such as HCC) and calls for more research[241]. Influenza and pneumococcal vaccines are recommended for patients with advanced liver disorders to avoid lower immunogenicity in liver disease patients[242,243].

Despite the lack of long-term safety evidence about liver diseases patients vaccinated by SARS-CoV-2 vaccines, it is crucial to balance the potential benefits of vaccination against any possible risks, especially considering the catastrophic implications of SARS-CoV-2 infection in at-risk groups. When new vaccines are introduced, evaluation of safety and immunological response to immunization in individuals with liver disease should be conducted[244]. National and international perspective registries should start as quickly as possible, ideally without governmental obstacles. Individuals at risk should prioritize SARS-CoV-2 infection prevention by vaccination, given the promising short-term safety results of the recently approved vaccines[245].

## CONCLUSION

Hepatic injuries have been approved to be associated with SARS-CoV-2 infection. Indeed, several factors have been embedded in the pathological forms of SARS-CoV-2 hepatic injuries, including viral tropism, cytokine storm, hypoxia, endothelial cells, and even some drugs that treat COVID-19. In addition, previous studies have proved that pregnant women and neonates with hepatic illness are risky for COVID-19 complications. Due to the fast spread of new SARS-CoV-2 strains, vaccines were administered and developed accordingly. In the present review, we believe that patients with CLDs especially those have severe cirrhosis, liver decompensation, and hepatobiliary cancer should be given a priority to get SARS-CoV-2 immunization. Since it is unknown whether vaccination gives sterilizing immunity and inhibits transmission from asymptomatic patients, preventative measures, such as wearing masks, proper hand washing, and social seclusion, remain of utmost relevance.

## FOOTNOTES

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*REVIEW*

# Seronegative spondyloarthropathy-associated inflammatory bowel disease

Chrong-Reen Wang, Hung-Wen Tsai

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## Abstract

Seronegative spondyloarthropathy (SpA) usually starts in the third decade of life with negative rheumatoid factor, human leukocyte antigen-B27 genetic marker and clinical features of spinal and peripheral arthritis, dactylitis, enthesitis and extra-articular manifestations (EAMs). Cases can be classified as ankylosing spondylitis, psoriatic arthritis, reactive arthritis, enteropathic arthritis, or juvenile-onset spondyloarthritis. Joint and gut inflammation is intricately linked in SpA and inflammatory bowel disease (IBD), with shared genetic and immunopathogenic mechanisms. IBD is a common EAM in SpA patients, while extraintestinal manifestations in IBD patients mostly affect the joints. Although individual protocols are available for the management of each disease, the standard therapeutic guidelines of SpA-associated IBD patients remain to be established. Nonsteroidal anti-inflammatory drugs are recommended as initial therapy of peripheral and axial SpA, whereas their use is controversial in IBD due to associated disease flares. Conventional disease-modifying anti-rheumatic drugs are beneficial for peripheral arthritis but ineffective for axial SpA or IBD therapy. Anti-tumor necrosis factor monoclonal antibodies are effective medications with indicated use in SpA and IBD, and a drug of choice for treating SpA-associated IBD. Janus kinase inhibitors, approved for treating SpA and ulcerative colitis, are promising therapeutics in SpA coexistent with ulcerative colitis. A tight collaboration between gastroenterologists and rheumatologists with mutual referral from early accurate diagnosis to appropriately prompt therapy is required in this complex clinical scenario.

**Key Words:** Seronegative spondyloarthropathy; Inflammatory bowel disease; Biologics; Anti-tumor necrosis factor monoclonal antibody; Small molecules; Janus kinases inhibitor

**Core Tip:** Seronegative spondyloarthropathy (SpA) with negative rheumatoid factor has spinal and peripheral arthritis, dactylitis, enthesitis and extra-articular manifestations (EAMs). It can be classified into ankylosing spondylitis, psoriatic arthritis, reactive arthritis, enteropathic arthritis, and juvenile-onset spondyloarthritis. Inflammatory bowel disease (IBD) is a common EAM in SpA, whereas extraintestinal manifestations in IBD mostly affect the joints. Anti-tumor necrosis factor monoclonal antibodies are effective medications with indicated use in SpA and IBD, a drug of choice for treating SpA-associated IBD. A tight collaboration between gastroenterologists and rheumatologists with mutual referral from early accurate diagnosis to prompt therapy is required in this complex clinical scenario.

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## INTRODUCTION

Spondyloarthropathy (SpA) usually starts in the third decade of life with a shared genetic marker human leukocyte antigen (HLA)-B27 and clinical features including spinal and peripheral arthritis, dactylitis (sausage-like swelling of the digits), enthesitis (inflammation at the attachment of tendons/ligaments and joints), tenosynovitis, and extra-articular manifestations (EAMs) mostly involving the eyes, intestine, and skin[1,2]. The prevalence of SpA in population-based studies from North America is estimated to be between 0.4% and 1.3%[3]. SpA is classified as axial type with or without radiographic sacroiliitis, predominantly involving the spine, and peripheral type with or without psoriasis (PsO), inflammatory bowel disease (IBD) or preceding infection, predominantly affecting the extremities[1,2,4]. The concept of seronegative SpA, established in 1974[5], describes a group of chronic arthritis patients who have negative rheumatoid factor and classically includes ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA), enteropathic arthritis (EnA), juvenile-onset SpA (JSpA), and undifferentiated SpA (USpA)[1,6-8]. Regarding the occurrences of disease, AS is the most common types of SpA, followed by PsA, ReA, EnA, and JSpA[3]. In particular, USpA is used to describe seronegative SpA patients with suggestive features but not fulfilling the diagnostic or classification criteria for any of the currently established aforementioned other five subtypes[9]. Table 1 demonstrates demographic, clinical, laboratory, therapeutic and prognostic characters of five seronegative SpA subtypes, including AS, PsA, ReA, EnA, and JSpA.

IBDs, mainly Crohn's disease (CD) and ulcerative colitis (UC), are chronic idiopathic inflammatory disorders of the intestinal tract with progressive disease course[10-12]. CD features chronic granulomatous transmural inflammation with discontinuous lesions involving any part of the intestine, ileum, and colon in particular, complicated by intestinal granuloma, obstruction, stricture, and fistula[11], whereas UC is characterized by continuous mucosal inflammation extending from the rectum toward the colon without the above complications[12]. Extraintestinal manifestations (EIMs) occur in 25% to 40% of IBD patients and mostly affect the joints, followed by the skin, eyes, and hepatobiliary tract[10,13]. Primary sclerosing cholangitis (PSC) is the most frequently observed hepatobiliary manifestation [13,14]. IBD has been identified in 60% to 80% of PSC patients. Up to 5% of UC patients have PSC, while it is less frequent in CD patients. Furthermore, CD and UC patients have an increased risk of intestinal malignancies, such as colorectal cancer[10,15]. IBD was initially thought to be a rare disease in Asia, contrary to the West[16]. Recent population-based data have revealed a rapidly rising incidence in eastern countries while plateauing or even declining in western nations[17]. The epidemiological evolution in IBD is supposedly linked to the Westernized lifestyle and industrialization, including dietary changes, antibiotics use, hygienic status, microbial exposure and pollution, as all are potential environmental risk factors. Furthermore, increased disease awareness, advances in diagnosis, and improved healthcare access can also contribute to the increasing trend of IBD incidence[17,18]. Table 2 shows the demographic, clinical, laboratory, therapeutic, and prognostic characters of the two main types of IBD.

An individual susceptibility to IBD is strongly conditioned by the interaction between intestinal microbiota and the host immune response[19]. Westernized lifestyle-associated dysbiosis, an individual loss of diversity in microbiome composition, has been observed in IBD, and there is a trend toward restored intestinal eubiosis in such patients responding to anti-tumor necrosis factor (TNF) therapy[20]. Accumulating evidence indicates that intestinal inflammation is linked to dysbiosis occurring in rheumatic diseases[21]. The interaction between dysbiosis and the intestinal immune system can lead to

**Table 1 Demographic, clinical, laboratory, therapeutic, and prognostic profiles in five seronegative spondyloarthropathy subgroups**

| Category | AS | PsA | ReA | EnA | JSpA |
|---|---|---|---|---|---|
| Demographic | | | | | |
| Sex, M:F | 3:1 | 1:1 | 5-10:1 | 1:1 | ERA 3:1, JPsA 1:2 |
| Age, yr | 20-40 | 35-45 | Any | 20-40 | < 16 |
| Laboratory | | | | | |
| HLA-B27 | > 90% | Axial 50%-70% | 60%-80% | Axial 50%-70% | ERA 40%-70% |
| | | Peripheral 20% | | Peripheral 20% | JPsA 10% |
| Clinical | | | | | |
| Affected joints | Spine, sacroiliitis | Any area | Peripheral, sacroiliitis | Peripheral | Peripheral, sacroiliitis |
| Peripheral | 30%, lower | Common, upper | Common, lower | Common, lower | Common, lower |
| Sacroiliitis | 100% | 50% | 30% in urogenital | 20% | 40%-60% in ERA |
| Dactylitis | Uncommon | Common | Common | Uncommon | 20% in JPsA |
| Enthesitis | Common | Common | Common | Uncommon | Uncommon |
| EAM common | Intestine, skin, uveitis | Intestine, skin, uveitis | Skin, uveitis | Intestine, skin, uveitis | Intestine, skin, uveitis |
| Treatment | Spinal physical therapy, NSAIDs/cDMARDs for peripheral SpA, biologics, JAKi | NSAIDs, avoid CS, cDMARDs for peripheral SpA, biologics, JAKi, PDE4i | NSAIDs, antibiotics for chlamydia-induced ReA, cDMARDs for peripheral SpA | Coxibs/cDMARDs for peripheral SpA, biologics, JAKi | Spinal physical therapy, NSAIDs/cDMARDs for peripheral SpA, biologics |
| Prognosis | Life-threatening EAMs with heart, intestine or neurological involvement | Comorbidities associated with more severe disease activity | Usually a self-limited disease | Rarely grave EnA in controlled intestinal activity | More spinal deformity and THR as compared with adult SpA or other JIA subtypes |

AS: Ankylosing spondylitis; cDMARD: Conventional synthetic disease-modifying anti-rheumatic drug; Coxib: Cyclooxygenase-2 inhibitor; CS: Corticosteroid; EAM: Extra-articular manifestation; EnA: Enteropathic arthritis; ERA: Enthesitis-related; F: Female; IBD: Inflammatory bowel disease; JAKi: Janus kinase inhibitor; JPsA: Juvenile psoriatic arthritis; M: Male; NSAID: Non-steroidal anti-inflammatory drug; PDE4i: Phosphodiesterase 4 inhibitor; PsA: Psoriatic arthritis; ReA: Reactive arthritis; SpA: Spondyloarthropathy; JSpA: Juvenile-onset spondyloarthropathy; THR: Total hip replacement.

the aberrant activation of immune cells that can recirculate from the gut to the EIM sites as observed in SpA[19,21]. Subclinical gut inflammation in SpA patients represents the repertoire in which immune cells are activated, and is correlated with the severity of spinal inflammation[22]. Genetic risk factors are shared between SpA and IBD, and changes in the composition of the intestinal microbiota are observed in both diseases, indicating that joint and gut inflammation is intricately linked in SpA[19,23].

Since SpA and IBD patients share common genetic and immunopathogenic mechanisms[23], SpA patients have an up to four-fold increased risk of IBD compared to the general population. Different forms of SpA can be associated with variable frequencies of intestinal involvement, whereas articular involvement is frequently observed in IBD. Nevertheless, the chronic medication history of patients' needs to be considered to appropriately evaluate gastrointestinal symptoms in SpA. In addition to direct gastrointestinal adverse reactions, it is necessary to rule out infectious complications with a detailed microbiological survey due to potential immunosuppressive effects. Furthermore, it has been suggested that SpA patients should be evaluated by gastroenterologists when suspected IBD symptoms are present, including rectal bleeding, perianal disease, and chronic diarrhea with organic characteristics [24]. Although individual protocols for managing each disease are available, the standard therapeutic guidelines of seronegative SpA-associated IBD patients remain to be established. In particular, some therapeutic options used to manage one disease might have a negative impact on another disease[25].

Herein, we provide a thorough overview on coexisting IBD in different subtypes of seronegative SpA patients.

## ANKYLOSING SPONDYLITIS

AS is a chronic autoimmune disease mainly involving spinal and sacroiliac as well as peripheral joints, with up to 50% of cases mainly affecting the hips and knees[1,2]. There is a similar pooled prevalence of 0.25% and 0.20% in AS from Caucasian-dominant Europe and North America, respectively[1,26]. Furthermore, this disorder has a prevalence of 0.25% and 0.20% in Taiwan and China, respectively, both

**Table 2 Demographic, clinical, laboratory, therapeutic, and prognostic profiles in two main types of inflammatory bowel disease**

| Category | Ulcerative colitis | Crohn's disease |
|---|---|---|
| Demographic | | |
| Sex, M:F | 1:1 | 1:1 |
| Age at onset in yr | 30-50 | 10-40 |
| Laboratory | | |
| ANCA | Common | Rare |
| ASCA | Rare | Common |
| Clinical | | |
| Origin/Location | Rectum/colon, rectum | Terminal ileum/any part |
| Distribution | Continuous | Skip lesions |
| Pathology | | |
| Inflamed thickness | Mucosa, submucosa | Transmural |
| Crypt abscess | Common | Uncommon |
| Granuloma | Rare | Common |
| Fissure | Uncommon | Common |
| Fibrosis | Rare | Common |
| Treatment | ASA, CS, IS, biologics, JAKi, S1PR modulator, surgery for refractory medical disease or malignancy | CS, IS, biologics, surgery for refractory medical disease, complication or malignancy |
| Prognosis | Complete remission in most patients, low surgical requirement | Prolonged remission in about 20% of patients, 10-yr surgical resection risk near 50% |

ANCA: Anti-neutrophil cytoplasmic antibody; ASA: Aminosalicylate; ASCA: Anti-*Saccharomyces cerevisiae* antibody; CS: Corticosteroid; IS: Immunosuppressant; JAKi: Janus kinase inhibitor; SIPR: Sphingosine-1-phosphate receptor.

with a Han Chinese-dominant population[27,28]. In the EAMs of AS patients, frequencies of about 30% have been found for anterior uveitis in both Caucasian and Han Chinese populations[29,30]. Typical attacks are abrupt and unilateral, with pain, photophobia and visual impairment, frequently alternating from one eye to another[1,2]. PsO occurs in more than 10% of Caucasians, more common than in Han Chinese patients[1,29]. There is a 5% to 10% incidence of IBD in AS patients from western countries[29], whereas frequencies of only 0.4% to 0.6% have been identified for IBD in Han Chinese AS populations [30,31]. In comparison with earlier years, there is a sharply increasing current incidence of IBD, without changes in AS prevalence from East Asia[16]. Despite a progressively narrowing gap between Asia and West, the prevalence of IBD remains much higher in Western countries compared to that in Asian nations. In the 21$^{st}$ century, the pooled prevalence of IBD in North America and Europe is estimated to be about 0.3% of the general population[17,18], whereas in the Han Chinese population, the recent prevalence of UC and CD per 100000 individuals has risen to 12.8 and 3.9 in Taiwan and 24.5 and 18.6 in Hong Kong, respectively[32,33]. Although genome-wide association studies have demonstrated shared risk alleles between the two disorders, the above-mentioned clinical observations suggest that ethnicity can be an important factor causing inconsistency in the coexistent frequencies of IBD in AS between Caucasian and Han Chinese populations. Further investigations in the gut-joint axis of inflammation in SpA should consider the issue of disconnection between the occurrence of IBD in AS on the basis of ethnicity, *i.e.*, Han Chinese or other races[23].

In addition to pharmacotherapy, physical therapy and regular exercise in AS patients, either with active or stable axial SpA, can improve the symptoms and functions by maintaining posture and spinal flexibility[34]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the drug of choice for initial therapy for axial SpA. There are frustrated outcomes regarding axial symptoms, spinal pain in particular, in AS patients receiving conventional disease-modifying anti-rheumatic drug (cDMARD) therapy, including methotrexate (MTX) and sulfasalazine (SAZ). Nevertheless, clinical evidence supports the use of cDMARDs for controlling peripheral arthritis in AS patients. With advances in the understanding of immunopathogenesis in AS[1,2,23], there are increasing numbers of novel medications, including biologics targeting TNF or interleukin (IL)-17 and small-molecule agents, Janus kinase inhibitors (JAKis) [34]. Such therapies have been associated with substantial improvements in disease activity and quality of life.

IBD manifestations in AS represent a clinical challenge by increasing the disease burden with difficulties in managing such patients[35]. Nevertheless, the introduction of new therapeutics targeting both articular and intestinal manifestations, TNF inhibitor (TNFi) in particular, has revolutionized the treatment of patients not responding to conventional medications[1,2,36,37]. In Table 3, the English-language literature is summarized for published reports related to the occurrences of IBD, flare-up or new-onset, in AS patients under the treatment of different TNF blockades, including adalimumab (ADA), certolizumab pegol (CZP), etanercept (ETA), golimumab (GOL), and infliximab (IFX)[38-62]. Notably, most of the enrolled cases were predominantly Caucasian. Since the dosages of TNFi for IBD therapy are higher than those used in AS, new-onset or flare events of IBD can occur in such patients during the therapeutic period, indicating the potential inefficacy of particular TNF blockade in the AS-associated IBD manifestation. Notably, monoclonal antibodies (mAbs) have better protective effects than recombinant soluble TNF receptor fusion proteins. Despite the lack of observed IBD events in AS patients during three GLO randomized clinical trials (RCTs), four cases were reported to have a flare at 2 mo to 5 mo after starting treatment[63].

Table 4 shows the demographic, clinical, laboratory, medication, course and outcome profiles in 4 AS-associated IBD patients, 3 UC patients, and 1 ulcerative proctitis (UP) patient with moderate to severe activity. All received endoscopic biopsy with characteristic histopathological changes (Figure 1A, B, G, and H). This 5-year observation enrolled 878 (86% male) Han Chinese AS patients by the authors. There was a 0.5% occurrence of IBD. At IBD onset, there was a long disease period (12 years to 25 years, 16.5 ± 5.8) with high-activity treated with NSAIDs and cDMARDs. For IBD therapy, corticosteroids (CSs) were prescribed in the acute stage with topical and systemic high-dose for case No. 1 and others, respectively, followed by aminosalicylate (ASA) or plus low-dose CS for maintenance. Nevertheless, all experienced a disease relapse, while case No. 3 had colonic perforation that required surgical intervention. Repeated endoscopic biopsy in case No. 1 showed chronic active rectitis (Figure 1C and D). Due to refractory activity, all started ADA injection with 40 mg biweekly. A relapse occurred in case No. 2 under the tapered dosage of 40 mg every 4 wk (Figure 1I and J); however, there were no more flares for 4.8 years after resuming a biweekly regimen. Altogether, all had no IBD flares under ADA 40 mg biweekly injection without CS, cDMARD, or immunosuppressants for 4.3 years to 5.8 years (5.1 ± 0.7). All had clinical IBD remission and only mild non-specific lymphocytic infiltration (Figure 1E, F, K, and L). Despite histopathological changes more resistant to resolution than clinical remission in IBD[64], whether microscopic healing provides additional outcome benefits remains to be determined.

Reactivation or development of IBD in AS patients receiving ETA therapy is thought to be caused by particular structure, administration mode, neutralizing effect, and/or pharmacokinetic characteristics of ETA[65]. Despite the indirect evidence based on the risks of IBD among AS patients during biologics therapy, the American College of Rheumatology (ACR)/Spondylitis Association of America/Spondyloarthritis Research and Treatment Network have recommended the treatment with anti-TNF mAbs over other biologics in adults with AS and coexisting IBD[66]. Notably, AS patients under IL-17 blockade therapy have increased risks of IBD development or exacerbation compared with the placebo-controlled group[67,68]. Moreover, according to the management recommendations of axial SpA from Assessment of Spondyloarthritis International Society/European League Against Rheumatism for the EAMs, anti-TNF mAbs are effective in IBD therapy and in the prevention of uveitis recurrence, whereas ETA has no effects on treating IBD and contradictory outcomes in uveitis prevention[69]. The use of a special mAb can be made in consultation with gastroenterologists due to different indications of mAbs in the IBD subtype, ADA and IFX for CD or UC, CZP for CD, and GOL for UC.

In the Han Chinese population, ADA is an effective biologic agent in controlling the articular activities in AS[70]. For ADA therapy in IBD, higher remission and response rates have been observed in China compared with those in Western countries[71]. Furthermore, its efficacy has also been demonstrated in moderate to severe IBD patients in Taiwan with more rigorous prescription criteria than in the West[72]. Interestingly, contradictory to our favorable therapeutic results without any UC flares in Han Chinese AS-associated IBD patients (Table 4), in an RCT of AS patients (97% Caucasian) under 40 mg ADA biweekly injection for 24 wk, 2 cases experienced a UC flare, 1.9 events per 100 patient-years *vs* none in the placebo group[54]. In systemic rheumatic disorders, clinical outcomes under the similar immunosuppressant treatment can be variable in different racial populations[73], while the ethnic factor has been considered to be involved in therapeutic responses to biologics therapy[74]. Further international collaborations in large-scale RCTs enrolling more ethnic groups might be needed to evaluate such an issue in AS-associated IBD.

UP patients with inflammation limited in the rectum can manifest as tenesmus, urgency, and rectal bleeding[75]. Such patients might fail to improve and require additional medications despite the beneficent effects of ASA and CS. Medical therapy in UP refractory to the standard treatment is challenging due to no evidence-based large-scale data of other medications[76]. In addition, UC patients limited to the rectum are usually excluded from the RCT on biologics therapy. Nevertheless, a recent referral cohort with 118 cases followed for up to 20 years revealed that UP resistant to conventional therapies could have clinical responses to anti-TNF mAbs[77]. Furthermore, long-term outcome in UP patients receiving biologics therapy was superior to azathioprine treatment, consistent with the results demonstrating beneficent efficacy of refractory UP under anti-TNF therapy from a retrospective cohort with 104 cases[77,78]. In our 5-year observation, a UP case (No. 1 in Table 4) resistant to ASA use

**Table 3 Inflammatory bowel disease manifestation in ankylosing spondylitis patients receiving approved tumor necrosis factor inhibitor or Janus kinase inhibitor therapy published in the English literature**

| No. | Clinical trials, *n* | Countries involved in clinical trials | Cases, *n* | TNFi or JAKi | IBD manifestation events, flare-up and new-onset | IBD manifestation events per 100 patient-yr[1] | Ref. |
|---|---|---|---|---|---|---|---|
| 1 | 7 | Canada, Germany, Netherlands | 366 | IFX | 1 CD | 0.2 | [38-44] |
| 2 | 9 | European nations, United Kingdom, United States | 724 | ETA | 14 (8 CD, 6 UC) | 2.0 | [45-52] |
| 3 | 5 | France, Germany, Netherlands, United States, *etc.* | 2026 | ADA | 14 | 0.7 | [53-55] |
| 4 | 3 | Canada, Germany, Netherlands, United States, *etc.* | 837 | GOL | 0 | 0 | [56-58] |
| 5 | 1 | Belgium, Canada, France, Germany, Netherlands, United States | 121 | CZP | 1 CD | 0.2 | [59,60] |
| 6 | 1 | Australia, Canada, European nations, United States, *etc.* | 133 | TOF | 0 | 0 | [61] |
| 7 | 1 | Australia, Canada, European nations, Israel, United States, *etc.* | 211 | UPA | 1 CD | 1.8 | [62] |

[1]One point six events per 100 patient-years in placebo groups by pooling 1015 ankylosing spondylitis patients under clinical trials[38,42,45,46,47,49,52,54,56,59,61,62].

ADA: Adalimumab; AS; Ankylosing spondylitis; CD: Crohn's disease; CZP: Certolizumab pegol; ETA: Etanercept; GOL: Golimumab; IBD: Inflammatory bowel disease; IFX: Infliximab; JAKi: Janus kinase inhibitor; No.: Number; Ref.: Reference; TNFi: Tumor necrosis factor inhibitor; TOF: Tofacitinib; UC: Ulcerative colitis; UPA: Upadacitinib.

showed a clinical remission under ADA therapy for more than 4 years.

A better understanding of the complex IBD pathogenesis has brought about a therapeutic approach focusing on clinical and histopathological remission with precise molecular targeting of inflammatory cascades. Since the successful results on the use of IFX in CD patients in 1997, three additional anti-TNF mAbs, two anti-integrin mAbs, three small-molecule agents including a sphingosine-1-phosphate receptor modulator and two JAKis, and two mAbs targeting the p40 subunit of IL-12/IL-23 and the p19 unit of IL-23 have been approved by the United States Food and Drug Administration (FDA), expanding the options for IBD treatment[79].

The signaling pathway of JAK-signal transducer and activator of transcription (STAT), including JAKs 1-3, STATs 1-6, and tyrosine kinase 2, can regulate miscellaneous cytokine receptors and has pathogenic roles in various autoimmune and inflammatory disorders[80]. Furthermore, individual cytokine receptors can recruit their own combined JAKs and STATs to activate distinct processes in different targeted cells, while antagonizing a specific JAK can inhibit diverse cytokine pathway, expanding the effects of JAKi on cytokine-targeted therapy[81]. Tofacitinib (TOF), a pan-JAKi targeting JAKs 1-3, and upadacitinib (UPA), a selective JAK1 inhibitor, have been approved by the FDA in adult UC with moderately to severely activity with intolerance or poor responses to TNF mAbs in 2018 and 2022, respectively, overcoming the challenges of using biologics to avoid immunogenicity induction and parenteral administration[79]. Furthermore, TOF and UPA have received an indication in adult AS with an inadequate response or intolerance to TNFi in 2021 and 2022, respectively (Table 3). In a recent phase III RCT enrolling 136 AS patients with more than 80% Caucasians, there were no observed IBD events under TOF 5 mg bid therapy for 16 wk[61], validating its expected effects for UC manifestation in TNFi-refractory AS patients. Nevertheless, there was an observed new-onset CD event under UPA 15 mg once daily treatment for 14 wk in another phase III RCT enrolling 209 AS patients dominant in Caucasians (1.8 events per 100 patient-years)[62].

IL-12 helps naïve T cells differentiate into type 1 T helper (Th1) cells secreting IL-6, interferon (IFN)-γ and TNF, while IL-23 stimulates Th17 cells to express IL-17, IFN-γ, TNF, granulocyte-macrophage colony-stimulating factor, and IL-21, all of which promote mucosal inflammation in IBD patients[79,82]. IL-12 is encoded by two separate genes, IL-12A (p35) and IL-12B (p40), to form an active heterodimer following protein synthesis with p35 and p40 chains, while IL-12 p40 chain can dimerize with IL-23 p19 chain to form IL-23[82]. Ustekinumab (UST), a p40 chain mAb, was approved for the treatment of moderately to severely active CD patients who failed or were intolerant to treatment with anti-TNF therapy in 2016, and for moderately to severely active UC in 2019[79]. Moreover, risankizumab (RIS), a

**Table 4 Demographic, clinical, laboratory, medication, course, and outcome profiles in 4 ankylosing spondylitis-associated inflammatory bowel disease patients from 2017 January to 2021 December[30]**

| No. | Age in yr and sex | [1]AS period in yr | Affected joints | Other EA | [3]BASDAI/[2] AS medication | HLA-B27/[3] ESR | IBD clinical manifestation | IBD entity/[6] severity | [4]IBD medication | Disease course, under ADA 40 mg q2w SCI | Final outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 42, F | 12 | SI, spine, hip | Uveitis | 7.6/NSAIDs | Positive/38 | Rectal bleeding, BWL, anemia | UP/ moderate, MS 9 | CS, mSAZ, ADA 40 mg q2w | No IBD relapse for 4.3 yr | AS in low activity with BASDAI 2.0-2.5, IBD in remission, MS 0 |
| 2 | 35, M | 15 | SI, spine, hip | Uveitis | 8.8/NSAIDs, SAZ | Positive/80 | Bloody diarrhea, BWL, fever, anemia | UC/severe, MS 12 | CS, mSAZ, ADA 40 mg q4 to q2w | No IBD relapse for 4.8 yr | AS in low activity with BASDAI 2.5-3.0, IBD in remission, MS 1 |
| 3 | 45, M | 14 | SI, spine, hip | Nil | 8.4/NSAIDs, SAZ | Positive/42 | Bloody diarrhea, BWL, anemia, [5] colon perforation | UC/severe, MS 11 | CS, SAZ, ADA 40 mg q2w | No IBD relapse for 5.8 yr | AS in low activity with BASDAI 2.5-3.0, IBD in remission, MS 2 |
| 4 | 45, F | 25 | SI, spine, shoulder hip | Nil | 8.1/NSAIDs, SAZ, MTX | Positive/35 | Bloody diarrhea, BWL, anemia | UC/severe, MS 11 | CS, SAZ, ADA 40 mg q4 to q2w | No IBD relapse for 5.3 yr | AS in low activity with BASDAI 2.5-3.0, IBD in remission, MS 1 |

[1]AS duration before the inflammatory bowel disease (IBD) development.
[2]Methotrexate 15 mg per wk, salazopyrin 2 to 3 g/d, nonsteroidal anti-inflammatory drugs only with Coxibs after IBD diagnosis.
[3]At disease onset of IBD.
[4]High-dose corticosteroids (CS, 1-2 mg/kg/d prednisolone equivalent doses) for acute ulcerative colitis (UC), topical CS for active ulcerative proctitis, low-dose CS for UC maintenance.
[5]Perforation at the splenic flexure, under double barrel colostomy and abdominal abscess drainage.
[6]MS: Mayo score, 11-12 severe, 6-10 moderate, 3-5 mild, 0-2 remission.
Age at diagnosis of inflammatory bowel disease. ADA: Adalimumab; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BWL: Body weight loss; CS: Corticosteroids; ESR: Erythrocyte sedimentation rate (normal value ≤ 15 mm/h); F: Female; IBD: Inflammatory bowel disease; M: Male; mSAZ: Mesalazine; MTX: Methotrexate; No.: Number; NSAIDs: Nonsteroidal anti-inflammatory drugs; q2w: Every 2 wk; SCI: Subcutaneous injection; UP: Ulcerative proctitis; SAZ: Salazopyrin; SI: Sacroiliac; UC: Ulcerative colitis; WNL: Within normal limit.

p19 chain mAb, was approved for the treatment of moderately to severely active CD patients who failed or were intolerant to treatment with TNF blockers in 2022. Nevertheless, both UST and RIS have no indication for treating TNFi-refractory AS patients. In a national cohort study evaluating the long-term UST effects in 152 CD patients including 17 associated with AS, efficacy was not identified in SpA symptoms[83].

## PSORIATIC ARTHRITIS

PsA, a chronic inflammatory arthritis with impaired function and reduced quality of life, develops in up to 30% of PsO patients[84]. Both axial and peripheral joints can be involved with five clinical patterns not mutually exclusive, including the most commonly observed asymmetric oligoarticular, symmetric polyarticular, distal interphalangeal joint-predominant, axial/SpA-predominant, and the rarely identified deforming/destructive subtype, *i.e.*, arthritis mutilans[4,84]. Cutaneous lesions can be found in most cases at the time of articular presentation; however, in up to 15% of PsA patients, arthritis can antedate the appearance of skin disease, *i.e.*, PsA sine PsO[85]. Dactylitis or enthesitis has been reported in up to 50% of patients. In addition, about 40% to 50% of patients are positive for HLA-B27, higher in axial than the peripheral-only type[84]. The prevalence of PsA is between 0.3% and 1.0% in the United States[84], whereas it is much lower in Han Chinese, with the prevalence ranging from 0.01% to 0.1%[86, 87].

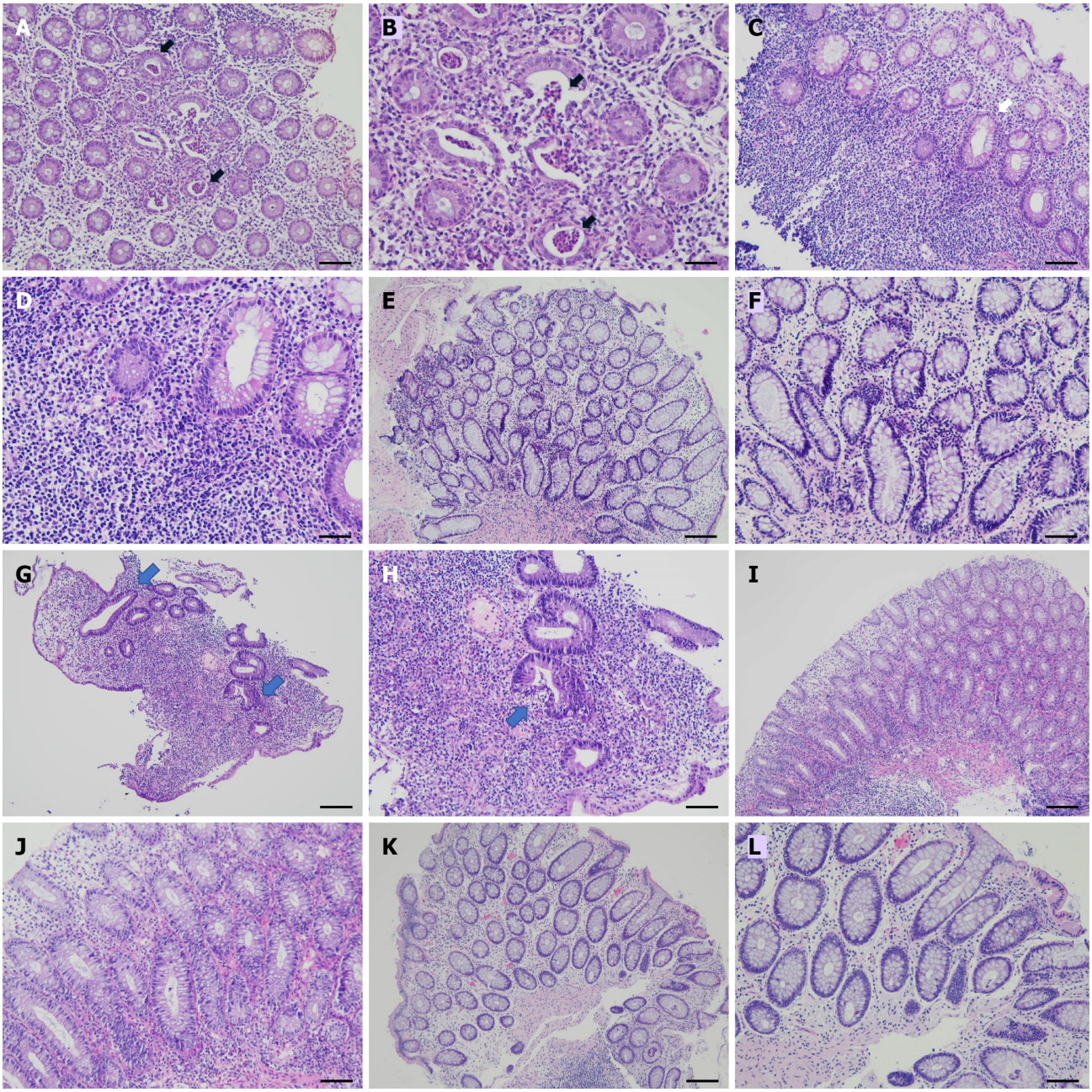


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**Figure 1 Serial histopathological findings of ulcerative proctitis before and after therapy from rectum biopsy specimens.** Hematoxylin and eosin stain. A and B: Rectal mucosa before therapy shows acute rectitis with neutrophilic infiltrates and crypt abscess in case No. 1 (arrows), 100 × (A) and 200 × (B); C and D: Rectal mucosa shows features of chronicity including dense lymphocytic infiltration, basal lymphoplasmacytosis and crypt distortion in case No. 1 (arrow), 100 × (C) and 200 × (D); E and F: Rectal mucosa after adalimumab therapy shows mild non-specific lymphocytic infiltration in case No. 1, 100 × (E) and 200 × (F); G and H: Colonic mucosa before therapy shows crypt distortion (arrows) and lymphoplasmacytic infiltration in the lamina propria in case No. 2, 100 × (G). The crypt shows distortion and neutrophilic infiltration (arrow), 200 × (H); I and J: Colonic mucosa shows less crypt distortion and lymphoplasmacytic infiltration as compared with (G and H) before adalimumab (ADA) therapy in case No. 2, 100 × (I) and 200 × (J); K and L: Colonic mucosa after ADA 40 mg injection once every 2 wk shows mild non-specific lymphocytic infiltration in case No. 2, 100 × (K) and 200 × (L). Bars shown on 100 × and 200 × photomicrographs correspond to 100 μm and 50 μm, respectively.

Uveitis has been identified in 8% of PsA patients, affecting the anterior and posterior poles of the eyes [84]. Comorbidities in PsA are associated with more severe disease activities, including diabetes, hypertension, hyperlipidemia, metabolic syndrome and fatty liver, while there is an increased risk of cardiovascular events[88]. It is estimated that 9.6% of CD patients have PsO (2.2% in the general population), while 0.5% of PsO patients have CD (0.2% in general population)[89]. Despite there being a lower occurrence than in CD, there is a similar trend between patients with UC and PsO[90]. In comparison with patients with PsO alone, patients with PsA have a higher risk of IBD coexistence[91]. Cohort studies have demonstrated an increased risk of concomitant CD[92] or UC[93] in PsA patients. Furthermore, IBD is more common in PsA patients with greater activities, and in the axial than the

peripheral-only subtype[94].

Based on high-quality, evidence-based, domain-focused recommendations for medication selection in PsA, the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis updated treatment recommendations for such patients in 2021[95]. Choice of therapy for an individual patient should ideally address all active disease domains, related EAMs, and comorbidities. For patients with axial involvement not responding to NSAID use, initiation of a targeted therapy is strongly recommended, including TNFi, IL-17i, and JAKi. For peripheral arthritis, cDMARDs such as MTX and SAZ can be used as first-line therapy. In dactylitis, enthesitis, nail or topicals-unresponsive PsO, cDMARD-refractory peripheral arthritis, recent evidence supports the use of IL17i (ixekizumab, SEK), IL23i (guselkumab, RIS), JAKi (TOF, UPA), phosphodiesterase 4 inhibitors (PDE4is, apremilast), TNFi (ADA, CZP, ETA, GOL, IFX), and UST. For PsA-related EAMs, MTX or TNF mAbs can be used for the treatment of anterior uveitis. TNF mAbs and UST have demonstrated their therapeutic efficacy in CD and UC. TOF and UPA are effective in treating UC, while RIS has efficacy in CD therapy. Notably, IL17i can increase the risk of IBD onset or exacerbation, and their use should be avoided in IBD, even in disease remission [25,96]. Since comorbidities are associated with greater PsA activity and reduced therapeutic responses, their recognition and monitoring with appropriate management is important for health-care providers caring for such patients[91,95].

## REACTIVE ARTHRITIS

ReA has a sterile, transient nature, typically with an asymmetric oligoarthritis of the lower limbs following a preceding genitourinary or gastrointestinal tract infection, ranging from several days to weeks [97-99]. Depending on the causative agents and other factors, after more than 6 mo, about one-quarter of patients can progress into chronic arthritis requiring long-term therapy. This disease shares the overlapping features of seronegative SpA, including HLA-B27 association, axial involvement, sacroiliitis, enthesitis, dactylitis, and EAMs. It is an uncommon disease that occurs in young adults, with a global prevalence of 0.02% to 0.04%[86,97-99]. Post-venereal ReA most commonly affects men, while post-enteric ReA affects men and women equally. Genital *Chlamydia trachomatis* is the most common cause of ReA, and other common responsible enteric strains include *Yersinia*, *Salmonella*, *Shigella*, and *Campylobacter*. Similar to human immunodeficiency virus-induced ReA, the development of ReA has been identified in post-coronavirus disease 2019 (COVID-19) illness, with negative results of synovial COVID-19 polymerase chain reaction test[99].

Eye involvement including anterior uveitis and conjunctivitis preceding arthritis occurs in one-fifth of patients[100], while up to half of cases have mucocutaneous lesions with characteristic keratoderma blennorhagicum and circinate balanitis[97-99]. Upon colonoscopic biopsies of the terminal ileum and colon, histological alterations mimicking IBD with the features of acute enterocolitis or early CD were found in two-thirds of ReA patients despite an asymptomatic condition in most cases[101]. Notably, there are no known reports of increased IBD occurrences in ReA patients.

Although the disease course of post-dysentery ReA is unaltered by antibiotics use, such therapy is indicated for the identification of *C. trachomatis* infection[98,99]. Due to a self-limited nature in most ReA patients, NSAIDs are prescribed as first-line therapy. In patients not responding to NSAIDs or with chronic ReA, cDMARDs are indicated with SAZ as the drug of choice and MTX as an alternative. In patients refractory to cDMARD treatment, off-label use of ETA has shown beneficial effects[102].

## ENTEROPATHIC ARTHRITIS

Musculoskeletal conditions with articular, periarticular, muscular, and skeletal manifestations are frequently observed, with an up to 50% frequency in IBD patients[103]. Rheumatological EIMs are associated with HLA-A2, DR1, and DQw5 alleles in CD, and with DRB1*0103, B27, and B58 alleles in UC[104]. Arthritis is the most common EIM in IBD involving axial (spondylitis, sacroiliitis), peripheral joints, or a combination. The prevalence of arthritis decreases with increasing age in IBD patients[105]. It occurs equally in both sexes, more commonly in CD with colon involvement than in UC, and can precede, be concomitant with, or follow the onset of IBD[106]. Peripheral arthritis can be classified into two entities: Type 1 pauciarticular and type 2 polyarticular (Table 5)[107-109]. Type 1 arthropathy is often acute, asymmetrical, and affecting less than five joints, commonly involving the large knee joint. It is usually related to IBD activity and is self-limiting, with a duration of no more than 10 wk. Treatment of the underlying intestinal inflammation is usually associated with improvement of arthritis. Type 2 arthropathy is a symmetrical arthritis involving five or more joints, commonly involving the small metacarpophalangeal joint. It is not related to IBD activity and may persist for years with articular erosion and destruction. There is an association of type 1 arthropathy with erythema nodosum, uveitis and HLA-DRB1*0103, B35 and B24, and type 2 arthropathy with uveitis and HLA-B44[108]. Notably, such a categorization of peripheral arthritis can be related more to the duration and progression of articular presentation, while the patients with a polyarticular manifestation can begin their clinical

**Table 5 Classification of inflammatory bowel disease-associated peripheral arthritis**

| Category | Type 1 pauciarticular | Type 2 polyarticular |
|---|---|---|
| Prevalence | 4% to 5% in IBD, higher in CD than UC | 3% in IBD, higher in CD than UC |
| Joint manifestation | | |
| Involved numbers | < 5 | ≥ 5 |
| Articular distribution | Large joint, asymmetric | Mainly small joint |
| Involved area with the decreasing frequencies | Knee, ankle, wrist, elbow, MCP, hip, shoulder, MTP, PIP | MCP, knee, PIP, wrist, ankle, elbow, hip, shoulder, MTP |
| Erosion/destruction | Absent | Present |
| Clinical course | Early in IBD disease course, acute and self-limiting (mostly under 10 wk) | Arthritis for months, episodic exacerbation for yr |
| Disease characters | | |
| IBD activity | Parallel with activity | Independent of activity |
| Other EIM | EN, uveitis | Uveitis |
| HLA association | HLA-B27, B35, DR*0103 | HLA-B44 |
| Treatment | Control of IBD activity, coxibs, CS, cDMARDs (SAZ 1$^{st}$ choice), TNF mAbs for refractory cases, JAKi for anti-TNF failure | Coxibs, CS, cDMARDs (SAZ 1$^{st}$ choice), TNF mAbs for refractory cases, JAKi for anti-TNF failure |

CD: Crohn's disease; cDMARD: Conventional disease-modifying antirheumatic drug; Coxib: Cyclooxygenase 2 inhibitor; EIM: Extra-intestinal manifestation; EN: Erythema nodosum; HLA: Human leukocyte antigen; IBD: Inflammatory bowel disease; JAKi: Janus kinase inhibitor; mAb: Monoclonal antibody; MCP: Metacarpophalangeal; MTP: Metatarsophalangeal; PIP: Proximal interphalangeal; PsA: Psoriatic arthritis; SAZ: Salazopyrin; S1PR: Sphingosine-1-phosphate receptor; TNF: Tumor necrosis factor; UC: Ulcerative colitis.

course with an oligoarticular involvement[104].

Axial involvement can be a part of IBD but independent of gut pathology. It is more common in CD than in UC, with an up to 25% frequency[110]. Most IBD patients with axial spondylitis are HLA-B27-positive despite a lower association rate than idiopathic AS, 50% to 70% *vs* more than 90%. There is a 5% to 10% occurrence of AS in IBD with a 1:1 sex ratio and a development at any age, rather than a 3:1 male to female ratio and onset before 40 years of age in idiopathic AS[111]. Although sacroiliitis can be detected by magnetic resonance imaging (MRI) in IBD patients, most of them are asymptomatic, HLA-B27-negative and without progression into AS[112]. The prevalence of symptomatic sacroiliitis is estimated to be less than 10%.

Dactylitis, enthesitis, and tenosynovitis also occur in IBD patients as musculoskeletal EIMs. Enthesitis presenting with Achilles tendinitis, plantar fasciitis, and chest wall pain can lead to structural changes of underlying bones with functional disability[109]. Ultrasonography or MRI examination of the affected area can help in earlier detection missed by clinical inspection[113].

NSAIDs are suggested as initial therapy for peripheral and axial SpA. Nevertheless, their use is controversial in IBD due to an association with the development of intestinal ulcerations and flares of IBD[114]. Although the safety of cyclooxygenase 2 inhibitors have been investigated[115,116], their use should be limited to a short course during the IBD remission. Systemic CS can be helpful for peripheral arthritis despite ineffectivity in controlling axial SpA and enthesitis, while intra-articular CS injection may be effective in cases with limited numbers of joint involvement[106,109]. SAZ, a formulation of ASA available in intestinal therapy, is an effective cDMARD in improving peripheral arthritis, but not axial arthritis or sacroiliitis in IBD patients[117]. MTX is an alternative cDMARD recommended for the treatment of IBD-associated peripheral SpA[118]. Furthermore, TNFi can be reserved for patients with IBD-associated axial or peripheral SpA not responsive to conventional therapies[119]; however, ETA should be avoided due to its inefficacy for IBD treatment and a potential clinical exacerbation[120]. Since the doses of TNF blockade for IBD therapy is higher than those used in treating SpA, it is recommended that high-dose regimen is preferred in IBD-associated SpA during active intestinal disease[121]. Two JAKis have been approved for treating TNFi-refractory UC and AS/PsA patients. Despite the lack of an evidence-based indication, such therapy might be considered in UC-related EnA patients lacking therapeutic responses to anti-TNF therapy. Although UST use is indicated for IBD and PsA therapy, it is only effective in CD/UC-associated peripheral rather than axial SpA[122].

At least 5% of IBD patients, more frequently in CD than UC, experience ocular EIM, with uveitis as the commonest manifestation, particularly in those cases associated with arthritis[123,124]. Anterior uveitis in patients with IBD is initially treated with CS eye drops, followed by systemic CS or IS if unsuccessful[104]. Anti-TNF mAbs have shown efficacy in IBD-associated uveitis, while their use can be

considered in cases refractory to the aforementioned treatment[125].

## JUVENILE-ONSET SPONDYLOARTHRITIS

JSpA, a distinct disease to adult SpA, constitutes up to one-third of juvenile idiopathic arthritis (JIA), and usually affects males and starts in early adolescence (before the age 16)[126-129]. This disease primarily affects children fulfilling the criteria for JIA categories of enthesitis-related arthritis (ERA) and juvenile psoriatic arthritis (JPsA) as well as undifferentiated arthritis with either features[127,130]. An approximate 20% prevalence of JSpA was found in JIA cohort studies[131], while approximately 10% of adult AS patients have an onset of disease in childhood[1,3]. There is HLA-B27 positivity detected in 40% to 60% of ERA, whereas only 10% of JPsA patients show HLA-B27 positivity[129]. JSpA commonly manifests with peripheral arthritis, usually asymmetric, oligoarticular, involving joints of the lower extremities including the hip, knee, ankle, and midfoot. Tender entheses are commonly present at insertions of the patellar ligament at the inferior patella, plantar fascia at the calcaneus, and the Achilles tendon[132]. About 40% to 60% of ERA cases have sacroiliitis, an early sign of axial involvement, in their disease course[129]. Nevertheless, children are known to have silent sacroiliitis without inflammatory back pain[127]. Dactylitis can be observed in 30% of patients with JPsA[133]. JSpA has a poorer outcome with more spinal deformity and need for total hip replacement, as compared with cases of other forms of JIA and their adult counterparts[127].

Similar to the adult-onset disease, common EAMs in JSpA include skin, eye, and bowel involvement [126-129]. The overall prevalence of uveitis, more common with acute anterior uveitis, is approximately 10%[134]. Two-thirds of children with SpA have been reported to have gastrointestinal symptoms[135]. Intestinal inflammation on ileocolonic biopsy has been identified in JSpA[136], while ERA with sacroiliitis had increased levels of fecal calprotectin, a gastrointestinal inflammation marker[137]. IBD in children might begin with arthritis before clinically evident intestine inflammation, while difficult-to-control arthritis, longstanding, vague gastrointestinal complaints and anemia might be helpful clues for earlier diagnosis[138]. In a large-scale survey of 3071 JIA patients, 11 with 4 JSpA had IBD (8 CD, 3 UC); furthermore, there was 1.31 case per 1000 patient-years, higher than the annual incidences of 10 cases per 100000 in pediatric populations of western countries[139]. In another large-scale investigation with 8942 JIA patients, 48 had IBD (22 CD, 13 UC, 13 indeterminate), showing a prevalence of 0.54%, much higher than the reported 0.02% in a Western pediatric population[140]. Furthermore, the occurrences of IBD were identified in 2% to 6% of ERA patients and 0.3% to 0.5% of JPsA patients[133,140,141].

According to the 2019 ACR guidelines for JIA treatment[142], initial therapy with a cDMARD is recommended over NSAID monotherapy, while MTX is suggested over other cDMARDs. Oral CS is only recommended as bridging therapy, with a limited course of less than 3 mo. Furthermore, initial biologic therapy (ADA, ETA, GOL, abatacept, tocilizumab) may be considered for patients with risk factors (seropositivity, articular damage), involvement of high-risk joints (cervical spine, wrist, hip), or high disease activity. For sacroiliitis and enthesopathy, NSAID therapy is recommended, while TNFi is suggested for refractory cases. Notably, UST and SEC have been approved for use in JPsA and ERA/JPsA patients, respectively, as an option for TNFi-resistant patients[143,144]. Although ADA and IFX are both approved in treating pediatric CD and UC, the incidence of IBD in JIA patients was increased in those receiving IFX but not ADA therapy[140]. Furthermore, IFX use is not approved in JIA patients. ADA appears to be a drug of choice for treating patients with JSpA-associated IBD. Nevertheless, there are scarce data regarding ADA use in pediatric patients with joint diseases associated with IBD[145].

Finally, Table 6 lists current FDA-approved indications of biologics and small molecules for seronegative SpA (AS, PsA), JIA and IBD (UC, CD) patients discussed in this review.

## CONCLUSION

Seronegative SpA usually starts in the third decade of life with the HLA-B27 genetic marker and clinical features of spinal and peripheral arthritis, dactylitis, enthesitis and EAMs. This group of patients who have negative rheumatoid factor can be classified into AS, PsA, ReA, EnA and JSpA cases. Joint and gut inflammation are intricately linked in SpA and IBD, with shared genetic and immunopathogenic mechanisms. IBD is a common EAM in SpA patients, while EIMs in IBD patients mostly affect the joints. Although individual protocols for managing each disease have been established, the standard therapeutic guidelines of SpA-associated IBD patients remain to be established. NSAIDs are recommended as initial therapy of peripheral and axial SpA, while their use is controversial in IBD due to associated disease flares. cDMARDs are beneficent for peripheral arthritis but ineffective in axial SpA or IBD therapy. Anti-TNF mAbs are effective medications with indicated use in SpA and IBD, being a drug of choice for treating SpA-associated IBD. JAKi, approved in treating SpA and UC, are promising therapeutics in SpA co-existent with UC. A tight collaboration between gastroenterologists and rheumatologists is needed in managing such complex clinical scenarios.

**Table 6 Generic names and currently approved indications of biologics and small molecules from the United States Food and Drug Administration for ankylosing spondylitis, psoriatic arthritis, juvenile idiopathic arthritis, and inflammatory bowel disease**

| Category | AS | PsA | JIA[1] | UC | CD |
|---|---|---|---|---|---|
| Biologics/TNFi | | | | | |
| Etanercept | X | X | X | | |
| Infliximab | X | X | | X | X |
| Adalimumab | X | X | X | X | X |
| Golimumab | X | X | X | X | |
| Certolizumab pegol | X | X | | | X |
| Biologics/IL-17i | | | | | |
| Ixekizumab | X | X | | | |
| Secukinumab | X | X | X | | |
| Biologics/IL-12/23i | | | | | |
| Ustekinumab | | X | X | X | X |
| Biologics/IL-23i | | | | | |
| Guselkumab | | X | | | |
| Risankizumab | | X | | | X |
| Biologics/IL-1i | | | | | |
| Canakinumab | | | X | | |
| Biologics/IL-6i | | | | | |
| Tocilizumab | | | X | | |
| Biologics/anti-integrin mAb | | | | | |
| Natalizumab | | | | | X |
| Vedolizumab | | | | X | X |
| Biologics/anti-CTLA-4 mAb | | | | | |
| Abatacept | | X | X | | |
| Small molecules/JAKi | | | | | |
| Tofacitinib | X | X | X | X | |
| Upadacitinib | X | X | | X | |
| Small molecules/PDE4i | | | | | |
| Apremilast | | X | | | |
| Small molecules/S1PR modulator | | | | X | |

[1]Tumor necrosis factor inhibitor, abatacept, tocilizumab and tofacitinib for polyarticular juvenile idiopathic arthritis (JIA), canakinumab and tocilizumab for systemic JIA, secukinumab and ustekinumab for juvenile psoriatic arthritis, secukinumab for enthesitis-related arthritis.

AS: Ankylosing spondylitis; CD: Crohn's disease; FDA: United States Food and Drug Administration; IL: Interleukin; JAKi: Janus kinase inhibitor; JIA: Juvenile idiopathic arthritis; PDE4i: Phosphodiesterase 4 inhibitor; PsA: Psoriatic arthritis; S1PR: Sphingosine-1-phosphate receptor; TNFi: Tumor necrosis factor inhibitor; UC: Ulcerative colitis.

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## FOOTNOTES

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*REVIEW*

# Review of ferroptosis in colorectal cancer: Friends or foes?

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## Abstract

Ferroptosis is a newly discovered type of cell-regulated death. It is characterized by the accumulation of iron-dependent lipid peroxidation and can be distinguished from other forms of cell-regulated death by different morphology, biochemistry, and genetics. Recently, studies have shown that ferroptosis is associated with a variety of diseases, including liver, kidney and neurological diseases, as well as cancer. Ferroptosis has been shown to be associated with colorectal epithelial disorders, which can lead to cancerous changes in the gut. However, the potential role of ferroptosis in the occurrence and development of colorectal cancer (CRC) is still controversial. To elucidate the underlying mechanisms of ferroptosis in CRC, this article systematically reviews ferroptosis, and its cellular functions in CRC, for furthering the understanding of the pathogenesis of CRC to aid clinical treatment.

**Key Words:** Ferroptosis; Colorectal cancer; Cell death; Therapy



**Core Tip:** Ferroptosis, a novel type of cell-regulated death, has diverse roles in the occurrence and development of colorectal cancers (CRCs). This article reviews the cellular functions of ferroptosis in CRC, providing potential therapeutic targets and treatment strategies for patients with CRC.

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## INTRODUCTION

Regulated cell death, including apoptosis, necroptosis, pyroptosis, ferroptosis, autophagy-dependent cell death, netotic cell death, and other forms, is an important mechanism for regulating the internal environment of the human body, and maintaining tissue function and morphology[1,2]. Ferroptosis, which was formally proposed in 2012, is a unique form of death that depends on the disorder of iron metabolism and accumulation of lipid reactive oxygen species (ROS). It differs from other forms of regulated cell death in terms of morphology, biochemical characteristics, and gene expression[2]. Especially in terms of morphology, ferroptosis involves unique mitochondrial alterations, concerning mitochondrial morphological disorder, membrane potential change, iron overload in the membrane and lipid ROS accumulation, that are different from other death forms[3]. The underlying mechanisms and pathways involved in ferroptosis include glutathione peroxidase/glutathione (GPx/GSH), system $Xc^-$ and p53 regulatory pathways. Usually, the pathways involved in ferroptosis ultimately regulate ROS accumulation through iron accumulation[2,4,5]. At present, the inhibitory effect of ferroptosis on tumor formation and development has been increasingly gaining attention, and its discovery has led to important progress in the diagnosis and treatment of tumors, as well as prognosis.

Colorectal cancer (CRC) is a malignant tumor of the digestive system and is associated with high morbidity and mortality. According to the 2018 GLOBOCAN assessment of global morbidity and mortality, CRC is the third most diagnosed cancer and the second leading cause of cancer-related death globally[6], and is characterized by multiple steps and stages during progression[7]. Currently, treatments for CRC patients include surgery, radiotherapy, chemotherapy, immunotherapy and biological targeted therapy[8]. However, due to the lack of highly specific biomarkers and the complex biological characteristics of CRC, the lack of drugs targeting colorectal stem cells, and chemoresistance or intolerance to current treatment methods continue to hamper treatment[9,10]. Ferroptosis, as a form of regulated cell death independent of other forms of cell death, could provide an effective strategy for CRC treatment. In addition, a large number of recent studies have shown that ferroptosis-related genes can be used to predict the prognosis of patients with CRC, which is of great significance for improving the clinical efficacy of cancer treatment and the survival of patients[11-13].

## THE MOLECULAR MECHANISM OF FERROPTOSIS

### *Discovery of ferroptosis*

Erastin, a compound with the ability to kill tumor cells expressing high levels of the *Ras* oncogene, was discovered to induce a novel cell-death form that differed from apoptosis in terms of nuclear morphology, DNA fragmentation and caspase 3 activation[3,14,15]. Although the form of cell-death induced by erastin was not well elucidated at that time, other Ras-selective-lethal compounds (RSLs), such as RSL3 and RSL5, have been shown to trigger the same process, accompanied by increases in ROS levels that could be suppressed by iron chelators[3,16].

Ras protein, encoded by the well-known *RAS* oncogene, binds guanosine 5'-diphosphate (GDP)/guanosine 5'-triphosphate (GTP) and possesses intrinsic GTPase activity[17]. Mutation of Ras is related to the loss of GTPase activity, providing a possible therapeutic strategy of recovering Ras GTPase function in RAS-mutant cancer cells as an effective means to combat cancer[18]. Based on previous findings, Dixon *et al*[2] defined the unique non-apoptotic cell death caused by erastin and RSLs as ferroptosis[2]. Ferroptosis, an iron-dependent form of cell death, is characterized by increases in intracellular ROS, but is distinguished morphologically, biochemically and genetically from other regulated cell death forms, such as apoptosis, necrosis and autophagy, in ways that will be specifically described in the following sections. Since the proposal of the concept of ferroptosis, the mechanism of ferroptosis has become an area of intense research, leading to progress in the study of anti-cancer drugs focused on ROS homeostasis.

### *The molecular mechanism of ferroptosis*

The imbalance between production and degradation of intracellular lipid ROS is the central mechanism of ferroptosis-mediated cell death[2,19]. If the antioxidant capacity of cells is decreased, excessive iron will initiate ferroptosis by producing lethal ROS *via* the Fenton reaction and cause ROS accumulation accordingly[2,5]. In addition, GSH depletion is also important for the induction of ferroptosis and subsequent nicotinamide adenine dinucleotide phosphate (NADPH)-dependent lipid peroxidation[5].

Thus, intracellular ROS accumulation due to iron excess is the key for initiating ferroptosis (Figure 1).

No matter whether using erastin or RSL, the induced ferroptosis is related to iron-dependent accumulation of ROS. In the normal intracellular environment, lipid oxidation and reduction are in a state of dynamic equilibrium. When cellular homeostasis is disrupted, gene expression related to lipid oxidation is up-regulated or that related to lipid reduction is inhibited, causing a high accumulation of intracellular oxidized lipid[20]. However, the sources of ROS are still unclear. Hassannia *et al*[21] pointed out that peroxidation of phospholipids containing polyunsaturated fatty acids (PUFAs) in cell membranes could also lead to ferroptosis[21]. During induction of ferroptosis, PUFAs can form phospholipid hydroperoxides (PLOOHs) through enzymatic or non-enzymatic oxidation reactions. PLOOHs combined with intracellular iron will generate toxic lipid free radicals, such as alkoxy radicals, causing cell damage. Furthermore, these free radicals can extract protons from adjacent PUFAs, initiating a new round of lipid oxidation and delivering further oxidative damage[21,22]. Overall, ROS-mediated cell lipid damage is required for ferroptosis.

Obviously, intracellular iron plays a vital role during the process of ferroptosis, involving the absorption and reduction processes of iron[23]. Iron ingested in food is mainly absorbed, into the blood, as the ferric ($Fe^{3+}$) form in the duodenum and upper jejunum, and transferred by plasma transferrin into cells, where it is converted to the reduced ferrous ($Fe^{2+}$) form by metalloreductases in the endoplasmic reticulum (ER). $Fe^{2+}$ is the main form of iron that participates in metabolic processes. Therefore, inhibition of iron absorption and reduction, such as silencing transferrin receptor expression, could inhibit erastin-induced ferroptosis, whereas the elevation of heme catabolism or iron supplementation could restore and accelerate ferroptosis[24-26].

Generally, $Fe^{2+}$ is transported into cells and stored as ferritin to protect cells from iron toxicity[27]. In order to exert biological activity, $Fe^{2+}$ has to be released into the active iron pool in the cytoplasm *via* iron pump solute carrier family 11 member 2/divalent metal transporter 1 (SLC11A2/DMT1), while the extra $Fe^{2+}$ will either be recycled or stored as ferritin[28,29]. Up-regulation of ferritin gene expression restricts iron overload, whereas knockout of the *SLC11A3* gene, which blocks iron transport out of the cells, aggravates erastin-induced ferroptosis in neuroma cells[30-32]. In the case of iron deficiency, ferritin is degraded by autophagy through the ATG5-ATG7-nuclear receptor coactivator 4 (NCOA4) signaling pathway, where NCOA4 binds to and transports ferritin to the lysosome, releasing $Fe^{2+}$ to abnormally increase the labile iron pool. Subsequently, through the Fenton reaction, excess hydroxyl and peroxy radicals can be generated to initiate ferroptosis. Deletion of ATG5, ATG7 or NCOA4 will prevent erastin-induced ferroptosis by limiting ferritin degradation and reducing intracellular ferrous iron levels[33-35].

### *Signaling pathways involved in ferroptosis*

The previous findings show that the key link causing ferroptosis involves increased lipid peroxidation and accumulation of ROS. Generally, the ferroptotic upstream pathways ultimately affect the activity of GPx directly or indirectly[4,36,37]. Consequently, GPx family members play an indispensable role in the process of ferroptosis. Among the 8 GPx family members, GPX4, a selenoprotein that inhibits lipid oxidation, has been shown to be the main regulator of ferroptosis[38].

GPX4, a selenoprotein capable of degrading small molecular peroxides and relatively complex lipid peroxides, is also able of reducing cytotoxic lipid hydroperoxides to non-toxic lipid alcohols, preventing the formation and accumulation of lethal ROS[39,40]. Knocking down GPX4 with siRNA results in cell sensitivity to ferroptosis, whereas up-regulating GPX4 induces resistance to ferroptosis[4,36,37]. In fact, RSL3, noted above as an important ferroptotic inducer, can directly suppress the activity of GPX4, thereby inducing ferroptosis[2,41,42]. The selenocysteine active site of GPX4 is covalently bound by RSL3, resulting in reduced cellular antioxidant capacity, increased lipid ROS and initiation of ferroptosis [2,4,43].

Additionally, the biosynthesis of GPX4 occurs through the mevalonate (MVA) pathway *via* interfering with the maturation of selenocysteine tRNAs[43,44]. Selenocysteine is one of the amino acids in the active center of GPX4, and its insertion into GPX4 requires a special selenocysteine tRNA. Isopentenyl pyrophosphate (IPP), a product of the MVA pathway, facilitates the maturation of selenocysteine tRNA by transferring an isopentenyl group to a selenocysteine tRNA precursor through isopentenyltransferase. Importantly, the MVA pathway influences the synthesis of selenocysteine by down-regulating IPP to further disrupt the activity of GPX4, finally causing ferroptosis[45]. Statins, such as cerivastatin, inhibit the MVA pathway and restrict GPX4 biosynthesis[43,44]. Coenzyme Q10 (CoQ10), an endogenous antioxidant produced by the MVA pathway, protects cells from ferroptosis by preventing lipid oxidation[4,37]. Recently, Hadian *et al*[46] implicated ferroptosis-suppressor protein 1 (FSP1) as a novel ferroptosis resistance factor that reduces the expression of CoQ10, leading to the accumulation of lipid peroxides in a process independent of the cysteine/GSH/GPX4 pathway[46].

GSH, a tripeptide antioxidant composed of glutamate, cysteine and glycine[47,48], is an essential cofactor for GPX4 to degrade hydroperoxide[49]. Yant *et al*[50] found that GSH depletion is an indirect way of inactivating GPX4, which further causes a reduction in cellular antioxidant capacity, and increases accumulation of lipid ROS and subsequent ferroptosis[50]. Overall, hindering the synthesis and absorption of GSH or accelerating its degradation provides another means to induce ferroptosis. For example, erastin can block the absorption of GSH by inhibiting system $Xc^-$ to initiate ferroptosis[23].

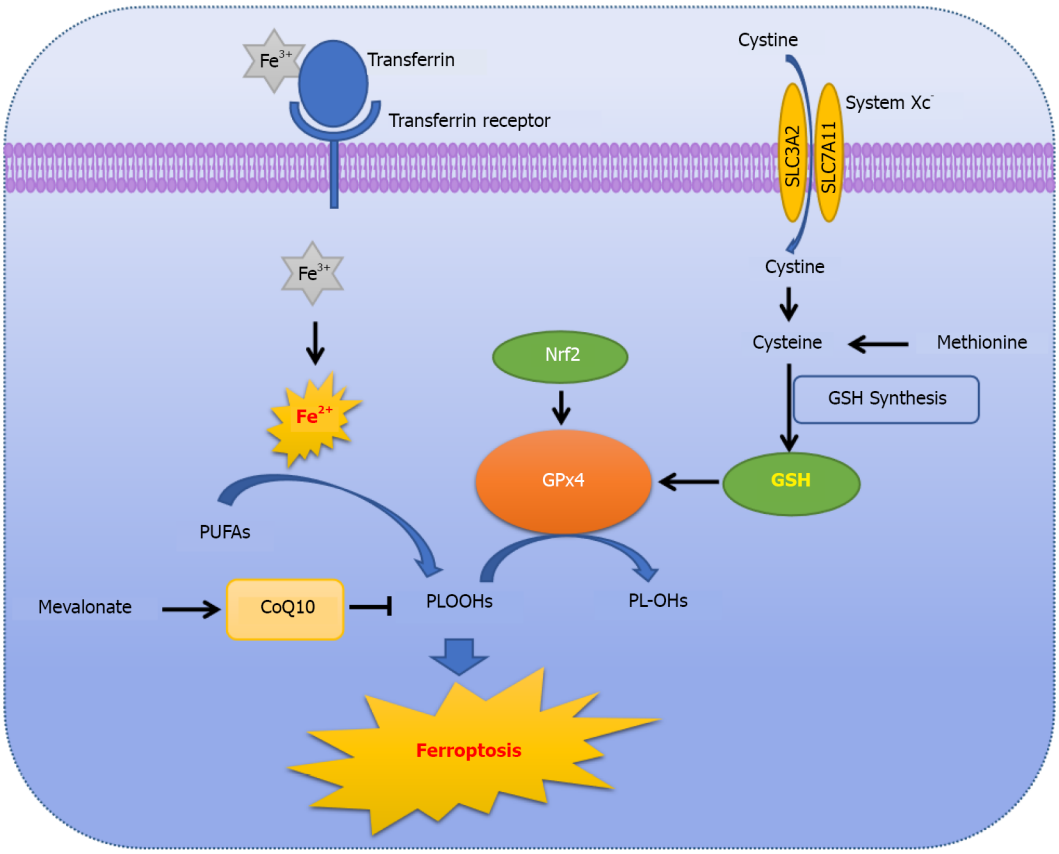


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**Figure 1 The schematic diagram of ferroptosis.** PUFAs: Polyunsaturated fatty acids; PLOOHs: Phospholipid hydroperoxides; GSH: Glutathione; Nrf2: Nuclear factor erythroid2-related factor 2; GPx4: Glutathione peroxidase 4.

System $Xc^-$ is a heterodimer composed of solute carrier family 3 member 2 (SLC3A2) and solute carrier family 7 member 11 (SLC7A11), embedded in the cell surface membrane. SLC7A11 is the main functional subunit, which can transport cystine into cells, reduce it to cysteine in the cytoplasm, and incorporate it in the synthesis of GSH[51-54]. Interestingly, inhibiting system $Xc^-$ results in compensatory transcriptional upregulation of SLC7A11 in erastin- and sulfasalazine-induced ferroptosis [54-56]. When system $Xc^-$ is restrained, the absorption of cystine will be hindered, decreasing the synthesis of intracellular GSH, which will interfere with the biological activity of GPX4[22]. Finally, erastin obstructs the absorption of GSH by inhibiting system $Xc^-$. However, GSH is a necessary cofactor for GPx, so the activity of GPx wanes and eventually results in cell ferroptosis[23]. Nevertheless, ferroptosis inducers that negatively regulate system $Xc^-$ are not effective in killing cells, since the cysteine involved in GSH synthesis can also be synthesized from methionine *via* trans-sulfation. Hayano *et al*[57] showed that inhibition of cysteine tRNA synthetase expression activates the trans-sulfuration pathway, further reducing cellular sensitivity to ferroptosis-inducing agents[57]. In addition, β-mercaptoethanol is able to promote cystine uptake without system $Xc^-$, thus significantly inhibiting erastin- and glutamate-induced cell death[58].

Another factor involved in ferroptosis is p53, as an important tumor suppressor encoded by *TP53* gene, which is mutated or inactivated in more than half of human cancers[59]. A large number of studies have shown that the tumor suppressing capacity of p53 is mainly derived from its typical functions, such as inducing cell cycle arrest, senescence, or apoptosis[60]. However, p53 also regulates metabolism, metastasis and invasion, and stem cell processes[61]. Recently, atypical functions of p53, such as controlling metabolism and redox status, have also been demonstrated to inhibit tumor development *via* regulating ferroptosis[62,63]. To verify whether p53 could induce ferroptosis, Jiang *et al* [56] showed treatment of p53-mutant non-small cell lung cancer cells with ROS had no significant effect on cell proliferation. However, following re-activation of p53, treatment with ROS dramatically induced 90% cell death, indicating that activation of p53 could dramatically reduce the antioxidant capacity of tumor cells[56]. Under the same conditions, addition of ferrostatin-1, an iron-death inhibitor, reduced the ROS-induced the cell death to 40%, indicating prevention of ROS-induced p53-dependent ferroptosis.

Under circumstances of oxidative stress, p53 can induce ferroptosis by transcriptional inhibition of *SLC7A11*, thereby inhibiting the absorption of cystine and reducing the production of GSH to enhance the sensitivity of cells to ferroptosis[56,64]. It is worth mentioning that acetylation of the p53 DNA-

binding domain plays a key role in the regulation of SLC7A11 expression[56,65]. Notably, mice harboring p53 (3KR), an acetylation-defective p53 due to a lysine-to-arginine mutation, did not form tumors spontaneously, suggesting that p53 (3KR) cells lose their typical functions of inducing apoptosis, senescence, and cell cycle arrest[15], but retain the ability to regulate SLC7A11 expression. This finding highlights the ability of p53 to restrain tumorigenesis by means of inhibiting SLC7A11 expression and triggering ferroptosis[15,56].

Moreover, p53 could promote ferroptosis through regulating its target genes, such as glutaminase 2 (GLS2), prostaglandin endoperoxidase synthetase 2, and spermidine/spermine N1 acetyltransferase 1 (SAT1)[4,56,66,67]. For example, SAT1 enhances the activity of arachidonic acid (AA) and oxidizes PUFAs, thus promoting lipid peroxidation. Knockout of SAT1 will reduce p53-mediated ferroptosis whereas overexpression of SAT1 has the opposite effect[66].

Thus, depending on the p53 mutation status and cellular environment, p53 can promote or inhibit ferroptosis in response to different oxidative stress scenarios[68]. Under high oxidative stress, p53 will promote ferroptosis, while under basal or low ROS stress, it can prevent ferroptosis[69]. On the one hand, activation of the p53-p21 transcriptional pathway enables wild-type p53 to inhibit cysteine deprivation and systemic $Xc^-$ inhibition in cancer cell lines[70], which may help normal cells survive under various metabolic stress conditions. By binding to dipeptidyl peptidase-4 (DPP4) in the nucleus, p53 can prevent the interaction of DPP4 with NADPH oxidase (NOX) in the cytoplasm, and then reduce the accumulation of intracytoplasmic lipid peroxides, thereby inhibiting ferroptosis. This results in p53-WT CRCs being resistant to erastin-induced ferroptosis[71].

Initiation of cystine deprivation-induced ferroptosis requires glutaminolysis[67]. To prevent glutamine hydrolysis and resist ferroptosis, it is possible to restrict the uptake of glutamine by inhibiting the SLC1A5 transporter, inhibit glutamine metabolism to glutamate by mitochondrial GLS2, or deter glutamate synthesis to α-ketoglutarate by aspartate aminotransferase 1[67,72].

FSP1, a novel GSH-independent ferroptosis suppressor, suppresses CoQ10-mediated ferroptosis through an FSP1-CoQ10-NAD(P)H pathway, in a parallel manner to GPX4[73,74]. NADPH, normally used as a biomarker of iron-death inducer sensitivity, is a GSH reductase that maintains reduced GSH [75]. NOX, an enzyme complex that produces superoxide anions and oxidative radicals by consuming NADPH, mediates cellular oxidation to provide an important source of oxidative radicals[2]. Overexpression of NOX causes depletion of intracellular NADPH and increases the level of oxidative free radicals, which significantly raises the sensitivity of cells to ferroptosis. In contrast, NOX inhibitors can down-regulate NOX expression, thereby inhibiting erastin-induced ferroptosis[76].

## FERROPTOSIS, A NON-APOPTOTIC CELL DEATH, IS ASSOCIATED WITH MITOCHONDRIAL ALTERATIONS

### *The characteristics of ferroptosis, compared with other forms of cell death*

Ferroptosis, a form of cell death that is different from apoptosis, necrosis and autophagy, depends on the accumulation of lipid ROS, resulting in a redox imbalance. To investigate the differences between ferroptosis and other forms of cell death in morphology, biochemical characteristics, gene expression, and bioenergetics, Dixon *et al*[2] used different inducers to individually induce apoptosis, necrosis and autophagy, and found that, following erastin-induced ferroptosis, cells did not show the morphological characteristics associated with apoptosis (chromatin condensation, plasma membrane blebbing, unique apoptotic bodies), necrosis (cytoplasmic and organelle swelling, cell rupture, cytoskeleton disintegration) or autophagy (formation of a classic closed bilayer structure)[2,14]. Notably, mitochondrial alterations, including small mitochondria, increased membrane density, and mitochondrial outer membrane disruption detected in erastin-treated cells, are unique features that distinguish ferroptosis from other forms of cell death[77-79].

Also, the biochemical characteristics of ferroptosis differ from other forms of cell death. During ferroptosis, $Fe^{2+}$ and ROS accumulate, the mitogen-activated protein kinase (MAPK) system is activated, and the uptake of cystine is reduced, resulting in an inhibitory effect on the $Xc^-$ system. At the same time, this process increases the activity of NOX and promotes the release of mediators, such as AA[1]. Regarding bioenergetics, a large reduction in intracellular ATP is found in $H_2O_2$-treated, but not in erastin-, STS-, or rapamycin-treated cells[2]. Regarding the characteristics of gene expression in the ferroptosis process, the intracellular Ras/Raf/MAPK and cystine transport pathways, and the activities of acyl-CoA synthetase long-chain family member 4 (ACSL4), NADPH oxidase 1 (NOX1), GPX4, and SLC7A11 were all involved in ferroptosis, which is one of the differences between erastin-induced ferroptosis and other forms of cell death[2].

To investigate the effect of existing cell death inhibitors on erastin-induced ferroptosis, Dixon *et al*[2] used a regulatory assay strategy to test 12 cell death inhibitors for their ability to prevent ferroptosis in cells, and found that compounds confirmed to inhibit apoptosis, necrosis, and autophagy were unable to modulate erastin-induced ferroptosis[2]. In contrast, other compounds, such as the iron chelator deferoxamine (DFO), the antioxidant Trolox, a MEK inhibitor, and the protein synthesis inhibitor cycloheximide, conversely were able to alleviate ferroptosis[3], demonstrating that these compounds are

involved in ROS production and exert a preventive effect on ferroptosis[2].

### *Unique features of ferroptosis: Mitochondrial alterations*

As mentioned above, mitochondrial morphological changes are the most significant feature of ferroptosis compared to other forms of cell death. Dixon *et al*[2] investigated the potential combination of erastin and the voltage-dependent anion channel 2/3 (VDAC2/3) on the mitochondrial membrane by affinity purification, demonstrating that VDAC2 and VDAC3 were necessary but not sufficient for erastin-induced cell death, which also suggests that mitochondria may be involved in the regulation of ferroptosis[3]. Under transmission electron microscopy, it was obvious that the number of mitochondria decreased and bilayer density increased in erastin induced BJeLR cells[2]. Mitochondrial swelling and mitochondrial crests decreased or disappeared in GPX4 ablated cells, and mitochondrial outer membranes rupture in RSL3 exposed Pfa1 cells in a time-dependent manner[37]. These abnormal mitochondrial structural changes are considered to be unique morphological features of ferroptosis[5]. In addition, the latest studies from Dr. Xuejun Jiang's laboratory have shown that cystine starvation-induced ROS accumulation and ferroptosis can be blocked by mitochondrial electron transport chain inhibitors, such as mitochondrial decoupling CCCP with mitochondrial membrane potential disruption [72]. However, in GPX4 knockout-induced ferroptosis, these electron transport chain inhibitors were unable to produce the blocking effect described above[37].

It is well known that iron overload and ROS accumulation are critical processes of ferroptosis in cells, and may be related to their induction of mitochondrial damage[80,81]. Iron overload would lead to mitochondrial morphological abnormalities, limit mitochondrial oxidative phosphorylation and antioxidant reactions, and impair mitochondrial function. Mitochondrial DNA (mtDNA) double-strand breaks, reduced mtDNA transcription, and decreased expression of respiratory chain subunits encoded by the mitochondrial genome have been observed in iron-overloaded mitochondria[82-85], whereas preserving mitochondrial structure and function protects cells from iron toxicity[86]. In addition, Carsten Culmsee and colleagues found a sharp increase in mitochondrial ROS in erastin-[87] and RSL3-treated[88] HT-22 and MEF cells, but not in erastin treated HT-1080 cells. Thus, the researchers speculated that the difference could be due to the use of different cells or different exposure times. The mitochondrial targeting ROS scavenger MitoQ (mito-quinone) prevents neuronal cells from undergoing RSL3-induced ferroptosis[88]. Other studies have indicated that lipid ROS accumulates in the mitochondria rather than the cytoplasm[89], while some reports suggest that ferroptosis is caused by lipid peroxidation outside the mitochondria[37].

In general, according to existing studies, the structural integrity of mitochondria becomes damaged, membrane potential is altered, and abnormal iron metabolism and lipid peroxidation have varying degrees of influence on mitochondrial function. However, the alterations in mitochondrial structure and function in ferroptosis still requires further exploration and verification.

### *The surefire way to ferroptosis: Lipid peroxidation*

Based on lipomics analysis, polyphosphorylated phosphatidylethanolamines (PEs) have been found to be key components in the induction of ferroptosis[90,91]. ACSL4, a key enzyme regulating lipid composition, catalyzes the addition of coenzyme A to AA and adrenic acid (AdA) to form PUFA coenzyme derivatives AA-CoA and AdA-CoA through an ER-associated oxygenation center. Subsequently, AA-CoA and AdA-CoA are esterified to AA-PE and AdA-PE by lysophosphatidylcholine acyltransferase 3 (LPCAT3) to take part in the synthesis of membrane phospholipids with negative charge[91-94]. In this situation, downregulation of ACSL4 for better conversion of AA to acylated AA, or inactivation of LPCAT3 to catalyze the insertion of acylated AA into membrane phospholipids, are also effective approaches to induce resistance to ferroptosis[90-92,95].

Additionally, free PUFAs such as AA-PE and AdA-PE will be selected as the preferred substrates for lipoxygenases (LOXs), lipid peroxidizing enzymes that catalyze the peroxidation of unsaturated fatty acids[96]. Knocking out LOX expression or treatment of cells with both tocotrienols and tocopherols have become an effective means to reduce erastin-induced ferroptosis[97,98]. With such treatment, LOX binds to phosphatidylethanolamine-binding protein 1 (PEBP1) to form a 15-LOX/PEBP1 complex that oxidizes AA-PE and AdA-PE to lipid hydroperoxides, thereby co-regulating the oxidation and reduction of esterified fatty acids with recombinant GPX4[99,100].

Recently, nuclear factor erythroid 2-related factor 2 (Nrf2), a well-known transcription factor, was found to be involved in the antioxidant process by inducing many antioxidant enzymes with antioxidant response elements in their promoters, such as GPX4 and GSH reductase, and its activity is strictly controlled by Kelch-like ECH-associated protein 1 (KEAP1)[101]. Under normal conditions, the binding of Nrf2 to KEAP1 causes the degradation and inactivation of Nrf2. However, when in a state of oxidative stress or with a large number of electrophiles, Nrf2 is released from the KEAP1 binding site and rapidly enters the nucleus to balance oxidative stress by activating transcriptional pathways and maintaining cellular redox homeostasis, ultimately inhibiting cellular oxidation and ferroptosis[74,102].

## FERROPTOSIS PARTICIPATES IN TUMOR OCCURRENCE AND PROGRESSION

### *Ferroptosis and the tumor microenvironment*

Ferroptosis can either inhibit or enhance tumorigenesis and development, with enhancement depending on the release of damage-associated molecular patterns in the tumor microenvironment (TME) and the inhibition of anti-tumor immune mechanisms, and inhibition depending on the activation of immune responses triggered by ferroptosis injury[103]. Therefore, understanding the interaction between ferroptosis and TME could provide new and effective anti-cancer strategies[104].

The TME is a complex environment within the tumor and enables tumor cells to survive and develop, serving as the 'soil' for non-cancerous cells (including stromal cells, immune cells, adipocytes, and endothelial cells) and extracellular matrix[105]. Theoretically, changes in tumor cytogenetics and epigenetics could enhance the ability of cancer cells to evade immune surveillance through various metabolic and biochemical mechanisms, ultimately promoting tumor initiation, progression, and metastasis[106]. Recently, Dai *et al*[107] found that ferroptosis caused by autophagic degradation releases cancer cells into TME and drives tumor-associated macrophage (TAM) polarization[107]. Yet ferroptosis inducers, such as erastin, RSL3 and sulfasalazine, have the capability to induce ferroptosis in cancer cells through different pathways, thus exerting anti-cancer effects, indicating the diverse role of ferroptosis in the process of cancer.

### *The regulatory effects of ferroptosis in malignancies*

Erastin-induced cell death can be inhibited by antioxidants and iron chelators, suggesting that erastin-triggers cell death *via* ferroptosis related to the accumulation of ROS and iron[2,3]. The reduced GSH levels caused by erastin results from direct inhibition of the $Xc^-$ system, activating the ER stress response, and attenuating the antioxidant effect of GPX4/GSH, thereby accelerating the accumulation of ROS in the cytoplasm[42]. Sulfasalazine, another inducer of ferroptosis, has the same mechanism and function as erastin[108]. Thus, treatment with sulfasalazine also induces ferroptosis in cancer cells[2,42]. Activation of the RAF/MEK/ERK signaling pathway also appears to be an important factor in erastin-treated cells with high Ras expression[3]. As mentioned before, changes in mitochondrial structure and morphology are detected in cells following treatment with erastin, which binds to the mitochondrial VDAC[3].

Other inducers of ferroptosis, such as RSL3 and ferroptosis-inducing agents (FINs), are also associated with ROS accumulation. RSL3, a direct inhibitor of GPX4, can inactivate GPX4 through direct binding, resulting in the accumulation of intracellular lipid peroxides and ferroptosis[4]. FINs, with the ability to generate ROS, are classified into two types according to their mechanism of action[109,110]. Class I FINs share the same mechanism as erastin and sulfasalazine, inducing GSH depletion[111,112]. Class II FINs, similar to RSL3, directly inhibit the activity of GPX4 without depleting GSH[4]. Yang *et al*[4] showed that GPX4 overexpression and knockdown modulated the lethality of 12 ferroptosis inducers, but not of 11 compounds that induced cell death by other mechanisms[4].

Nevertheless, erastin- and RSL3-induced ferroptosis could be inhibited by ferrostatin, a lipophilic iron chelator[2]. Ferrostatin can cross the cell membrane and chelate free intracellular iron, or act directly on enzymes containing iron, to prevent the formation of iron-catalyzed lipid free radicals and inhibit the degradation of PUFAs. Lipoxygenase can be directly inactivated by iron chelators and thus most likely mediates iron-dependent lipid ROS formation[113]. Unlike lipophilic iron chelators, DFO is a non-membrane-permeable chelator that can accumulate on cell lysosomes, following endocytosis, and interact with iron in lysosomes to prevent the generation of lipid ROS[113-115]. Liproxstatin-1, a clinical drug, acts by preventing the accumulation of ROS and cell death in $GPX4^{-/-}$ cells[37]. More importantly, liproxstatin-1 is able to inhibit FIN-induced ferroptosis *in vitro*[37].

## MECHANISMS OF FERROPTOSIS IN CRC

### *Signaling pathways of ferroptosis involved in CRC*

GPX4 is a key factor in the regulation of ferroptosis. Molecules that inhibit GPX4 activity, either directly or indirectly, are involved in ferroptosis. RSL3, a confirmed ferroptosis-inducer, has anti-cancer effects in CRC that can be enhanced by aspirin through suppressing mechanistic target of rapamycin (mTOR)/sterol regulatory element-binding protein 1 (SREBP-1)/stearoyl-CoA desaturase-1 (SCD1)-mediated lipogenesis in PIK3CA-mutant CRC[116]. Not surprisingly, genetic ablation of SREBP-1 or SCD1 expression enhances CRC cell sensitivity to RSL3-induced ferroptosis, supporting the molecular mechanism of aspirin on RSL3-induced cytotoxicity[116].

In addition, reducing the synthesis of intracellular GSH by inhibiting SLC7A11, which is also the target of erastin and sulfasalazine, is also an effective way to induce ferroptosis in CRC[117]. 2-Imino-6-methoxy-2H-chromene-3-carbothioamide, a benzopyran derivative, has also been reported to have anti-cancer activity and was first discovered to exert ferroptotic anti-CRC activity through down-regulating SLC7A11 expression in the AMP-activated protein kinase/mTOR/p70S6K pathway in CRC[118].

Importantly, cancer stem cell (CSC)-regulated phenotypic plasticity and protection of metastasized cancer cells from lipid peroxidation and ferroptosis are related to the increased expression of SLC7A11 [119]. In CRC, a remarkably low level of ROS is found in colorectal CSCs high in cysteine, GSH and SLC7A11 compared with CRC cells, while targeting SLC7A11 could induce ferroptosis through specifically suppressing the progression of colorectal CSC. Erastin exerts a dramatically strong cytotoxic effect on colorectal CSCs *in vitro* and *in vivo*[120]. By comparing the stemness of CRC cells and drug-resistant cells, it was found that the higher the stemness of CRC tumors, the more obvious the anti-ferroptosis characteristics. Correspondingly, the higher the stemness of CRC, the higher the expression of SLC7A11, suggesting that SLC7A11 is a potential target for colorectal CSC resistance to ferroptosis [120,121]. Knockdown of SLC7A11 expression in CSCs induced down-regulation of ALDH1, and tumor sphere size, as well as decreased cysteine and GSH and increased ROS levels, indicating decreased tumor stemness and increased ferroptotic characteristics. Similar results were obtained with erastin treatment, suggesting that erastin can effectively induce ferroptosis in drug-resistant CRC cells, thus achieving a therapeutic effect by targeting drug-resistant CSCs[120,121], providing a potential solution for drug resistance in CRC.

Doll *et al*[91] performed a genome-wide CRISPR-based genetic screen and microarray analysis of ferroptosis-resistant cell lines, and identified ACSL4 as an essential component for the synthesis of PE and execution of ferroptosis[91]. Another study analyzed the signaling pathway and miRNA profile of Kras mutant human CRC cells, and found that ACSL4 expression is high in CRC cells. Bromelain, a plant extract derived from pineapple, stimulated the expression of ACSL4, induced the cells to undergo ferroptosis and inhibited tumor progression[122], suggesting that the Kras gene may be an upstream regulator of ferroptosis.

TP53, an important tumor suppressor, plays a tumor suppressor role through transcriptional or non-transcriptional mechanisms in cancer cells[71]. In CRC cells, wild-type p53 restrains ferroptosis by blocking DPP4 activity, while deletion of wild-type p53 increases the anti-cancer activity of erastin in tumor-bearing mice[71]. In the nucleus, wild-type p53 binds to DPP4, preventing the translocation of DPP4 from the nucleus to the cytoplasm and the formation of the DPP4-NOX1 complex, responsible for preventing lipid peroxidation and ferroptosis, thereby restoring erastin-induced sensitivity in CRC cells [64,71]. On the contrary, p53 can also stimulate the expression of SLC7A11 in CRC, thereby protecting CRC cells from ferroptosis[71]. Therefore, it is desirable to modulate p53 to achieve efficacy in future CRC treatments.

Although ferroptosis is markedly different from other forms of cell death, there is a link between them in CRC. Hong *et al*[123] treated cancer cells with a combination of an apoptotic agent [tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)] and ferroptotic agents (erastin or artesunate) and found molecular crosstalk between ferroptosis and apoptosis[123]. The combined treatment remarkably promoted TRAIL-induced apoptosis due to the expression of ER stress-induced p53-independent up-regulation of apoptosis regulator PUMA. Further experiments found that the ER stress-response mediated by death receptor 5, one of the TRAIL receptors, also plays a significant role in the combined synergistic cytotoxic effect on multiple cell lines[124]. On the other hand, iron autophagy promotes ferroptosis in various types of cancer cells through regulating NCOA4, and inhibition of ferritin degradation inhibits the ferroptosis of these cells[31,35]. Interestingly, knockdown of NCOA4 did not change the ferroptosis of CRC cells[125], which could be explained by cell line differences or NCOA4 functional compensation, but further studies are needed.

### *Recent progress in CRC ferroptosis*

In addition to the classical pathways mentioned above, mechanistic studies on ferroptosis in CRC are also increasing. A recent study revealed TP53-induced glycolysis and apoptosis regulator (TIGAR) to be a potential inhibitor of ferroptosis during CRC development[126]. TIGAR is highly expressed in CRC cell lines, and knockdown of TIGAR unexpectedly increases erastin-induced growth inhibition and death, indicating that low levels of TIGAR increase the sensitivity of CRC cells to erastin-induced ferroptosis and that TIGAR is a potential negative regulator of ferroptosis. Increased levels of lipid peroxidation and malondialdehyde are associated with knockdown of TIGAR in CRC, without obvious changes of iron level, suggesting TIGAR is a potential target for iron-death-based therapy for CRC through regulating ROS[126]. Similarly, cytoglobin (CYGB), a regulator of ROS, has been shown to be an inhibitor of ferroptosis *via* the p53-YAP1 pathway. In the same study, CYGB suppression, first shown in CRC, promoted ROS production and increased the sensitivity of cancer cells to RSL3- and erastin-induced ferroptosis, thus inhibiting the growth of CRC cells in a YAP1-dependent manner[126].

As GPX4 also plays crucial roles in ferroptosis, factors regulating GPX4 are predicted to be involved in the regulation of ferroptosis. Lipocalin-2 (LCN2), a protein siderophore that regulates iron homeostasis, is upregulated in several types of tumors, including CRC. Overexpression of LCN2 reduces the level of ferroptosis through reducing intracellular iron levels and stimulating the expression of GPX4 and system Xc$^-$[127]. Serine and arginine rich splicing factor 9 was identified as a key factor promoting GPX4 expression and correspondingly decreased lipid peroxide damage, thereby driving CRC tumorigenesis, and thus providing another target for enhancing the sensitivity of CRC to erastin[128]. In addition, miR-15A-3p was found to positively regulate ferroptosis by directly targeting and suppressing GPX4 in CRC[129].

GTP cyclohydrolase 1 (GCH1), a rate-limiting enzyme in the synthesis of the free radical trapping antioxidant tetrahydrobiopterin (BH4) was found to suppress ferroptosis in a GPX4-independent manner[130]. Blocking GCH1/BH4 promoted erastin-induced but not RSL3-induced ferroptosis, suggesting that GCH1 inhibitors combined with erastin provide a novel treatment strategy for CRC [130]. Interestingly, autophagy inhibitors could reverse erastin resistance in GCH1-knockdown cells, suggesting that GCH1/BH4 may act through ferritin phagocytosis[130]. Another novel inducer of ferroptosis, talaroconvolutin A was found to strongly induce ferroptosis in a dose- and time-dependent manner, but not apoptosis. Surprisingly, talaroconvolutin A was far more effective in inhibiting CRC by ferroptosis than erastin, and thus has become a potential treatment option for inducing ferroptosis in CRC[131].

## CLINICAL IMPLICATIONS OF FERROPTOSIS INDUCTION IN CANCER TREATMENT

Cancer remains one of the most threatening diseases to human health. Although traditional treatments, such as medication, surgery, radiation and chemotherapy, and comprehensive treatment, as well as immune therapy and targeted therapy, have been applied in the clinic, but the complexity of cancer pathogenesis, drug resistance, and patient intolerance have severely limited the efficacy of these approaches[132]. Therefore, further investigation is needed to explore the molecular changes and mechanisms involved in tumorigenesis and prognosis. Ferroptosis, a novel form of death, could play an indispensable role in inhibiting tumor growth and may therefore become an emerging strategy for anti-cancer therapy.

### *Reversing chemotherapeutic drug resistance*

Chemotherapy has remained a necessary means to treat cancer, but drug resistance also remains one of the reasons for the poor prognosis of patients with malignancies. According to the molecular mechanism of ferroptosis, the pathways that reduce chemotherapeutic drug resistance are mainly involved in the lipid metabolism, iron metabolism and classical GPX4 pathways. The resistance of chemotherapeutic drugs in CRC also involves these processes.

In the process of lipid metabolism, ACSL4 is involved in the lipid oxidation pathway through the conversion of AA and AdA in PUFAs into coenzyme derivatives, and then producing oxidized lipid molecules[91]. Wu *et al*[133] reported that inhibiting ADP ribosylation factor 6 (ARF6), functions downstream of the Kras/ERK signaling pathway, and can activate ACSL4 and endow cancer cells with sensitivity to oxidative stress, especially RSL3-induced lipid peroxidation. ARF6 has a profound effect on the development of pancreatic cancer. Abrogation of ARF6 promotes RSL3-induced ferroptosis and alleviates gemcitabine resistance[133]. Another key enzyme in lipid metabolism, LOX, can directly oxidize PUFAs and mediate ferroptosis in a non-enzymatic manner[97]. Wu *et al*[134] demonstrated that arachidonate lipoxygenase 15 (ALOX15) is closely related to the inhibition of ferroptosis in gastric cancer. Decreasing miRNA-522 and increasing ALOX15, to induce ferroptosis, has become a novel treatment strategy to reverse drug resistance in gastric cancer, especially resistance to cisplatin/paclitaxel[134].

In iron metabolic pathways, dihydroartemisinin (DHA), a safe and promising therapeutic agent that preferentially induces ferroptosis of cancer cells, was found to intensively enhance the cytotoxicity of cisplatin through impairing mitochondrial homeostasis and increasing mitochondrial-derived ROS, as well as promote ferroptosis with catastrophic accumulation of free iron and unrestricted lipid peroxidation. Depleting the free iron reservoir prevents death and triggers tolerance to DHA/cisplatin-induced ferroptosis, whereas supplementation of iron accelerates ferroptotic cell death[135]. Blocking lysosomal iron translocation out of lysosomes can be caused by the inhibition of DMT1 in CSC, resulting in iron accumulation in lysosomes, production of ROS and cell death in the form of ferroptosis[136].

Chen *et al*[137] discovered that androgen receptors could induce tumor cell drug resistance during the treatment of glioblastoma with temozolomide. Curcumin analogues reverse temozolomide resistance through ubiquitinating androgen receptors, which can be achieved by inhibition of GPX4 followed by induction of ferroptosis[137]. The reduction of oxaliplatin resistance in CRC occurs through a similar mechanism. CRCs induce ferroptosis by disrupting the KIF20A/NUAK1/PP1β/GPX4 pathway, in which high expression of KIF20A has been shown to be associated with oxaliplatin resistance[138]. In addition to the direct inhibition of GPX4 activity, blocking the synthesis of GSH also triggers ferroptosis indirectly. It is reported that ent-kaurane diterpenoids overcome cisplatin resistance by targeting peroxiredoxin I/II and consuming GSH to induce ferroptosis[139]. In head and neck cancer, cisplatin resistance can also be overcome by inhibiting system $Xc^-$[140], and in gastric cancer, inhibition of the Nrf2/Keap1/system $Xc^-$ signaling pathway can induce the same effect[141].

### *Reversing targeted-therapy resistance*

However, chemotherapy as a major means of cancer treatment has the undesirable side-effect of killing normal cells. In contrast, targeted therapy has gradually become an effective treatment. About half of patients with metastatic CRC have *RAS* mutations, which greatly limit the efficacy of cetuximab, an

anti-epidermal growth factor receptor antibody. A natural product β-elemene, isolated from the Chinese herb turmeric, in combination with cetuximab, confers high cytotoxicity toward metastatic CRC cells with Kras mutations, and works by inducing ferroptosis and inhibiting epithelial-mesenchymal transition[9]. Olaparib, a well-known inhibitor of poly (ADP-ribose) polymerase, promotes ferroptosis by inhibiting SLC7A11-mediated GSH synthesis. A synergistic effect with FINs can sensitize BRCA-activated ovarian cancer cells and xenograft cells[142]. Triple-negative breast cancer (TNBC) cells are resistant to clinical doses of gefitinib. Inhibition of GPX4 and induction of ferroptosis can enhance the sensitivity of TNBC to gefitinib[143]. Sorafenib is the first approved systemic medicine for advanced hepatocellular carcinoma, but acquired resistance limits its usefulness in the clinic. Inhibition of metallothionein-1g expression can enhance the anti-cancer activity of sorafenib by inducing ferroptosis *in vitro* and *in vivo*[144]. Artesunate, a drug derived from traditional Chinese medicine, inhibits the growth of sunitinib-resistant renal cell carcinoma by cell cycle arrest and induction of ferroptosis[145]. Similar to erastin, GSH depletion accompanied by GPx inactivation is the underlying mechanism of cisplatin, and cisplatin combined with erastin has enhanced anti-tumor activity compared to cisplatin alone[10]. The combination of erastin and cisplatin may be a useful strategy to improve the efficacy of cisplatin for the reason that the mechanisms used by these two compounds are different[10,146].

### *Reversing immunotherapy resistance*

Over the past decade, immunotherapy with immune checkpoint inhibitors has shown promising efficacy in various malignancies. Even so, there has been some resistance to its use. Based on research advances, it has been proposed that stimulating the adaptive immune system by promoting immunogenic cell death may change the immune cold state into a checkpoint blockade response state, and ferroptosis happens to be immunogenic[147,148]. Therefore, induction of ferroptosis in cancer cells may induce vaccine-like effects and stimulate anti-tumor immunity, thereby overcoming immunotherapy resistance[149-151]. On the other hand, immunosuppressive cells in the TME also contribute to immunotherapy resistance, such as regulatory T cells (Tregs) and TAMs[152]. These findings suggest that induction of ferroptosis in Tregs by GPX4 inhibition may reverse immunotherapy resistance[153]. Moreover, Jiang *et al*[154] found that reprogramming of TAMs, due to in-tumor cell ferroptosis, resensitizes the tumor cells to immunotherapy[154].

## CLINICAL PROGNOSTIC MODEL FOR FERROPTOSIS IN CRC

In recent years, numerous studies have focused on genetic screening for colon cancer and the establishment of polygenic prognostic models associated with ferroptosis. Owing to the lack of reliable and accurate biomarkers, the diagnosis, treatment and prognosis of colon cancer are faced with great challenges. Therefore, the establishment of a sound prognostic model and the mining of key biomarkers are effective ways to accurately predict the prognosis of CRC patients. In addition to their anti-cancer potential, ferroptosis-related genes also play an important role in the construction of prognostic models. Recent studies have shown that a new ferroptosis-related 10-gene prognostic model effectively predicts the prognosis and overall survival of patients with CRC, providing a reference value for targeted therapy and immunotherapy[11]. Xiang *et al*[12] established a prediction model based on regression analysis of CRC differentiation-related genes (CDRGs), and found that down-regulation of CDRGs was closely related to ferroptosis and immune metabolism, thus showing that molecular subtypes based on cell differentiation can successfully predict the prognosis of CRC patients undergoing chemotherapy and immunotherapy[12]. Moreover, a prognostic model based on EMT and ferroptosis-related genes predicted the ability of colorectal adenocarcinoma to invade and metastasize, where four genes involved in ferroptosis were potential prognostic biomarkers, thus providing important guidance for the individualized treatment and clinical decision-making of CRC[13]. Additionally, ferroptosis-related lncRNA signatures have proved to be promising biomarkers. Wu *et al*[155] constructed a robust prognostic model with only 4 ferroptosis-related lncRNA signatures, and the signature-based risk score showed a stronger ability to predict survival than traditional clinicopathological features, contributing to the prediction of clinical outcomes and treatment responses in patients with colon cancer[155] (Table 1). In future studies, the construction of CRC prognostic models and the discovery of potential biomarkers are expected to enhance and improve the survival and prognosis of CRC patients.

## CONCLUSION

In summary, ferroptosis mainly involves the accumulation of intracellular lipid ROS resulting from a disorder of iron metabolism[2]. Its unique form of death provides potential targets for the treatment of tumors. Since ferroptosis exerts different effects through different mechanisms in different tumor types, this article focuses on CRC to elaborate the molecular mechanisms and pathways of ferroptosis.

**Table 1 Prognostic model of ferroptosis-related genes in colorectal cancer patients**

| Model | Related genes | Ref. |
|---|---|---|
| 10-Gene prognostic model | *TFAP2C, SLC39A8, NOS2, HAMP, GDF15, FDFT1, CDKN2A, ALOX12, AKR1C1, ATP6V1G2* | [11] |
| Prediction model based on CDRG[1] regression analysis | *ACAA2, SRI, UGT2A3, KPNA2, MRPL37* | [12] |
| Prognostic model based on EMT[1] and FRGs[1] | *MMP7, YAP1, PCOLCE, HOXC11* | [13] |
| Prognostic model of 4-FRL[1] signatures | *AP003555.1, AC104819.3, LINC02381, AC005841.1* | [155] |

[1]CDRG: Colorectal cancer differentiation-related gene.
EMT: Epithelial-mesenchymal transition; FRG: Ferroptosis-related gene; FRL: Ferroptosis-related lncRNA.

Many studies have proposed possible pathways of ferroptosis in CRC, but the specific mechanisms involved in the occurrence, development and metastasis of CRC remains unclear. In the classical pathway, GPX4 can be used as a target for tumor therapy, but the inhibition of GPX4 may cause side effects due to its protective role against β-amyloid toxicity in neurons[156,157]. Additionally, p53 has contradictory effects on ferroptosis, but the mechanism in CRC is unique. Future studies can explore how to achieve the switch between "brake" and "accelerator" in the regulation of ferroptosis[71]. Generally, the role of ferroptosis in disease may be to promote[158,159] or inhibit, but the induction of ferroptosis, undoubtedly, has an inhibitory effect on the occurrence, development and metastasis of CRC. In addition to the classical mechanism, other potential regulatory pathways need to be discovered. As mentioned above, these regulated types of cell death may share common pathways and key regulators, which could provide new directions for combined therapeutic interventions. Furthermore, the occurrence of ferroptosis is cell-type dependent, so cancer treatment based on ferroptosis will not necessarily be suitable for all cancer types, or even for different clinical stages of the same type. Although these treatments can be expected to be affected by the development of tumor cell drug resistance, with the gradual development of research, reducing drug resistance by inducing ferroptosis is gradually becoming a reality[10], and the construction of prognostic disease models by screening ferroptosis-related genes has become a focus of clinical research[13]. However, translating the multiple basic research discoveries in ferroptosis to clinical treatment will be another difficult problem we will soon face.

## FOOTNOTES

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*MINIREVIEWS*

# Clinical implications of COVID-19 in patients with metabolic-associated fatty liver disease

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## Abstract

People across the world are affected by the "coronavirus disease 2019 (COVID-19)", brought on by the "SARS-CoV type-2 coronavirus". Due to its high incidence in individuals with diabetes, metabolic syndrome, and metabolic-associated fatty liver disease (MAFLD), COVID-19 has gained much attention. The metabolic syndrome's hepatic manifestation, MAFLD, carries a significant risk of type-2-diabetes. The link between the above two conditions has also drawn increasing consideration since MAFLD is intricately linked to the obesity epidemic. Independent of the metabolic syndrome, MAFLD may impact the severity of the viral infections, including COVID-19 or may even be a risk factor. An important question is whether the present COVID-19 pandemic has been fueled by the obesity and MAFLD epidemics. Many liver markers are seen elevated in COVID-19. MAFLD patients with associated comorbid conditions like obesity, cardiovascular disease, renal disease, malignancy, hypertension, and old age are prone to develop severe disease. There is an urgent need for more studies to determine the link between the two conditions and whether it might account for racial

differences in the mortality and morbidity rates linked to COVID-19. The role of innate and adaptive immunity alterations in MAFLD patients may influence the severity of COVID-19. This review investigates the implications of COVID-19 on liver injury and disease severity and vice-versa. We also addressed the severity of COVID-19 in patients with prior MAFLD and its potential implications and therapeutic administration in the clinical setting.

**Key Words:** Metabolic-associated fatty liver disease; COVID-19; Metabolic syndrome; Non-alcoholic steatohepatitis; Angiotensin converting enzyme 2



**Core Tip:** The severity of coronavirus disease 2019 (COVID-19) symptoms and sequelae positively correlate with high rates of hepatic decompensation and elevated transaminases in patients with chronic liver disease and cirrhosis. Implicated mechanisms linking cirrhosis with severe COVID-19 symptoms include cirrhosis-related immune dysregulation, systemic inflammation, coagulopathy, and metabolic derangements. Metabolic-associated fatty liver disease (MAFLD) is characterized as the hepatic manifestation of the metabolic syndrome and therefore is highly associated with other comorbidities such as obesity, diabetes, and hyperlipidemia. Those comorbidities are also risk factors for severe COVID-19. The hepatic distribution of the angiotensin-converting enzyme 2 receptor, the main viral entry receptor for SARS-CoV-2, may determine the severity of hepatic involvement. In addition, moderate hepatic dysfunction could alter the severity of COVID-19, as well as the safety profile, and the therapeutic efficacy of antiviral drugs metabolized in the liver. Therefore, it is of high clinical priority to enhance our understanding of COVID-19 infection-associated liver injury in MAFLD patients to treat both of these conditions effectively.

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## INTRODUCTION

A substantial hazard to public health has suddenly emerged from the "severe acute respiratory syndrome (SARS)" global pandemic caused by the coronavirus SARS-CoV-2 "[coronavirus disease 2019 (COVID-19), *Sarbecovirus subgenus*, *Betacoronavirus genus*, *Coronaviridae family*]"[1-5]. Up until the September 2, 2022, the total infected cases were 607013841; total deaths were 6508326 and total vaccinated were 12185442365[2,6]. The most common and important clinical manifestation of COVID-19, alternating from moderate respiratory symptoms to severe pneumonia, is respiratory involvement, even though many people still show no symptoms. The severe Corona virus infection, however, is a systemic illness that can cause myocardial injury, heart failure, vascular inflammation, myocarditis, cardiac arrhythmias, hypoxic encephalopathy, multi-organ failure, and eventually death[1-5,7-11].

Though severe liver damage is rare, the liver remains a potential target for Coronavirus. This infection poses a novel challenge for hepatologists because it may harm the liver by direct (viral translocation from the gastrointestinal tract to the liver) or indirect pathways (systemic inflammation, hepatic ischemia and hypoxia, effects on pre-existing liver illnesses, and drug-related liver injury)[12-17].

Notably, nonalcoholic fatty liver disease (NAFLD), a chronic dysmetabolic pandemic with a prevalence rate of > 30% in the global population, has become the most widespread liver disease in the world. Furthermore, NAFLD is a "fellow traveler" with a number of risk factors, metabolic syndrome, and diseases rather than a stand-alone disorder. Along with this viewpoint, the term "metabolic-associated fatty liver disease" (also known as "MAFLD") has recently given the acronym NAFLD a second look[18,19]. Therefore, NAFLD/MAFLD may impact how COVID-19-infected "patients" fare. Additionally, in situations of chronic injury, the liver itself is more vulnerable to medicines.

In this setting, individuals with NAFLD/MAFLD and COVID-19 infections exhibit inflammatory response pathways, particularly those involving cytokines that may aggravate the clinical result by causing an increase in liver inflammation or by serving as a marker of metabolic risk factors. A precise understanding of the behavior of the virus and the risk factors contributing to the initiation and progression of COVID-19 will be crucial in the near future to predict virus-related events around the globe due to the pandemic characteristics and high mortality rate of SARS-CoV-2 infection.

According to Wang *et al*[20], analysis of COVID-19-infected patients revealed independent risk factors for hypertension, diabetes, chronic obstructive pulmonary disease, cardiovascular disease, and cerebrovascular disease [odds ratio (OR): 2.29-5.97]. A recent study on COVID-19-infected individuals who were hospitalized in New York reported that 48.7% of the patients had a BMI > 40 kg/m$^2$, suggesting that BMI is one of the strongest predictors of hospitalization (OR: 6.2), only being surpassed by ages ≥ 75 years (OR: 66.8) and age 65-74 years (OR: 10.9)[21]. Finally, MAFLD was found to be independently linked with COVID-19 progression in a study of 202 consecutive individuals with confirmed COVID-19[22]. Acute COVID-19 epidemic and chronic MAFLD, which is a member of a larger group of metabolic illnesses, are the two pandemic conditions that are the subject of this article's discussion. The underlying MAFLD may contribute to more severe hepatic and metabolic consequences during COVID-19 infection and may develop into another prognostic indicator of the viral illness[21-26].

## COVID-19 AND MAFLD

In contrast to the hepatocytes which are the predominant liver cells and of which only 3% express angiotensin I converting enzyme 2 (ACE2) receptors, about 60% of the cholangiocytes expresses ACE2 receptors even though they occupy only 3% to 5% of the liver cell population[27]. Acute liver injury was common in 15.4% of Chinese patients with COVID-19 illness[1]. However, it has been noted that the liver is involved in roughly 60% of cases, and the likelihood of liver malfunction appears to rise with age. A report by Ji *et al*[22] on 202 COVID-19-positives showed that 50% of the patients had some form of liver abnormalities upon admission, and 75% of patients developed liver dysfunction during the course of their stay in the hospital. Most of the liver injury was mild, and only 3% of the patients had ductular or mixed patterns of liver abnormalities. Male gender, older age > 60 years, a high BMI, underlying comorbidities, and MAFLD were all linked to COVID-19 development[28]. MAFLD was identified as having an OR of 6.4 with a 95% confidence interval (CI) of 1.5 to 31.2 in this study by multivariate logistic regression analysis[29]. However, this survey has limitations due to the small number of cases that were available, various severity criteria, underlying comorbidities, and unclear liver disorders[22,30-32]. In contrast, the presence of intermediate or high fibrosis-4 (FIB-4) scores significantly and independently enhanced the probability of severe COVID-19 illness in a sample of 310 individuals with COVID-19 and MAFLD[33]. Due to their increased metabolic risk, patients with MAFLD exhibit a distinct risk[23-26,34].

ACE2 receptors are required for the spike viral proteins to attach to the target cells in order for the COVID-19 disease to progress to its first stage[12]. These receptors are mainly expressed on alveolar epithelial cells (type II) and ciliated cells in the human lung, as well as on the epithelia of the upper respiratory tract (nasopharynx), which is a key site of replication[12]. The vascular endothelium, the brush border of intestinal enterocytes, and cholangiocytes express the ACE2 receptor[14]. Therefore, COVID-19 may cause symptoms to appear in the gastrointestinal tract[15].

According to a recent United States survey, 61% of people who tested positive for COVID-19 had clinically obvious gastrointestinal symptoms[16]. Because ACE2 receptors are found in the glandular cells of the stomach, duodenum, and distal enterocytes, their presence may cause malabsorption, imbalanced intestinal secretion, and enteric nervous system activation, all of which can result in gastrointestinal symptoms[12].

## COVID-19 AND LIVER BIOCHEMISTRY PATTERNS AND FREQUENCY

Although the specific impact of COVID-19 on the liver is yet unknown, patients with COVID-19 frequently experience liver biochemistry abnormalities, affecting 15%-65% of SARS-CoV-2-infected people[35-42]. The large variation in these reported frequencies may be due to various interpretations of what constitutes the upper limit of normal, variable lab results regarded as liver enzymes and regional variations in the prevalence and nature of the underlying chronic liver disease (CLD). An estimated 29%-39% and 38%-63% of patients, respectively, have been reported to have mild (1-2 times the upper limit of normal) elevations of their serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, which characterize liver biochemistry abnormalities in COVID-19[36,38,43]. Although severe liver damage, increased blood bilirubin levels, and hepatic synthetic malfunction are all uncommon in SARS-CoV-2 patients, hypoalbuminemia, a non-specific index of illness severity, has been linked to worse COVID-19 outcomes. Non-specific findings from liver biopsies in SARS-CoV-2 patients have included steatosis, moderate lobular and/or portal inflammation, and vascular pathology [10,44,45].

The majority of the time, abnormal biochemistries are likely multifactorial, with direct infection of hepatocytes as well as immune-mediated inflammatory response, drug-induced liver injury, hepatic congestion, and extrahepatic release of transaminases all having a potential role. Elevations in serum AST levels among COVID-19 hospitalized patients positively correlate with levels of ALT but not with

markers of systemic inflammation [such as C-reactive protein (CRP) and ferritin] or muscle breakdown (such as creatinine kinase)[21]. Despite the rarity of reports of rhabdomyolysis (muscle breakdown) related to COVID-19[46], these findings suggest that high liver enzymes in COVID-19 are the result of direct hepatic injury. Finally, during COVID-19, AST is frequently higher than ALT, which is unusual for a classic hepatocellular pattern of liver injury outside of specific situations like alcohol-related liver disease, some drug-induced liver injuries (like those caused by lamotrigine), ischemic hepatitis, and cirrhosis. The causes of an AST-predominant aminotransferase increase are not fully understood, although they may include mitochondrial failure linked to COVID-19, hepatic steatosis brought on by SARS-CoV-2, and altered hepatic perfusion brought on by microthrombotic disease[34,47,48].

## EFFECTS OF COVID-19 ON MAFLD DISEASE PROGRESSION

### *Comorbidities associated with MAFLD*

MAFLD is a serious public health issue and a leading cause of CLD globally. MAFLD has been identified as a hepatic manifestation of an insulin resistance-related metabolic syndrome. A growing body of research evidence suggests that systemic disorders like type 2 diabetes, obesity, metabolic syndrome, chronic kidney disease, and cardiovascular disease are all linked to MAFLD. The primary cause of death in MAFLD patients is cardiovascular disease. Rather than just being steatosis, these findings are intricately linked to nonalcoholic steatohepatitis (NASH). MAFLD should be seen as an early mediator of systemic disease in addition to being a liver-specific condition. In relation to other medical illnesses, the pathophysiology, and underlying processes of MAFLD are still poorly understood. Future therapeutic approaches for MAFLD require more research[49]. The various risk factors associated with severe COVID-19 in patients with MAFLD are enumerated in Table 1.

### *Systemic inflammation and hypoxia*

Patients with COVID-19 infection who have chronic liver disorders may express more ACE2 receptors and hypoxia-inducible factors (HIFs), a class of transcription factors triggered by hypoxia[50]. The progression of metabolic illnesses like MAFLD may be accelerated by such changes[31,35-37,39,51-55]. Clinically, biliary ductal abnormalities are uncommon in COVID-19-infected patients; as a result, the ACE2-mediated liver injury may primarily result from the localization of these receptors in endothelial cells. Additionally, the progression of MAFLD involves increased production of reactive oxygen species and nitric oxide derivatives, inflammatory pathways that result in cellular communication with Kupffer cells, and upregulation of HIF through suppression of fatty acid oxidation. This theory is somewhat corroborated by liver histology from patients who died from severe COVID-19, which showed minor lobular and portal activity as well as moderate microvesicular steatosis, presumably as a direct result of SARS-CoV-2 infection or drug-induced liver injury[51,55,56].

### *Altered liver response*

Lipids, which are part of the cell membrane, exosomes, and energy storage components, are strongly associated with the viral life cycle. Infected cells typically have changes in their metabolism of circulating lipids[41,43]. In order to facilitate their replication, viruses alter lipid metabolism, including the expression and activity of crucial enzymes involved in lipid biosynthesis. Changes in lipid metabolism may also be linked to the host's reaction to an infection. SARS-CoV-2 is not an exception and causes significant modifications in lipid metabolism after infection[42,43,57]. SARS-CoV-2 infection specifically causes a general down-regulation of approximately 100 serum lipids, including fatty acids, sphingolipids, and glycerophospholipids. Lipids are not only altered in COVID-19, but they are also linked to pathophysiology and the development of the illness. Changes in bilirubin and bile acids provide evidence that the observed down-regulation of lipids during SARS-CoV-2 infection is related to liver damage. Many of the COVID-19 lipid and lipoprotein changes that have been reported are connected to hepatic activities. The investigation of plasma lipidomic analysis was conducted during COVID-19. Sphingomyelin and monosialodihexosyl ganglioside levels were upregulated, and diacylglycerol levels were downregulated, accounting for the majority of the significantly altered lipids. The severity of the condition was positively linked with higher monosialodihexosyl ganglioside levels. Again, disruption of the normal circulating lipid profiles may be caused by inflammation and infection. Unsaturated fatty acids may be released as a defense mechanism in response to a cytokine storm. When COVID-19 illness is present, proinflammatory lipids and lipid mediators may modify the immunological response[43,57,58]. In addition to the lipid metabolism, liver detoxification and protein synthesis are significantly impaired in the COVID-19 patient. In an autopsy study, looking at the transcriptome of the severe COVID-19 patient with non-covid patient, the cytochrome P450 gene - *ACAD11, CIDEB, GNMT* and *GPAM* were significantly down regulated[59]. This consequently affects the detoxification of drugs and metabolites through the CYP 450 system. The liver is the powerhouse of protein synthesis. It does not only synthesize the anabolic proteins but it also synthesizes proteins involved in both innate and acquired immune responses. This is very much reduced in the MAFLD patient who are in a state of immune dysregulation but the exact role of each component of hepatic immune dysregulation to

**Table 1 risk factors associated with severe coronavirus disease 2019 in metabolic-associated fatty liver disease patients**

| Common risk factors for severe COVID-19 infections | |
|---|---|
| Obesity | High serum IL-6 at admission |
| Advanced age > 65 yr | Male gender |
| Black race | High ferritin level at admission |
| Liver fibrosis | High EWS at admission |
| Dyslipidemia | Type 2 diabetes mellitus |

EWS: Early warning score; IL-6: Interleukin-6; COVID-19: Coronavirus disease 2019.

COVID-19 severity is difficult to delineate[3].

### *Liver steatotic state/ lipid derangement*

The development of steatosis and liver fibrosis in MAFLD patients is facilitated by active innate immunity in the infectious state, which not only directly causes and intensifies liver inflammation but also interferes with the control of lipid metabolism. In COVID-19 patients, proteomic and metabolomic analysis identified dyslipidemia, including lipid build-up and downregulation of apolipoproteins[57]. In turn, it was discovered that SARS-CoV-2 infection can alter lipid synthesis and absorption pathways, increasing the accumulation of lipid droplets (LD) in human cells[43]. SARS-CoV-2 can also highjack LDs to increase its ability to replicate. Recent research has shown that ACE2 is crucial for maintaining metabolic homeostasis from a mechanistic perspective. A SARS-CoV-2 infection reduces ACE2 expression, which leads to aberrant metabolic processes. Patients with COVID-19 may experience MAFLD development as a result of the metabolic imbalance brought on by ACE2 deficiency[27,42,43,57,58,60].

## ELEVATED FIB-4 AND POOR COVID-19 OUTCOMES

A straightforward, thoroughly tested point-of-care measure called the FIB-4 index is used to categorize individuals with suspected MAFLD according to their likelihood of developing liver fibrosis. It uses a combination of patient's age, ALT, AST, and platelet count, all of which may be quickly determined by front-line healthcare professionals[33]. FIB-4 is helpful in identifying liver disease patients who are more likely to experience a negative clinical outcome connected to the liver. FIB-4 has also been demonstrated to predict non-liver clinical outcomes in MAFLD patients, such as cardiovascular mortality or risk of atrial fibrillation. Similarly, FIB-4 has been used to predict mortality in the general population as well as clinical outcomes in clinical situations unrelated to the liver. In the study of Ibáñez-Samaniego *et al*[33], increased FIB-4 Levels were linked to a poor outcome in COVID 19 patients.

The chance of developing an enhanced inflammatory response, a feature of severe COVID-19, may be increased by advanced hepatic fibrosis. Advanced liver disease is actually characterized by a persistent stimulation of immune cells by pathogen-associated molecular patterns and damage-associated molecular patterns[58]. This stimulation causes immune cells to become activated and increases the production of cytokines, chemokines, and growth factors. These growth factors are then released to attract and activate additional inflammatory cells, maintaining a state of chronic low-grade systemic inflammation[33]. Patients with obesity and insulin resistance have been noted to experience a similar level of low-grade inflammation. In fact, the degree of obesity and the likelihood of developing type-2 diabetes mellitus have been linked to increased serum levels of Interleukin-6 (IL-6)[32]. Activated macrophages release IL-6 during an acute infection, which is a significant inducer of the creation of acute phase reactant proteins in hepatocytes (CRP, ferritin, complement, clotting factors). The hepatocytes' acute phase proteins have a direct effector role on innate immunity, facilitating pathogen clearance[21,33,46,61-65].

### *Increased hepatic decompensation rates in cirrhotic patients*

Data on decompensated cirrhosis and COVID-19 is limited. In the first 152 cases of clinically and laboratory-confirmed COVID-19 infections with CLD in two international reporting registries ($n$ = 103 with cirrhosis and $n$ = 40 with chronic liver disease) (COVID-Hep.net and COVIDCirrhosis.org)[66], the probability of death after hepatic decompensation during COVID-19 was significantly higher in those with new decompensation: 63.2% died compared to 26.2% in those without new decompensation. Notably, 24.3% of people with new hepatic decompensation at the time of diagnosis had no pulmonary symptoms of COVID-19. Therefore, decompensated liver disease is a significant risk factor for mortality in COVID-19 patients. As a result, all patients with decompensated liver disease should be hospitalized,

and any recent decompensation in a cirrhotic patient should be tested for COVID-19 at this time[13,66]. In a metanalysis of observational studies of COVID-19 infection with cirrhosis, the patient with cirrhosis not only had higher rate of decompensation but the odds for mortality has been 2.48 (CI 2.2-3.04) when compared to the non-cirrhotic patients[67].

## EFFECTS OF PRE-EXISTING MAFLD ON COVID-19 DISEASE SEVERITY

### *Coagulopathy*

Proinflammatory cells may produce cytokines, which can increase the synthesis of procoagulant molecules like tissue factor and von Willebrand factor. This can result in a hypercoagulable condition, which can lead to widespread micro-/macrovascular thrombosis[63]. In addition to elevated levels of tissue factor and von Willebrand factor in the bloodstream, MAFLD patients also have increased levels of platelet activation and plasminogen activator inhibitor type 1 concentration. Patients with COVID-19 who have MAFLD have greater levels of circulating D-dimer than patients without MAFLD, indicating that the pro-coagulant condition associated with MAFLD may be a factor in the severity of COVID-19 [65]. According to findings from a retrospective investigation on a group of COVID-19 patients, people who presented with deep vein thrombosis, confirmed by Doppler ultrasound, had a greater prevalence of MAFLD[46,62]. In addition, COVID-19 patients with MAFLD had a higher mean admission and peak serum D-dimer concentrations than those without MAFLD[46]. In MAFLD individuals, COVID-19 may potentially further boost the production of proinflammatory cytokines, resulting in the activation of the coagulation cascade and thrombosis. In fact, a pathologic analysis of the pulmonary arteries in COVID-19 patients revealed extensive thrombosis with microangiopathy in addition to hepatic steatosis affecting 50%-60% of the liver parenchyma[68]. Hepatic steatosis and pulmonary thrombi were discovered in 55% and 73%, respectively, of COVID-19 patients, according to an Italian post-mortem examination, which corroborated this report[32]. These findings strongly imply a connection between these disorders, with the proinflammatory hypercoagulable state acting as a common pathogenic pathway to severe COVID-19, which promotes thrombosis and the spread of the disease[62-64].

### *Cytokine production*

Prolonged and significant lymphopenia, an abnormal inflammatory response related to aberrant and uncontrolled cytokine activation, and lung mononuclear cell infiltration are all associated with COVID-19[52]. The prognosis of an illness depends on the degree of involvement of additional organs. In fact, observational studies showed that increased levels of inflammatory markers in the blood (CRP, ferritin, and D-dimer), a higher neutrophil-to-lymphocyte ratio, as well as elevated levels of inflammatory cytokines and chemokines were linked to the disease severity and a poor prognosis[21]. One element of liver damage in COVID-19 may be dysregulation of the innate immune response. Inflammatory indicators, such as abnormally high levels of CRP, lymphocytes, neutrophils, and cytokines, are usual in COVID-19 patients[3]. Due to the loss of control over cytokine regulation, pulmonary and extrapulmonary damage occurs. During the early stages, this control could help to slow the evolution of the disease[62]. Hypercytokinemia, that is deadly or fulminant, may set off a series of events that damage or fail many organs, including the liver. Jaundice, hepatic encephalopathy, hepatomegaly, and increased blood transaminase levels could be brought on by the inflammatory response[62]. Since COVID-19 is associated with cytokine storm there is overlap of cytokines involved in both the disorders, however, it will be difficult to point out that these are sole causative agents for hepatic decompensation in MAFLD as there are more factors in play than the cytokines alone. It is also interesting to note that MAFLD patients had a distinct cytokine profile with higher concentrations of IL-6, IL-8, IL-10, and IFN-β when compared to the patients without MAFLD[69]. Higher levels of IL-8 and IL-10 are associated with the worst prognosis and delayed time to recovery[69].

### *Obesity*

Apart from diabetes, the presence of an "overfat" condition (excess body fat that harms health) has become a global pandemic and can occur in obese, overweight, and even normal weight subjects with excess fat involving the liver in the form of steatosis[70]. Several abnormalities can cluster together with overfat, including obesity, overweight, chronic "metabolic" inflammation, and insulin resistance, ultimately configuring the metabolic syndrome[18,20,30,32,44,45]. As seen by the higher prevalence of both autoimmune and immunological illnesses, excess body fat may impede the immunity[41]. Adiposity underlies a compromised immune response (mostly mediated by T cells and macrophages) that increases the risk of infections and chronic respiratory illnesses. Notably, being overweight appears to increase the risk of contracting contagious viral infections[47,48,71,72]. In particular, being overfat may have a negative impact on the immune system performance and host defense mechanism, while being overfat causes hosts to respond improperly to viral and bacterial attacks[48].

The association between obesity and COVID-19 severity persisted after adjusting for age, sex, smoking habits, diabetes, hypertension, and dyslipidemia. In MAFLD patients, obesity was associated with a 6-fold increased risk of severe infection[72]. Patients with MAFLD, especially those who are

obese, have been found to have higher levels of IL-6, which has been linked to an aggravation of the COVID-19 infection[72-74].

### *Pre-existing CLD*

Investigations on COVID-19 rarely include patients with pre-existing liver illness, and these patients' features according to their Child's status or model for end-stage liver disease (MELD) score have not been independently assessed in these studies. The innate immune response against the virus likely caused important changes in the liver enzymes and coagulation profile in 63 patients with severe COVID-19 disease, and CLD was not proven to affect the severity of COVID-19[3]. Cirrhosis patients are now recognized as an independent predictor of COVID-19 severity and a higher hospitalization risk [61]. According to the Child Turcotte Pugh score or MELD score, patients with cirrhosis have a greater mortality rate, and this rate increased as the severity of the liver disease grew[66]. Patients with cirrhosis had a significant 30-d death rate and a 20%-30% chance of decompensation manifesting as acute on chronic liver failure[66]. Data on patients with COVID-19-associated autoimmune hepatitis, chronic viral hepatitis, and alcoholic liver disease are few. Immunosuppressive medications should not be reduced for immunosuppressed patients, whether for autoimmune hepatitis or post-transplantation, out of concern for COVID-19[75]. Data on post-transplant patients are scarce, although there is no evidence of significant COVID-19-related mortality during the peri-transplant period[76]. Routine endoscopy and liver biopsy should be avoided, but urgent procedures for variceal bleed and cholangitis should be carried out according to the correct protocols designed for COVID-19 patients. Various drug combinations are being used with varying degrees of success in treating COVID-19 in patients with cirrhosis. According to the combined findings of the preliminary COVID-19 data, CLD had a negligible impact on patient progression to the severe stage of the disease. However, further research conclusively demonstrated that underlying CLD was associated with worse outcomes and more severe COVID-19 illness[48].

## HEPATIC IMMUNE MODULATION

Disconcerting, the inherent chronic activation of inflammatory pathways in MAFLD appears to increase liver damage in patients with COVID-19, perhaps worsening outcomes in those with prior comorbid metabolic disorders. The likelihood of developing more severe types of COVID-19 infection has also been reported to be increased in people with pre-existing chronic liver disorders[23]. Patients with severe COVID-19 infection had high levels of inflammatory markers like CRP, serum ferritin, lactate dehydrogenase, D-dimer, and interleukins (IL-6, IL-2)[36]. Individuals with MAFLD have been found to have elevated IL-6 Levels. When individuals with COVID-19 infection experience a "cytokine storm," IL-6 is a key player. Particularly, IL-6 seems crucial in the beginning and development of the "cytokine storm" seen in COVID-19-infected patients[52]. Elevated IL-6 Levels are linked to MAFLD, which may be a marker or mediator of the comorbidities and related atherosclerosis that are typically observed in COVID-19-infected patients[23]. The cytokine, monocyte chemoattractant protein (MCP-1), is commonly raised in patients with COVID-19 infection, which exacerbates steatohepatitis[23,24,77]. The interplay between COVID-19 and MAFLD in modulating the pathophysiology and outcomes of either disease is shown in Figure 1.

## DIAGNOSTIC CHALLENGES

An accurate clinical history, radiographic and laboratory tests, and histologic data are all necessary to diagnose MAFLD. In the absence of significant alcohol intake, other hepatic steatosis-causing factors, and the presence of other liver illnesses, hepatic steatosis must be present in order to diagnose the disease. MAFLD, diagnosed with abdominal imaging, reduces the need for invasive tests like liver biopsies[38]. A liver biopsy may be helpful when deciding between basic steatosis (NAFL) and NASH. Also, a liver biopsy can help in assessing the likelihood of other conditions that will worsen the MAFLD. Patients with COVID-19: Liver biopsy should be postponed in most patients because: (1) Liver biopsy may pose a risk for viral transmission (although the virus has not yet been detected in the liver tissue), the expression of its receptor on cholangiocytes suggests that the virus might be present[49]; (2) COVID-19 treatment/care outweighs diagnosis of concurrent liver disease; and (3) Systemic inflammation associated with COVID-19 will obscure etiology-specific histologic characteristics.

The patient's clinical history must be considered while interpreting test results (Table 2). The "World Gastroenterology Organization" suggests the following guidelines for treating people with liver disease generally in the COVID-19 era[49]: (1) Routine outpatient testing of liver biochemistry is not advised in the COVID-19 era; (2) Discard viral hepatitis in patients with increased ALT or AST levels. Due to the possibility that patients in developing nations have never undergone testing, this may be very crucial; (3) Local context and availability should be considered throughout routine investigation to rule out

**Table 2 Analysis of liver test results in coronavirus disease 2019 patients**

| Test | Comments |
|---|---|
| Prolonged INR or thrombocytopenia | In one-third of sick patients |
| | Spontaneous coagulopathy/DIC may be present |
| | Thromboembolic incidents are probably frequent |
| | There may be a chance of ACLF |
| Imaging | Where chest-CT is frequently performed: Assessing liver/biliary tract disease might be helpful |
| | Do US, if necessary, but refrain from using US for superfluous imaging (not formally investigated) |
| Hypoalbuminemia | Common in people with systemic inflammatory response |
| | May also be a sign of acute hepatic decompensation or acute liver failure in people with pre-existing liver cirrhosis |
| High transaminases or bilirubin (> 3 × ULN) | Although not typical for COVID-19, ACLF may be present in patients with cirrhosis who already have liver disease |
| Dyselectrolytemia | Diarrhea and other GI problems might result in numerous electrolyte abnormalities |
| Anemia | Consider bleeding due to variceal hemorrhage in the context of MAFLD cirrhosis, portal hypertensive gastropathy or stress mucosal GI ulcer |

ACLF: Acute on chronic liver failure; CT: Computed tomography; DIC: Disseminated intravascular coagulation; GI: Gastrointestinal; ULN: Upper limit of normal; US: Ultrasonography; COVID-19: Coronavirus disease 2019; MAFLD: Metabolic-associated fatty liver disease.

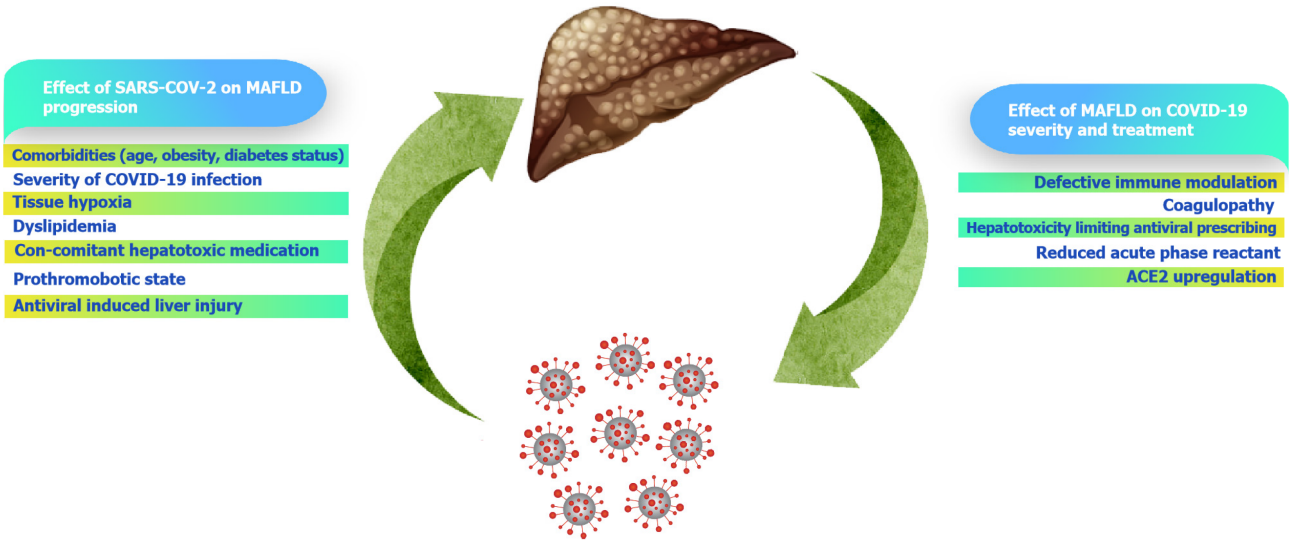


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**Figure 1 Interplay between coronavirus disease 2019 and metabolic-associated fatty liver disease.** COVID-19: Coronavirus disease 2019; MAFLD: Metabolic-associated fatty liver disease; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

other etiologies; and (4) Regular imaging should only be done if it will change management.

## NONINVASIVE MARKERS FOR FIBROSIS DETECTION

The increasing prevalence of MAFLD, the limits of liver biopsy, and the lack of consensus regarding clinical predictors of NASH have generated a market for next-generation noninvasive biomarkers and imaging modalities to aid in the distinction between MAFLD and NASH. Aminotransferases, cytokeratin-18, and numerous scoring systems that incorporate laboratory indicators such as the AST/platelet ratio index, NAFLD fibrosis score, FIB-4 index, and Fibrotest are some examples of indirect markers. The extracellular matrix contains direct fibrosis indicators such as fibronectin, elastin, laminin, and hyaluronic acid which develop in the presence of prolonged hepatocyte damage and have also been included in certain ratings[33,78].

## ENDOSCOPY FOR PATIENTS WITH COVID-19

Patients who are at risk of variceal bleeding, such as those with a history of variceal bleeding or symptoms of significant portal hypertension (ascites, low thrombocyte count, ALT > 5 × ULN) of unknown etiology, should be considered for esophago-gastro-duodenoscopic variceal screening (in case of suspected autoimmune liver disease, treatment without a histological diagnosis can be considered based on individual risk-benefit considerations)[49,75].

## DETECTION OF ACE2 POLYMORPHISM

Studies on whether *ACE1/ACE2* genetic variability influences the clinical course of COVID-19 in diverse ethnic communities remain elusive[14]. Between Asians and Caucasians, *ACE2* demonstrated significant minor allele frequency differences due to four missense mutations[71]. 64 K26R and I468W, two of these variations, may influence how the S protein of SARS-CoV-2 binds to the hACE2 receptor[55]. A difference in male and female individuals was found in *ACE2* expression between Asians and others [79]. The *ACE2* variant rs2285666 was not connected to the course of the disease when *ACE2* genetic variation was examined in the COVID-19 progression[71]. Nevertheless, numerous studies have shown a substantial correlation between COVID-19 and *ACE1*-insertion/deletion (I/D)[71,77,80]. When compared to *ACE1-II* people, *ACE1-DD* carriers had higher blood levels of ACE-I that are roughly twice as high and have been linked to hypertension, ARDS, and in-hospital mortality[81]. As a result, although the *ACE1-II* genotype negatively correlates with infection rate and mortality, the deletion allele positively corresponds with COVID-19 progression and SARS-CoV-2 infection rate and mortality. However, *ACE1-I/D* allele frequency ratio was substantially linked to the rise in recovery rate but not to mortality in a meta-analysis of 48758 healthy adults from 30 different nations[82]. Additionally, *ACE1-I/D* polymorphisms may help to explain how COVID-19 manifests in different ethnic populations. African Americans (29%, 60%, and 11%, respectively), Indians (19%, 50%, and 31%, respectively), and Whites (29%, 40%, and 31%, respectively) all had statistically different distributions of the *D/D*, *I/D*, and *I/I* genotype frequency ratios[82]. Additionally, there was a statistically significant difference in the frequency of the deletion allele among African Americans, Indians, and whites (0.59, 0.49, and 0.44, respectively)[79,81]. More research is necessary to determine whether these indications could explain COVID-19 progression in various populations. Overall, more *in vitro* and functional research are needed to fully understand the importance of the *ACE* and *ACE2* allele frequency ratio findings and how they relate to the COVID-19 studies[71]. These investigations ought to look into the morbidity and mortality hazards linked to COVID-19 and MAFLD in these racially varied genetic variants.

## CLINICAL INTERVENTIONS AND MANAGEMENT

### *Antivirals and monoclonal antibodies*

There is a paucity of information on the safety and effectiveness of new and existing COVID-19 treatments in patients with MAFLD, CLD, and cirrhosis. Clinical professionals' key worries are around adverse immune-related events, long-term effects, and drug interactions. On the basis of the presumption that dysregulated immune responses need to be suppressed; a number of medicines have been evaluated in COVID-19. Steroids, such as dexamethasone, prednisolone, methylprednisolone, or intravenous hydrocortisone, which act through the glucocorticoid receptor and effector genes, are one of the principal treatments. According to the World Health Organization recommendations; systemic corticosteroid medication is not recommended for everyday usage[5,83]. Only patients who have cytokine storm, ARDS, severe cardiac failure, acute kidney injury, and high serum D-dimer levels should receive it. Janus Kinase JAK inhibitors, IL-1 and IL-6 inhibitors, anti-tumor necrosis factor-alpha (often referred to as anti-TNF-alpha) medications, corticosteroids, colchicine, and intravenous immunoglobulin are other immunomodulators studied in COVID-19 infection[83]. Chloroquine and hydroxychloroquine have been shown to lessen COVID-19-mediated damage by stopping the cytokine storm, activating CD8+ cells, or blocking the virus from being taken up by endocytosis[84]. By building up in lysosomes and raising the pH of the endosome, chloroquine and hydroxychloroquine block the entry and departure of viruses from cells. Additionally, these medications block the ACE2 receptor, inhibiting SARS-CoV-2 entrance. Chloroquine and hydroxychloroquine may lessen the ACE2 receptor's glycosylation, preventing the virus from attaching to and infecting new cells. Chloroquine and hydroxychloroquine have been known to cause QT prolongation due to a delay in the cardiomyocyte depolarization rate[85]. There have been reports of patients developing torsades de pointes with the use of chloroquine[86]. Major studies, however, failed to demonstrate any alleged COVID-19 prophylactic and therapeutic benefit, and these medications have subsequently fallen out of favor due to their serious cardiovascular complication risk[87]. Other direct antivirals, like remdesivir and favipiravir, similarly did not demonstrate any appreciable efficacy or survival advantage[83]. In COVID-19, tocilizumab, a

**Table 3 Drugs and vaccines used in the management and prevention of coronavirus disease 19**

| Classification | Drugs |
|---|---|
| Antiviral agents | Favipiravir, molnupiravir, paxlovid, remdesivir |
| Immunomodulatory agents | |
| JAK inhibitors | Baricitinib, ruxolitinib, tofacitinib |
| Monoclonal antibodies to IL-6 | Sarilumab, tocilizumab |
| Corticosteroids | Cortisol, dexamethasone, methylprednisolone |
| Monoclonal antibodies to SARS-CoV-2 | Bamlanivimab, casirivimab, etesevimab, imdevimab, sotrovimab |
| COVID-19 vaccines | |
| mRNA | BNT162b2 [Pfizer-BioNTech], mRNA-1273 [Moderna] |
| Adenovirus vector | ChAdOx1-S [AstraZeneca, Oxford]; Ad26.COV2.S [Johnson and Johnson, Janssen], Sputnik-V-Gam - COVID Vac Ad5+Ad26 [Gamleaya] |
| Recombinant nanoparticles | NVX-CoV2373 [Novavax] |
| Miscellaneous agents | Azithromycin, chloroquine, dexamethasone, fluvoxamine, hydroxychloroquine, ivermectin |

COVID-19: Coronavirus disease 19; IL-6: Interleukin-6; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

humanized IgG1 monoclonal antibody to the IL-6 receptor, has shown only patchy success. However, the adverse effects can include hepatotoxicity, diverticulitis, hypertriglyceridemia, and increased susceptibility to infection[88]. Cytokine dialysis, utilizing blood ultrafiltration, diffusion, and adsorption circuits in dialysis machines, has also been tested as an alternative to medications that directly decrease the immune response[44]. Theoretically, restoring immunological IL-6/IL-1 levels and other proinflammatory molecules protect against organ failures, but the clinical effectiveness of this protection is still unknown, and immune dysregulation is just one issue among many[89]. It has been suggested to use immunomodulators based on mesenchymal stem cells to prevent and control the cytokine storm. Mesenchymal stem cell transplantation intravenously was proven successful in COVID-19 patients in a study[90].

Of the drugs listed in Table 3, the commonly used agents and their hepatoxicity profile are shown in Table 4. The appropriate selection of drugs in MAFLD patients depends on the severity of COVID-19 infection, duration of the disease, ALT level, and potential drug interactions with other medications. Our approach is in line with the Infectious Disease Society of America 2022 guidelines and is shown in Figure 2[91]. Hence when these drugs are used, liver function tests should be routinely monitored, and manufacturers' advice regarding dose adjustment should be followed until more studies are available in MAFLD patients. A very rare case of acute severe hepatitis with the use of Tocilizumab was noted in a patient who had previous lopinavir and ritonavir exposures[88]. In general, the management of these drug induced liver injury is usually symptomatic and in severe cases, when the ALT is more than six times the upper limit of normal, the medication may need to be temporarily stopped[44].

### *Vaccines*

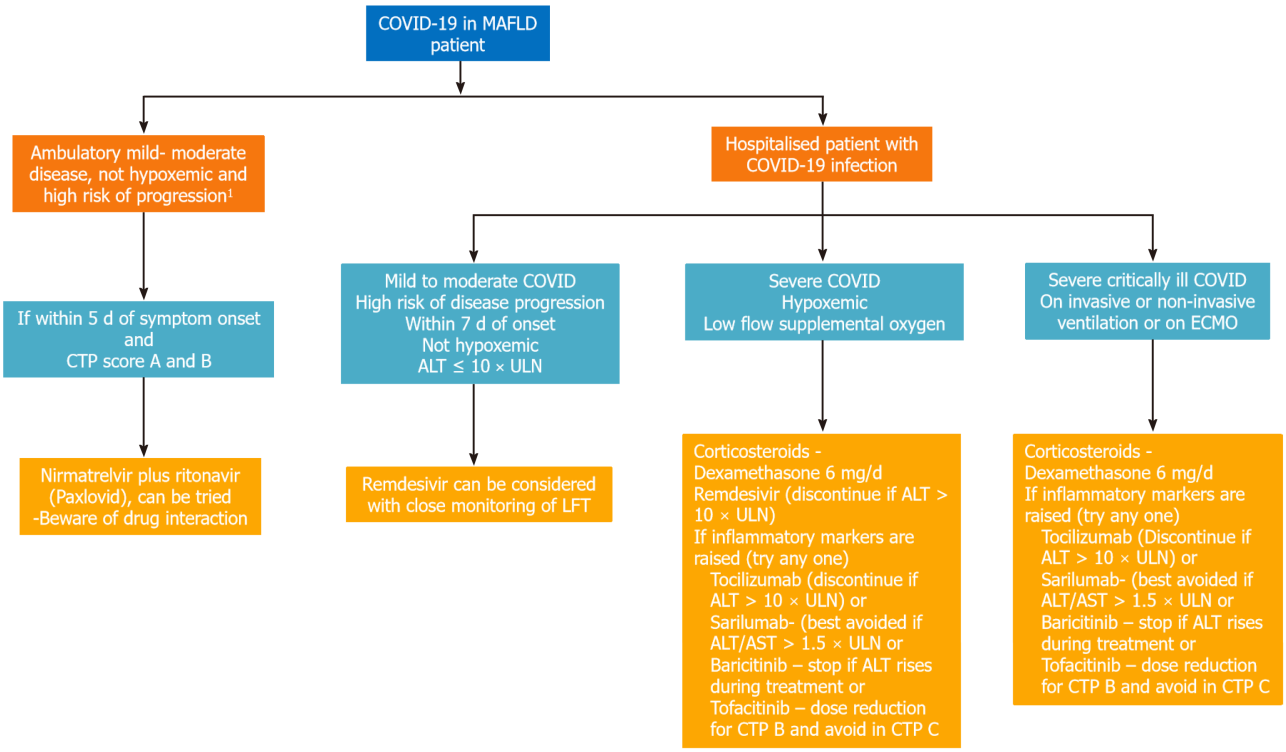
At least 85 vaccine proposals were being researched in clinical trial phases, and 184 vaccines were being evaluated in pre-clinical stages, according to the most recent version of the WHO report from April 2, 2021[28]. Other vaccines, including the plant-derived vaccine and the *Bacillus Calmette-Guérin* vaccine, have also been proven in tests to potentially aid in the management of the COVID-19 pandemic[92].

The currently commercially available vaccines include Oxford-AstraZeneca, Pfizer-BioNTech, Moderna, Sinopharm-Beijing, Gamaleya (Sputnik V), Sinovac, Sinopharm-Wuhan, Johnson & Johnson, Bharat Biotech (Covaxin), CanSino and Vector Institute (EpiVacCorona). In the multicenter study conducted by Wang *et al*[93], in patients with MAFLD who had two doses of inactivated vaccine against SARS-CoV2 without a history of SARS-CoV-2 infection, these vaccines were found to be safe with good immunogenicity. In a multicentric study from China, the inactivated vaccine induced adequate antibody titer against SARS-CoV-2 in 95% of the patients with MAFLD. The adverse event profile was similar to the individuals without MAFLD and hence the vaccine is safe and equally immunogenic as in the normal population[93].

**Table 4 Hepatoxicity profile of the commonly used drugs to treat coronavirus disease 2019 infection**

| Medication | Hepatotoxicity pattern |
|---|---|
| Dexamethasone | None reported at the dose given for COVID |
| Protease inhibitors (*e.g.*, lopinavir, ritonavir) | Mostly hepatitis pattern with ALT raise up to 6 times the normal, but rarely cholestatic pattern reported[43] |
| Nucleoside analogue: Remdesivir | Hepatitis pattern with mild to moderate ALT raise (up to 6 times the normal)[77] |
| Monoclonal antibodies to IL-6: Tocilizumab | Rarely can cause acute severe hepatitis in patients on concomitant or previous hepatotoxic drug usage[83] |

ALT: Alanine transaminase; COVID: Coronavirus disease; IL-6: Interleukin-6.




**DOI:** 10.3748/wjg.v29.i3.487 

**Figure 2 Selection of coronavirus disease 2019 therapy in metabolic-associated fatty liver disease patient.** [1]High risk of progression - advanced age ≥ 65-yr-old, immunocompromised state or multiple medical co-morbidities. Hypoxemia: $SpO_2$ ≤ 94% on room air; ECMO: Extracorporeal membrane oxygenation; Mild disease: Cough, upper respiratory tract symptom and absence of dyspnea; Severe: Hypoxemia or need for supplemental oxygen; Moderate: Dyspneic patient and absence of severe disease features. ALT: Alanine transaminase; AST: Aspartate transaminase; COVID: Coronavirus disease; CTP: Child Pugh score; LFT: Liver function test; ULN: Upper limit of normal.

## CONCLUSION

SARS-CoV-2 infection's pandemic traits and high mortality rate have sparked worries about the processes causing harm to vulnerable patients. The people most susceptible to COVID-19 had pre-existing illnesses. As a result of metabolic irregularities, the accumulation of metabolically active fat (also known as the "overfat state") coexists with chronic inflammatory alterations, the emergence of insulin resistance, the buildup of fat in the liver, and perhaps even hepatic fibrosis in the long run. This interplay between the numerous inflammatory pathways constantly present in MAFLD can dramatically increase the risk for COVID-19 infection and intensify liver damage. MAFLD should therefore be considered as a prognostic indication during COVID-19, while on the other hand, close long-term monitoring of individuals with MAFLD who experienced COVID-19 may be required. Finally, reducing the vulnerability to non-communicable diseases and boosting personal resistance to future epidemics are additional challenges in diagnosing and treating individuals with MAFLD.

## FOOTNOTES

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*MINIREVIEWS*

# Potential role of the microbiome in liver injury during COVID-19: Further research is needed

Marcos Roberto Tovani-Palone, Paolo Pedersini

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## Abstract

Although different studies have associated coronavirus disease 2019 (COVID-19) with the occurrence of liver injury, the hepatic injury route during the COVID-19 course is not yet fully understood. In order to better understand the mechanisms of the disease, the human gut microbiota has been the subject of extensive discussion in the context of COVID-19 pathophysiology. However, many questions remain, including the risks of liver injury due to COVID-19 specific populations. Further research in this field could allow the discovery of new personalized treatment strategies aimed at improving the microbiota composition, thereby reducing COVID-19 severity and its complications in different populations. In this article, we discussed basic mechanisms of severe acute respiratory syndrome coronavirus 2 infection and recent evidence on the relationship between COVID-19, the gut microbiome and liver injury as well as proposed recommendations for further research.

**Key Words:** COVID-19; Gut microbiota; Coronavirus; Gut microbial-host-immune axis; Gut-lung axis; Liver injury



**Core Tip:** Although different studies have associated coronavirus disease 2019 (COVID-19) with the occurrence of liver injury, the hepatic injury route during the COVID-19 course is not yet fully understood. Further research is needed to better understand the impacts of changes of the gut microbiota and immunology of COVID-19.

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## INTRODUCTION

The gut-liver axis is a well-described bidirectional relationship where a mutual interaction between gut and liver microbiota occurs. It has attracted significant attention in the context of coronavirus disease 2019 (COVID-19). This close anatomical and functional relationship between the gut and its microbiota and liver function results from an interaction between genetic and environmental factors, including diet, medicine use and diseases[1]. Although the human gut microbiota is recognized to have an important role for immunity and protection against pathogens, its diversity decreases in old age, which is the age group with the highest mortality from COVID-19[2]. This suggests a potential protection of balanced gut-liver axis against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which should be of interest to prevent and reduce the number of fatal cases of COVID-19. On the other hand, any imbalance of this microbiome should affect immunity as well as viral activity against SARS-CoV-2 [3]. Moreover, different studies have also reported the occurrence of liver injury to varying degrees in COVID-19 patients, which could be associated with important changes in both the gut-liver axis microbiota and responses at the cellular and molecular level[4,5]. However, research on the risks of liver injury due to COVID-19 in many specific populations is still scarce. Here we discussed basic mechanisms of SARS-CoV-2 infection and recent evidence on the relationship between COVID-19, the gut microbiome and liver injury as well as proposed recommendations for further research.

## CELLULAR ENTRY OF SARS-COV-2 AND GENERAL IMPLICATIONS

There is a consensus among most scientists that the cellular entry of SARS-CoV-2 primarily occurs *via* high-affinity interactions between the receptor-binding domain of the SARS-CoV-2 spike protein and the angiotensin converting enzyme 2 (ACE2) receptor, in addition to other molecules[4-7]. This receptor has been identified in different and important organs, including the surface of respiratory tract epithelium, epithelial cells of the upper esophagus, enterocytes of the ileum and colon, in the heart, testicles, cells of smooth muscles, the endothelium of pancreatic, brain and kidney blood vessels[4], and in bile duct epithelial cell and liver[5]. The resulting downregulation of ACE2 activity may lead to an increase in angiotensin 2 through ACE. This is due to the fact that the decrease in ACE2 is associated with a lower conversion of angiotensin to angiotensin 1-7 vasodilator. Thus, there is a gradual tendency towards an increase in plasma concentrations of angiotensin I and angiotensin II, causing an imbalance in the renin-angiotensin system as well as a consequent deregulation of systemic homeostasis[6,8].

## COVID-19 AND GUT

According to general statistics, about half of COVID-19 patients are expected to have at least one of these gastrointestinal symptoms: diarrhea, nausea, vomiting, and abdominal pain[4,5]. Research has shown that the ACE2 receptor is the main gateway for SARS-CoV-2 into epithelial cells of the gastrointestinal tract. This receptor is in turn highly expressed on epithelial cells in the small intestine. In addition to the decrease in ACE2 receptor expression due to the invasion of SARS-CoV-2, important changes in the gut microbiota involving different microorganisms (dysbiosis) may also occur (Figure 1), affecting the function of the intestinal barrier and the permeability and homeostatic balance of metabolites in the gut lumen[8,9].

It is also hypothesized that SARS-CoV-2 infection of epithelial cells in the gut, especially in the small intestine, could result in malnutrition as well as potentiate the associated dysbiosis, leading to impaired gut barrier function and systemic inflammation. This in turn may create a positive feedback loop for increased translocation of gut microbes into the systemic circulation and potentiation of inflammation, culminating in systemic inflammation and cytokine storm that may contribute to both worsening gut and systemic damage as well as increasing the severity of COVID-19[8,9]. Therefore, in addition to the classic gastrointestinal disorders and symptoms of COVID-19, accessory digestive organs such as the liver can be affected, as a result of the worsening infection[4].

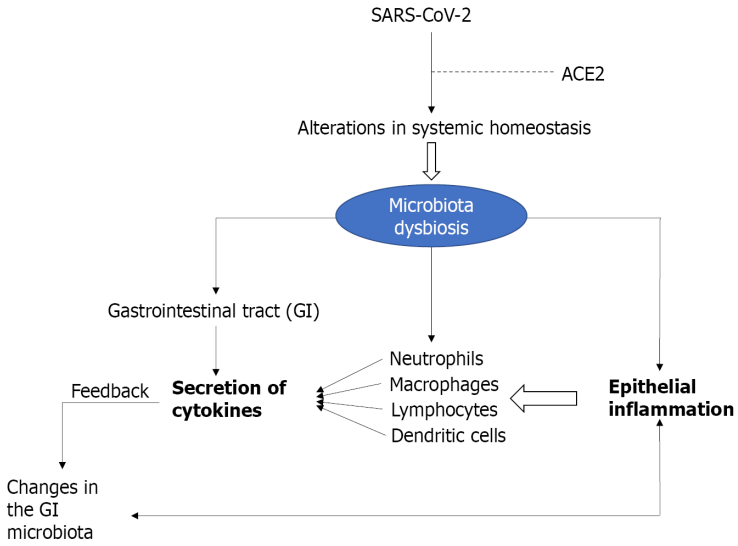


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**Figure 1 Pathway of severe acute respiratory syndrome coronavirus 2 to gastrointestinal microbiota imbalance.** ACE2: Angiotensin converting enzyme 2; GI: Gastrointestinal tract; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

## COVID-19 AND LIVER INJURY

Although COVID-19 has been associated with liver injury, the hepatic injury route during the COVID-19 course is not yet fully understood. It is believed that such injury is due to specific pathogenic mechanisms of the virus or even the use of hepatotoxic drugs[3,4]. Among the different etiological hypotheses described in the literature in order to advance knowledge about this topic the following stand out: (1) Liver injury resulting from a direct virus cytopathic effect by lysis or by inducing apoptosis; (2) Immune-mediated liver injury from proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor, chemokines, and inflammatory cells produced against SARS-CoV-2); (3) Liver injury resulting from viral-induced cytotoxic T cells (CD8); (4) Liver injury due to the use of drugs including antivirals, anti-inflammatory drugs, anticoagulants, antibiotics, and drugs used for chronic diseases during SARS-CoV-2 infection; (5) Liver injury caused by hypoxia resulting from pneumonia[4,5]; and (6) Liver injury resulting from the gut vascular barrier and dysbiosis due to the indirect effect of toxic compounds from opportunistic microorganisms[5].

## COVID-19, THE GUT MICROBIOME AND LIVER INJURY

More specifically, researchers in this field believe that the occurrence of prolonged gut microbiome dysbiosis in COVID-19 patients may be associated with two important phenomena: fecal shedding of the virus into the environment and disease severity. Evidence for this pathophysiological mechanism is based on the hypothesis that dysbiosis may lead to epithelial inflammation and an increase in ACE2 expression. Given that ACE2 plays a key role in dietary amino acid homeostasis, patients can be severely affected. In this connection, SARS-CoV-2 binds to ACE2, leading to microflora imbalance. This is because the possible downregulation of ACE2 may reduce the secretion of antimicrobial peptides and in turn lead to increased pathogen survival and gut dysbiosis[5]. It is also worth noting that some drugs used to treat COVID-19, such as corticosteroids, have been shown to interact with the gut microbiome. This is also true for chloroquine, which has been equivocally administered to many patients[3,5] as well as different medicinal herbs[10].

Despite this, in the current context of the ongoing pandemic, although a large amount of research has been published on liver injury due to COVID-19[4,5], there remain many questions to be answered (Table 1), including the risk of this type of injury in specific populations. Important research has demonstrated a greater vulnerability to alterations in the composition of the gut microbiota in different populations. This is true for example for the population of individuals with cleft lip and palate[11] and Hashimoto's thyroiditis[12]. Therefore, knowing more about interactions between the human microbiota and the host cytokine pathway should be of great relevance. One of the justifications for carrying out further research in this field includes the need to discover new personalized treatment strategies to improve the composition of the gut microbiota in order to more effectively reduce the severity of COVID-19 and its complications[3,5]. This in conjunction with a healthy lifestyle could have positive impacts on both COVID-19 prevention and treatment[13,14].

**Table 1 Other important questions to be answered by new research**

| No. | Major questions |
|---|---|
| 1 | Mean duration of dysbiosis associated with liver injury due to COVID-19 |
| 2 | Differences in the magnitude of liver injury and changes in the microbiota associated with COVID-19 in patients with varying degrees of disease severity |
| 3 | The impact of different drugs metabolized in the liver on the worsening of liver injury associated with COVID-19 and changes in the gastrointestinal microbiota |
| 4 | Whether changes in the gastrointestinal microbiota and liver injury associated with COVID-19 are also related to long-COVID-19 symptoms |
| 5 | Effective medical protocols and/or treatments to prevent changes in the gastrointestinal microbiota and prevent or treat this type of liver injury |
| 6 | The impact of healthy habits on the prevention of changes in the gastrointestinal microbiota and recovery of liver injury in COVID-19 patients |

COVID-19: Coronavirus disease 2019.

## CONCLUSION

In view of the development of new COVID-19 vaccines, another important point to consider is that the microbiome may affect the immune response to vaccines. This is due to the fact that the immunogenicity can be impaired with dysbiosis[5]. Moreover, due to the likely global endemic situation of COVID-19, further microbiological and immunological research may be critical to determine the impact of changes to the balance of the human microbiota and immunology related to COVID-19 in order to achieve better predictions in the fight against possible new SARS-CoV-2 variants.

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## FOOTNOTES

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*MINIREVIEWS*

# Artificial intelligence and inflammatory bowel disease: Where are we going?

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## Abstract

Inflammatory bowel diseases, namely ulcerative colitis and Crohn's disease, are chronic and relapsing conditions that pose a growing burden on healthcare systems worldwide. Because of their complex and partly unknown etiology and pathogenesis, the management of ulcerative colitis and Crohn's disease can prove challenging not only from a clinical point of view but also for resource optimization. Artificial intelligence, an umbrella term that encompasses any cognitive function developed by machines for learning or problem solving, and its subsets machine learning and deep learning are becoming ever more essential tools with a plethora of applications in most medical specialties. In this regard gastroenterology is no exception, and due to the importance of endoscopy and imaging numerous clinical studies have been gradually highlighting the relevant role that artificial intelligence has in inflammatory bowel diseases as well. The aim of this review was to summarize the most recent evidence on the use of artificial intelligence in inflammatory bowel diseases in various contexts such as diagnosis, follow-up, treatment, prognosis, cancer surveillance, data collection, and analysis. Moreover, insights into the potential further developments in this field and their

effects on future clinical practice were discussed.

**Key Words:** Inflammatory bowel disease; Artificial intelligence; Machine learning; Crohn's disease; Ulcerative colitis; Computer-aided diagnosis



**Core Tip:** Management of patients with inflammatory bowel disease is complex and costly. Therefore, in this field being able to improve clinical efficiency and optimize healthcare resources is of paramount importance. In this regard, artificial intelligence appears to be an extremely promising tool with a significantly wide range of potential applications that encompass diagnosis, treatment, and follow-up. This review summarized the most recent significant scientific findings regarding the application of artificial intelligence in inflammatory bowel diseases, providing a picture of the current state of the field and future perspectives.

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## INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic and relapsing conditions that affect the gastrointestinal tract and are labelled as inflammatory bowel diseases (IBD)[1,2]. In the last few decades, IBD has emerged as a global disease with a conspicuous burden on public health and healthcare costs [3].

The diagnosis and management of IBD is complex and implies the interplay and synergy of various specialists, including clinical gastroenterologists, gastrointestinal endoscopists, radiologists, pathologists, surgeons, and clinical nutritionists[4].

Along with histological assessment, endoscopy has an important role in the diagnosis and the follow-up of IBD while also being the mainstay for colorectal cancer surveillance[5]. Due to the essential role of endoscopy and imaging in gastroenterology, and particularly in IBD, artificial intelligence (AI)-based image analysis can be utilized in numerous applications such as evaluation of endoscopic lesions, cancer detection, and assessment of disease activity (*e.g.*, prognosis and response to treatment)[6].

## ARTIFICIAL INTELLIGENCE

AI is an umbrella term that encompasses any cognitive function developed by machines for learning or problem solving. A particular subset of AI is represented by machine learning (ML), a discipline that uses large datasets as an input in order to identify patterns of interaction among variables, allowing the possibility to apply these findings to new data[7].

A further subset and evolution of ML is deep learning (DL), which mimics the neuronal interaction in the human brain to develop artificial neural networks and subsequently convolution neural networks (CNN) that are then able to use the input data in an autonomous fashion with the aim of assessing predictive factors of a specific outcome through the development of multiple levels of abstractions[8].

Among the numerous implementations of AI in medicine, a promising field is that of automatic collection of complex and nuanced clinical data from electronic medical records through natural language processing, the subset of AI that studies the interpretation of the human language made by the computer[9,10].

Another fertile ground of application of AI is the interpretation of radiologic imaging. A computer model based on computed tomography enterography allows for accurate characterization of intestinal fibrosis in CD, in some instances outperforming human radiologists[11].

In the context of gastrointestinal endoscopy, AI has found application in two main fields, namely in the detection of mucosal lesions, with computer-aided detection (CADe) and in the characterization of mucosal lesions, with computer-aided diagnosis, along with the evaluation of the quality of the endoscopic procedure itself, with computer aided monitoring[12-20].

Therefore, implementation of AI in IBD is a promising tool for improving the assessment of disease activity and reducing the interobserver variability in grading such activity[21]. In addition, similar to what is already happening in the general population, CADe systems could eventually improve detection of IBD-associated dysplasia[22]. Finally, AI may also allow for application of precision medicine through the analysis of large databases, correlating differences in the biology of the patient with differences in the susceptibility to develop IBD, the activity of the disease, and the response to specific therapies[23] (Figure 1). Such considerations are further supported by the growing number of clinical studies on the application of AI for IBD in recent years (Figure 2).

Table 1 summarized the most impactful studies on AI in several fields of IBD that were identified after a literature review using PubMed (MEDLINE) from inception to November 6, 2022. The impact measure of the articles cited was assessed *via* the *Reference Citation Analysis* (RCA; Baishideng Publishing Group Inc.) tool.

## AI IN ETIOLOGY, PATHOGENESIS, AND DIAGNOSIS OF IBD

Although it is not yet thoroughly understood, the etiology of IBD is known to depend upon the complex interplay of genetic, microbial, and environmental factors and the immune system[24]. In this context, AI allows for more effective data analysis to evaluate the role of specific genes in the predisposition and development of IBD.

Genome-wide association studies have been employed in order to identify sequence variations related with specific conditions, with over 200 genes found to be potentially implicated in IBD etiology [25]. Such complex and large genomic data, however, are difficult to assess with standard analytical tools. In this context, studies have shown that ML and DL can effectively analyze genome-wide association study data and overcome part of the inherent limitation of such methodology through algorithms of minimum Redundancy-Maximum Relevance and incremental feature selection[26-28].

Gut microbiota is thought to play a relevant role in the complex etiology and pathogenesis of IBD, partly concurring in providing a substrate that may range from protective to proinflammatory[29]. Moreover, in the context of dysbiosis, the taxonomic composition appears to vary between patients affected by IBD and unaffected subjects and between IBD subtypes, UC, and CD. Promising studies in this field show that the application of ML algorithms on the analysis of gut microbiome data may assist the clinician in diagnosing IBD[30,31].

AI has also been employed in the attempt of supporting the conventional diagnosis of IBD. In this regard, isolated and combined endoscopic and histological parameters were used to develop ML models, which was accurate in the classification of pediatric patients affected by IBD[32].

Additional models based on confocal laser endomicroscopy were developed and by quantitative analysis through cryptometry were able to provide a diagnosis of IBD with high sensitivity and specificity. Moreover, these models managed to successfully differentiate between UC and CD[33].

## AI APPLICATIONS FOR ENDOSCOPIC ASSESSMENT IN IBD

In order to rigorously define endoscopic disease activity through specific parameters and limit interobserver variability, a plethora of endoscopic scores have been developed in the field of IBD. The most relevant scores are the CD endoscopic index of severity and the simple endoscopic score for CD, the Mayo endoscopic score, the UC endoscopic index of severity, and the UC colonoscopic index[34-38].

Implementation of AI in this field might be a further step towards reproducibility and homogeneity of endoscopic findings. The first successful attempts in identifying the presence of mucosal remission or activity *via* AI were made using a dedicated CADe system based on CNN trained on large datasets of endoscopic still images in patients affected by UC[39,40].

A subsequent development was represented by the implementation of neural networks in order to assess disease activity not only on still images but on the entirety of colonoscopy videos in real time. Also in this field, AI was efficient in recognizing active disease, calculating scores, predicting the risk of clinical relapse, and supporting clinicians in real time decision making for treatment[41-44].

Moreover, deep neural networks trained on endoscopic images and histological reports of UC managed to identify patients in endoscopic remission and histological remission with such an accuracy that it may potentially obviate the need for biopsy collection and analysis to identify patients in remission[45].

With regards to prediction of *in vivo* histological activity, the first dedicated CADe system was recently developed for application in endocytoscopy, yielding a high accuracy when compared with the gold standard of the pathologist's assessment[46].

Finally, in order to overcome the fact that conventional DL systems train CNN based on the subjective scoring of images done by clinicians, novel approaches have been developed based on algorithms that only analyze parameters such as pixel color data and vascular pattern recognition. Objective computer-based and operator-independent tools provided a dedicated score that significantly

**Table 1 Main studies of artificial intelligence application in the various fields of inflammatory bowel diseases**

| Ref. | Field | Study features | Main finding |
|---|---|---|---|
| Isakov *et al*[26], 2017 | IBD genetics | ML model to assess 16390 genes in IBD and healthy patients | Identified 347 IBD-risk genes (67 newly identified) |
| Cheng *et al*[27], 2019 | IBD genetics | Software analysis to assess the genetics of 32713 IBD patients | Identified several genes potentially involved in UC; identification of 11 common Gene Ontology terms for UC |
| Yuan *et al*[28], 2017 | IBD genetics | Software analysis to assess 12754 genes in IBD and healthy patients | Identified 41 genes closely associated with IBD |
| Mihajlović *et al*[30], 2021 | IBD and microbiota | ML classification algorithm to identify IBD from 1638 fecal samples | Confirmed strong connection between IBD and specific fecal microbial species |
| Manandhar *et al*[31], 2021 | IBD and microbiota | ML model analysis of fecal microbiota from 729 IBD patients and 700 healthy controls | Identified of 117 bacterial taxa with a potential role in diagnostic screening of IBD |
| Mossotto *et al*[32], 2017 | IBD diagnosis | ML model to assess 287 pediatric patients with IBD | Accuracy of 83.3% of the combined endoscopy-histology ML model in the classification of pediatric IBD patients |
| Quénéhervé *et al*[33], 2019 | IBD diagnosis | AI analysis of CLE images from 50 IBD patients and 9 healthy controls | AI analysis had 100% sensitivity and specificity for IBD diagnosis, 92% sensitivity and 91% specificity of IBD differential diagnosis |
| Ananthakrishnan *et al*[9], 2013 | IBD diagnosis and data collection | NLP model trained and validated on 700 UC patients and 700 CD patients to improve case definition and identification from EMRs | NLP model provided better accuracy (AUC 0.94-0.95) than models using only the International Classification of Diseases 9th revision for IBD case definition and identification |
| Stidham *et al*[39], 2019 | IBD endoscopy | DL model for UC severity trained on 16514 endoscopic images | Similar performance of the DL model and experienced human reviewers in grading UC endoscopic severity |
| Ozawa *et al*[40], 2019 | IBD endoscopy | CNN-based CADe system for UC severity trained on 26304 endoscopic images | CADe system had AUCs of 0.86 and 0.98 in the identification of Mayo score 0 and 0-1, respectively |
| Maeda *et al*[41], 2021 | IBD endoscopy | Endoscopic AI model used in real time on 135 UC patients in clinical remission | Endoscopic applications of real time AI predicted clinical relapse of UC with statistical significance |
| Gottlieb *et al*[42], 2021 | IBD endoscopy | DL algorithm to assess UC severity on 795 full-length endoscopy videos | DL algorithm showed significant inter-rater agreement to human central readers for prediction of UC severity |
| Yao *et al*[43], 2021 | IBD endoscopy | Endoscopic AI model (CNN) to assess UC grading used on 169 endoscopy videos and compared to dual central reader review | AI model approximated the scoring of experienced reviewers for grading of UC endoscopic activity |
| Byrne *et al*[44], 2021 | IBD endoscopy | DL model (CNN) to detect and assess UC activity leveraged on > 375000 frames | DL model resulted in well aligned scoring guidelines and experts' performances |
| Takenaka *et al*[45], 2020 | IBD endoscopy | DL algorithm trained on endoscopic images and biopsy results and tested on 875 UC patients | DL model identified with an accuracy > 90% patients in endoscopic and histologic remission |
| Maeda *et al*[46], 2019 | IBD endoscopy (endocytoscopy) | CADe system to predict persistent histologic phlogosis from endocytoscopy validated on 100 UC patient | CADe system provided a diagnostic accuracy of 91% with perfect reproducibility for identification of persistent histologic inflammation |
| Bossuyt *et al*[47], 2020 | IBD endoscopy | AI algorithm based on pixel color data and pattern recognition from endoscopic images tested on 55 patients | AI algorithm ("red density") provided an objective computer-based assessment of UC disease activity with good correlation with endoscopic and histological scoring systems |
| Aoki *et al*[51], 2019 | IBD endoscopy (VCE) | AI system (CNN) tested on 10440 small bowel images for detection of erosions and ulcers in CD | AI system showed an accuracy of 90.8% for detection of erosions and ulcers |
| Klang *et al*[52], 2020 | IBD endoscopy (VCE) | DL algorithm applied on 17640 VCE images for ulcer detection in CD | DL algorithm provided an accuracy ranging from 95.4% to 96.7% with an AUC of 0.99 for ulcer detection |
| Klang *et al*[53], 2021 | IBD endoscopy (VCE) | DL model applied on 27892 VCE images for identification of intestinal strictures in CD | DL model showed an accuracy of 93.5% in stricture identification and excellent differentiation between strictures and other lesions |
| Ferreira *et al*[54], 2022 | IBD endoscopy (VCE) | DL model trained and validated on 8085 VCE images for detection of erosions and ulcers in CD | DL model provided an accuracy of 92.4% and a precision of 97.1% for lesion detection |
| Aoki *et al*[55], 2020 | VCE | Comparison between standard endoscopist reading and reading after AI model screening of 20 full-length VCE videos | The mean VCE video reading time was significantly shorter after AI model (CNN) screening compared to standard reading |
| Maeda *et al*[56], 2021 | IBD endoscopy | Case report of dysplasia detection by AI | AI system (EndoBRAIN) identified 2 colonic lesions that |

| | | | |
|---|---|---|---|
| | (surveillance) | system in a patient with long standing UC | harbored low-grade dysplasia upon histological examination |
| Fukunaga *et al*[57], 2021 | IBD endoscopy (surveillance) | Case report of dysplasia detection by AI system in a patient with long standing UC | AI system (EndoBRAIN) identified rectal lesions that harbored high-grade dysplasia upon histological examination |
| Reddy *et al*[72], 2019 | IBD prognosis | ML model employed on 82 CD patients' EMRs to predict disease course | ML model predicted inflammation severity with high accuracy (AUC 92.8%) from EMR data |
| Takenaka *et al*[73], 2022 | IBD prognosis | ML model validated on endoscopic images and biopsy results from 875 UC patients to predict disease course | Histologic remission detected by the ML model correlated with a significant reduction in clinical relapse, steroid use, hospitalization, and colectomy |
| Li *et al*[75], 2021 | Response to treatment | ML model employed on 174 CD patients to predict response to infliximab | ML model based on clinical and serological parameters showed an accuracy of 0.85 for prediction of response to infliximab |
| Waljee *et al*[76], 2018 | Response to treatment | AI model employed on 472 CD patients to predict response to vedolizumab | AI model based on clinical and serological parameters was able to identify patients that achieved a corticosteroid-free biologic remission at week 52 of vedolizumab |
| Waljee *et al*[77], 2018 | Response to treatment | ML algorithm employed on 491 UC patients to predict response to vedolizumab | ML algorithm based on clinical and serological parameters was able to identify patients that achieved a corticos-teroid-free biologic remission at week 52 of vedolizumab |
| Doherty *et al*[78], 2018 | Response to treatment | AI model to assess response to treatment with ustekinumab in 306 patients with CD | AI model detected patients in remission based on clinical data and fecal microbiota at week 6 and 22 of ustekinumab |

AI: Artificial intelligence; AUC: Area under the curve; CADe: Computer-aided detection; CD: Crohn's disease; CLE: Confocal laser endomicroscopy; CNN: Convolution neural networks; DL: Deep learning; UC: Ulcerative colitis; EMR: Electronic medical record; IBD: Inflammatory bowel disease; ML: Machine learning; NLP: Natural language processing; VCE: Video capsule endoscopy.

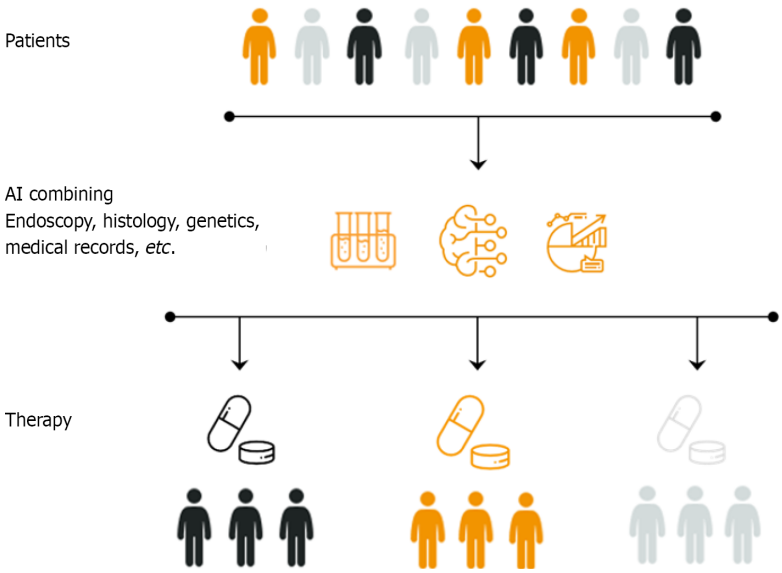


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**Figure 1 Application of artificial intelligence in inflammatory bowel diseases for precision medicine.** AI: Artificial intelligence.

correlated with both endoscopic and histological scoring systems[47].

Video capsule endoscopy (VCE) represents one of the main modalities of investigating the small bowel in suspect or established CD[48]. The main scoring systems used for disease activity quantification are the Lewis score and the capsule endoscopy CD activity index, which consider parameters such as extension, grading of inflammation, and presence of strictures[49,50].

VCE video review, however, is time-consuming and requires a high level of attention during the observation of thousands of frames. In order to simplify this task, AI has been implemented with the objective of reducing the time needed for image assessment by selecting the most relevant frames or portions of the video. Several CNN models have been developed in order to recognize pathologic findings such as erosions, ulcers, and strictures with very high accuracy[51-54]. Moreover, a study that assessed the review time for VCE showed how the employment of AI systems allowed clinicians to complete the task in a fraction of the time with no differences in overall accuracy[55].

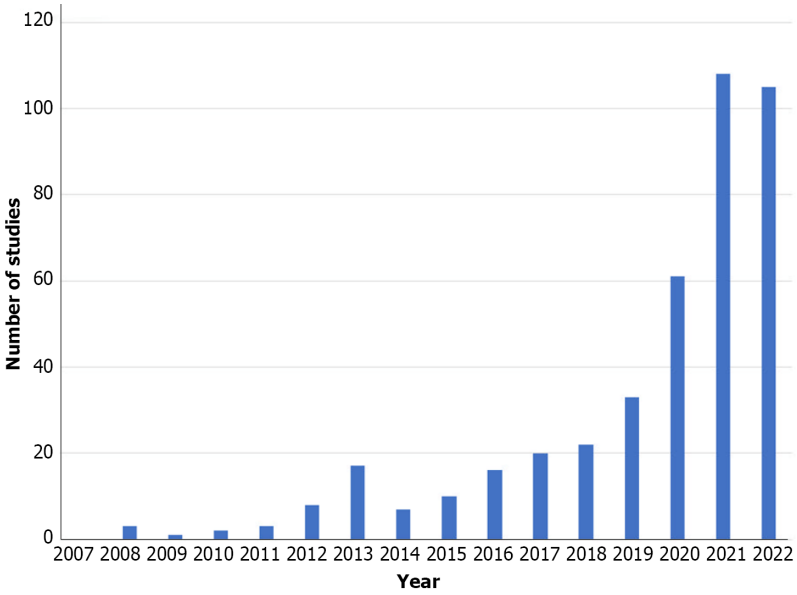


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**Figure 2 Clinical studies on the application of artificial intelligence in inflammatory bowel diseases in the last 25 years (as of November 6, 2022).**

In addition to recognition of disease activity, AI may find a relevant role in the surveillance of colorectal neoplasia in patients with IBD since a history of long-standing disease is a significant risk factor in developing colorectal cancer[5]. While AI is proving increasingly useful in the detection of colonic neoplasia in the general population with dedicated CADe systems, first attempts at developing specific applications for surveillance in IBD are being made[13]. As of today, successful detection of dysplasia has been described in case reports regarding the application of EndoBRAIN, a CADe system, in endoscopy and endocytoscopy[56,57].

Along with CADe systems, AI may prove to be an effective and safe tool for real-time quality improvement of the examination as well (*i.e.* withdrawal time, checking for blind spots), thereby potentially increasing the adenoma detection rate also in IBD patients[58].

## AI IN IBD HISTOLOGY ASSESSMENT

Histological remission is now considered an adjunct target of treatment in UC and arguably the most stringent way to assess remission[59]. Growing evidence shows that persistence of histologic disease activity, even in the absence of macroscopic endoscopic inflammation, is associated with worse clinical outcome and risk of relapse[60]. More than 30 histological scores have been proposed to grade UC histological activity, but their application in clinical practice remains minimal, mainly due to the impracticality of the scores[61,62]. Even when the scores are applied, for example in clinical trials, the interobserver variability is very high, limiting comparison and reproducibility. Indeed, clinical trials increasingly resort to expensive central reading systems so that all biopsies are evaluated by few highly-qualified pathologists to reduce variability. Therefore, AI-based systems to automatically read UC biopsies would be of great help standardizing the assessment and reducing interobserver variability[63].

Trials in this field are ongoing, and initial results are promising. The first attempt to develop a CADe model to assess UC biopsies focused on eosinophils. The system had a good agreement compared to the manual count performed by human pathologists (interclass correlation coefficient = 0.81-0.92) but did not demonstrate an association between eosinophils counted and overall inflammatory activity[64]. More recently, Gui *et al*[65] proposed to simply assess UC activity by taking into consideration the sole presence or absence of neutrophils, the hallmark of active inflammation. They proposed a simplified score, the PICaSSO histologic remission index that was then embedded into a CADe system that was able to distinguish histological activity from remission in biopsies of UC with good accuracy. Further improvements to the same CADe have been recently presented showing that the neutrophil-only assessment by the CADe is largely consistent with mainstay scores such as Robarts and Nancy histological indexes[66].

Other studies on CADe systems for assessment of UC are ongoing and preliminary results are promising[67,68] (Table 2). Further applications of CNN in computational pathology have been studied in order to empower the pathologist's accuracy and efficiency, with models trained on whole slide

**Table 2 Artificial intelligence application for histological assessment of ulcerative colitis**

| Ref. | Study design | Population | Outcome | Results |
|---|---|---|---|---|
| Vande Casteele *et al*[64], 2022 | Cohort study | Colonic biopsies from 88 UC patients with histologically active disease | To assess a DL machine in quantifying eosinophils in colonic biopsies and validate against a pathologist's count | The AI system highly agreed with manual eosinophil count by pathologists (ICC 0.81-0.92) |
| Peyrin-Biroulet *et al*[67], 2022 | Cohort study | 200 histological images of UC biopsies | To evaluate an AI algorithm in assessing histological disease activity according to the Nancy index | The CNN model had an excellent agreement with pathologists in the assessment of the Nancy index (ICC 0.84) |
| Villanacci *et al* [66], 2022 | Cohort study | 614 biopsies from 307 UC patients | To test a CNN-based CADe system for evaluating HR based on PHRI, Robarts, and Nancy indexes | The CADe system accurately assessed HR (sensitivity 89%, specificity 85% for PHRI) and similar performance for Nancy and Robarts |

AI: Artificial intelligence; CADe: Computer-aided detection; CNN: Convolution neural networks; DL: Deep learning; HR: Histological remission; ICC: Interclass correlation coefficient; PHRI: PICaSSO histologic remission index; UC: Ulcerative colitis.

images that were able to effectively support the pathologists by excluding non-diagnostic slides, while retaining optimal sensitivity[69].

## AI IN PROGNOSIS AND PREDICTION OF RESPONSE TO TREATMENT

Being able to predict the course of disease with regards to severity and progression is of the utmost importance in IBD patients in order to implement specific management strategies accordingly[70].

Studies have showed that by employing natural language processing and ML algorithms trained on several clinical data from electronic medical records (*i.e.* demographics, laboratory tests, endoscopy reports) it was possible to predict disease severity and surrogate markers of disease flare such as outpatient steroid use or hospitalization[9,71,72].

Furthermore, initial studies on the use of ML to predict the prognosis of patients affected by UC have shown that the endoscopic mucosal healing predicted by a deep neural network model was associated with prognostic features (reduced risks of hospitalization and colectomy) with statistical significance [73].

Lack of response to biological therapies (around 1 out of 3 patients with anti-TNF-alpha therapy) or progressive decline in response over time is an issue of paramount importance in IBD, with a significant economic toll on healthcare costs[74]. For this reason, the possibility to anticipate the likelihood of a patient responding to a specific biological drug prior to its start represents a cost-effective approach to treatment individualization.

ML has been implemented to deal with the complexity of such topics with encouraging results. Through the use of ML on clinical data from trials, random forests were developed to predict the response to biologics such as infliximab, vedolizumab, and ustekinumab, both in CD and in UC with encouraging results[75-78]. The integration and analysis of patients' molecular and clinical features *via* ML algorithms therefore appears to be a promising field with great potential in terms of patient management and optimization of healthcare costs[79].

## AI IN PRECLINICAL SETTINGS AND DRUG DISCOVERY

Design and discovery of new drugs are complex processes hampered by high costs and time consumption. Furthermore, especially in recent years, the process of drug discovery has to deal with the increasing wealth and complexity of data from various "omics" (*i.e.* genomics, proteomics)[80].

AI, however, is proving to be a crucial tool in the development of novel drug candidates modernizing this field as well. Among the various implementations of ML and DL in the drug discovery process we can list peptide synthesis, virtual screening (structure-based and ligand-based), prediction of toxicity, drug monitoring and release, quantitative structure-activity relationship, and drug repositioning[81].

In the field of IBD AI has already been employed in drug discovery with encouraging initial results. Computational approaches have been used to identify metabolite-target interactions using the dataset of the IBD cohort from the Human Microbiome Project 2, followed by ML analyses aimed at ranking metabolites according to their importance in IBD and identifying possible human targets through virtual ligand-based screening. Overall, 983 high quality connections between metabolites from the gut microbiota and human proteins possibly relevant to IBD were identified, thus providing multiple novel drug targets for potential immune therapies[82].

Another relevant application of DL was the construction of a scaffold-based molecular design workflow aimed at developing drug candidates targeted towards the discoidin domain receptor 1. Through a deep generative model, molecular docking and virtual profiling, a high-quality scaffold-based molecular library was established, subsequently leading to the synthesis of a molecular compound that effectively inhibited the expression of proinflammatory cytokines, showing extraordinary kinase selectivity and significant therapeutic protection in *ex vivo* and *in vivo* animal models, respectively[83].

## CONCLUSION

Based on the numerous advances in the field of IBD in recent years, it is foreseeable that AI will gain an ever-greater role in the standard patient care, ranging from evaluation of the risk of developing IBD to assistance in the assessment of mucosal activity or detection of dysplasia to support in histopathological reporting, prediction of disease course, and treatment efficacy (Table 1). At the same time, the integration of AI in daily practice will lead to changes in clinical practice itself, getting us closer to the concept of precision medicine and its subsequent improvement in the quality of care and optimization of healthcare costs (Figure 3).

As for research, AI is proving to be an irreplaceable aid in the collection and analysis of large data, while also limiting subjectiveness and interobserver variability, simplifying standardization and providing a feasible alternative to the need of independent central reading in clinical trials. The implementation of AI in everyday practice is expected to improve diagnostic accuracy and reproducibility by allowing for a better standardization of lesion features and classification and by increasing the detection rate of small and subtle mucosal abnormalities or lesions. Moreover, evidence shows how AI may have a role in advanced endoscopic techniques, such as confocal laser endomicroscopy or molecular imaging. Through these advanced application AI will support the clinician by detecting microscopic and even molecular details otherwise invisible, thereby paving the way to the potential incorporation of ultrastructural and molecular endpoints in IBD endoscopy.

Nonetheless, with regard to future applications of AI, there are several issues that will need solving, such as how to provide more transparency of the AI algorithms, how to assess and choose among different models with similar purposes, and how to effectively allocate resources in the plethora of models that will be available, to only list a few[84]. To the best of our knowledge, this is the first review on the roles and the future perspectives of AI in IBD that considers the first clinical applications in a real world setting that are now available from the most recent clinical studies. Nevertheless, the chief limitation is represented by the limited amount of evidence that support our review, which is inevitably due to the scarcity of data currently available for the topic itself from the literature. Undoubtedly further studies are direly needed to build a more robust and comprehensive foundation for future analyses. In brief, while the human component is unlikely to be substituted altogether in future clinical practice and while a few questions still await an answer, AI is an extremely promising means of improvement in patient care and resource optimization.

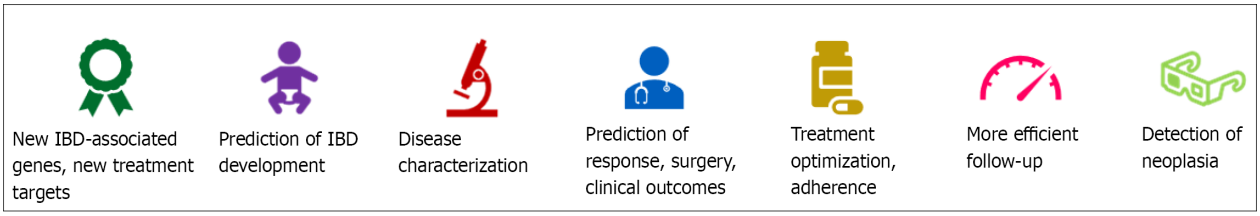


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**Figure 3 Fields of application of artificial intelligence in inflammatory bowel diseases.** IBD: Inflammatory bowel disease.

## FOOTNOTES

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**L-Editor:** Filipodia A
**P-Editor:** Chen YL

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*MINIREVIEWS*

# Role of advanced imaging techniques in the evaluation of oncological therapies in patients with colorectal liver metastases

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## Abstract

In patients with colorectal liver metastasis (CRLMs) unsuitable for surgery, oncological treatments, such as chemotherapy and targeted agents, can be performed. Cross-sectional imaging [computed tomography (CT), magnetic resonance imaging (MRI), 18-fluorodexoyglucose positron emission tomography with CT/MRI] evaluates the response of CRLMs to therapy, using post-treatment lesion shrinkage as a qualitative imaging parameter. This point is critical because the risk of toxicity induced by oncological treatments is not always balanced by an effective response to them. Consequently, there is a pressing need to define biomarkers that can predict treatment responses and estimate the likelihood of drug resistance in individual patients. Advanced quantitative imaging (diffusion-weighted imaging, perfusion imaging, molecular imaging) allows the *in vivo* evaluation of specific biological tissue features described as quantitative parameters. Furthermore, radiomics can represent large amounts of numerical and statistical information buried inside cross-sectional images as quantitative parameters. As a result, parametric analysis (PA) translates the numerical data contained in the voxels of each image into quantitative parameters representative of peculiar neoplastic features such as perfusion, structural heterogeneity, cellularity, oxygenation, and glucose consumption. PA could be a potentially useful imaging marker for predicting CRLMs treatment response. This review describes the role of PA applied to cross-sectional imaging in predicting the response to oncological therapies in patients with CRLMs.

**Key Words:** Colorectal cancer metastases; Prediction response; Computed tomography; Magnetic resonance imaging; Positron emission tomography; Parametric imaging

**Core Tip:** Chemotherapy and targeted agents can be administered to patients with colorectal liver metastasis (CRLM) unsuitable for surgery. The risk of toxicity requires identification of imaging biomarkers that can estimate the likelihood of response and drug resistance before starting therapy. Clinical validation may aid clinicians in tailoring their individual treatment regimens. In this setting, parametric analysis applied to cross-sectional imaging plays a crucial role in evaluating *in vivo* peculiar neoplastic features, such as perfusion, structural heterogeneity, cellularity, oxygenation, and glucose consumption. However, there is no consensus on the most promising imaging quantitative parameter to predict therapy response in CRLMs patients.

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## INTRODUCTION

Colorectal cancer is one of the most common cancers worldwide and the fourth leading cause of cancer death[1]. Unfortunately, up to 19% of colorectal cancer patients present with liver metastasis at diagnosis, while up to 13% develop it within the 5-year follow-up[1]. Surgery plays a crucial role in improving the prognosis of colorectal liver metastasis (CRLMs), but only 20% of them are initially suitable for this approach[2]. Hence, systemic chemotherapy is the treatment of choice for the remaining 80% of patients with the aim of rendering metastases resectable and/or prolonging survival[3]. In clinical practice, systemic chemotherapy based on a combination of fluoropyrimidines with oxaliplatin and/or irinotecan is usually associated with targeted agents. The assessment of *KRAS*, *NRAS*, and *BRAF* genes status influences the choice of the most appropriate targeted agents: If they are wild-type, panitumumab or cetuximab, epidermal growth factor receptor (EGFR) antibodies, are preferred, whereas if they are mutated, bevacizumab, a vascular endothelial growth factor (VEGF) antibody, is chosen[4]. In this setting, the cross-sectional imaging evaluation, represented by computed tomography (CT), magnetic resonance imaging (MRI) and 2-[$^{18}$F]fluoro-2-deoxy-D-glucose positron emission tomography (2-[$^{18}$F]FDG-PET) associated with CT or MRI (2-[$^{18}$F]FDG-PET/CT or MRI), is fundamental in the assessment of treatment response based on dimensional evaluation of tumour burden in consecutive scans through the application of standardised criteria, known as "Response Evaluation Criteria in Solid Tumours (RECIST, Figure 1)"[5-7]. These criteria have been very useful in the assessment of treatment response to cytotoxic chemotherapeutic agents, but the introduction of targeted agents with predominant cytostatic effects, such as anti-EGFR and anti-VEGF, makes them insufficient for adequate response imaging evaluation. Indeed, solid tumours may respond to these new agents by developing intra-tumoural necrotic areas and/or cystic, fibrotic, or myxoid degeneration, resulting in an overall increased, decreased, or unchanged size. Thus, the assessment of treatment response during follow-up based purely on dimensional evaluation of the tumour burden seems no longer sufficient. Furthermore, targeted agents are more expensive than cytotoxic agents and are burdened by hepatic toxicity (steatosis, hepatitis, sinusoidal obstruction syndrome, and impaired liver function). Considering these issues, identification of imaging biomarkers that can estimate the likelihood of response and drug resistance in individual patients before or immediately after starting therapy is mandatory. This critical point represents not only a clinician request to avoid unnecessary drug toxicity and the starting delay of alternative therapies, potentially more effective, but also an economic requirement to reduce futile health care costs.

The growth of neoplastic tissue is characterised by the activation of several biological processes, such as neoangiogenesis and anarchic cellular proliferation, which determine neoplastic heterogeneity for the coexistence of high cell density, necrotic, hypoxic, haemorrhagic, and necrotic areas. Neoplastic cells are characterised by increased metabolism and glucose consumption. Currently, these biological neoplastic processes as well as the neoplastic heterogeneity can be analysed *in vivo* applying several post-processing imaging analyses to different cross-sectional imaging techniques, such as diffusion-weighted imaging (DWI) on MRI, perfusion imaging on CT and MRI, and molecular imaging on 2-[$^{18}$F]FDG-PET/CT and MRI. The *in vivo* structural, functional, and molecular information obtained from imaging is expressed through parametric parameters, which represent potentially useful biomarkers in clinical practice. Parametric analysis (PA) allows the extraction of numerical data contained in the voxels of each image and converts the extracted numerical data into quantitative parametric maps, which are

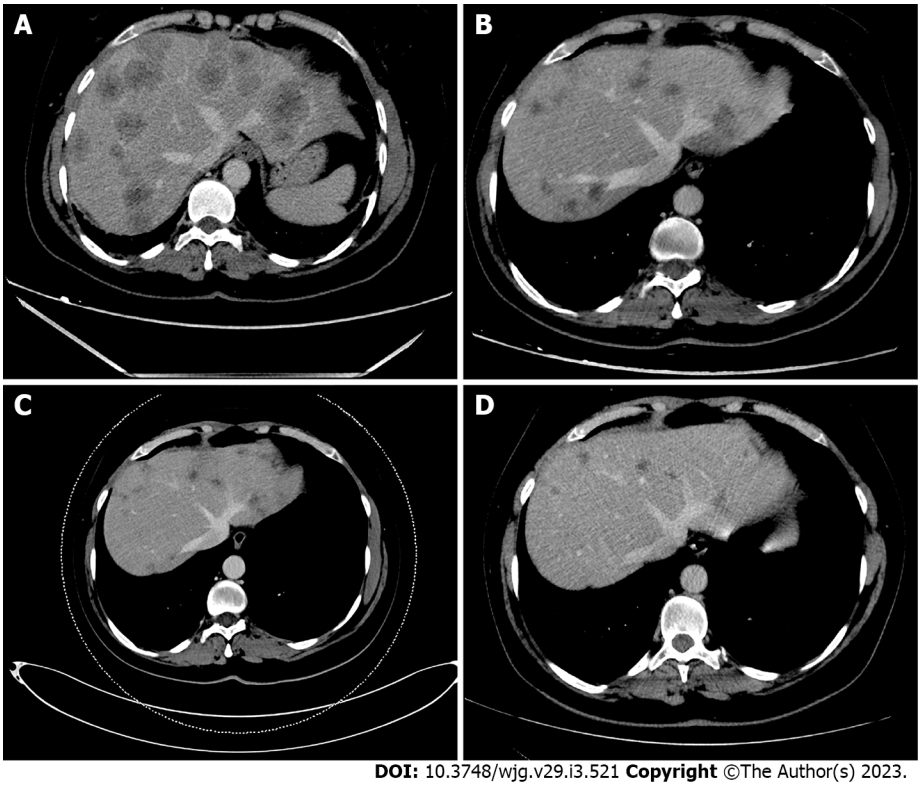


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**Figure 1 Contrast enhanced computed tomography images from a patient with colon cancer.** A: Baseline computed tomography (CT) demonstrates the presence of multiple liver metastases; B: After four cycles of combined chemotherapy (folinic acid + fluorouracil + irinotecan + cetuximab) the CT scan shows a reduction in both size and number of liver metastases, which was classified as a partial response with Response Evaluation Criteria in Solid Tumours criteria; C and D: The partial response was then confirmed after (C) 8 and (D) 12 chemotherapy cycles.

representative of peculiar neoplastic features, such as perfusion, structural heterogeneity, cellularity, oxygenation, and glucose consumption, depending on the imaging modalities and techniques used. PA requires the drawing of a region of interest (ROI) or volume of interest that includes the target tissue for analysis. In recent years, PA has been enriched with radiomics, a complex multi-step process that allows the extraction of a huge amount of computational quantitative features from digital medical images, thereby increasing the potential role of cross-sectional imaging in the oncological field. Radiomics has recently emerged as a promising tool for discovering new imaging biomarkers by extracting and analysing numerous quantitative image features representative of tumour heterogeneity and phenotype. Radiomics combines quantitative imaging biomarkers with clinical reports and laboratory test values in statistical models[8].

Finally, the response to chemotherapeutic agents is influenced by their delivery to neoplastic tissues, which is influenced by the tumour microenvironment and cellular characteristics, such as uptake, retention, metabolic activation, and catabolism of drugs, as well as genetic factors such as DNA repair mechanisms[9]. The *in vivo* knowledge of peculiar neoplastic features through cross-sectional imaging may provide imaging biomarkers aiding in the prediction of treatment response and drug resistance. To date, several researchers have investigated the role of quantitative imaging parameters in the pre-treatment response prediction of CRLMs patients using MRI, CT, and 2-[$^{18}$F]FDG-PET/CT or MRI[10, 11], but there is no clear consensus about which is the most promising imaging technique as well as the most promising quantitative imaging parameter. Therefore, this review aimed to describe the role of PA in predicting the response to oncological therapies in patients with CRLMs.

## PA BASED ON MRI DIFFUSION TECHNIQUE

### *DWI with apparent diffusion coefficient maps*

DWI is a functional MRI technique that measures the Brownian motion of water molecules in biological tissues, which is restricted by an increase in cellularity and architectural tissue changes[12]. Consequently, in tumour tissues, the dense cellularity associated with fibrosis, necrosis, neovascularization, and haemorrhages reduces the intercellular space, altering water diffusion properties and restricting Brownian motion. Diffusion-weighted MR images measure the apparent diffusion coefficient (ADC), which is inversely proportional to the cell density, presumably resulting from the tortuosity of

the interstitial space and the consequential limitation of water movement. Tumours with high cellularity tend to present low ADC values on diffusion-weighted MRI because of their high cellularity, characteristically presenting with restriction in these lesions. Therefore, using DWI, it is possible to obtain a parametric ADC map, which is composed of the ADC values calculated for each voxel and represents a quantitative measure of water molecule diffusion expressed as $10^{-3}$ mm$^2$/s. ADC is inversely related to tumour cellularity and is strongly affected by molecular viscosity, permeability of the membrane separating the intra- and extracellular compartments, as well as active transport and flow[13]. During treatment, the increase in necrosis, loss of cell membrane integrity, decrease in tumour cellularity, and increase in extracellular spaces determine the increase in water diffusion and, consequently, the increase in ADC values[14]. However, it should be mentioned that a transient decrease in ADC may occur during the first 36–48 h after starting therapy with vascular targeting agents, and the rationale may be the activation of a local immune response, as demonstrated in animal models[15]. Since DWI/ADC provides *in vivo* structural information of tissue composition at any time, and tissue composition influences the treatment response, several authors have investigated the role of this MRI technique in predicting or assessing very early therapy response in patients with CRLMs[16-21]. Most of them are concordant that pre-treatment lower ADC is associated with a better response in CRLMs patients, while a higher ADC is associated with a poorer response[16-18,20]. In particular, Cui *et al*[17] evaluated 11 patients with CRLMs and found that the pre-chemotherapy mean ADC values was significantly lower in responding lesions than those in non-responding lesions ($0.948 \pm 0.147 \times 10^{-3}$ mm$^2$/s *vs* $1.185 \pm 0.275 \times 10^{-3}$ mm$^2$/s; $P = 0.003$). Furthermore, Koh *et al*[16] analysed 20 patients with 40 CRLMs and observed that high pre-treatment ADC was predictive of poor response to oxaliplatin- and 5-fluorouracil-base chemotherapy (non-responders: $1.55 \times 10^{-3}$ mm$^2$/s; responders: $1.36 \times 10^{-3}$ mm$^2$/s; $P < 0.001$). These results were confirmed by Tam *et al*[18] and Fouladi *et al*[20]. The former conducted a study on a larger population composed of 102 patients with CRLMs treated with chemotherapy alone or associated with surgery/radiofrequency ablation (non-responders: $1.40 \times 10^{-3}$ mm$^2$/s; responders: $1.16 \times 10^{-3}$ mm$^2$/s; $P = 0.024$)[18]. The latter tested the usefulness of baseline 3D ADC to identify the potential responding CRLMs (non-responders: $1332.3 \pm 384.6 \times 10^{-6}$ mm$^2$/s; responders: $1150.2 \pm 272.9 \times 10^{-6}$ mm$^2$/s; $P = 0.04$)[20]. Recently, Uutela *et al*[22] investigated the correlation between ADC values at baseline and the RECIST response in a prospective study conducted in 52 patients with CRLM. ADC values below the median of $1.20 \times 10^{-3}$ mm$^2$/s at baseline were associated with partial response according to the RECIST criteria 8–12 wk after starting therapy[22].

The biological rationale of these results can be postulated as follows: A higher ADC is observed in necrotic tissues, whereas a lower ADC is observed in viable areas. Necrosis before therapy may indicate a more aggressive phenotype and compromise the delivery of chemotherapeutic drugs; therefore, necrotic areas are usually poorly perfused and tumour cells are exposed to a more hypoxic and acidic environment. These factors reduce the effectiveness of the therapy[23]. However, it should be highlighted that coagulative necrosis does not increase the ADC, which could explain non-responding lesions with lower ADC[16]. In contrast, viable areas are usually well-perfused, facilitating the delivery and retention of anticancer agents.

Currently, there is no consensus regarding the optimal cutoff point ADC value for predicting response to treatment. The most important factors that influence the identification of a generally accepted ADC threshold are several, such as different scanners, methods of acquisition, sequence parameters, and choice of b values. The b-value is a factor that reflects the strength and timing of the gradients used to generate DWI images: The higher the b-value, the stronger the diffusion effect. Koh *et al*[16] identified a mean pre-treatment ADC value of $1.69 \times 10^{-3}$ mm$^2$/s for CRLMs that did not respond to chemotherapy with a sensitivity of 60% and a specificity of 100%, whereas Fouladi *et al*[20] proposed the baseline 3D-ADC value as the optimal cut-off point of $1.006 \times 10^{-3}$ mm$^2$/s with a sensitivity of 77.4% and a specificity of 91.3%. In this setting, Drewes *et al*[11] conducted a meta-analysis and identified a practical ADC threshold value of $1.2 \times 10^{-3}$ mm$^2$/s, below which nearly all responders are situated and no simultaneous overlap with non-responders exists.

Although, according to the aforementioned results, ADC could appear to be a promising predictive biomarker, some studies contradict these previous results[19,21]. Matsushima *et al*[19] did not find a significant difference in ADC values between responders and non-responders to CRLMs treated with bevacizumab. Boraschi *et al*[21] correlated pre-chemotherapy ADC values of 58 CRLMs with histological tumour regression grade (TRG). TRG is a histological descriptive system aimed at grading fibrotic transformation induced in tumours by neoadjuvant therapy. In detail, TRG 1 is represented by fibrosis with no evidence of residual tumour (*i.e.* complete regression), TRG 2 is represented by fibrosis with single cells or rare groups of residual tumour cells, TRG 3 is represented by fibrosis and residual tumour with a dominance of fibrosis, TRG 4 is represented by fibrosis and residual tumour with a dominance of tumour, and TRG 5 is represented by extensive tumour without evidence of regression[24,25]. A non-linear distribution was observed between pre-ADC values and TRG; lower pre-ADC values correlated with TRG 2-3, but an overlap was observed between TRG 1 (complete response) and TRG 4-5 (no response). The heterogeneous structure of liver metastases in terms of cellularity, necrosis, and/or calcification may explain these results.

Finally, the potential role of ADC in the early prediction of therapy response in CRLMs was also evaluated. Cui *et al*[17] observed an increase in ADCs on day 3 or 7 after initiating chemotherapy in responders, suggesting a very early change in tissue composition from a more cellular pre-treatment phenotype to a less cellular or necrotic posttreatment phenotype. Knowledge of therapy effectiveness as soon as possible allows clinicians to prevent overtreatment of non-responder patients, thus avoiding adverse effects.

### *Intravoxel incoherent motion and diffusion kurtosis imaging*

The ADC value is calculated by a mono-exponential relationship between the DWI signal and b-value. Hence, ADC is influenced by tumour heterogeneity and the Gaussian movement of water molecules. The heterogeneity of tumour tissues affects the non-Gaussian diffusion behaviour of water molecules; therefore, new non-mono-exponential diffusion models, such as intravoxel incoherent motion (IVIM) and diffusion kurtosis imaging (DKI), have been proposed to better characterise neoplastic tissues. IVIM assesses both diffusion and microcapillary perfusion changes in tissues by analysing the signal decay curve obtained from multiple b values and provides both diffusion-related parameters, such as the true diffusion coefficient (D) and ADC, and perfusion-related parameters, such as the pseudodiffusion coefficient (D*) and perfusion fraction (f)[26]. DKI estimates and quantifies the skewed distribution of water diffusion based on a probability distribution function[27]. In addition to the diffusion coefficient, DKI extracts the kurtosis value (K) that results from the probability of the diffusion displacement distribution, which is a dimensionless metric. These advanced MRI diffusion techniques have been investigated for predicting the treatment response in patients with CRLM[26,28,29]. Zhang *et al*[29] conducted a prospective study of 40 patients with CRLMs to evaluate the performance of DWI, IVIM, and DKI in predicting therapy response. Their results confirmed the promising role of ADC and suggested the potential role of IVIM and DKI. At baseline, lower ADCs and D on the IVIM parameter map, mean diffusion values, and higher K values on the DKI parameter map correlated with a better response ($P$ = 0.001, < 0.001, = 0.003, = 0.002, respectively), with areas under the curve (AUCs) of 0.845, 0.832, 0.819, and 0.787, respectively. ADC reached the highest AUC (0.845) with a sensitivity of 73.3%, specificity of 84.0%, and cut-off value of $1.107 \times 10^{-3}$ mm$^2$/s. In the literature, D is reported to positively correlate with the degree of tumour necrosis as well as ADC; hence, lower D is expression of poor presence of necrosis and better response to chemotherapy[30]. The combination of the aforementioned parameters by logistic regression yielded an AUC of 0.867[29]. Furthermore, Zhou *et al*[28] found that K values were higher in patients with non-responding CRLMs (responders 0.77 ± 0.15 *vs* non-responders 0.90 ± 0.15; $P$ = 0.015), as expression of more complex microstructure, composed of micro-necroses, fibroses, and cystic changes. Finally, Kim *et al*[26] observed a significant change in diffusion parameters of IVIM, such as ADC and D, after the first cycle of therapy in responder patients, whereas perfusion-related IVIM parameters did not change significantly in both groups, suggesting that diffusion-related IVIM parameters are more useful than perfusion-related parameters in differentiating early responders from non-responders, avoiding overtreatment of patients who may not benefit from chemotherapy.

## PA BASED ON SPECTROSCOPY

MR spectroscopy (MRS) is an advanced imaging technique that allows for the non-invasive measurement of the levels of some molecules *in vivo*, using the magnetic properties of certain atomic nuclei, such as protons ($^1$H), phosphorus ($^{31}$P), and carbon-13 ($^{13}$C)[31]. Therefore, MRS can provide information on tumour pathophysiology and metabolism, potentially influencing treatment planning[32]. Currently, very few studies have investigated the role of MRS in the assessment and prediction of treatment response in patients with CRLMs with poor and discordant results[22,31,33]. In $^{31}$P MRS, an increased ratio of phosphomonoesters and nucleoside triphosphate is associated with tumour progression, while it decreases with tumour regression, even in the absence of changes in standard imaging[31]. These results may encourage the use of MRS in monitoring treatment response. Similarly, Kamm *et al*[33] observed a correlation between the maximum levels of 5-FU catabolites on $^{19}$F-MRS and the response to treatment in patients with larger CRLMs, suggesting a potential role of MRS in the prediction of therapy response. In contrast, Uutela *et al*[22] did not find a significant association between baseline levels of free choline on $^1$H-MRS and treatment response according to the RECIST criteria. Therefore, the role of this technique in patients with CRLMs remains to be investigated. Currently, MRS is not yet applied in clinical settings because of technical issues such as the relatively long scan times needed for a good signal-to-noise ratio, as well as the need for additional hardware and expertise in spectral interpretation.

## PA BASED ON CONTRAST-ENHANCED CT OR MRI

Neoangiogenesis is induced by the upregulation of vascular growth factors and is required for tumour growth. This leads to the development of a new, altered, and immature microcirculatory network inside

the tumour lesions. The irregular vascular pattern promotes the coexistence of areas of low vascular density and areas of high angiogenic activity; consequently, regions of high cell density and necrotic, haemorrhagic, and myxoid changes are observed. The use of contrast medium and the acquisition of CT or MRI images before and after its intravenous injection allows the assessment of the vascularity of biological tissues *in vivo*, hence the tumours' neoangiogenesis. Tissue contrast enhancement can be evaluated using two different CT or MRI imaging modalities. One is dynamic and is based on repeated high-frequency image acquisition, which allows the assessment of changes in density on CT or signal intensity on MRI over time. The other is based on image acquisition at a fixed time point to obtain at least two or three phases (arterial, portal, and delayed). A broad spectrum of quantitative parameters can be extracted using dynamic acquisition images, which reflect tumour vessel features (perfusion, permeability, and density), extracellular-extravascular space composition, and plasma volume. A summary of the main quantitative parameters used in the assessment of treatment response in patients with CRLM is presented in Table 1. The development of targeting agents with angiogenesis-inhibiting effects, such as bevacizumab, has encouraged studies to examine the correlation between angiogenesis and quantitative imaging parameters. Currently, several potential predictive imaging biomarkers have been identified in different types of cancers, such as renal cell carcinoma, hepatocellular carcinoma, hypopharyngeal carcinoma, and colorectal cancer[10,34-36].

### Contrast-enhanced MRI

Few studies have been published regarding the predictive role of MRI quantitative perfusion parameters in patients with CRLMs[37-41]. In particular, Coenegracht *et al*[37] observed in 10 patients with CRLMs a significant difference of $K^{ep}$ values between responders and non-responders (0.09852 *vs* 0.07829; $P < 0.001$); O'Connor *et al*[41] also noticed a high ratio of enhancing tumour voxels to overall tumour voxels in patients with better tumour response. The pathophysiological basis of these results should be as follows: Higher baseline $K^{ep}$ values indicate higher exchange of contrast medium between the blood and the extracellular extravascular space; similarly, a higher exchange of chemotherapy may occur. For this reason and for the presence of an oxygen-rich environment, highly perfused CRLMs at baseline are more likely to respond well to treatment. Furthermore, the role of MRI quantitative perfusion parameters in the prediction of treatment response in patients with CRLMs after the first cycle of a chemotherapy regimen containing bevacizumab has also been investigated. Hirashima *et al*[38] observed a correlation between a higher response and the decrease in $K^{trans}$ ratio ($\Delta K^{trans}$) and $K^{ep}$ ratio ($\Delta K^{ep}$), calculated at baseline and after the first cycle ($P < 0.0001$). De Bruyne *et al*[40] found a correlation between worse response and an increase of at least 40% in $K^{trans}$ after the first cycle of treatment. These results suggest a potential role for quantitative dynamic contrast-enhanced MRI (DCE-MRI) parameters in the early prediction of therapy response and in the assessment of drug resistance[38,40]. On the other hand, Kim *et al*[39] observed discordant results; no significant change in perfusion parameters, such as $K^{trans}$, $K^{ep}$ and $V_e$, was found after the first cycle of chemotherapy between responders and non-responders, questioning their role in predicting early therapy response in CRLMs patients.

In clinical practice, gadoxetic acid, a hepatobiliary contrast agent incorporated into hepatocytes by the transporter OATP1B3, is used to better assess CRLMs because of the excellent lesion-to-liver contrast of the hepatobiliary phase (HBP). Murata *et al*[42] investigated the role of gadoxetic acid-enhanced MRI in predicting treatment response in patients with CRLMs. The authors calculated the pre-treatment relative tumour enhancement of the HBP ($RTE_{HBP}$) in 26 patients with CRLMs using the following formula: RTE values (%) = $[(SI_H - SI_P)/SI_P] \times 100$, where $SI_H$ and $SI_P$ are the signal intensities in the hepatobiliary and pre-contrast phases, respectively. The mean pre-treatment $RTE_{HBP}$ values were significantly higher in responders than in non-responders (37.2% ± 10.9% *vs* 17.9% ± 10.5%; $P = 0.0006$), suggesting a potential association between chemotherapeutic response and OATP1B3 expression. OATP1B3 is an organic anion transporter that is incorporated into hepatocytes, not only in gadoxetic acid, but also in endogenous and exogenous molecules, such as bile acids and chemotherapeutic agents.

### Contrast-enhanced CT

CRLMs are generally hypovascular lesions in the portal phase that obtain their blood supply primarily from the hepatic artery; hence, they are arterialised tumours with increased blood flow (BF) and vascular permeability[43]. Based on this assumption, Joo *et al*[44] investigated the haemodynamic features of liver metastases using quantitative colour mapping of the arterial enhancement fraction (AEF) to explore its potential role in the prediction of therapeutic response in patients with CRLMs. The Authors observed a higher mean AEF value of metastatic tumour (58.9 ± 18.8) than that of tumour-adjacent parenchyma (35.5 ± 15.4) and tumour-free parenchyma (26.4 ± 7.5) (all $P < 0.0001$), confirming the arterial vascularisation of liver metastases. Similarly, Kim *et al*[45] extracted some perfusion parameters from perfusion CT of 17 patients with CRLMs and noticed that BF, $K^{trans}$, and portal liver perfusion were significantly lower in metastatic lesions than in background normal liver parenchyma (41.2 *vs* 50.8, 25.9 *vs* 41.2, 19.3 *vs* 40.9 mL/100 mL/min, respectively), while arterial CRLM perfusion indices were significantly higher than those of hepatic perfusions (28.0 *vs* 22.9 mL/100 mL/min and 57.1% *vs* 26.6%, respectively) ($P < 0.05$). The metastatic blood supply from the hepatic artery and their increased arterial perfusion may be due to the development of a microcirculatory network caused by the neoangiogenesis process. As a consequence, responding lesions of CRLMs patients showed significantly

**Table 1 Main quantitative parameters extracted from perfusion computed tomography and magnetic resonance imaging imaging techniques to predict treatment response in patients with colorectal liver metastasis**

| Parameter name | Parameter definition | Parameter significance |
|---|---|---|
| Transfer constant ($K^{trans}$) | Rate of contrast extraction from the blood to the interstitium | It reflects the balance between capillary permeability and BF in a tissue |
| Tissue interstitial volume ($V^e$) | Volume of extravascular and extracellular contrast agent in a certain tissue, expressed as a percentage | It is a measure of cell density |
| Rate contrast ($K^{ep}$) | Rate at which the contrast agent returns from the extravascular-extracellular space to the vascular compartment ($K^{ep} = K^{trans}/V^e$) | It reflects the tissue microcirculation and contrast agent permeability |
| Regional BF | BF *per* unit volume or mass of tissue (mL of blood/min/100 mL of tissue) | It expresses the rate of the delivery of nutrients and oxygen to a certain tissue |

BF: Blood flow.

higher AEF values than that non-responding (65.5 ± 9.6 *vs* 51.3 ± 13.2; $P = 0.005$)[44]. These results are concordant with those of Osawa *et al*[46], who investigated the predictive role of contrast-enhanced CT in patients with CRLM treated with chemotherapy with or without bevacizumab. The authors found a significant correlation between a higher composite endpoint (CE) ratio (ratio of CT value during the arterial phase to unenhanced CT value) at baseline and higher tumour shrinkage after four cycles of chemotherapy associated with bevacizumab ($R^2 = 0.24$, $P = 0.03$), unlike in patients not treated with bevacizumab. Furthermore, among CRLM patients with a high CE ratio at baseline, an increase of 29.6% in the tumour shrinkage rate was observed in those treated with bevacizumab compared with a decrease of 1.46% in those not treated with bevacizumab ($P = 0.03$). Among the CRLMs patients with a low CE ratio at baseline, no significant tumour shrinkage was noted. The rationale for these results is unclear, but we can hypothesise that the presence of higher microvessel density at baseline, evaluated on CT as higher AEF values or higher CE ratios, promotes greater delivery of chemotherapeutic agents. Hence, the assessment of these parameters at baseline could be useful in the prediction of treatment response and drug resistance in patients with CRLMs.

Finally, although Kim *et al*[45] did not find any significant difference in perfusion parameters at baseline between responder and non-responder patients with CRLMs, they observed a significant decrease in BF and $K^{trans}$ after the first cycle of chemotherapy (BF: 28.3% *vs* 5.2%, $P = 0.036$, AUC: 0.806; $K^{trans}$: 18.7% *vs* 13.0%, $P = 0.027$, AUC: 0.819). The early reduction in perfusion parameters may reflect the inhibiting effect of neo-angiogenesis by anticancer drugs and should encourage clinicians to continue the chosen chemotherapy regimen. Furthermore, the Authors identified a cut-off value for the reduction rate of $K^{trans}$ of 15.0% after the first cycle of chemotherapy, with a sensitivity of 66.7% and specificity of 87.5%[45].

## PA BASED ON HYBRID IMAGING

The 2-[$^{18}$F]FDG-PET/CT and 2-[$^{18}$F]FDG-PET/MRI are molecular and morphological imaging techniques associated with metabolic and anatomical evaluation of tumour lesions[47,48]. The tracer 2-[$^{18}$F]FDG, an analogue of glucose, is injected intravenously, transported into cells through membrane glucose transporter proteins, and tends to accumulate in malignant cells because of increased glucose consumption[49]. The uptake of 2-[$^{18}$F]FDG detected by PET can be quantitatively assessed using different parameters; the main parameters used in the prediction of therapy response in CRLMs patients are shown in Table 2[45,48].

Currently, the quantitative evaluation of tumour metabolism using 2-[$^{18}$F]FDG-PET integrated with CT or MRI plays a crucial role in the routine management of oncological patients. In patients with colorectal cancer, hybrid imaging aids in the detection of extrahepatic distant metastasis in the evaluation of therapy response, as well as in the follow-up of treated patients with rising serum carcinoembryonic antigen (CEA) levels and no detectable disease on morphological imaging[48]. To date, the role of this hybrid technique in the prediction of treatment response in CRLMs patients remains under investigation. Several studies have shown that standardised maximum uptake value ($SUV_{max}$) is significantly lower in responders than in non-responders before chemotherapy with or without bevacizumab[10,40,45,50-52]. In detail, Byström *et al*[50] found a mean baseline $SUV_{max}$ of 5.6 in responders and 7.4 in non-responders treated with irinotecan-based chemotherapy ($P = 0.02$). Similarly, De Bruyne *et al*[40] observed a mean baseline $SUV_{max}$ of 3.77 in responders and 7.20 in non-responders treated with FOLFOX/FOLFIRI and bevacizumab followed by surgery ($P = 0.012$). In addition, De Bruyne *et al*[40] did not find any correlation between DCE-MRI parameters, $SUV_{max}$ and anatomical tumour response, suggesting that tumour BF, glucose metabolism, and shrinkage are potentially

**Table 2 Main quantitative parameters extracted from the 18-fluorodexoyglucose positron emission tomography used in the prediction of treatment response in patients with colorectal liver metastasis**

| Parameter name | Parameter definition |
|---|---|
| $SUV_{max}$ | Uptake value of the pixel with the highest activity inside an ROI divided by the injected dose, which must be corrected for decay and normalised to the patient's weight or body surface |
| $SUV_{mean}$ | Average of all the uptake values of the pixels within an ROI |
| MTV | Volume of tumour tissues included in a tridimensional ROI with pathological FDG uptake *via* threshold represented by a settled absolute value or percentage of the $SUV_{max}$ or $SUV_{mean}$ |
| | It includes both volumetric data and metabolic activity of the tumour |
| TLG | The product of multiplying $SUV_{mean}$ by MTV |
| SAM | A marker of total lesion glycolysis, calculated by drawing a volume of interest [VOI(1)] around the tumour and a larger VOI [VOI(2)] around VOI(1) |
| | SAM = Total SUV VOI1 − (mean BG × volume VOI1) |
| | Mean BG (background activity) = (total SUV VOI2 − total SUV VOI1)/(volume VOI2 − volume VOI1) |

$SUV_{max}$: Standardised maximum uptake value; $SUV_{mean}$: Standardised mean uptake value; MTV: Metabolic tumour volume; TLG: Total lesion glycolysis; SAM: Standardised added metabolic activity; FDG: Fluorodexoyglucose; PET: Positron emission tomography; ROI: Region of interest; VOI: Volume of interest.

independent predictors[40]. Mertens *et al*[53] introduced a new metabolic parameter, the standardised added metabolic activity (SAM), which is a marker of total lesion glycolysis that measures the total excess tumoural SUV above the tumour background (Table 2). The authors found a significant difference in both SAM and $SUV_{max}$ at baseline between responders and non-responders (34 *vs* 211, $P$ = 0.002; 3.8 *vs* 7.2, $P$ = 0.021, respectively)[52].

In addition to $SUV_{max}$, other metabolic parameters have been proposed as predictors of therapy response in patients with CRLMs, such as standardised mean uptake value ($SUV_{mean30}$), metabolic tumour volume ($MTV_{30}$) and 30% lesion glycolysis ($LG_{30}$). $SUV_{mean30}$ was defined as the average value of the SUV of the voxels that showed $SUV_{max} \geq 30\%$. $MTV_{30}$ was defined as the tumour volume segmented *via* the threshold $SUV_{mean30}$ of the lesion. Finally, $LG_{30}$ was obtained by multiplying $MTV_{30}$ by $SUV_{mean30}$. Kim *et al*[45] observed a higher mean $SUV_{mean30}$ in responder than in non-responder patients with CRLMs on prechemotherapy 2-[$^{18}$F]FDG-PET/CT (5.2 ± 2.3 *vs* 3.5 ± 1.0, $P$ = 0.046; AUC: 0.792) and a significant difference in the reduction rate of $MTV_{30}$ and $LG_{30}$ between responders and non-responders (18.1% *vs* -5.5%, $P$ = 0.015, AUC: 0.847; 37.9% *vs* 10.7%, $P$ = 0.008, AUC: 0.868, respectively) on 2-[$^{18}$F]FDG-PET/CT performed 2 or 3 wk after the first cycle of chemotherapy. These results suggest the role of these other 2-[$^{18}$F]FDG-PET/CT quantitative parameters in the prediction of treatment response in patients with CRLMs. In the same population, Kim *et al*[45] did not observe a significant reduction in the $SUV_{max}$ after the first cycle of therapy between responders and non-responders. A possible explanation is that $SUV_{max}$ is based only on a single pixel and does not consider the entire heterogeneous tumour volume, whereas MTV and LG are volume-based parameters and could be more accurate in the early prediction of treatment response. Similarly, Hendlisz *et al*[51] investigated the potential role of 2-[$^{18}$F]FDG-PET/CT in the prediction of early response after the first cycle of chemotherapy in patients with CRLMs. To avoid metabolic imaging-based rejection of potentially beneficial therapy, the lowest possible reliable response threshold for FDG uptake changes, the $\Delta SUV_{max} < 15\%$, was applied. Using this threshold, the predictive performance of the metabolic assessment for RECIST response showed a sensitivity of 100%, specificity of 57%, positive predictive value of 43%, and negative predictive value of 100%.

Finally, in the assessment of early response to chemotherapy with 2-[$^{18}$F]FDG-PET, the flare phenomenon should be considered because it might interfere with the measurement of quantitative parameters. It occurs–1–2 wk after the initiation of chemotherapy and consists of a marked increase in 2-[$^{18}$F]FDG metabolism in lesions that respond later. Hence, it is recommended to avoid the first two weeks after chemotherapy when performing 2-[$^{18}$F]FDG-PET/CT evaluation[54].

## PA BASED ON RADIOMICS

Radiomics represents a multi-step post-processing technique that can be applied to any medical image to convert it into mineable high-dimensional data (radiomics features). The assumption is that biomedical images contain information that reflects tissue heterogeneity and pathophysiology[8]. Radiomics data can be used alone or with other clinical data to build predictive models and decision-

support tools to aid physicians in clinical practice, potentially improving diagnostic, prognostic, and predictive accuracy. Radiomics analyses represent the pursuit of precision medicine to choose the right treatment for the right patient at the right time. To extract the radiomics features, the first step is image acquisition; the second step is image segmentation, which consists of the 2D or 3D delineation of the ROI, represented by the largest cross-section of the tumour, the whole tumour, or tumour sub-regions; and the third step is the radiomics data extraction (Figure 2). Radiomics features are obtained from the drawn ROI using specifically designed formulae conveying different quantitative parameters, such as first-order (based on histogram analysis of the distribution of individual voxel values without concern for spatial distribution) and second- and higher-order statistical descriptors (accounting for pixel intensity spatial distribution)[55]. Second-order statistical descriptors are generally defined as "texture" features, which describe the statistical interrelationship between voxels with similar or dissimilar contrast values, providing a measure of heterogeneity.

The huge amount of radiomics data extracted from medical images can be more easily handled by artificial intelligence (AI) than traditional statistical methods, and machine learning (ML) is a branch of AI focused on algorithms that can be trained for a task they were not specifically programmed to perform[56]. The algorithms are decision support tools and are mainly used for classification problems. In the oncological field, they are applied to detect and characterise tumour lesions as well as to predict and monitor therapy response. A family of ML algorithms of particular interest is represented by neural networks (NN), a complex model composed of nodes (called neurones) that contribute to the creation of deep, multi-layered networks. The use of NNs with such architectures is commonly referred to as deep learning (DL), which autonomously learns the best features for performing data classification[56]. The texture features utilised were a grey-level co-occurrence matrix (GLCM) and grey-level run length (GLRL). Some features were extracted after pre-processing with a wavelet transform. Supervised classification was achieved using ML approaches: Support vector machine (SVM), k-nearest neighbours, and Random Forest. Texture analysis (TA) is a technique that enables the quantification of variations in pixel intensity, including those imperceptible to the human visual system. TA includes the quantification of grey-level patterns, pixel interrelationships, and the spectral properties of an image[57,58].

Few studies have been published on the role of radiomics applied to CT and MRI images to predict therapy response in patients with CRLMs[59-64].

### MRI

Zhang *et al*[63] conducted an analysis of T2-weighted images of liver MRI in 26 patients with CRLMs (a total of 193 liver metastases) and extracted five histogram features (mean, variance, skewness, kurtosis, and entropy) and five GLCMs, including angular second moment (ASM), entropy, contrast, correlation, and inverse difference moment. Among the former parameters, only variance was significantly different between CRLMs responders and non-responders ($P < 0.001$), whereas among the latter, all parameters were significantly different ($P \leq 0.001$). Furthermore, when tested using multivariable logistic regression analysis, the association of variance and ASM showed the most potential predictive value for discriminating responders from non-responders with an AUC of 0.814, a sensitivity of 71%, and a specificity of 84.9. These parameters correlate with the complexity and non-uniformity of the image texture, and hence with tumour heterogeneity. According to Zhang *et al*[63], heterogeneous tumours seem to have a more favourable response to therapy. This observation may be related to irregular angiogenesis, greater distribution of tumour blood vessels, and extracellular vascular permeability. Since the effect of drugs relies on their delivery to the tumour site, tumours with greater heterogeneity should theoretically have a better response.

Furthermore, Liang *et al*[62] performed a histogram analysis using ADC maps of 53 patients with CRLMs before starting chemotherapy and observed that the mean, 1$^{st}$, 10$^{th}$, 50$^{th}$, 90$^{th}$, and 99$^{th}$ percentile values of the ADC maps were significantly lower in responders than in non-responders ($P$ = 0.000-0.002) with AUCs of 0.79, 0.76, 0.76, 0.79, 0.80, and 0.82, respectively. The 99$^{th}$ percentile of ADC showed the highest diagnostic performance for predicting response to chemotherapy, with an AUC of 0.82. These results are concordant with those mentioned above: ADC values included in the 99$^{th}$ percentile are predictive of a good response to chemotherapy, suggesting their association with viable neoplastic tissues, whereas the remaining 1% of ADC values are predictive of insensitivity to chemotherapy, suggesting their association with areas of fluid resulting from necrotic tissues. The authors also investigated histogram-derived CE-MRI parameters extracted from arterial and portal venous phases, such as mean, variance, skewness, kurtosis, and 1$^{st}$, 10$^{th}$, 50$^{th}$, 90$^{th}$, and 99$^{th}$ percentiles; however, they did not find any significant difference between responders and non-responders in patients with CRLMs. These results may reflect the fact that tumour enhancement is non-specific and influenced by several factors, including BF, capillary permeability, blood volume, and extravascular leakage space[62].

### CT

Ahn *et al*[59] conducted a study of 235 patients (145 in the training cohort and 90 in the validation cohort) with CRLMs treated with FOLFOX and FOLFIRI to evaluate several parameters on contrast-enhanced CT, including histogram, volumetric, and morphological features. In multivariate analysis, lower skewness [odds ratio (OR): 6.739, $P$ = 0.003] in 2D analysis, higher mean attenuation (OR: 2.587, $P$ = 0.017), and narrower standard deviation (SD) (OR: 3.163, $P$ = 0.002) in 3D analysis attained statistical

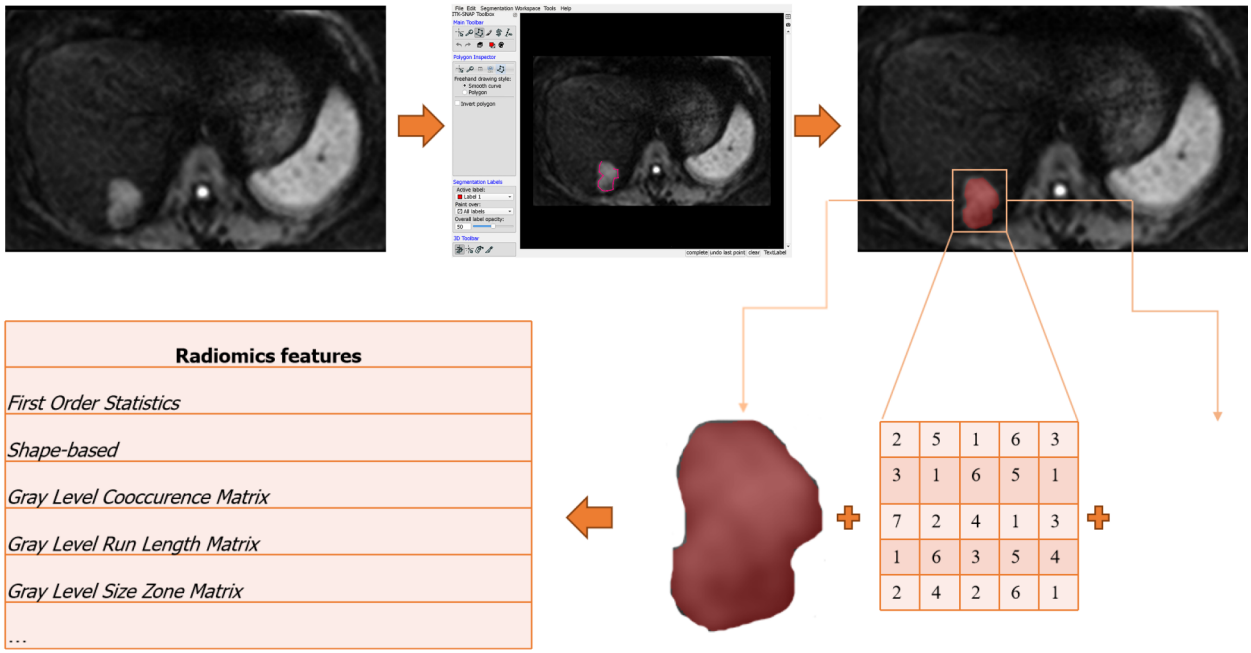


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**Figure 2 Schematic diagram showing how radiomics features can be extracted from medical images using a diffusion-weighted imaging image from an magnetic resonance imaging scan of a patient with colorectal liver metastasis as an example.** The process begins on the left upper corner with image acquisition, followed by lesion segmentation on a dedicated software leading to a region of interest. The shape of the region of interest as well as the distribution and spatial relation of intensity values of each pixel are computationally analysed to extract radiomics features of different order.

significance for predicting the response of CRLMs to chemotherapy in the training cohort. The lower skewness on 2D images and the narrower SD on 3D images showed good performance in the validation cohorts (AUC: 0.797 and 0.785, respectively). In contrast, Rabe *et al*[64] conducted a CT TA on 29 patients with non-necrotic CRLMs and did not observe a significant correlation between SD and the prediction of response. A possible explanation for these contradicting results could be the exclusion of necrotic lesions in the analysis conducted by Rabe *et al*[64]; indeed, necrosis increases SD. Furthermore, among the several first- and second-order radiomics features extracted by Rabe *et al*[64], eight, such as minimum histogram gradient intensity, skewness, discretised skewness, volume at intensity fraction 10, three GLRL indicators (long run low grey level emphasis, low grey level run emphasis, short run low grey level emphasis), and low grey level count emphasis, were significantly associated with treatment response in univariate analysis. Due to strong correlations within these radiomics features, only two, minimum histogram gradient intensity and long-run grey level emphasis, were included in the multivariate analysis. The AUC of the multivariate model using minimum histogram gradient intensity and long-run grey level emphasis was 0.80, with a sensitivity of 0.73 and a specificity of 0.79 reached with the best threshold of the linear predictor of 0.42. In addition, Ravanelli *et al*[60] investigated the role of contrast-enhanced CT TA in predicting treatment responses to chemotherapy ± bevacizumab in 43 patients with CRLMs. Uniformity was lower in responders than in non-responders ($P < 0.001$) in the bevacizumab-containing chemotherapy group, and in the multivariate analysis, this parameter was independently correlated with radiological CT response at three months (OR: 20, $P = 0.01$). The CT texture parameters were not significantly different between responders and non-responders in the group of patients treated with chemotherapy alone. The correlation between lower uniformity and responders to chemotherapy regimens containing bevacizumab seems to contradict the concept that higher heterogeneity reflects greater aggressiveness. However, it should be highlighted that lesion uniformity is mainly influenced by the presence of angiogenesis, which is triggered and promoted by the upregulation of VEGF, the molecular target of bevacizumab; indeed, the leakage of contrast medium from newly formed and highly permeable tumour microvessels into the extracellular space could account for the small areas of hyper-enhancement that are quantified by the variable uniformity. Hence, the assessment of uniformity should be useful in clinical practice for identifying patients who may benefit from bevacizumab. Low contrast-enhanced CT lesion density was significantly associated with no response in patients with CRLMs treated with chemotherapy with or without bevacizumab ($P = 0.03$ and 0.02, respectively), reflecting poor vascularisation, and thus poor local bioavailability of chemotherapy. Creasy *et al*[61] evaluated, for the first time, the prediction of volumetric response to systemic chemotherapy alone or in association with hepatic artery infusion (HAI), extracting 272 radiomics features from the largest hepatic metastases of 157 colorectal cancer patients. Thirty of the 271 analysed CT radiomics features were selected based on the univariate analysis and used as inputs for

the multivariate regression model. This model was constructed to calculate the percentage of tumour responses. The mean absolute prediction error (MAPE), which represents the mean difference between the predicted response from the model and actual radiographic response, was calculated. MAPE was 16.5% for the training set and 21.5% for the validation set. Furthermore, they conducted a secondary analysis in the validation set stratified by HAI utilisation, demonstrating a MAPE of 19.5% for patients with CRLM treated with HAI and 25.1% for those treated with chemotherapy alone. Since HAI chemotherapy is an expensive treatment with potential complications, predicting the response before starting therapy is very useful for clinicians to choose the most appropriate treatment strategy for each patient.

In the era of personalised medicine, Giannini *et al*[65] developed and validated an ML algorithm to predict the response of individual liver metastases in 24 colorectal cancer patients with a total of 123 lesions, extracting 22 radiomics features on pre-treatment portal CT scans and using an SVM classifier. Their ML algorithm achieved accuracies of 80.9% and 61.5% with sensitivities of 85.7% and 72.7%, and specificities of 66.7% and 47.1%, in the training and test sets, respectively. The prediction of response for each metastasis is crucial in treatment planning because the detection of one or more metastases that will respond differently than others can suggest clinicians to treat them differently[65]. The same group of researchers developed another ML algorithm to predict response in a subgroup of patients with CRLMs and who express HER2 amplification and undergo HER2-targeted therapy[66]. These patients may exhibit a heterogeneous response because some metastases shrink, while others progress[67]. Giannini *et al*[66] extracted 24 radiomics features from a 3D-ROI drawn on baseline portal phase CT and used a Gaussian naïve Bayesian classifier. The radiomics score of individual metastases reached a per-lesion sensitivity of 90% and specificity of 42% in the validation set; thus, the ML algorithm was more accurate in predicting responders than non-responders.

Wei *et al*[68] developed and validated a DL-based radiomics model based on contrast-enhanced CT to predict the response to chemotherapy in patients with CRLMs. The authors compared the diagnostic accuracy of four predictive models based on clinical data and contrast-enhanced CT qualitative features, such as tumour margin, enhanced rim, and target lesion size, DL-based radiomics model, and a combined model. The model that reached the highest AUC was constructed using a combination of CEA level and DL-based fusion radiomics signature (AUC of 0.935 in the training cohort and 0.830 in the validation cohort). Considering that the scanning CT parameters may influence the grey level values and, consequently, the radiomics features, Ahn *et al*[59] compared data acquired with four different CT scanners and did not find any significant differences.

## SYNTHESIS OF CURRENT KNOWLEDGE, LIMITS AND FUTURE PERSPECTIVES

The accurate prediction of therapy response in patients with CRLMs is a clinical requirement. In this setting, different imaging techniques such as MRI, CT, and 2-[$^{18}$F]FDG-PET/CT have been investigated.

PA imaging plays a crucial and promising role. However, evidence is limited, and reproducibility is a major concern. First, most of the studies were retrospective, monocentric, and conducted on small samples, and their results were not validated in the external population. Therefore, prospective, multicentre studies with a larger patient population should be conducted. On the other hand, the identification of a universally accepted cut-off value for each imaging quantitative parameter would be desirable, but it represents a great and ambitious challenge. Indeed, scanners and protocols may influence the value of some quantitative parameters such as ADC and radiomics features.

Regarding contrast-enhanced MRI and CT, perfusion parameters showed the most promising results for predicting therapy response in patients with CRLM. Unfortunately, perfusion techniques have not yet been introduced in routine clinical practice, possibly because of the complexity of the parameter measurements and acquisition protocols. In addition, the quantification of contrast agent concentration is difficult because of the complex relationship between density on CT and signal intensity on MRI and contrast medium concentration, influenced by many factors, such as contrast agent dose, injection rate, time of circulation, and scanner parameters. Finally, the current tumour ROI analysis utilises mean quantitative vascular parameters, which do not accurately reflect the spatial heterogeneity of tumour perfusion.

Regarding hybrid imaging techniques, only a few studies have been published, and they recognise a promising role of 2-[$^{18}$F]FDG-PET/CT in the prediction of treatment response in patients with CRLM, although this is still under investigation. This technique is not routinely performed in clinical practice, and FDG uptake is influenced by different factors such as tumour grade and histological type.

Recently the emergency of radiomics opens new horizons about the potential role of imaging techniques in predicting tumour response in patients with CRLMs, but currently a lot of issues have to be solved. First, the biological correlation of radiomics features is unclear, and second, imaging acquisition and post-processing may influence the values of radiomics features, hence their reproducibility between different centres. Radiomics represents an ongoing topic of investigation, but its clinical effectiveness remains to be defined.

Hence, considering the great potential of PA in the prediction of therapy response in patients with CRLMs, some issues should be solved. To overcome the lack of reproducibility of quantitative imaging parameters, centre-specific solutions could be hypothesised (*i.e.* each centre could identify its own threshold using the same protocol and the same scanner every time).

Finally, considering that PA is time consuming, the real effect on patient management and outcomes must be defined accurately before introducing it in clinical practice.

## CONCLUSION

In an oncological setting, PA applied to cross-sectional imaging allows the extraction of numerical data from neoplastic tissues, which are correlated with morphological, structural, functional, and metabolic features. *In vivo* evaluation of parametric imaging biomarkers can estimate the likelihood of response and drug resistance in individual patients before or immediately after starting chemotherapy and targeted agent therapy. Although the potential role of different imaging quantitative parameters in the prediction of therapy response in patients with CRLMs has been investigated, there is no consensus about which is the most promising parameter; moreover, sometimes the results are controversial. This critical point depends in part on the need for standardisation of the acquisition protocols to obtain data of good quality and reproducibility among different scanners and operators, a well-defined cut-off value, and a clear knowledge of the clinical significance of each imaging quantitative parameter. Therefore, further investigation should be conducted in this field. The identification of a shared quantitative predictive imaging parameter can be of clinical value because it avoids the risk of toxicity in patients who may not benefit from treatment as well as an economic utility to reduce unnecessary healthcare costs.

## FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

# Magnetic resonance imaging-based deep learning model to predict multiple firings in double-stapled colorectal anastomosis

Zheng-Hao Cai, Qun Zhang, Zhan-Wei Fu, Abraham Fingerhut, Jing-Wen Tan, Lu Zang, Feng Dong, Shu-Chun Li, Shi-Lin Wang, Jun-Jun Ma

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## Abstract

### *BACKGROUND*

Multiple linear stapler firings during double stapling technique (DST) after laparoscopic low anterior resection (LAR) are associated with an increased risk of anastomotic leakage (AL). However, it is difficult to predict preoperatively the need for multiple linear stapler cartridges during DST anastomosis.

### *AIM*

To develop a deep learning model to predict multiple firings during DST anastomosis based on pelvic magnetic resonance imaging (MRI).

### *METHODS*

We collected 9476 MR images from 328 mid-low rectal cancer patients undergoing LAR with DST anastomosis, which were randomly divided into a training set ($n$ = 260) and testing set ($n$ = 68). Binary logistic regression was adopted to create a clinical model using six factors. The sequence of fast spin-echo T2-weighted MRI of the entire pelvis was segmented and analyzed. Pure-image and clinical-image integrated deep learning models were constructed using the mask region-based convolutional neural network segmentation tool and three-dimensional convolutional networks. Sensitivity, specificity, accuracy, positive predictive value (PPV), and area under the receiver operating characteristic curve (AUC) was calculated

for each model.

***RESULTS***

The prevalence of ≥ 3 linear stapler cartridges was 17.7% (58/328). The prevalence of AL was statistically significantly higher in patients with ≥ 3 cartridges compared to those with ≤ 2 cartridges (25.0% *vs* 11.8%, $P$ = 0.018). Preoperative carcinoembryonic antigen level > 5 ng/mL (OR = 2.11, 95%CI 1.08-4.12, $P$ = 0.028) and tumor size ≥ 5 cm (OR = 3.57, 95%CI 1.61-7.89, $P$ = 0.002) were recognized as independent risk factors for use of ≥ 3 linear stapler cartridges. Diagnostic performance was better with the integrated model (accuracy = 94.1%, PPV = 87.5%, and AUC = 0.88) compared with the clinical model (accuracy = 86.7%, PPV = 38.9%, and AUC = 0.72) and the image model (accuracy = 91.2%, PPV = 83.3%, and AUC = 0.81).

***CONCLUSION***

MRI-based deep learning model can predict the use of ≥ 3 linear stapler cartridges during DST anastomosis in laparoscopic LAR surgery. This model might help determine the best anastomosis strategy by avoiding DST when there is a high probability of the need for ≥ 3 linear stapler cartridges.

**Key Words:** Deep learning; Image-reading artificial intelligence; Magnetic resonance imaging; Predictive model; Double stapling technique; Linear stapler; Rectal cancer; Laparoscopic surgery; Low anterior resection; Anastomotic leakage



**Core Tip:** Multiple linear stapler firings during double stapling technique (DST) anastomosis are associated with an increased risk of anastomotic leakage after laparoscopic low anterior resection. This retrospective study developed a deep learning model to predict the use of ≥ 3 linear stapler cartridges during DST anastomosis. With the help of the artificial intelligence to identify and extract information from pelvic magnetic resonance imaging, we developed a clinical-image integrated model with satisfactory accuracy. This model might help preoperatively to determine the anastomosis strategy for rectal cancer patients (suggesting not to perform DST when the risk for ≥ 3 firings is high).

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## INTRODUCTION

Anastomotic leakage (AL) is the most common postoperative complication after laparoscopic low anterior resection (LAR) for mid and low rectal cancer[1]. The consequences of AL include higher mortality, need for remedial re-operation, unplanned stoma, delay before adjuvant therapy, and compromised long-term oncological outcomes[2-4]. Although several techniques have been designed to prevent AL[5-9], the prevalence of this complication has hardly improved over the past 20 years[10,11].

Of these techniques, the double stapling technique (DST) has facilitated bowel reconstruction but failed to eliminate AL[2]. During this procedure, the distal margin of the tumor-bearing specimen is transected by one or more linear stapler firings to create the rectal stump. Several publications have identified multiple linear stapler firings as an independent risk factor for AL[1,6,12-16]. Both the Chinese Expert Consensus Statement on the Diagnostic, Prevention and Treatment of the AL for Rectal Cancer (2019) and the United States Food and Drug Administration have suggested limiting the number of stapler firings to two in the DST procedure[17,18]. A recent review of DST suggested that alternative anastomotic techniques to avoid multiple firings on the rectal stump might lower the AL rate[11].

If the number of stapler cartridges used during surgery were predictable before operation, we could predetermine whether DST would be the ideal method for reconstruction. Several studies have reported the association between pelvimetry findings and the technical difficulties (including the use of ≥ 3 linear stapler cartridges) in LAR for mid-low rectal cancer[19-21]. However, previous studies only considered the dimension of pelvic bone landmarks in pelvimetry but ignored mesorectum thickness, tumor size, or tumoral infiltration to nearby organs (prostate, seminal vesicle, uterus). Based on our subjective

experience, we speculated that the narrow (male) pelvis, thick mesorectum, aggressive tumor infiltration, and low transection margin might be associated with the need for ≥ 3 linear stapler cartridges to close the rectal stump. Besides, a simple comparison of one or several measurements of pelvimetry is insufficient to reveal the difficulty of the pelvic procedure. For a lean female patient or a heavy male patient, the same interspinous distance has a vast difference in clinical significance. Furthermore, manual measurement of pelvimetry indicators is time-consuming and labor-intensive.

These shortcomings of existing predictive methods prompted us to design and develop a new model to predict more precisely and effectively the need for ≥ 3 linear stapler firings during DST. Pelvic magnetic resonance imaging (MRI), a routine and first-choice tool for preoperative staging of rectal cancer[22], can capture mesorectal or nearby tissue infiltration characteristics in addition to bony structures. On the other hand, machine learning and deep learning models have been widely applied in health care because of their high ability to predict and make decisions[23]. Owing to the recent technological development[24-25], image-reading artificial intelligence (AI) programs can be used to recognize target features, and then interpret images or provide diagnoses based on these target features[26-30].

In this study, we aimed to create a deep learning pre-warning model for the use of multiple linear stapler cartridges during DST anastomosis by adopting AI to identify, extract and integrate image information from pelvic MRI.

## MATERIALS AND METHODS

### Patients

We retrospectively analyzed the records of 328 patients who underwent laparoscopic LAR for mid-low rectal cancer at Ruijin Hospital, Shanghai, China, between 2016 and 2021. Clinicopathological data were collected from our prospective institutional database and the study was approved by Ruijin Hospital Ethics Committee (Approval No. 2019-82). Informed consent was waived by the committee because of the retrospective nature of the study. The study was registered at clinicaltrials.gov with the registry number: NCT05498506.

The inclusion criteria were: (1) Rectal carcinoma confirmed by histopathological evaluation; (2) Tumor located in the mid-low rectum (< 10 cm from the anal verge); (3) Performance of DST anastomosis; and (4) Pelvic MRI obtained within 14 d before surgery.

The exclusion criteria were: (1) Other anastomotic techniques (*e.g.*, trans-anal rectal excision); (2) Hartmann's operation or other procedures without anastomosis; (3) Robotic surgery; and (4) The number of linear stapler cartridges was not traceable in the operative report. By using an unbiased random sampling method with a split ratio of 4:1, the patients were divided into a training set ($n$ = 260) and testing set ($n$ = 68).

### Surgical procedure

Laparoscopic LAR was performed by one operating team who treated > 200 cases of rectal cancer *per* year. The surgical procedure followed the national guidelines for laparoscopic radical resection of colorectal cancer (2018 edition). Distal rectal transection was performed with an endoscopic linear stapler (Endo-GIA™ Ultra Universal Stapler Reload with Tri-staple™ Technology; Covidien Limited Liability Company, Minneapolis, MN, USA), fired manually through the right lower quadrant 12-mm trocar. The 60-mm purple cartridges containing three different staple heights (3.0 mm, 3.5 mm, and 4.0 mm) were routinely used. However, the 45-mm purple cartridges could be used when the stapler could not be placed perpendicularly to the rectum with the 60-mm cartridges.

### Clinical variables and clinical model

We collected and analyzed baseline characteristics [sex, age, body mass index (BMI)], laboratory analysis [hemoglobin, albumin, carcinoembryonic antigen (CEA)], and tumor features [distance from the anal verge, circumferential resection margin (CRM), tumor size, tumor stage]. For the clinical model, we created a multivariate binary logistic regression model based on clinical variables that might be associated with the number of linear stapler cartridges during surgery: Three binary variables [sex (male, female), CEA level [normal, elevated (> 5 ng/mL)], and CRM (positive, negative)] and three continuous variables (BMI, distance from the anal verge, and tumor size).

### MRI protocol and labeling of target region

Pelvic MRI was performed by a Philips INGENIA™ MR scanner with a field strength of 3.0 T and the patient in the supine position. The scanning parameters included: Repetition time = 3565 ms; echo time = 80 ms; layer thickness = 5 mm; image matrix = 312 × 357, field of view = 250 × 340 × 166 mm.

The sequence of fast spin-echo (FSE) T2-weighted MRI with a large field of view with fat suppression obtained in the axial plane of the entire pelvis was retrieved from the Picture Archiving and Communication System for image segmentation. A total of 9476 T2-weighted MR images were collected from the enrolled patients. Fifteen patients in the training set were randomly selected by random

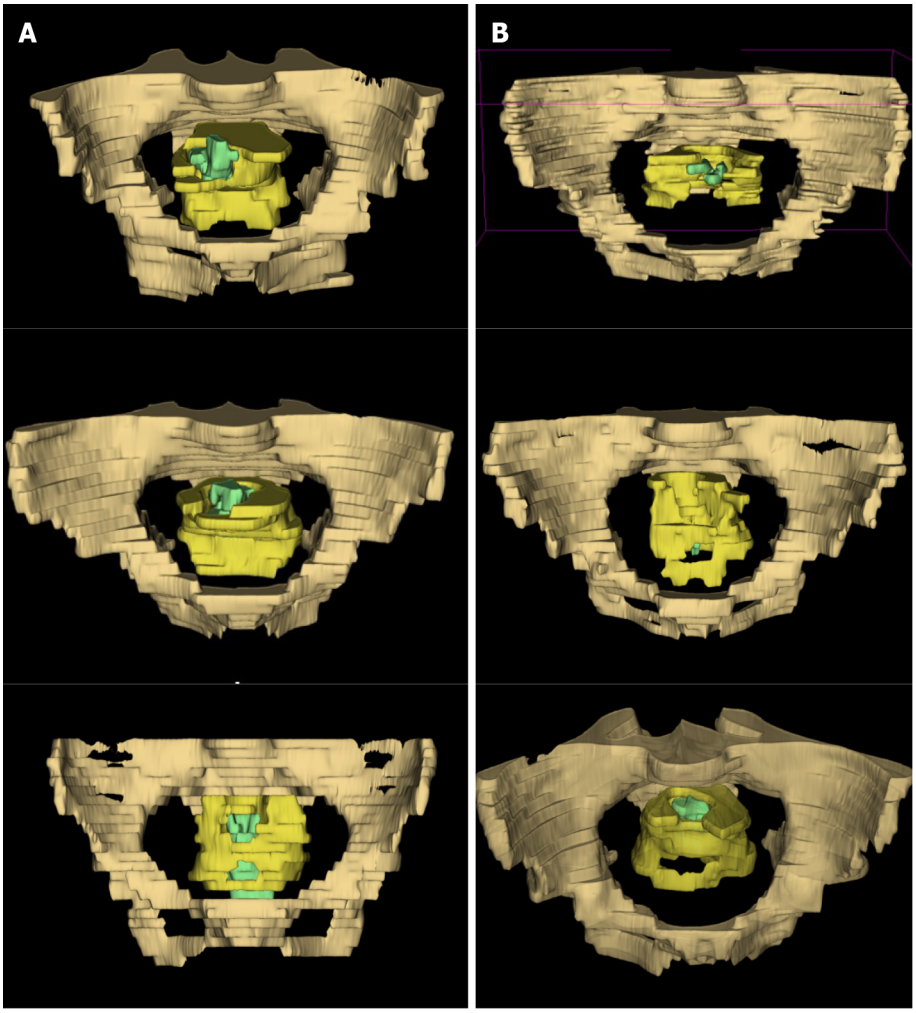


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**Figure 1 Examples of three-dimensional model of the target regions.** A: Models from patients with the use of ≥ 3 linear stapler cartridges; B: Models from patients with the use of ≤ 2 cartridges. The regions of pelvis, mesorectum, and tumor body were represented by drab, yellow, and green, respectively.

number tables and 367 images from these patients served for manual labeling. A radiological expert with > 15 years of experience in pelvic MRI labeled three target regions (pelvis, mesorectum, and tumor body) on each of the consecutive T2-weighted images. These regions were represented by drab, yellow, and green, respectively (Supplementary Figure 1), using an open annotation tool named Labelme (available at labelme.csail.mit.edu)[31]. Data were transformed into the Common Objects in Context (COCO) dataset format[32].

### *Segmentation model*

Mask region-based convolutional neural network (Mask R-CNN)[24] was used to detect and segment the three target regions (Supplementary Figure 2).

The entire Mask R-CNN network was trained on the training set, and the performance of the testing set was evaluated using the mean Average Precision (mAP). When mAP was > 50, we considered the segmentation model to have performed well[24].

To visualize intuitively the segmentation of the target region, 3D Slicer software (available at www.slicer.org) was adopted to reconstruct a three-dimensional visualization model for each patient (Figure 1).

### *Deep learning model*

A three-dimensional convolutional networks (C3D)-based model was used to generate the probability of multiple linear stapler cartridges after segmentation[25]. We used all the images of one patient as the input whereas the output was the probability of ≥ 3 linear stapler cartridges. When the probability was greater than a preset threshold (set to 0.5 empirically), the sample was judged as positive. We trained the C3D network on the training set for 100 epochs and obtained the final C3D model.

Two deep learning models were used in our study, a pure image model using only T2-weighted MR images segmented by Mask R-CNN and an integrated model using MR images as well as six above-

mentioned clinical variables. The flow chart of the design of these pre-warning models is shown in Figure 2. Our source code is publicly available (https://github.com/suli609/MRI-DST).

Finally, one clinical model and two deep learning models were evaluated on the testing set. A receiver operating characteristic (ROC) curve was plotted for each model. Sensitivity, specificity, accuracy, positive predictive value (PPV), and area under the curve (AUC) were calculated for each curve. AUC > 0.70 indicated an acceptable model.

### Statistical analysis

Statistical Package for the Social Sciences (SPSS 13.0, Chicago, IL, USA) was used for statistical analysis. The statistical methods of this study were reviewed by Shuang Wu from China Novartis Institutes for BioMedical Research Co. Ltd. Numerical variables were examined by non-parametric Wilcoxon rank-sum test. Pearson's Chi-Square or Fisher's exact test was adopted to analyze categorical data. Multivariate analysis was performed by binary logistic regression model. The difference was considered statistically significant if two-sided *P* values were < 0.05.

## RESULTS

### Clinicopathological characteristics of patients

The entire study population included 328 patients, 227 male and 101 female with a median age of 63 (range 24 - 87) years. The prevalence of use of ≥ 3 linear stapler cartridges was 17.7% (58/328). The training set ($n$ = 260) consisted of 48 cases with ≥ 3 cartridges and 212 cases with ≤ 2 cartridges. The testing set ($n$ = 68) consisted of 10 cases with ≥ 3 cartridges and 58 cases with ≤ 2 cartridges.

When clinicopathological characteristics were compared between the patients with ≥ 3 cartridges and those with ≤ 2 cartridges in the training set (Table 1), there was no statistically significant difference between the two groups with respect to sex, age, BMI, diabetes mellitus, preoperative CEA serum level, and the percentage of patients undergoing neoadjuvant chemoradiotherapy. No statistically significant difference was found in the distance from tumor to the anal verge, tumor size, tumor stage, operation time, or insufficient distal resection margin (≤ 5 mm). The incidence of AL was statistically significantly higher in the patients with ≥ 3 cartridges compared to those with ≤ 2 cartridges ($P$ = 0.018).

Univariate and multivariate analysis revealed two independent risk factors for use of ≥ 3 Linear stapler cartridges: Preoperative CEA level > 5 ng/mL (OR = 2.11, 95%CI 1.08-4.12, $P$ = 0.028) and tumor size ≥ 5 cm (OR = 3.57, 95%CI 1.61-7.89, $P$ = 0.002) (Table 2). All these clinicopathological features were comparable between the training set and testing set (Table 3).

### Visualization of target regions

Of the three-dimensional reconstruction models presented in Figure 1, those in Figure 1A, 1C, and 1E were models from patients with the use of ≥ 3 linear stapler cartridges while those in Figure 1B, 1D, and 1F were models from patients with the use of ≤ 2 cartridges. Characteristics potentially relevant to the use of ≥ 3 cartridges were narrow pelvis (Figure 1A, drab part), thick mesorectum (Figure 1C, yellow part), and large tumor size with low distal margin (Figure 1E, green part), as can be seen in the models in the left column.

### Performance of pre-warning models

The mAP of the segmentation model was 57.2 for the object detection task and 53.7 for the instance segmentation task.

The sensitivity, specificity, and accuracy of the clinical model were 70.0%, 81.0%, and 79.4%, respectively (Youden index = 0.51, PPV = 38.9%). The relevant technical indicators of the image model were as follows: Sensitivity = 50.0%, specificity = 98.3%, accuracy = 91.2%, Youden index = 0.48, and PPV = 83.3%. The integrated model showed the best pre-warning performance: Sensitivity = 70.0%, specificity = 98.3%, accuracy = 94.1%, Youden index = 0.68, and PPV = 87.5%. Finally, the AUC was 0.72, 0.81, and 0.88 for the clinical model, the image model, and the integrated model, respectively (Figure 3).

## DISCUSSION

Our deep learning model can predict the probability of using ≥ 3 linear stapler cartridges in the DST anastomosis during laparoscopic LAR surgery. Compared with the clinical model and the pure image model, the integrated model, which combined both the clinical variables and pelvic MR images, had a better Youden index (0.68) and AUC (0.88). Our results suggest that clinical or imaging information alone is insufficient to predict the use of ≥ 3 cartridges during surgery and an MRI-based integrated deep learning model might help determine the best anastomotic strategy for mid-low rectal cancer patients.

**Table 1 Clinicopathological characteristics of patients in the training set**

| Number of linear stapler cartridges | ≥ 3 | ≤ 2 | *P* value |
|---|---|---|---|
| | *n* = 48 (18.5%) | *n* = 212 (81.5%) | |
| Sex, *n* (%) | | | 0.125 |
| Male | 38 (79.2) | 144 (67.9) | |
| Female | 10 (20.8) | 68 (32.1) | |
| Age (y), median (quartile) | 62 (55-71) | 63 (55-68) | 0.749 |
| BMI (Kg/m$^2$), median (quartile) | 23.5 (21.1-25.3) | 22.9 (21.3-25.1) | 0.942 |
| Diabetes mellitus, *n* (%) | | | 0.801 |
| Yes | 7 (14.6) | 28 (13.2) | |
| No | 41 (85.4) | 184 (86.8) | |
| Hemoglobin (g/L), median (quartile) | 136 (124-143) | 133 (124-144) | 0.540 |
| Albumin (g/L), median (quartile) | 39 (36-41) | 40 (37-42) | 0.015 |
| CEA (ng/mL), median (quartile) | 4.27 (2.11-7.08) | 3.05 (2.11-5.61) | 0.147 |
| nCRT, *n* (%) | | | 0.865 |
| Yes | 13 (27.1) | 60 (28.3) | |
| No | 35 (72.9) | 152 (71.7) | |
| Distance from anus (cm), median (quartile) | 7.2 (5.9-8.4) | 7.0 (5.6-8.7) | 0.842 |
| CRM evaluated by MRI, *n* (%) | | | 0.103 |
| Positive | 16 (33.3) | 47 (22.2) | |
| Negative | 32 (66.7) | 165 (77.8) | |
| Operation time (min), median (quartile) | 139 (111-180) | 143 (116-175) | 0.526 |
| Length of cartridges used, *n* (%) | | | 0.113 |
| Only 60 mm | 42 (87.5) | 200 (94.3) | |
| 45 mm ± 60 mm | 6 (12.5) | 12 (5.7) | |
| Anastomotic leakage, *n* (%) | | | 0.018 |
| Yes | 12 (25.0) | 25 (11.8) | |
| No | 36 (75.0) | 187 (88.2) | |
| Tumor size (cm), median (quartile) | 3.7 (3.1-5.1) | 3.5 (2.9-4.2) | 0.091 |
| T stage, *n* (%) | | | 0.213 |
| T ≤ 2 | 11 (22.9) | 68 (32.1) | |
| T 3-4 | 37 (77.1) | 144 (67.9) | |
| N stage, *n* (%) | | | 0.879 |
| N0 | 25 (52.1) | 113 (53.3) | |
| N+ | 23 (47.9) | 99 (46.7) | |
| DRM, *n* (%) | | | 0.395 |
| ≤ 5 mm | 4 (8.3) | 27 (12.7) | |
| > 5 mm | 44 (91.7) | 185 (87.3) | |

BMI: Body mass index; CEA: Carcinoma embryonic antigen; nCRT: Neoadjuvant chemoradiotherapy; CRM: Circumferential resection margin; MRI: Magnetic resonance imaging; DRM: Distal resection margin.

The safety, feasibility, and oncological outcomes of laparoscopic LAR surgery for mid-low rectal cancer have been confirmed by a series of high-quality randomized controlled trials[33,34]. During laparoscopic LAR, the DST method is considered to be difficult in some patients because the size and

**Table 2 Risk factors of ≥ 3 linear staplers in the training set**

| Factors | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95%CI) | *P* value | OR (95%CI) | *P* value |
| Sex (M/F) | 0.56 (0.26, 1.18) | 0.125 | NA | NA |
| Age (yr) (≥ 70/< 70) | 1.60 (0.76, 3.29) | 0.205 | NA | NA |
| BMI (Kg/m$^2$) (≥ 25/ < 25) | 1.24 (0.63, 2.44) | 0.542 | NA | NA |
| Diabetes mellitus (Y/N) | 1.12 (0.46, 2.75) | 0.801 | NA | NA |
| Albumin (g/L) (< 35/≥ 35) | 2.42 (0.92, 6.37) | 0.074 | NA | NA |
| CEA (ng/mL) (> 5/≤ 5) | 1.99 (1.04, 3.81) | *0.038* | 2.11 (1.08, 4.12) | *0.028* |
| nCRT (Y/N) | 0.94 (0.47, 1.90) | 0.865 | NA | NA |
| Distance from anus (cm) (< 5/≥ 5) | 0.60 (0.20, 1.79) | 0.358 | NA | NA |
| CRM evaluated by MRI (+/-) | 1.76 (0.89, 3.47) | 0.103 | NA | NA |
| Length of cartridges (mm) (45/60) | 0.42 (0.15, 1.18) | 0.113 | NA | NA |
| Tumor size (cm) (≥ 5/< 5) | 3.38 (1.55, 7.37) | *0.002* | 3.57 (1.61, 7.89) | *0.002* |

OR: Odds ratio; CI: Confidence interval; NA: Not applicable; BMI: Body mass index; CEA: Carcinoma embryonic antigen; nCRT: Neoadjuvant chemoradiotherapy; CRM: Circumferential resection margin; MRI: Magnetic resonance imaging.

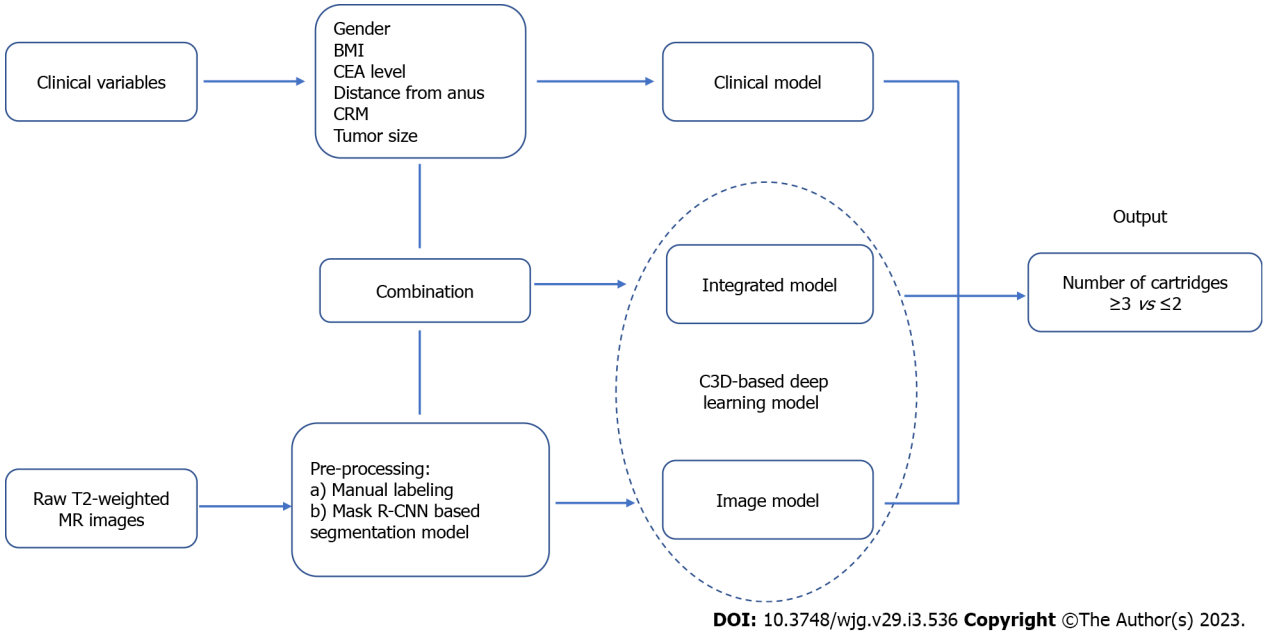


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**Figure 2 Flow chart of the design of pre-warning models.** BMI: Body mass index; CEA: Carcinoembryonic antigen; CRM: Circumferential resection margin; MR: Magnetic resonance; Mask R-CNN: Mask region-based convolutional neural network.

angle of linear staplers are limited in laparoscopy[14,35]. Consequently, multiple stapler firings are often needed. Two mechanisms might give rise to AL: Either space is left between two adjacent staple lines, or crossing the staple line with another row of staples or crushing the first staple line with the jaws can dislodge, break or deform the staples[6,12,13,18].

This has prompted surgeons to modify anastomosis techniques, which have been described as follows: Transanal transection of the rectal stump with transanal anastomosis[36,37]; intra-luminal transection of the rectal stump with manual purse-string sutures (*e.g.*, trans-anal total mesorectal excision technique)[37,38]; vertical rectal division using a linear stapler after making an additional skin incision above the pubic symphysis[6]; transverse rectal division using a Contour® stapler during laparoscopic surgery[7]; lateralization of the stump by Nelaton catheter pulling method[8]; side-to-end anastomosis (Baker technique)[9]; trans-anal reinforcement of anastomosis[39]; or removing the "dog ears"/ crossing staple lines[40,41].

**Table 3 Comparison between the training set and the testing set**

| | Testing set | Training set | *P* value |
|---|---|---|---|
| | *n* = 68 | *n* = 260 | |
| Sex, *n* (%) | | | 0.543 |
| Male | 45 (66.2) | 182 (70.0) | |
| Female | 23 (33.8) | 78 (30.0) | |
| Age (yr), median (quartile) | 63 (57-71) | 63 (55-68) | 0.322 |
| BMI (Kg/m$^2$), median (quartile) | 23.7 (22.0-25.0) | 22.9 (21.3-25.1) | 0.248 |
| Diabetes mellitus, *n* (%) | | | 0.303 |
| Yes | 6 (8.8) | 35 (13.5) | |
| No | 62 (91.2) | 225 (86.5) | |
| Albumin (g/L), median (quartile) | 39 (36-41) | 40 (37-42) | 0.111 |
| CEA (ng/mL), *n* (%) | | (Missing=5) | 0.863 |
| > 5 | 21 (30.9) | 76 (29.8) | |
| ≤ 5 | 47 (69.1) | 179 (70.2) | |
| nCRT, *n* (%) | | | 0.081 |
| Yes | 12 (17.6) | 73 (28.1) | |
| No | 56 (82.4) | 187 (71.9) | |
| Distance from anus (cm), median (quartile) | 7.1 (5.8-8.7) | 7.0 (5.6-8.7) | 0.828 |
| CRM evaluated by MRI, *n* (%) | | | 0.051 |
| Positive | 9 (13.2) | 63 (24.2) | |
| Negative | 59 (86.8) | 197 (75.8) | |
| Tumor size (cm), *n* (%) | | | 0.340 |
| ≥ 5 | 6 (8.8) | 34 (13.1) | |
| < 5 | 62 (91.2) | 226 (86.9) | |
| Number of linear stapler cartridges, *n* (%) | | | 0.470 |
| ≥ 3 | 10 (14.7) | 48 (18.5) | |
| ≤ 2 | 58 (85.3) | 212 (81.5) | |
| Length of cartridges used, *n* (%) | | | 0.603 |
| Only 60 mm | 62 (91.2) | 242 (93.1) | |
| 45 mm ± 60 mm | 6 (8.8) | 18 (6.9) | |
| Anastomotic leakage, *n* (%) | | | 0.686 |
| Yes | 11 (16.2) | 37 (14.2) | |
| No | 57 (83.8) | 223 (85.8) | |

BMI: Body mass index; CEA: Carcinoma embryonic antigen; nCRT: Neoadjuvant chemoradiotherapy; CRM: Circumferential resection margin; MRI: Magnetic resonance imaging.

Thus, if there is a high probability of using ≥ 3 cartridges according to preoperative data, one of these other anastomosis methods might be more suitable than the DST method. Foo *et al*[21] reported a pre-warning model to predict the likelihood of transecting the rectum with ≥ 3 stapler cartridges, which included the following parameters: Sex, pelvic inlet, interspinous distance, intertuberous distance, and tumor height. Two other studies investigated the technical difficulty in LAR surgery with DST anastomosis but they used other indicators, such as operative time, pelvic operative time, blood loss, conversion rate, complications, or specimen quality[42,43]. The factors associated with technical difficulty were BMI, tumor height, interspinous distance, intertubercle distance, pelvic inlet, and pubic tubercle height. The similarity of these studies with ours is that we combined clinical information with

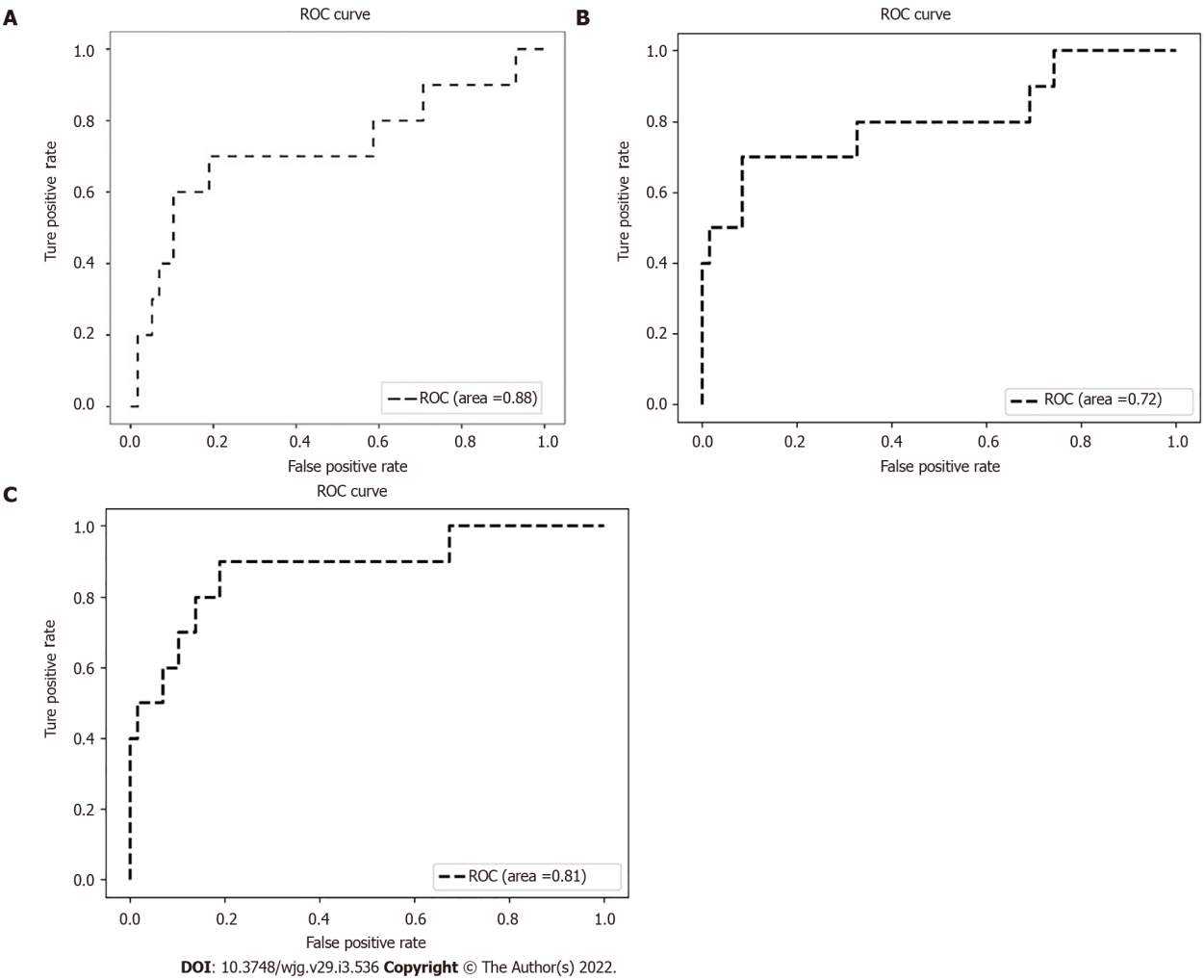


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**Figure 3 Receiver operating characteristic curves of the pre-warning models.** A: Clinical model; B: Image model; C: Integrated model.

pelvic anatomical factors and the pelvimetry was conducted in pelvic MRI. However, the strengths of our pre-warning model are mainly featured as follows: (1) By using AI-based segmentation of images, the pelvimetry is recognized as a whole instead of isolated measurements; (2) All parameters considered in the above-mentioned clinical models (sex, pelvic measurement, BMI, tumor size/height/stage) were synthesized in our image-reading models. This is why we performed segmentation of three different target regions (bony, fatty, and tumoral) in our study; and (3) This AI-based pre-warning model can shorten the prediction time to 100ms. The only data needed are six clinical factors and the sequence of FSE T2-weighted MR images.

Compared with other segmentation algorithms, such as faster R-CNN, the implementation process of Mask R-CNN is simpler, and the segmentation accuracy is higher. The mAP achieved by our model met the needs of most application scenarios[24]. The actual segmentation effect is close to the target regions manually segmented by radiologists (Supplementary Figure 2). The C3D network structure has good versatility, and the overheads of training the model are small, which is suitable for scenarios with limited training samples[25,44].

Our study had several limitations. First, the small sample size in the testing set lowered the statistical power of our analysis. With this sample size, the statistical difference between the three ROC curves might have been underestimated. Second, the lack of cases made it impossible to validate this model in an external set. Further prospective multi-center studies are needed to verify the validity of this model. Third, deep learning was only conducted on FSE T2-weighted sequences with specific scanning parameters. Further studies could focus on other MRI sequences or contrast-enhanced MRI. Fourth, the number of cartridges was not the only factor involved in AL. The intersection of staple lines[45], the precompression before stapler firings[2], and the distance between the linear staple line and the circular end-to-end anastomosis[35] might also have been implicated in addition to the number of firings. However, we could not include these factors in our analysis because of the retrospective nature of our study. Finally, apart from those factors mentioned above, the number of linear stapler cartridges depended on other factors that were difficult to assess, such as the proper lateralization of the intestinal tube[8] and the precise placement of the trocar through which the linear stapler was fired[2,35]. Thus,

none of our three models achieved 100% accuracy in the testing set. However, the PPV increased to 87.5% in the integrated model compared with 38.9% in the clinical model, indicating that the transabdominal DST method would be unsuitable for positive cases predicted by the integrated model.

## CONCLUSION

With the goal of predicting the use of ≥ 3 linear stapler cartridges during DST anastomosis in laparoscopic LAR surgery, our pelvic MRI-based deep learning model might be helpful in the preoperative determination of the best anastomosis strategy for mid-low rectal cancer patients, and, in particular, in avoiding the DST technique when there is a high probability of the need for ≥ 3 linear stapler cartridges. In this setting, another anastomotic technique without staple line crossing should be chosen. Larger studies are needed to validate its clinical value and determine if this strategy can help lower the AL rate.

## ARTICLE HIGHLIGHTS

### *Research background*

The need for multiple (≥ 3) linear stapler firings during double stapling technique (DST) is associated with an increased risk of anastomotic leakage (AL) after laparoscopic low anterior resection (LAR).

### *Research motivation*

Current methods using clinical data cannot predict precisely the use of ≥ 3 linear stapler firings before surgery.

### *Research objectives*

This study aimed to develop a pelvic magnetic resonance imaging (MRI)-based deep learning model to predict the multiple firings during DST anastomosis.

### *Research methods*

Clinical data and 9476 MR images from 328 mid-low rectal cancer patients undergoing LAR with DST anastomosis were retrospectively collected. A pure-image model and a clinical-image integrated model were constructed using image-reading deep learning technologies, respectively.

### *Research results*

The clinical-image integrated model showed better predictive performance compared with the clinical model and the pure image model with the highest accuracy (94.1%) and area under the curve (0.88).

### *Research conclusions*

Our deep learning model might help determine the anastomosis strategy for mid-low rectal cancer patients (suggesting not to perform the DST when the risk for ≥ 3 linear stapler firings is high).

### *Research perspectives*

The clinical value of this clinical-image integrated model will be validated in further prospective studies. The incidence of AL is expected to be decreased with this strategy.

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## FOOTNOTES

**Author contributions:** Cai ZH, Zhang Q, Fu ZW, Fingerhut A, Tan JW, Zang L, Dong F, Li SC, Wang SL, Ma JJ contributed to the study conception and design; Cai ZH, Zhang Q, Zang L, Dong F, Li SC, Tan JW, and Ma JJ contributed to material preparation; Cai ZH, Fu ZW and Tan JW contributed to data collection; Fu ZW, Fingerhut A, and Li SC contributed to data analysis; The first draft of the manuscript was written by Cai ZH, Zhang Q, Wang SL, and Fu Z; The final version and revisions of the manuscript were performed by Cai ZH, Zang L, Tan JW, Zhang Q, Li SC, Ma JJ, Fu ZW, and Fingerhut A; All authors read and approved the final manuscript and accept to be responsible for the contents.

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**Informed consent statement:** Informed consent was waived by Ruijin Hospital Ethics committee due to the retrospective nature of the study. The analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at marsnew1997@163.com. Consent was not obtained but the presented data are anonymized and risk of identification is low.



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**P-Editor:** Liu GL

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SYSTEMATIC REVIEWS

# Metabolic dysfunction associated fatty liver disease: The new nomenclature and its impact

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## Abstract

***BACKGROUND***

In 2020, an international expert panel proposed a new definition of fatty liver: Metabolic dysfunction-associated fatty liver disease (MAFLD). The MAFLD added the criteria for defining metabolic dysfunctions, which are high-risk factors for liver-related and cardiovascular events. Contrary to the non-alcoholic fatty liver disease (NAFLD) definition, it allows the coexistence of MAFLD and significant alcohol use in the same patient.

***AIM***

To review the existing data that evaluate the clinical profile and long-term outcome difference between the patients identified as MAFLD and NAFLD.

***METHODS***

Databases MEDLINE *via* PubMed and EMBASE were searched and relevant publications up to June 28, 2022 were assessed. Studies were included if they involved human participants diagnosed with MAFLD.

***RESULTS***

A total of 2324 records were reviewed, of which 1575 duplicate citations were removed. Of the 2324 records screened, 207 articles were excluded, and 542 articles were assessed for their eligibility, for which 511 were excluded. The remaining 31 articles were selected for review. MAFLD diagnostic criteria were able to identify more individuals with fatty liver. Studies have shown that patients included using the MAFLD criteria were associated with higher risks of hepatic fibrosis when compared to NAFLD. All-cause mortality, cardiovascular disease-related, and cancer-related mortality were shown to be higher in MAFLD patients. MAFLD patients also had higher baseline metabolic derangement, and risks of

developing obesity, diabetes, and cardiovascular events. Of the 3 subtypes, diabetes mellitus has the strongest association with negative outcomes, followed by metabolic dysfunction and elevated body mass index. Within the subtypes of MAFLD, patients with more metabolic conditions at the time of diagnosis had worse hepatic and liver injury compared to those with a single metabolic condition.

*CONCLUSION*
MAFLD is a new definition of fatty liver disease that is gaining increasing acceptance. It is based on empirical clinical practice on positive inclusion of metabolic risk factors and recent evidence suggests that it helps to identify patients with higher risk for liver-related as well as cardiovascular events.

**Key Words:** Hepatic steatosis; Liver fibrosis; Cardiovascular events; Alcohol liver disease; Obesity



**Core Tip:** Metabolic dysfunction-associated fatty liver disease (MAFLD) is a new definition of fatty liver disease that is based on positive inclusion of metabolic risk factors. Studies have shown that patients included using the MAFLD criteria were associated with higher risks of hepatic fibrosis and all cause mortality when compared to non-alcoholic fatty liver disease.

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## INTRODUCTION

The global prevalence of fatty liver has been rising in recent times, along with metabolic syndrome which are both independently significant contributors to mortality and morbidity worldwide. Since 2020, experts have suggested the change of terminology from non-alcoholic fatty liver disease (NAFLD) to metabolic dysfunction-associated fatty liver disease (MAFLD)[1]. The shift connotes a transition from subtyping patients with hepatic steatosis and no discernible cause of fatty liver, to inclusion criteria characterized by metabolic dysfunction and associated risk factors. NAFLD is an independent disease entity that does not take into account alcohol intake and other causes of pre-existing liver diseases (Figure 1A: Flowchart for the diagnostic criteria of NAFLD).

Metabolic dysfunction in our paper will follow the 1999 World Health Organization definition of metabolic syndrome, which consists of insulin resistance, high fasting glucose, and at least 2 of the following: High-density lipoprotein (HDL)-cholesterol, triglycerides (TG), blood pressure and the presence of obesity. The new proposed MAFLD diagnostic criteria are as follows in Figure 1B (flowchart for the diagnostic criteria for MAFLD): Since the conception of new diagnostic criteria for MAFLD, there have been numerous debates regarding whether this new term should be adopted. There is still a lack of awareness regarding the new terminology and diagnostic criteria amongst many healthcare professionals across the world. This study aims to summarize existing data that evaluate the long-term outcome differences of the change from NAFLD to MAFLD. The study also evaluated the classification of hepatic steatosis by the new MAFLD diagnostic criteria, histopathological classification, as well as risk factors and pathophysiological mechanisms of the new proposed disease entity.

## MATERIALS AND METHODS

### *Eligibility criteria*

We included studies ranging from case reports to randomized control trials that have been published till June 28, 2022. We excluded abstracts in this review and have restricted to only studies in English. We excluded studies with insufficient information concerning our outcomes of interest and areas of comparison, *e.g.*, survival, incidence of liver steatosis and severity of fibrosis. A PRISMA checklist was also used to guide the development of the systematic review.

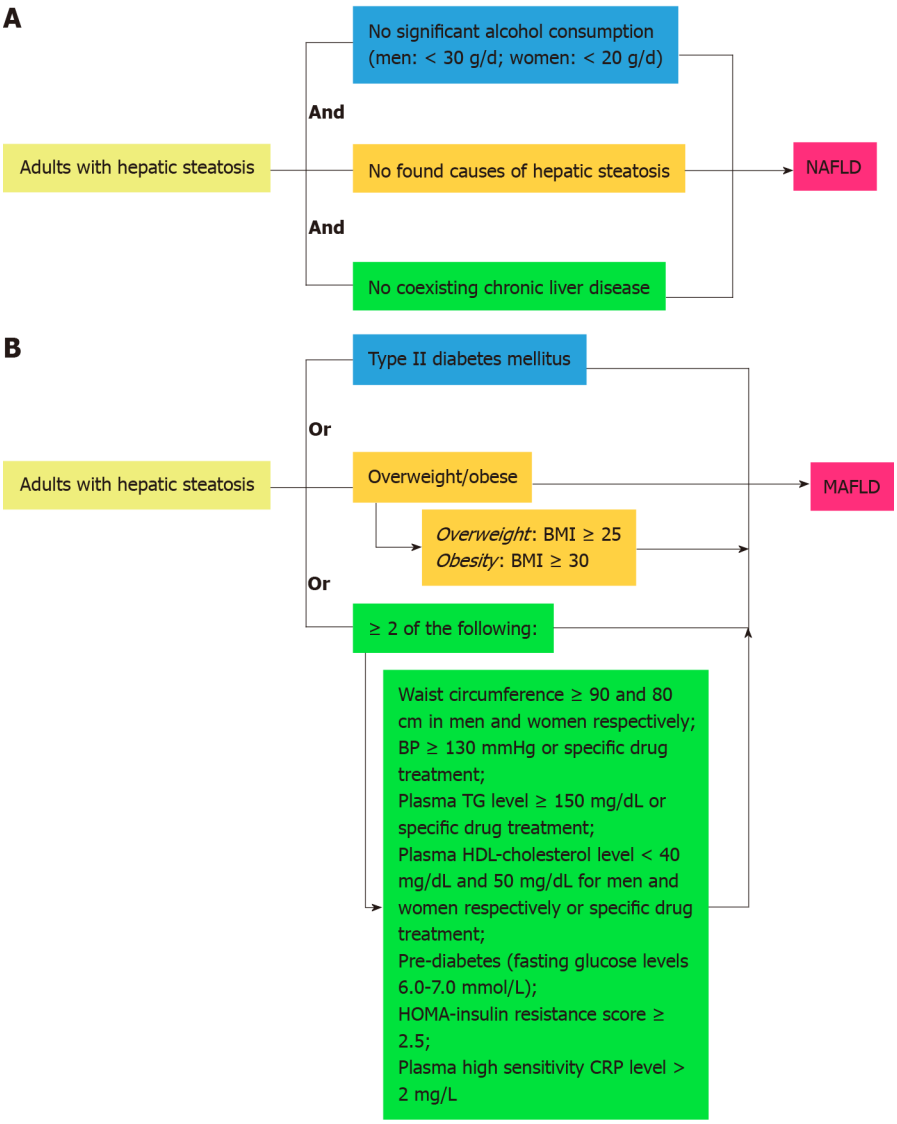


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**Figure 1 Diagnosis of non-alcoholic fatty liver disease and metabolic dysfunction-associated fatty liver disease[1].** A: Flowchart for the diagnostic criteria for non-alcoholic fatty liver disease; B: Flowchart for the diagnostic criteria for metabolic dysfunction-associated fatty liver disease. NAFLD: Non-alcoholic fatty liver disease; MAFLD: Metabolic dysfunction-associated fatty liver disease; BP: Blood pressure; TG: Triglycerides; HDL: High-density lipoprotein; CRP: C-reactive protein; BMI: Body mass index.

### *Information sources*

A comprehensive systematic search of databases and conference proceedings was conducted to identify all relevant studies up to June 28, 2022. The following electronic databases were searched: MEDLINE *via* PubMed, and EMBASE, with reference to PRISMA guidelines. We used both text words and medical subject heading terms. The literature search strategy was adapted to suit each database. Our search terms included: "Metabolic-Associated Fatty Liver disease" OR "Metabolic dysfunction-associated fatty liver disease" OR "MAFLD *vs* Non-Alcoholic Fatty Liver disease" or "MAFLD *vs* Non-alcoholic Steatohepatitis" OR "Metabolic Associated Steatohepatitis". The methods for data collection and analysis were based on the Cochrane Handbook of Systematic Reviews for Interventions. Where clarification of information in published data was required, corresponding authors were contacted through electronic mail for clarification.

### *Study selection*

Two authors (Tan JS and Pang XZ) independently selected potentially eligible studies using the data management software Rayyan QCRI. The initial screening was based on title and abstract, while final inclusion was based on full texts where available. After reading the titles and abstracts of the identified articles, full-text articles of all citations deemed to meet the inclusion criteria were sought. Duplicates were excluded. Each article was independently inspected to verify that they meet the pre-specified inclusion criteria. The study selection process is summarized in Figure 2 (summary of study selection

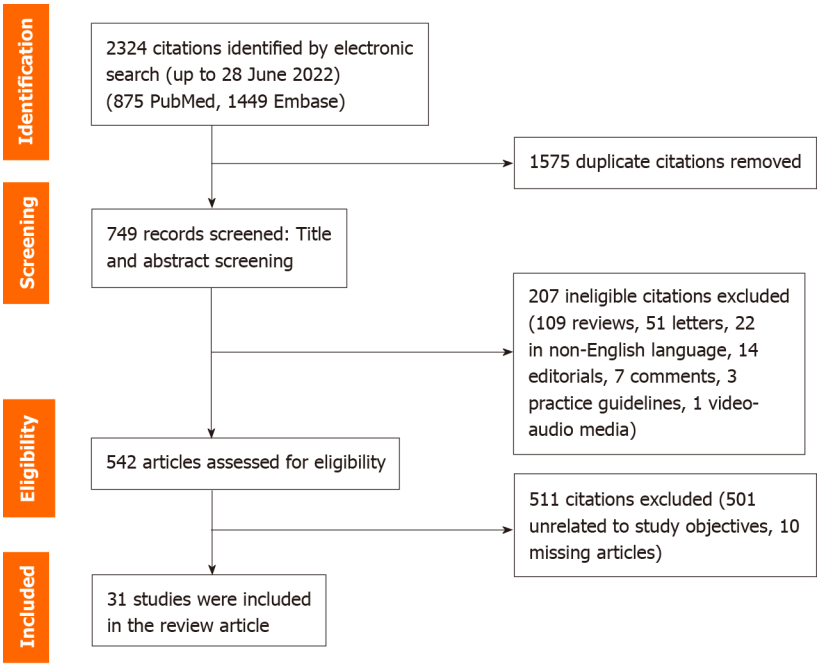


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**Figure 2 Summary of study selection process.**

process). Studies that were included in this systematic review are included in Tables 1 and 2 and Supplementary Tables 1-4. The authors included observational studies reporting the implications of MAFLD *vs* NAFLD.

## RESULTS

### Search results

A total of 2324 records were reviewed, of which 1575 duplicate citations were removed. Of the 2324 records screened, 207 articles were excluded, and 542 articles were assessed for their eligibility, for which 511 were excluded. The remaining 31 articles that were selected explored various themes, such as the long-term outcome differences of using the MAFLD criteria as compared to the NAFLD criteria, the fibrosis burden in MAFLD as compared to NAFLD, the correlation of MAFLD with other diseases, the histopathological characteristics of MAFLD, as well as risk factors and pathophysiological mechanisms of the new proposed disease entity. Articles that did not compare MAFLD and NAFLD criteria were excluded.

### Identification of hepatic steatosis and liver fibrosis

In capturing subjects with hepatic steatosis, the majority of the studies reviewed display a preference for the new MAFLD diagnostic criteria compared with the previous NAFLD, with the new definition being able to identify individuals with dual liver disease etiologies on top of all previously diagnosed NAFLD subjects[2-5]. Results from the Plinio Study also demonstrated that applying the MAFLD criteria reduces the unexplained form of lean NAFLD by identifying the presence of metabolic risk factors in these patients[6]. The Rotterdam Study was also able to identify more individuals with fatty liver disease by applying the MAFLD criteria, where the prevalence of modified MAFLD was higher than NAFLD (34.4% and 29.5%) in their population[7]. MAFLD criteria are also useful in determining the disease severity of patients with diagnosed hepatic steatosis; people with hepatic steatosis who do not fulfil MAFLD criteria are less likely to have significant liver disease as compared to those who are diagnosed with MAFLD (Table 1 and Supplementary Table 1).

In detecting subjects with liver fibrosis, MAFLD criteria also proved superior or concordant with NAFLD in many studies included in this paper[3-5,8],. Results show that the prevalence of significant fibrosis and liver stiffness is considerable in the MAFLD-only group, with marginal differences between the NAFLD-only group and metabolically healthy subjects. One study reported that liver stiffness was higher in MAFLD participants compared to NAFLD participants (7.7 *vs* 6.8 kPa, $P$ = 0.0010)[5]. Compared to NAFLD participants, MAFLD participants also had higher serum liver enzymes (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase), fatty liver index, and fibrosis scores including aminotransferase/platelet ratio index (APRI) and NAFLD fibrosis scores. In MAFLD

**Table 1 Overall evaluation of the clinical profile and long-term outcome difference between the patients identified as metabolic dysfunction-associated fatty liver disease and non-alcoholic fatty liver disease (also see Supplementary Tables 1-4 for more details on individual study)**

| Main outcome | Number of studies | Sample | Conclusion |
|---|---|---|---|
| **Hepatic steatosis and fibrosis identification in MAFLD terminology change** | | | |
| Steatosis and fibrosis | 10 | 38686 subjects | MAFLD definition is able to capture more subjects with fatty liver disease<br>MAFLD group showed either no difference or higher in fibrosis or liver stiffness compared to NAFLD group |
| **Long-term outcome differences in MAFLD terminology change** | | | |
| All cause mortality risks and cause specific mortality | 4 | 183380 subjects | MAFLD is associated with an increased risk of mortality compared to NAFLD<br>MAFLD mortality is largely contributed by the presence of metabolic disorders |
| All cause mortality risks | 1 | 12878 subjects | MAFLD and NAFLD share similar all-cause mortality risk<br>MAFLD mortality is hence likely caused by ALD, while NAFLD mortality seems to be caused by metabolic abnormalities |
| **MAFLD and correlation to non-liver diseases** | | | |
| CVD, ASCVD, cardiovascular events | 3 | 2458240 subjects | The risk of CVD is higher in MAFLD compared to NAFLD<br>MAFLD is superior over NAFLD in predicting ASCVD risk, contributed by the presence of metabolic risk factors |
| **Clinical and histopathological features of MAFLD** | | | |
| Risk factors, steatosis, advanced fibrosis | 9 | 237679 subjects | T2DM and obesity are significant drivers of MAFLD pathogenesis<br>MAFLD patients had higher BMI, LDL-C and prevalence of T2DM as compared to NAFLD patients<br>Older age, females and menopausal status are risks factors for developing MAFLD |

CVD: Cardiovascular disease; ASCVD: Atherosclerotic cardiovascular disease; NAFLD: Non-alcoholic fatty liver disease; MAFLD: Metabolic dysfunction-associated fatty liver disease; T2DM: Type 2 diabetes mellitus; BMI: Body mass index; LDL-C: Low-density lipoprotein cholesterol.

participants with excessive alcohol intake (≥ 30 g/d for males and ≥ 20 g/d for females), it was found that they have a significantly higher APRI score compared to those without excessive alcohol intake[2] (Table 1 and Supplementary Table 1).

However, all the studies reviewed could only provide an estimate of fibrosis and steatosis as the gold-standard technique for diagnosis (liver biopsy) was not done in these large population-based studies. The definition of fibrosis also differed among the studies with one study[7] using liver stiffness ≥ 8.0 kPa as the definition of fibrosis while another[9] defined fibrosis by liver stiffness measure ≥ 9.7 kPa and controlled attenuation parameter ≥ 274 dB/m (Table 1 and Supplementary Table 1).

Park *et al*[10] categorized MAFLD subjects into metabolic health - MAFLD group (≤ 1 risk factor and no diabetes) and metabolic unhealthy MAFLD group (having diabetes and/or ≥ 2 metabolic risk abnormalities) and found that the MH - MAFLD group showed no difference in the prevalence of significant or advanced hepatic fibrosis or carotid artery plaque formation compared with the healthy control group. Between the groups, there were marked differences in comorbidities and hepatic fibrosis burden, suggesting that the MAFLD definition involves an inhomogeneous population at risk of hepatic fibrosis and hence the need for a more elaborate definition (Table 1 and Supplementary Table 1).

There is also a gap in the literature surrounding the application of MAFLD criteria in the pediatric population. Although Ciardullo *et al*[11] managed to find the MAFLD criteria being fulfilled in most of their population (United States adolescents with evidence of hepatic steatosis), it did not affect the prevalence of significant fibrosis and liver stiffness between MAFLD patients and non-MAFLD steatotic patients. This might be due to the inherent chronicity in the progression of hepatic steatosis to liver fibrosis; more time should be granted to investigate the correlation between the new diagnostic criteria and long-term outcomes prospectively in the pediatric population (Table 1 and Supplementary Table 1).

### *Prediction of long-term outcomes and all-cause mortality*

Prospectively, many of the included studies show that individuals with MAFLD demonstrate higher all-

**Table 2 Studies included for study of metabolic dysfunction-associated fatty liver disease pathophysiology**

| Ref. | Type of study | Sample | Main outcomes | Results | Conclusion |
|---|---|---|---|---|---|
| Taheri *et al*[29] | Case-control study | 968 subjects from Iran | DIS, LIS | Risks of MAFLD (OR): High LIS and DIS > high LIS > high DIS (2.56 *vs* 1.96 *vs* 1.84; $P < 0.001$) | Pro-inflammatory dietary and lifestyle exposures are associated with higher risk of MAFLD regardless of gender. Inflammation may be a primary pathogenic mechanism behind dietary risks of MAFLD development |
| Mu *et al*[30] | Case-control study | 564 subjects from Xinjiang Uygur Autonomous Region, China | SNP | Risks of MAFLD (OR): PNPLA3 rs738409 CC genotype > MBOAT7 rs64173 TT genotype > STAT3 rs74416 AA genotype (1.402 *vs* 1.299 *vs* 0.738; $P < 0.005$) | The CC genotype of PNPLA3 rs738409 and TT genotype of MBOAT7 rs64173 genes are associated with higher risks of MAFLD. The AA genotype of STAT3 rs744166 gene is associated with lower risks of MAFLD. The genes TM6SF2 rs58542926 and GATAD2A rs4808199 show no significant correlation with MAFLD |
| Panera *et al*[31] | Cohort study-retrospective | 1111 subjects from Milan, Italy | Hepatic fibrosis | Associations of KLB rs17618244 variant (OR): Hepatic fibrosis (1.23; $P = 0.04$) | The KLB rs17618244 variant was associated with hepatic fibrosis ($P = 0.04$) but showed no statistical significance in the correlation with steatosis, inflammation and ballooning ($P = 0.37$, 0.12, 0.16 respectively) |
| Oses *et al*[32] | Cross-sectional study | 115 children (8-12 years old) | Fasting blood biochemical parameters, SNP | TG, insulin, HOMA-IR, ALT, AST, GGT, ferritin: MAFLD > non-MAFLD ($P < 0.05$). Percentage of risk of allele carriers: PNPLA3 rs4823173 > PPARG rs1801282 > PPARG rs13081389, HFE rs1800562 (46% *vs* 33% *vs* 21%; $P < 0.05$) | The genetic risk score based on 4 SNPs associated with MAFLD showed limited discriminatory capacity (67% sensitivity and 65% specificity) and did not improve the accuracy of the prediction protocol for MAFLD developed in the study |

DIS: Dietary inflammation score; LIS: Lifestyle inflammation score; OR: Odds ratio; NAFLD: Non-alcoholic fatty liver disease; MAFLD: Metabolic dysfunction-associated fatty liver disease; SNP: Single nucleotide polymorphisms; TG: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase.

cause, cardiovascular-related and cancer-related mortality as compared to individuals with NAFLD, or individuals with neither MAFLD or NAFLD[12-15]. A United States study that analyzed 7761 participants with a median follow-up of 23 years, noted that MAFLD patients who do not meet NAFLD criteria have a 1.7-fold higher risk of all-cause mortality, an association not demonstrated in patients with NAFLD or simple hepatic steatosis[15]. Even among MAFLD patients, individuals who meet all 3 criteria of its definition seem to exhibit higher all-cause mortality than those only fulfilling 1 or 2 of the criteria. Individuals who fulfilled all 3 MAFLD criteria had the highest hazard ratio [hazard ratio (HR)] for all-cause mortality risk (HR = 2.05), followed by individuals with metabolic dysfunction and type 2 diabetes mellitus (T2DM) (HR = 1.83), and lastly individuals with only metabolic dysfunction (HR = 1.30)[12] (Table 1 and Supplementary Table 2).

All-cause mortality in MAFLD patients is postulated to be driven by its individual metabolic constituents. Of which, T2DM has the strongest association, followed by metabolic dysfunction and elevated body mass index (BMI)[12-14]. In a United States population study[12], participants with MAFLD were sub-grouped into 1 of the 3 MAFLD criteria and were subsequently analyzed. Interestingly, the overweight (BMI ≥ 25.0 kg/m$^2$) subgroup was not associated with cancer-related mortality while the metabolic dysregulation subgroup (lean individuals with ≥ 2 metabolic risk factors among non-diabetic participants) was only associated with all-cause mortality, suggesting that T2DM is the most multifaceted cause of mortality in MAFLD patients. A similar study conducted in Kailuan, China showed similar results in that T2DM and metabolic dysfunction have the highest mortality risks (HR = 2.16, 1.79 respectively) among the MAFLD subtypes[14]. A suggested explanation is that on top of proinflammatory, pro-atherogenic and diabetogenic mediators released by livers of patients with NAFLD, the constant exposure to hyperglycaemia and raised concentrations of circulating insulin stimulated cancer progression[12] (Table 1 and Supplementary Table 2).

Age and gender seem to play a role in the mortality risks of MAFLD patients too. Among Kailuan Chinese adults, mortality risks have also been found to be higher in younger adults with MAFLD, with risks declining with age regardless of gender[14]. This association seems to suggest that early-onset metabolic comorbidities are more deleterious in MAFLD patients than when presented at later ages. It is also worth noting that the same study found that obesity has a negative association with mortality risks in older age groups (males above 40 years of age and females above 50 years of age). The non-concordant results could be explained by the obesity paradox, whereby excess adipose tissue could serve as an energy reserve, which could grant a survival advantage in older patients. This might be particularly significant in cancer-related mortality in older MAFLD patients, who are more likely to suffer from malnutrition or poor appetite. A study using LASSO regularisation for variable adjustment found that MAFLD association with cardiovascular-related and cancer-related mortality lost

significance once age, gender and ethnicity were accounted for[13], signifying that age and gender are secondarily important in mortality pathways in MAFLD patients (Table 1 and Supplementary Table 2).

A study of contention points out that the MAFLD definition has failed to capture the impact of metabolic dysfunction on long-term mortality outcomes, attributing the cause of increased all-cause mortality in the MAFLD group to the inclusion of alcoholic liver disease[16] rather than predisposing metabolic derangements. The study demonstrated good concordance between MAFLD and NAFLD groups with similar clinical characteristics except in components of each definition (*e.g.*, alcohol use for MAFLD) and concluded that there was no difference in cumulative all-cause and cause-specific mortality. In another study, individuals with MAFLD, advanced fibrosis was also associated with a higher risk of all-cause mortality [HR = 1.95; 95% confidence interval (CI): 1.46-2.60; $P < 0.001$], while individuals with NAFLD and advanced fibrosis were not significantly associated with all – cause mortality (HR = 1.33; 95%CI: 0.91-1.94; $P = 0.144$)[15]. These findings suggest that MAFLD's strong association with all-cause mortality is independent of known metabolic risk factors, though a point to consider is that mortality risk factors were only retrospectively available for NHANES III data set[15] and not for NHANES 2017-2018 data set reported in the study, which led to fibrosis being used as a surrogate marker for mortality. Contrarily, a study conducted using the Third National Health and Nutrition Examination Survey showed that MAFLD participants had a higher mortality risk regardless of excessive alcohol consumption status over a median follow up of 23.2 years[12] (Table 1 and Supplementary Table 2).

### Correlation with cardiovascular and metabolic diseases

NAFLD is tied very closely to cardiovascular diseases (CVD), with CVD being the most important cause of death in NAFLD patients. Hepatic steatosis is independently associated with coronary plaques and both hepatic steatosis and fibrosis are significantly associated with diastolic heart dysfunction. Multiple reports have shown that MAFLD is largely superior to NAFLD in the identification of high-risk patients for atherosclerotic cardiovascular diseases[17-19]. In a retrospective cohort study of 2,452,949 Japanese patients, of which the prevalence of MAFLD was estimated to be 9.7% ($n$ = 237242), the overall prevalence of hypertriglyceridemia, DM and both were 13.6%, 4.3% and 1.1% in non MAFLD patients, compared to 64.1%, 20.6% and 12.9% respectively, in the MAFLD group[17]. The same study also demonstrated that risks of coronary artery disease and CVD were higher in the MAFLD group than in the non-MAFLD group, but the CVD risks were almost the same in NAFLD and non-NAFLD group (HR = 1.02) after adjustments for metabolic syndrome factors, low-density lipoprotein cholesterol (LDL-C), statin use, age, gender, and smoking (Table 1 and Supplementary Table 3).

A single-center cohort study in Japan demonstrated that MAFLD, but not NAFLD, was an independent risk factor for the worsening of atherosclerotic disease[18]. It also identified that the presence of metabolic dysfunction might be the main risk factor for developing cardiovascular disease in MAFLD, instead of alcohol consumption. This suggests that the MAFLD criteria were superior to NAFLD in identifying patients at risk of CVD (Table 1 and Supplementary Table 3).

Patients diagnosed with the MAFLD criteria, but not fulfilling the NAFLD definition, had higher baseline metabolic derangements, except low HDL, compared to patients diagnosed with NAFLD but not fulfilling MAFLD criteria[19]. The same group of patients was also found to have a higher risk of developing general obesity, DM, and cardiovascular events at the end of a 7-year follow (Table 1 and Supplementary Table 3).

### Clinical and histopathological characteristics

With the new MAFLD definition gaining traction, many studies have explored methods to characterize the typical patient profile. MAFLD patients tend to be older, have higher BMI, and have more metabolic comorbidities as compared to healthy controls[20]. Unsurprisingly, the presence of metabolic traits meant a higher likelihood of inclusion into the MAFLD population. Compared to NAFLD, the MAFLD population has higher metabolic traits, including high TG, overweight or obesity, glucose intolerance and higher liver enzymes[21]. This result was similar to a study conducted in Fujian, China, where it was found that the MAFLD had higher BMI, LDL-C and T2DM prevalence as compared to NAFLD patients or healthy controls[22] (Table 1 and Supplementary Table 4).

It seems that the number of co-existing metabolic characteristics play an important role in defining the clinical characteristics of MAFLD patients. Patients with two or more metabolic conditions at diagnosis, had a higher grade of hepatic and renal injury compared to those with only one metabolic condition. As the number of concomitant metabolic comorbidities increased, MAFLD patients tended to be older, females, had renal impairment clinically and were more likely to have advanced fibrosis[23] (Table 1 and Supplementary Table 4).

The peak prevalence of MAFLD in the female population is older as compared to the male population [24,25]. This could be due to menopausal factors, where estrogen is postulated to have a protective effect on metabolic disorders. Post-menopausal, lower estrogen levels can lead to fat redistribution and hence result in metabolic disorders such as glucose intolerance, dyslipidemia and MAFLD[24]. It was also found that the odds ratio (OR) of MAFLD was 1.74 times higher for females over 50 years old, than those under 50 years old[26]. On the other hand, older men had a lower prevalence of MAFLD than middle aged men with the prevalence rising rapidly between the age of 18-39, and more slowly after the

age of 40 years with a peak prevalence at 42% in the 50-54 age before declining[25] (Table 1 and Supplementary Table 4).

Among the metabolic subtypes, DM superseded metabolic dysfunction and obesity in prevalence, as well as risks and severity of advanced fibrosis. Among Shanghai Chinese adults, the prevalence of MAFLD and advanced fibrosis was greatest in patients with T2DM, followed by obese and then overweight individuals[20]. In terms of severity, an NHANES III study population found higher fibrosis-4 index (FIB4) scores among MAFLD patients with DM, as compared to metabolic dysfunction and obesity[23]. Similarly, a Taiwanese study found that DM was second to hepatitis B Virus (HBV) infection in its risk of advanced fibrosis in its local MAFLD population, before hypertension or dyslipidemia[27]. More cases of hepatic steatosis and advanced liver fibrosis were found in MAFLD individuals as compared to NAFLD or healthy control groups[21], which might corroborate previous discussions on MAFLD efficacy in identifying liver disease and adverse liver outcomes (Table 1 and Supplementary Table 4).

Different conclusions were made in studies from Fujian, China and Korea. While the former drew similar conclusions in that MAFLD had a higher prevalence of moderate-severe hepatic steatosis than steatotic patients with no metabolic risks, the correlation could not be said the same for the prevalence of advanced fibrosis. However, it is worth considering that many of its participants are selected from a single center with a high proportion of HBV infection and low BMI, which might not adequately capture the relationship between metabolic dysfunction on advanced fibrosis in isolation[22]. In the Korean study, it showed that while metabolic dysfunction did have a positive correlation with risks of liver fibrosis, obesity seemed to be a more contributory factor than DM[28]. An important point worth bringing up is that the mentioned studies used different definitions of advanced fibrosis. While most of the studies collected biopsy-proven liver fibrosis, the definition of advanced differed slightly; the Korean study used defined advanced fibrosis as LSM value ≥ 7.0 kPa[28], the Fujian study as having a score of ≥ 3 on the Scheuer scale[22], the Taiwanese study as stage 3-4 on the NASH CRN fibrosis staging system[27]. To complicate things, some studies used FIB4 scoring as a marker of fibrosis[20,23], which is a measurement done clinically rather than histologically (Table 1 and Supplementary Table 4).

There are few studies comparing the histological profile in NAFLD and MAFLD due to the invasiveness of liver biopsy. One study of 1217 cases did not identify any significant differences in inflammation, advanced fibrosis, and grade of steatosis between MAFLD and NAFLD patients on histology[22]. The same study identified a third group of patients without obesity, T2DM or metabolic dysregulation but with liver steatosis on liver biopsy (non-metabolic related steatosis). Non-metabolic related steatosis patients demonstrated the similar extent of inflammation and degree of fibrosis as MAFLD and NAFLD patients despite being healthier from the metabolic syndrome point of view, hence suggesting that the MAFLD criteria may still miss out on some steatotic patients with significant liver injury (Table 1 and Supplementary Table 4).

### *Pathophysiology*

To date, the exact pathophysiology of MAFLD is not exactly well-understood. Many studies have, however, explored its correlations with genetic variants and modifiable lifestyle practices. Among Iranian adults, higher inflammatory scores secondary to dietary and lifestyle exposures such as smoking and sedentary lifestyles are associated with higher risks of MAFLD. The study suggests that inflammatory mechanisms are intrinsic in the pathophysiologic pathways in MAFLD development and progression[29]. Genetic variants have also been proven to show a link with MAFLD. Among the wide array of variants associated with higher risks of MAFLD include PNPLA3 rs738409 and MBOAT7 rs64173, while variants such as STAT3 rs74416 had been shown to have a protective effect instead. TM6SF2 rs58542926 did not show a significant correlation with MAFLD in the same study[30]. It is worth noting that the three single nucleotide polymorphisms are associated with NAFLD, which implies some degree of shared genetic predisposition to liver disease development. A variant KLB rs17618244 has emerged recently among Italian patients, and results show a predilection for hepatic fibrosis but no correlation to liver steatosis and inflammation[31]. However, the clinical practicality of genetic variant is not yet well-founded; in a pediatric MAFLD population, the genetic risk scores associated with PNPLA3 and PPARG single nucleotide polymorphisms showed little discriminatory value in predicting MAFLD patients[32]. Currently, many studies around MAFLD pathophysiology are limited by small subject groups, and more research should aim toward gaining a deeper and clinically relevant understanding of disease biomechanisms. In comparison, MAFLD shares similar genes as NAFLD, such as PNPLA3, MBOAT7 and TM6SF2[33], although most variants differ between the 2. A meta-analysis found that the PNPLA3 rs738409, also found in MAFLD, showed a positive association with NAFLD, with its G allele being frequently observed in NAFLD individuals (GG *vs* CC OR = 4.01 and GC *vs* CC OR = 1.88)[34] (Table 2).

## DISCUSSION

The proposed change of the term from 'NAFLD' to 'MAFLD' aims to better reflect and focus on the

underlying metabolism-related etiology of the disease and not just on the exclusion of alcohol intake or other liver diseases. Our review noted that the MAFLD diagnostic criteria were able to identify more individuals with fatty liver. In terms of advanced fibrosis, the MAFLD criteria were superior or concordant with NAFLD in many studies. All-cause mortality, cardiovascular disease-related and cancer-related mortality were shown to be higher in MAFLD patients. MAFLD patients also had higher baseline metabolic derangement, and risks of developing obesity, diabetes, and cardiovascular events.

Within the subtypes of MAFLD, patients with more metabolic conditions at the time of diagnosis had worse hepatic and liver injury compared to those with a single metabolic condition. This highlights the importance of individualized treatment in MAFLD patients. Non-modifiable risk factors identified for MAFLD include older age, female, post menopause, lower education level, and urban residence and modifiable risk factors include physical activity and BMI. While there are preliminary studies to suggest genetic variants associated with MAFLD, more investigations should be done to explore the mechanism behind them.

From the start, the level of acceptance for the proposal of MAFLD had been varied. So far, the Middle East and North Africa consensus panel and the Latin American Association for the Study of the Liver had endorsed the renaming of NAFLD to MAFLD[35,36]. The Latin American association had also indicated that a change in terminology could increase patients' willingness to openly discuss their disease, as the term "alcohol" leads to stigmatization. The Asian Pacific Association for the Study of the Liver had published clinical practice guidelines for the diagnosis and management of MAFLD[37], noting that dual etiology liver diseases, particularly a combination of MAFLD with viral hepatitis or alcohol, are common in this region. The change in terminology is still being debated in North America and Europe, even though the original expert consensus proposing MAFLD criteria was published in the *Journal of Hepatology*. Recently, it has been proposed that changing the terminology requires a new understanding of the molecular basis of the disease entity and new insights into risk stratification or other important aspects of this liver disease[38]. Central to the debate about the new nomenclature is whether NAFLD is an appropriate name as the term 'non-alcoholic' overemphasizes the absence of alcohol use and underemphasizes the importance of the metabolic risk factors which are the main drivers of disease progression. Further, several investigators have suggested that MAFLD but not NAFLD is associated with increased fibrosis and mortality. The opponents to "MAFLD" raised the concern that there is a lack of a general consensus on the definition of 'metabolic health'. Younossi *et al* [38] reported excess alcohol use was documented in approximately 15% of patients with MAFLD in an NHANES cohort, and contribute to liver-specific mortality for MAFLD (HR = 4.50; 95%CI: 1.89-10.75) but not NAFLD. In the same study, insulin resistance predicted liver-specific mortality in NAFLD (HR = 3.57; 95%CI: 1.35-9.42) but not MAFLD (HR = 0.84; 95%CI: 0.36-1.95). However, as seen, most of the publication to date do report higher fibrosis score.

The major limitation of our study Is, to date, most published studies on MAFLD are retrospective or cross-sectional, with very few prospective studies (which are really "retrospective-prospective", designed before the MAFLD was defined). This is not surprising since the consensus statement was only published in 2020. Second, many large database studies contain data obtained more than 10 years ago. The subjects were unlikely to have been screened comprehensively using the metabolic risk tests as listed in Figure 1B, or received the pharmacotherapies available today. Also, as MAFLD overlaps with NAFLD patients, the use of student *t*-tests and most parametric tests for comparison between the two groups is inappropriate as they are not independent groups. Publishing bias may exist as published studies are mostly positive studies and negative studies may not be reported. Lastly, most of the studies that have been included are over-represented by the Western population, and the generalizability of the results to the rest of the world can be questioned.

## CONCLUSION

In conclusion, MAFLD is a new definition of fatty liver disease that is gaining wide acceptance, especially in Asia, Latin America, and Africa. There are still questions in hot debates. The concept is based on empirical clinical practice on positive inclusion of metabolic risk factors and recent evidence suggests that it helps to identify patients with higher risk for liver-related as well as cardiovascular events. MAFLD also consists of three subtypes, each with a unique metabolic dysfunction, which may be useful for the development of new pharmacotherapy. The nomenclature and metabolic risk factor criteria will likely evolve with time. However, the principle of having "positive criteria" for metabolic dysfunction as an etiology for fatty liver disease, independent of alcohol intake, will probably prevail. More high-quality scientific evidence is still required before the widespread acceptance of this new definition.

## ARTICLE HIGHLIGHTS

### *Research background*

Metabolic dysfunction-associated fatty liver disease (MAFLD) was proposed in 2020 as the new definition of fatty liver. Compared to nonalcoholic fatty liver disease (NAFLD), MAFLD consists of inclusion criteria characterized by metabolic dysfunction and associated risk factors. There is still a lack of awareness regarding this new MAFLD terminology and its impact on clinical practice.

### *Research motivation*

There have been numerous debates regarding whether the new term MAFLD should be adopted. The definition of MAFLD reflects a shift in the focus from sub typing patients with hepatic steatosis and no discernible cause of fatty liver to the underlying metabolism - related etiology of the disease.

### *Research objectives*

This study summaries existing data that evaluate the long-term outcome differences of the terminology change from NAFLD to MAFLD, classification of hepatic steatosis, histopathological classification, risk factors and pathophysiological mechanisms of the new proposed terminology.

### *Research methods*

A systemic search of database MEDLINE *via* PubMed and EMBASE were conducted to identify relevant studies up to June 28, 2022.

### *Research results*

Of the 2324 records screened, 1575 duplicates were removed, following which 207 articles were excluded and a remaining 542 articles were assessed for eligibility. 511 articles were excluded and a remaining 31 articles were selected for review. Studies show that MAFLD patients were able to identify more patients with fatty liver compared to NAFLD. MAFLD criteria was also superior or concordant in terms of advanced fibrosis. MAFLD is also associated with higher all-cause mortality, cardiovascular disease - related and cancer - related mortality compared to NAFLD patients.

### *Research conclusions*

MAFLD is gaining acceptance as a new definition of fatty liver disease. The nomenclature and definition of MAFLD highlights the metabolic risk factor which are main drivers of disease progression.

### *Research perspectives*

MAFLD consists of 3 subtypes, each with a unique metabolic dysfunction profile that may be useful for development of new pharmacotherapy. However, further understanding is required to determine the molecular basis of MAFLD as a disease entity and new insights into risk stratification.

## FOOTNOTES

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CASE REPORT

# Small intestinal angiosarcoma on clinical presentation, diagnosis, management and prognosis: A case report and review of the literature

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## Abstract

*BACKGROUND*

Angiosarcoma is a highly malignant soft-tissue sarcoma derived from vascular endothelial cells that mainly occurs in the skin and subcutaneous tissues. Small-intestinal angiosarcomas are rare, and the prognosis is poor.

*CASE SUMMARY*

We reported a case of primary multifocal ileal angiosarcoma and analyze previously reported cases to improve our understanding of small intestinal angiosarcoma. Small intestinal angiosarcoma is more common in elderly and male patients. Gastrointestinal bleeding, anemia, abdominal pain, weakness, and weight loss were the common symptoms. CD31, CD34, factor VIII-related antigen, ETS-related gene, friend leukemia integration 1, and von Willebrand factor are valuable immunohistochemical markers for the diagnosis of small-intestinal angiosarcoma. Small-intestinal angiosarcoma most commonly occurs in the jejunum, followed by the ileum and duodenum. Radiation and toxicant exposure are risk factors for angiosarcoma. After a definite diagnosis, the mean and median survival time was 8 mo and 3 mo, respectively. Kaplan-Meier survival analysis showed that age, infiltration depth, chemotherapy, and the number of small intestinal segments invaded by tumor lesions were prognostic factors for small intestinal angiosarcoma. Multivariate Cox regression analysis showed that

chemotherapy and surgery significantly improved patient prognosis.

*CONCLUSION*
Angiosarcoma should be considered for unexplained melena and abdominal pain, especially in older men and patients with a history of radiation exposure. Prompt treatment, including surgery and adjuvant chemotherapy, is essential to prolonging patient survival.

**Key Words:** Angiosarcoma; Small intestine; Pathological features; Diagnosis; Prognosis; Case report



**Core Tip:** Small intestinal angiosarcoma is a rare malignant soft tissue tumor. We report a primary multifocal ileal angiosarcoma with metastases to the adrenal gland and lumbar spine. The patient died 4 mo after surgical resection. Further, we collected relevant case reports and analyzed statistically. We concluded that small intestinal angiosarcoma tend to occur in elderly men. Melena and anemia were the most common symptoms. The diagnosis depended on microscopic morphology and immunohistochemistry. CD31, CD34, factor VIII-related antigen, ETS-related gene, friend leukemia integration 1, and von Willebrand factor were valuable diagnostic markers. Surgery and chemotherapy could improve the prognosis of patients.

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## INTRODUCTION

Angiosarcoma is a rare malignant mesenchymal sarcoma that arises from vascular or lymphatic endothelial cells and accounts for only 1%-2% of all soft tissue sarcomas[1]. Angiosarcoma can invade any location in the body due to the widespread distribution of the blood and lymphatic systems[2]. Angiosarcoma has skin, visceral, and soft tissue subtypes, with visceral angiosarcoma accountings for 15%-47% and being more challenging to diagnose than the other subtypes[3]. Small intestinal angiosarcoma has a low incidence and presents with atypical abdominal pain, weight loss, nausea, vomiting, and gastrointestinal bleeding[4]. Various factors, including trauma, vinyl chloride, and radiation, have been implicated in the development of angiosarcoma. However, morbidity following exposure to these risk factors is rare. For example, a previous follow-up study showed that the overall risk of angiosarcoma after radiotherapy ranged from 0.01%-0.30%[5]. Timely diagnosis of small intestinal angiosarcoma is challenging owing to the diversity and non-specificity of the clinical symptoms, signs, and limited diagnostic methods, resulting in poor prognosis[6].

In this study, we report a case of primary small-intestinal angiosarcoma with lumbar and bilateral adrenal metastases. Furthermore, we retrospectively analyzed previously reported cases to explore the clinicopathological factors, diagnosis, treatment, and prognosis of small-intestinal angiosarcoma to further optimize the management and treatment of the disease.

## CASE PRESENTATION

### *Chief complaints*

A 70-year-old Chinese man presented with abdominal pain and melena for 4 mo.

### *History of present illness*

The patient's symptoms had started four months earlier, accompanied by distension, constipation, poor appetite. There was no apparent cause. The patient had lost 15 kg.

### *History of past illness*

The patient had a history of hypertension but no history of abdominal surgery, toxicity, or radiation exposure.

### *Personal and family history*

The patient denied any family history of malignant tumors.

### *Physical examination*

Physical examination revealed a chronically ill man. In addition, his abdomen was mildly swollen, with tenderness around the navel.

### *Laboratory examinations*

Laboratory data revealed hemoglobin, hematocrit, and C-reactive protein levels of 10.1 g/dL, 30.8%, and 17.18 mg/L, respectively. The tumor marker levels were not elevated.

### *Imaging examinations*

Electron gastroscopy and colonoscopy revealed no abnormalities. Computed tomography (CT) revealed that the part of the lower abdominal intestinal wall was significantly thickened with different degrees of enhancement in the arterial phase. In addition, bilateral adrenal masses and multiple soft-tissue nodules were noted in the right perirenal fascia (Figure 1). Magnetic resonance imaging (MRI) showed local abnormally enhanced nodules in the cauda equina at the L1/L2 Level; thus, metastasis was considered. Moreover, multiple nodules with abnormal signals in the bilateral adrenal area and right kidney were apparent, also leading to the consideration of metastasis (Figure 2). Electron enteroscopy revealed continuous periannulus ulcers 2.4-2.5 m above the ileocecal valve (Figure 3). The ulcer surface was covered with mucous moss, and the surrounding mucosa showed an irregular eminence, bled easily when touched, and had a hard texture.

## PATHOLOGIC FINDINGS

A laparotomy was performed that revealed multiple grayish-red ulcerative tumors in the mucosa of the ileum, with a thin film of foul moss on the surface (Figure 4). An 8 cm × 6 cm ulcerative mass was also detected 2 m distal to the ligament of Treitz, resulting in intestinal obstruction. All lesions were resected and sent for pathological examination. Microscopically, the tumor tissues were hemorrhagic and necrotic. Spindle tumor cell infiltration was observed with round or spindle nuclei, thick chromatin, and mitotic images. Giant tumor cells were arranged in cords or scattered singly. Some tumor cells formed vascular channels with erythrocytes in the center, and parts of the lumen anastomosed with each other (Figure 5). Tumor cells infiltrated the subserosal layer. No tumor tissue was detected at the resection margins or perienteral lymph nodes. Immunohistochemistry results showed that the tumor cells were positive for CD31, vimentin, ETS-related gene (ERG) and p53, but negative for CK (Pan), epithelial membrane antigen (EMA), CD34, SMA, CD117, DOG1, S100, Melan A, HMB45, and MyoD1. The Ki-67 proliferation index was 40%.

## FINAL DIAGNOSIS

According to the pathological findings, the patient was definitely diagnosed with small intestinal angiosarcoma.

## TREATMENT

The patient received R0 resection of small intestinal sarcoma with D2 Lymph node dissection and further functional end-to-end anastomosis. General supportive treatment was provided postoperatively. Further chemotherapy and molecular targeted therapy were suggested, but the patient declined owing to financial constraints.

## OUTCOME AND FOLLOW-UP

Following surgery, the patient's symptoms were relieved and there was no further melena. The patient was discharged following an improvement in his general condition. Shortly after discharge, the patient developed anorexia and diarrhea. However, the patient did not visit the hospital for review. He died four months later.

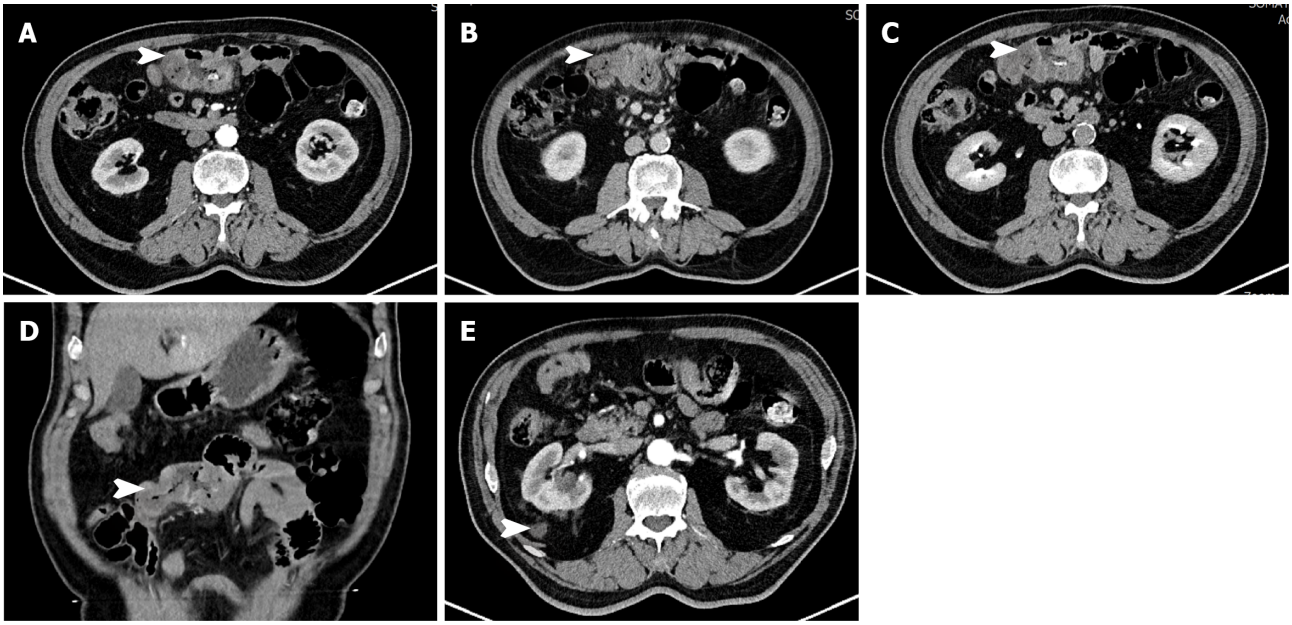


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**Figure 1 Computed tomography showed segmental thickening of the small intestine (white arrow), with lesion enhancement in the arterial phase.** A: Arterial phase; B: Venous phase; C: Balanced phase; D: Coronal plane; E: Adrenal masses.

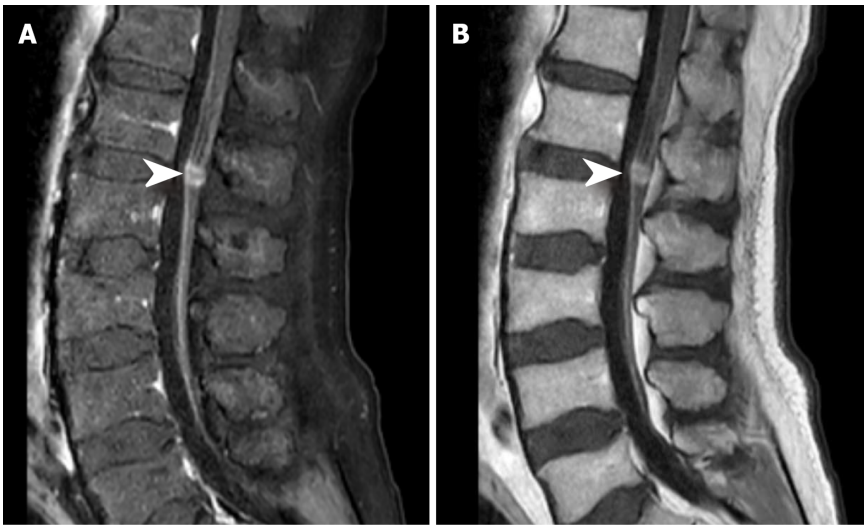


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**Figure 2 Magnetic resonance imaging showed local abnormally enhanced nodules (white arrow) at the L1/L2 Level in the cauda equina.** A: T1 phase; B: T2 phase.

## DISCUSSION

### *Literature review*

We searched PubMed, Embase, Web of Science, and CNKI for cases of small intestinal angiosarcoma (updated until August 01, 2022). Only original reports published in English language were included. The search terms were ("small bowel" OR "small intestine" OR "small intestinal") AND ("adenocarcinoma" OR "hemangiosarcoma" OR "angiomatous sarcoma"). Including our case, a total of 82 cases was collected[1-4,6-77]. Of all the cases, 62 were primary, and 14 were secondary. The primary site of 6 cases could not be determined as the tumor lesions were found at multiple sites simultaneously. The basic clinical data of the patients are presented in Table 1. Prognostic information was available for 62 cases and an endpoint event (death) was observed in 52. SPSS software was used for statistical analyses. Survival curves were obtained using the Kaplan-Meier method (log-rank test). Univariate and multivariate analyses were performed using the Cox proportional hazards model. Categorical variables were compared using the chi-squared test. All tests were two-tailed, and statistical significance was set

**Table 1 The basic clinicopathological factors of 82 collected cases**

| Characteristics | Number of patients | % |
|---|---|---|
| Gender | | |
| Male | 55 | 67.07 |
| Female | 27 | 32.93 |
| Age | | |
| ≤ 65 | 34 | 41.46 |
| > 65 | 47 | 57.32 |
| NA | 1 | 1.22 |
| Race | | |
| North America | 35 | 42.68 |
| European | 17 | 20.73 |
| Asia | 26 | 31.71 |
| other | 4 | 4.88 |
| Year | | |
| ≤ 2000 | 20 | 24.39 |
| > 2010 | 62 | 75.61 |
| Tumor origin | | |
| Primary | 62 | 75.61 |
| Secondary | 14 | 17.07 |
| NA | 6 | 7.32 |
| Radiation history | | |
| With | 21 | 25.61 |
| Without | 61 | 74.39 |

NA: Not available.

at $P < 0.05$ unless otherwise stated. Data are presented as mean ± SD.

### Age and gender characteristics of the patients

Of the 82 patients, 55 were men, and 27 were women, with a male-to-female ratio of 2.04:1.00. The ages of 55 men and 26 women were available (Table 2). The mean age of men was 64.44 years ± 14.92 years, with a range of 25-87 years; the mean age of women was 60.85 years ± 22.73 years, with a range of 20-92 years. The patients' age distribution is shown in Figure 6. There was no significant difference in age distribution between men and women ($P = 0.339$, Chi-square test).

### Clinical symptoms and complications

The most frequent clinical symptoms (in order of frequency) were gastrointestinal bleeding (62.20%), anemia (57.32%), abdominal pain (37.80%), weakness (23.17%), weight loss (18.29%), shortness of breath (15.85%), nausea (13.41%), abdominal distention (12.20%), and loss of appetite (9.76%) (Table 3). Symptoms caused by angiosarcoma are challenging to distinguish from those of patients with gastrointestinal tumors, ulcers, and inflammatory diseases. The possibility of angiosarcoma should be considered in patients with unexplained gastrointestinal bleeding. The common abdominal complications were intestinal obstruction (18.29%), intestinal perforation (13.41%), intussusception (4.88%), and intraperitoneal hemorrhage (2.44%), which can result in an acute abdomen requiring emergency surgical management.

### Detection of angiosarcoma lesions

The examination of the small intestine is difficult because of its anatomical location and structure. In recent years, the diagnostic rate of small-intestinal diseases has improved with the development of capsule endoscopy and enteroscopy. Among the cases collected, small bowel lesions or abnormalities were detected first by endoscopy in 23 cases, by CT in 12 cases, and capsule endoscopy in 6 cases. In

**Table 2 Age distribution characteristics of patients**

| Gender | Number | Mean age | SD | Median age | Range of age |
|---|---|---|---|---|---|
| Female | 26 | 60.85 | 22.73 | 70 | 20-92 |
| Male | 55 | 64.44 | 14.92 | 68 | 25-87 |
| All | 82 | 63.28 | 17.74 | 68 | 20-92 |

**Table 3 Patients' symptoms during the disease**

| Symptoms | Number | Percentage (%) |
|---|---|---|
| Gastrointestinal bleeding | 51 | 62.20 |
| Anemia | 47 | 57.32 |
| Abdominal pain | 31 | 37.80 |
| Weak | 19 | 23.17 |
| Loss of weight | 15 | 18.29 |
| Short of breath | 13 | 15.85 |
| Nausea | 11 | 13.41 |
| Abdominal distention | 10 | 12.20 |
| Loss of appetite | 8 | 9.76 |
| Dizziness | 5 | 6.10 |
| Fever | 3 | 3.66 |
| Constipation | 3 | 3.66 |
| Chest pain | 3 | 3.66 |
| Back pain | 3 | 3.66 |
| Diarrhea | 2 | 2.44 |
| Syncope | 2 | 2.44 |
| Drowsiness | 1 | 1.22 |
| Peripheral edema | 1 | 1.22 |
| Lower limb weakness | 1 | 1.22 |

addition, digestive tract radiography, MRI, barium meal, positron emission tomography (PET), and other examinations helped to detect lesions. In 26 cases, lesions were found by exploratory laparotomy, including those with an acute abdominal disease requiring emergency surgery and those in whim imaging and endoscopy examinations did not detect the lesion. In three cases, lesions were found on autopsy. The morphology of small intestinal angiosarcoma varies. Endoscopically, the tumors appear as deep or shallow ulcers, polyps, fungating lesion[22], nodules, huge masses, superficial elevations, depressions[48], or thickening and congestion of the small bowel wall[35]. Some lesions show varying degrees of hemorrhage[21,32,44,59] or are covered with filthy moss. On the CT scan, small intestinal angiosarcoma was characterized by segmental wall thickening of the small intestine[10,12,58,75,76], apple core lesion[11] and occupying lesion[6,20,33,67,70], with enlargement of the surrounding lymph nodes[52]. Necrosis was observed in the center of some lesions[33]. Contrast-enhanced CT scans showed different degrees of enhancement[4,57,61].

### *Diagnosis of small intestinal angiosarcoma*

The diagnosis of angiosarcoma depends mainly on morphological characteristics and immunohistochemistry. Abnormal and malignant endothelial cells are the hallmarks of angiosarcoma and can be round, polygonal, spindle-shaped, or epithelioid in appearance. Well-differentiated angiosarcoma presents as well-formed vessels, papillary vascular spaces, or anastomotic narrow vascular channels with visible red blood cells in the lumen[29]. Poorly differentiated angiosarcomas are solid tumors characterized by continuous sheets of malignant cells[29]. Local necrosis and bleeding of tumor tissue are common. Table 4 presents the expression of immunohistochemical markers in the collected cases. CD31, CD34, factor VIII-related antigen (VIII), ERG, friend leukemia integration 1 (Fli-1), and von

**Table 4 Characteristics of immunohistochemistry results**

| Pathological markers | Total | Positive | Negative |
|---|---|---|---|
| CD31 | 49 | 49 | 0 |
| CD34 | 40 | 30 | 10 |
| Vimentin | 28 | 28 | 0 |
| VIII | 29 | 26 | 3 |
| ERG | 10 | 10 | 0 |
| FLI-1 | 6 | 6 | 0 |
| von Willebrand factor | 2 | 2 | 0 |
| EMA | 17 | 1 | 16 |
| SMA | 10 | 0 | 10 |
| CD117 | 10 | 0 | 10 |
| Desmin | 11 | 0 | 11 |
| S100 | 28 | 1 | 27 |

VIII: Factor VIII-related antigen; ERG: ETS-related gene; FLI-1: Friend leukemia integration 1; EMA: Epithelial membrane antigen; SMA: Smooth muscle actin.

Willebrand factor (vWF) are important immune-positive markers of angiosarcoma. CD31 (49/49), ERG (10/10), FLI-1 (6/6), and vWF (2/2) were all positive in the stained cases. CD34 had a sensitivity of 75% (30/40), and VIII had a sensitivity of 89.7% (26/29). Besides, the immunohistochemistry results for EMA (16/17), SMA (10/10), CD117 (10/10), desmin (11/11), and S100 (27/28) were mostly negative in the collected angiosarcoma cases.

### *Distribution, characteristic, and metastasis of the lesions*

The location of the small intestinal angiosarcoma, in descending order, was jejunum (28.0%), ileum (19.5%), duodenum (12.2%), whole small intestine (12.2%), duodenum/jejunum (11.0%), jejunum/ileum (6.1%), and unspecified small intestine (11.0%) (Table 5). Of the 76 cases that reported a definite location of the lesion, 42 cases invaded the jejunum (55.3%, A + D + E), 40 cases invaded ileum (52.6%, B + D + E + F) and 29 cases invaded duodenum (38.2%, C + D + F). There were 49 cases (64.5%) involving a single segment of the small intestine (A + B + C), 14 cases (18.4%) involving two segments of the small intestine (E + F), and 10 cases (13.2%) involving the entire small intestine. The characteristics of angiosarcoma lesions are shown in Table 6. Angiosarcomas of the small intestine tend to be multifocal (multifocal/single focal = 1.8). The size of the lesions varied, with a maximum diameter of < 40 mm for 38 Lesions and > 40 mm for 14 Lesions. The largest reported lesions can be up to 240 mm in diameter [33]. In two cases, the tumor lesions showed diffuse distribution[23,40]. Angiosarcoma lesions present as ulcer type (15.66%), superficial type (9.64%), diffuse infiltration type (7.23%), and protrusion (49.4%) types, including mass, polyp, mushroom type, and so on. Microscopically, the lesions invaded the mucosa in 3 cases, submucosa in 4 cases, muscularis propria in 5 cases, and serosa in 7 cases. In one case, the tumor lesion was located under the serosa. Among the 62 cases of primary small intestinal angiosarcoma, 35 cases (56.5%) had distant metastasis, 23 cases (37.1%) had no distant metastasis, and the other 4 (6.5%) were not specified (Table 7). The most frequent metastatic sites were the lung (22.6%), liver (21.0%), large intestine (21.0%), spleen (8.1%), bone (8.1%), pleural (6.5%) and stomach (6.5%). Of the 14 sary cases, 4 were primary in the skin of the head and face, 4 in the liver, 4 in the spleen, and one each in the pleuropulmonary, thyroid, sternocleidomastoid muscle, and rectum. Systemic examination and careful exploration are necessary for patients with angiosarcoma to prevent missing multiple or metastatic lesions.

### *Risk factors*

A total of 21 cases had a clear history of radiation, including 15 women and 6 men (Table 8). There were 20 cases with a history of radiation therapy for tumors, and the remaining 1 had 30 years of severe occupational exposure to radiation and polyvinyl chloride. The time from radiation exposure to diagnosing small intestinal angiosarcoma fluctuated from 7 years to 45 years, with an average of 16.12 years ± 10.05 years. The radiation sites were located in the pelvis in 16 cases, chest in 2 cases, abdomen in 1 case, and neck in 1 case. The radiation dose ranged from 15 Gray to 60 Gray, with a mean of 48.03 Gray ± 14.18 Gray. Among the female patients, 10 were treated with radiation for uterine tumors, 2 for breast cancer, 2 for ovarian tumors, and 1 for colon cancer; of the male patients, 2 for prostate cancer, 1

**Table 5 Location of small intestinal angiosarcoma**

| Tag | Location | Number | Percentage (%) |
|---|---|---|---|
| A | Jejunum | 23 | 28.0 |
| B | Ileum | 16 | 19.5 |
| C | Duodenum | 10 | 12.2 |
| D | Duodenum, Jejunum and Ileum | 10 | 12.2 |
| E | Duodenum and Jejunum | 9 | 11.0 |
| F | Jejunum and Ileum | 5 | 6.1 |
| G | Unspecified small intestine | 9 | 11.0 |

**Table 6 Characteristics of small intestinal angiosarcoma lesions**

| Characteristics | Number | % |
|---|---|---|
| Size | | |
| ≤ 40 mm | 38 | 45.78 |
| > 40 mm | 14 | 16.87 |
| Diffuse | 2 | 2.41 |
| NA | 29 | 34.94 |
| Small intestinal lesions | | |
| Single focal | 26 | 35.6 |
| Multifocal | 47 | 64.4 |
| NA | 9 | |
| Pathological morphology | | |
| Ulcerative | 13 | 15.66 |
| Protuberant | 41 | 49.4 |
| Superficial | 8 | 9.64 |
| Diffuse infiltrating | 6 | 7.23 |
| NA | 15 | 18.07 |
| Infiltration depth | | |
| Mucosa | 3 | 3.61 |
| Submucosa | 17 | 20.48 |
| Muscularis propria | 7 | 8.43 |
| Serosa | 17 | 20.48 |
| Under the serosa | 1 | |
| NA | 39 | 46.99 |

NA: Not available.

for abdominal lymphoma, 1 for tonsil cancer, and 1 for pelvic chondrosarcoma. In addition, a 45-year-old male patient had a history of hemodialysis for up to 21 years due to chronic renal insufficiency[46], and a 72-year-old male patient worked in the construction industry and may have had a long history of toxicological exposure[9].

### *Treatment*

Treatment modalities were available for 74 cases among the reported cases. Among them, 42 patients (51.2%) underwent surgical resection only; 12 patients (14.6%) underwent surgical resection and chemotherapy; 11 patients (13.4%) received conservative treatment or no treatment; 5 patients (6.1%)

**Table 7 Distant metastatic site of primary small intestinal angiosarcoma**

| Distant metastasis | Number | % |
|---|---|---|
| No metastasis | 23 | 37.1 |
| Lung | 14 | 22.6 |
| Liver | 13 | 21.0 |
| Large intestine | 13 | 21.0 |
| Spleen | 5 | 8.1 |
| Bone | 5 | 8.1 |
| Pleural | 4 | 6.5 |
| Stomach | 4 | 6.5 |
| Bladder | 3 | 4.8 |
| Kidney | 2 | 3.2 |
| Vein | 2 | 3.2 |
| Abdominal wall | 2 | 3.2 |
| Gallbladder | 2 | 3.2 |
| Pancreas | 1 | 1.6 |
| Heart | 1 | 1.6 |
| Adrenal gland | 1 | 1.6 |
| Pelvic cavity | 1 | 1.6 |
| Brain | 1 | 1.6 |
| Oropharynx | 1 | 1.6 |
| Diaphragm | 1 | 1.6 |
| NA | 4 | 6.5 |
| All | 62 | 100% |

NA: Not available.

received chemotherapy only; one underwent surgical resection, chemotherapy, and radiation therapy; one was treated with chemotherapy, radiotherapy, and immunotherapy; and one was treated with argon plasma coagulation. Of the patients who received chemotherapy, 2 with doxorubicin; 1 with paclitaxel plus carboplatin; 1 with adriamycin, vincristine, dacarbazine and Cytoxan; 1 with liposomal non-pegilated doxorubicin and Ifosfamide. Bevacizumab was the immunotherapy drug. Some patients required repeated blood transfusion treatment owing to anemia caused by chronic gastrointestinal blood loss[3,28].

### *Prognostic factors*

We collected the survival time and status of 62 patients and performed a prognosis analysis. The mean survival of patients with small intestinal angiosarcoma was 234.77 d ± 41.88 d, with a range of 3 d to 3 years. Median survival time was 90.00 d ± 20.56 d. Respiratory failure, hemorrhagic shock, and multiple metastases were common causes of death. Kaplan-Meier survival analysis showed that age ($P$ = 0.033), infiltrating depth ($P$ = 0.038), chemotherapy ($P$ = 0.025), and the number of small intestinal segments tumor involved ($P$ = 0.020) were prognostic factors for small intestinal angiosarcoma (Figure 7). Sex, risk factors, acute abdomen, tumor origin, tumor size, number of tumor lesions, and distant metastasis had no significant effect on patient prognosis ($P$ > 0.100). In the COX regression survival analysis, infiltration depth was eliminated owing to a large amount of missing data. Univariate COX regression analysis showed that age > 65 years ($P$ = 0.047) and tumor lesions involving three whole segments ($P$ = 0.020) of the small intestine, without chemotherapy ($P$ = 0.032) were risk factors for small intestinal angiosarcoma (Table 9). We included factors with $P$ < 0.100 in the univariate COX regression analysis into the multivariate analysis to avoid missing important influencing factors. The results showed that chemotherapy [$P$ = 0.038, HR: 0.442 (0.205-0,956)], and surgery [$P$ = 0.028, HR: 0.407 (0.182--0.908)] effectively improved patient prognosis (Table 10).

**Table 8 Analysis of risk factors for small intestinal angiosarcoma**

| Gender/age (yr) | Time[1] (yr) | Cause of radiation | Radiation area | Dose (Gray) |
|---|---|---|---|---|
| F/66 | 20 | Uterus cancer | Pelvis | NA |
| F/69 | 7 | Adenocarcinoma of uterus | Pelvis | NA |
| F/50 | 14 | Adenocarcinoma of uterine body | Pelvis | 55.6 |
| F/76 | 7 | Adenocarcinoma of uterine body | Pelvis | 45.1 |
| F/78 | 10 | Endometrial cancer | Pelvis | 55.5 |
| F/51 | 9 | Adenocarcinoma of cervix | Pelvis | 50 |
| F/80 | 20 | Squamous cell carcinoma of cervix | Pelvis | 55 |
| F/61 | 20 | Squamous cell carcinoma of cervix | Pelvis | NA |
| F/NA | 8 | Cervical cancer | Pelvis | NA |
| F/72 | 24 | Leiomyosarcoma of uterus | Pelvis | NA |
| F/26 | 14 | Dysgerminoma of ovary | Pelvis | 48 |
| F/66 | 8 | Ovarian cancer | Pelvis | 60 |
| F/88 | 18 | Breast cancer | Chest | NA |
| F/37 | NA | Breast cancer | Chest | NA |
| F/92 | 12 | Colon cancer | Pelvis | NA |
| M/82 | NA | Prostate cancer | Pelvis | NA |
| M/80 | NA | Prostate cancer | Pelvis | NA |
| M/73 | NA | Squamous cell carcinoma of tonsil | Neck | NA |
| M/63 | 45 | Left lower abdominal lymphoma | Abdomen | 15 |
| M/57 | 8 | Chondrosarcoma of right hemipelvis | Pelvis | NA |
| M/68 | 30 | Occupational exposure | NA | Heavy |

[1]Time: Duration from exposure to risk factors to diagnosis of small intestinal angiosarcoma.
M: Male; F: Female; NA: Not available.

## DISCUSSION

Primary small intestinal malignancies are rare, accounting for < 2% of gastrointestinal tumors[36]. Small intestinal malignant tumors are often discovered late, due to their nonspecific symptoms and limited examination methods, resulting poor prognosis[51]. Gastrointestinal bleeding caused by small intestinal angiosarcoma is difficult to detect using routine gastroscopy and electronic colonoscopy[17]. CT, capsule endoscopy[25], PET[29], tagged red blood cell scanning[18], and push enteroscopy[28] may aid in the detection of small intestinal lesions. However, lesions were not detected in some patients after multiple examinations, thus necessitating surgical exploration. Even with endoscopic tissue biopsies, definitive diagnosis requires several attempts in some patients[9]. Thus, small intestinal angiosarcoma should be considered in patients with early abdominal symptoms, especially in older adults with melena, to avoid rapid disease development due to missed diagnosis.

Angiosarcoma is an aggressive tumor with high lymph node and peripheral metastases[51]. In our literature review, primary small intestinal angiosarcoma had a distant metastasis rate of at least 56.5%. Small intestinal angiosarcoma often metastasizes to the lungs, liver, large intestine, and spleens. Respiratory failure due to pulmonary metastases is a common cause of death in patients with small intestinal angiosarcoma (15 cases). There was one case of metastasis to an uncommon site, the right atrial appendage and right ventricular septum, with a survival of only 12 d[34]. Therefore, for patients diagnosed with angiosarcoma of the small intestine, systemic examinations, such as PET-CT, are recommended, with attention paid specifically to pulmonary metastases.

Depending on the degree of differentiation, angiosarcoma can develop and range from being a well-differentiated vascular form to a poorly differentiated solid tissue. The solid growth pattern of angiosarcoma consists of two cell types: Sheets of spindle-shaped or large, polygonal epithelioid-type cells with a high mitotic rate[29]. The specific angiosarcoma subtype consisting of epithelioid tumor cells is called epithelioid angiosarcoma[32]. Epithelioid morphology is typical, but it can also express endothelial-related markers, such as cytokeratin, leading to confusion with other entities, such as

**Table 9 Results of univariate COX regression analysis**

| Factors | HR (98%CI) | *P* value |
|---|---|---|
| Gender (male/female) | 1.395 (0.772-2.521) | 0.270 |
| Age (> 65/≤ 65) | 1.803 (1.007-3.227) | 0.047 |
| Tumor origin (secondary/primary) | 1.708 (0.789-3.696) | 0.174 |
| Tumor size(> 40 mm/≤ 40 mm) | 1.265 (0.578-2.767) | 0.557 |
| Tumor lesion (multifocal/single focal) | 1.365 (0.722-2.579) | 0.338 |
| Distant metastases (yes/no) | 1.140 (0.573-2.269) | 0.708 |
| Acute abdominal disease (yes/no) | 0.780 (0.424-1.435) | 0.424 |
| Surgery (with/without) | 0.570 (0.296-1.100) | 0.094 |
| Chemotherapy (with/without) | 0.473 (0.238-0.940) | 0.032 |
| Gastrointestinal bleeding (yes/no) | 1.076 (0.604-1.915) | 0.803 |
| Tumor distribution | | |
| Two segments/one segment | 2.116 (0.975-4.593) | 0.058 |
| Whole intestine/one segment | 2.473 (1.156-5.289) | 0.020 |

**Table 10 Results of multivariate COX regression analysis**

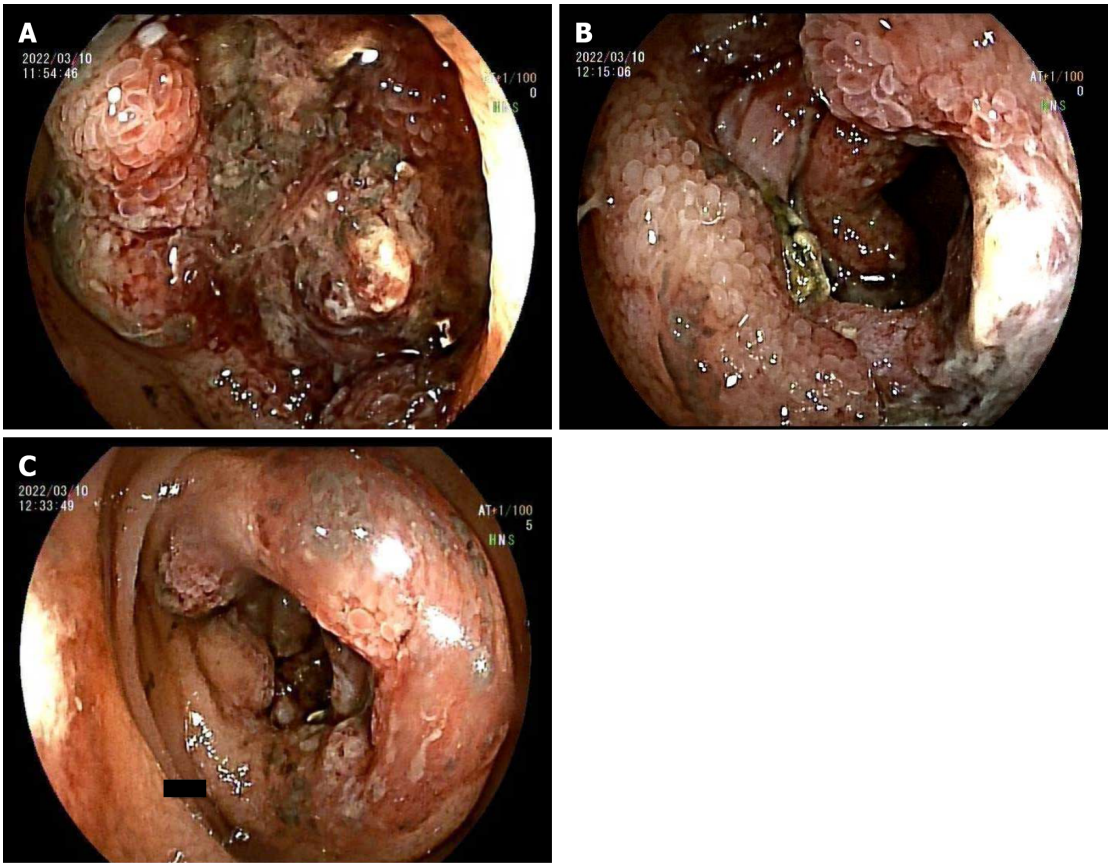
| Factors | HR (98%CI) | *P* value |
|---|---|---|
| Chemotherapy (with/without) | 0.442 (0.205-0.956) | 0.038 |
| Surgery (with/without) | 0.407 (0.182-0.908) | 0.028 |
| Age (> 65/≤ 65) | 1.944 (0.969-3.902) | 0.061 |
| Tumor distribution | | |
| Two segments/one segment | 0.434 (0.194-0.969) | 0.042 |
| Whole intestine/one segment | 0.820 (0.323-2.086) | 0.677 |

malignant melanoma, fibrosarcoma, mesothelioma, or sarcoma with epithelioid features (particularly gastrointestinal stromal tumors)[10,28].

Immunohistochemistry is essential for the diagnosis of angiosarcoma. Positive expression of endothelial markers, including CD31, CD34, factor VIII, ERG, Fli-1, and vWF, help define the vascular nature of the tumor[3]. CD31 and ERG show the highest positive detection rates. The specificity of CD34 is relatively low and is positively expressed in 60%-70% of gastrointestinal stromal tumors[78]. Vimentin is a marker of epithelial-mesenchymal transition, and its overexpression in tumors is closely related to accelerated growth, invasion, and poor prognosis[52]. Vimentin is also widely expressed in other tumors, including melanoma, malignant mesothelioma, and epithelioid sarcoma, thus lacking reliability in the differential diagnosis of angiosarcoma[3]. As a negative marker in angiosarcoma, S-100 proteins help differentiate angiosarcoma from carcinoma and melanoma[10]. EMA cannot be used definitively in the differential diagnosis of angiosarcoma as it can be positive for epithelioid angiosarcoma[3,52]. CD117 is commonly used to diagnose gastrointestinal stromal tumors[78]. However, previous studies have shown that > 50% of angiosarcomas are positive for CD117[79]. Additionally, epithelioid and some non-epithelioid angiosarcoma cases may express keratin[3,76].

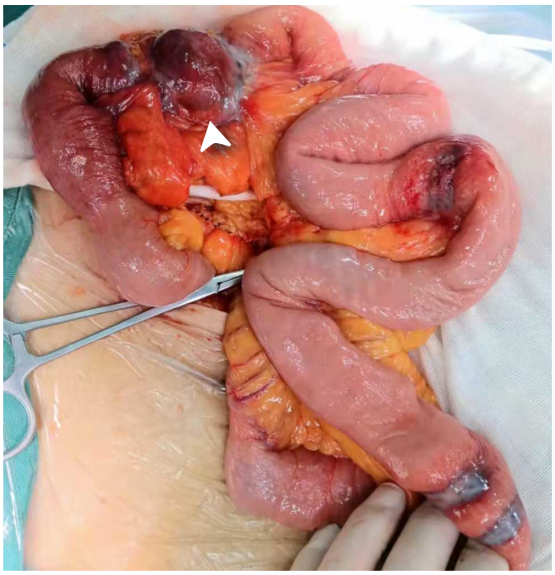
The prognosis for small intestinal angiosarcoma is poor, and the one-year survival rate was only 20.8% among the cases reviewed in the present study. Old age, infiltration depth, and involvement of two or all segments of the small intestine are risk factors for poor prognosis. Multivariate Cox regression analysis showed that surgery and chemotherapy can significantly improve the prognosis of patients with small intestinal angiosarcoma. In addition to surgical resection and chemotherapy, nutritional support, medication or endoscopic hemostasis, blood transfusion, and other treatments are also important. Local radiotherapy is also an alternative treatment.

With the development and clinical application of molecular targeted drugs, molecular targeted therapy for tumors has become a research hotspot in medical oncology. Studies have shown that vascular endothelial growth factor (VEGF) and its receptor (VEGFR) are highly expressed in angiosarcoma. VEGF and VEGFR inhibitors or multi-tyrosine kinase inhibitors, including bevacizumab and pazopanib, are potential drug targets for angiosarcoma. Malignant vascular tumors, including




**DOI:** 10.3748/wjg.v29.i3.561 

**Figure 3 Electronic double-balloon enteroscopy.** A-C: Electronic double-balloon enteroscopy showed continuous periannulus ulcers 2.4-2.5 m above the ileocecal valve, covered with mucous moss.



**DOI:** 10.3748/wjg.v29.i3.561 

**Figure 4 During the operation, multiple grey-red ulcerative tumors were observed in the ileum mucosa covered with moss.** In addition, an 8 cm × 6 cm ulcerative mass (white arrow) resulted in intestinal obstruction.

angiosarcoma, express high levels of adrenergic receptors. Targeting these receptors with drugs such as protamine inhibited tumor growth in mouse vascular cell lines[80]. In addition, a few cases with cutaneous angiosarcoma showed significant responses to checkpoint inhibitors, including pembrolizumab, anti-PD-L1 antibody, and anti-CTLA-4 antibody[81]. However, existing immunotherapy

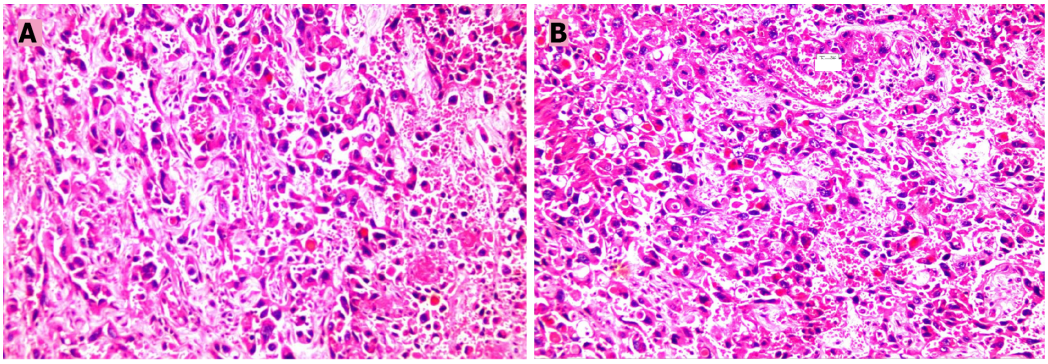



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**Figure 5 Pathologic findings.** A and B: Microscopically, spindle cell infiltration was observed with round or spindle-shaped nuclei. In some areas, tumor cells formed vascular channels with red blood cells in the middle (× 100).

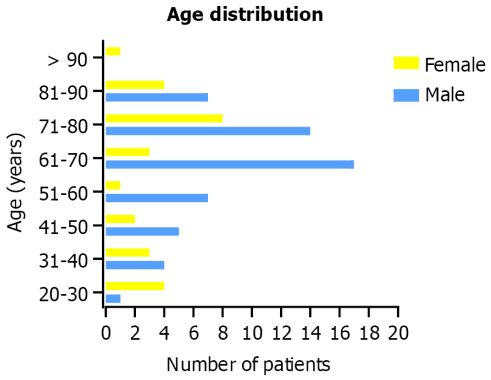


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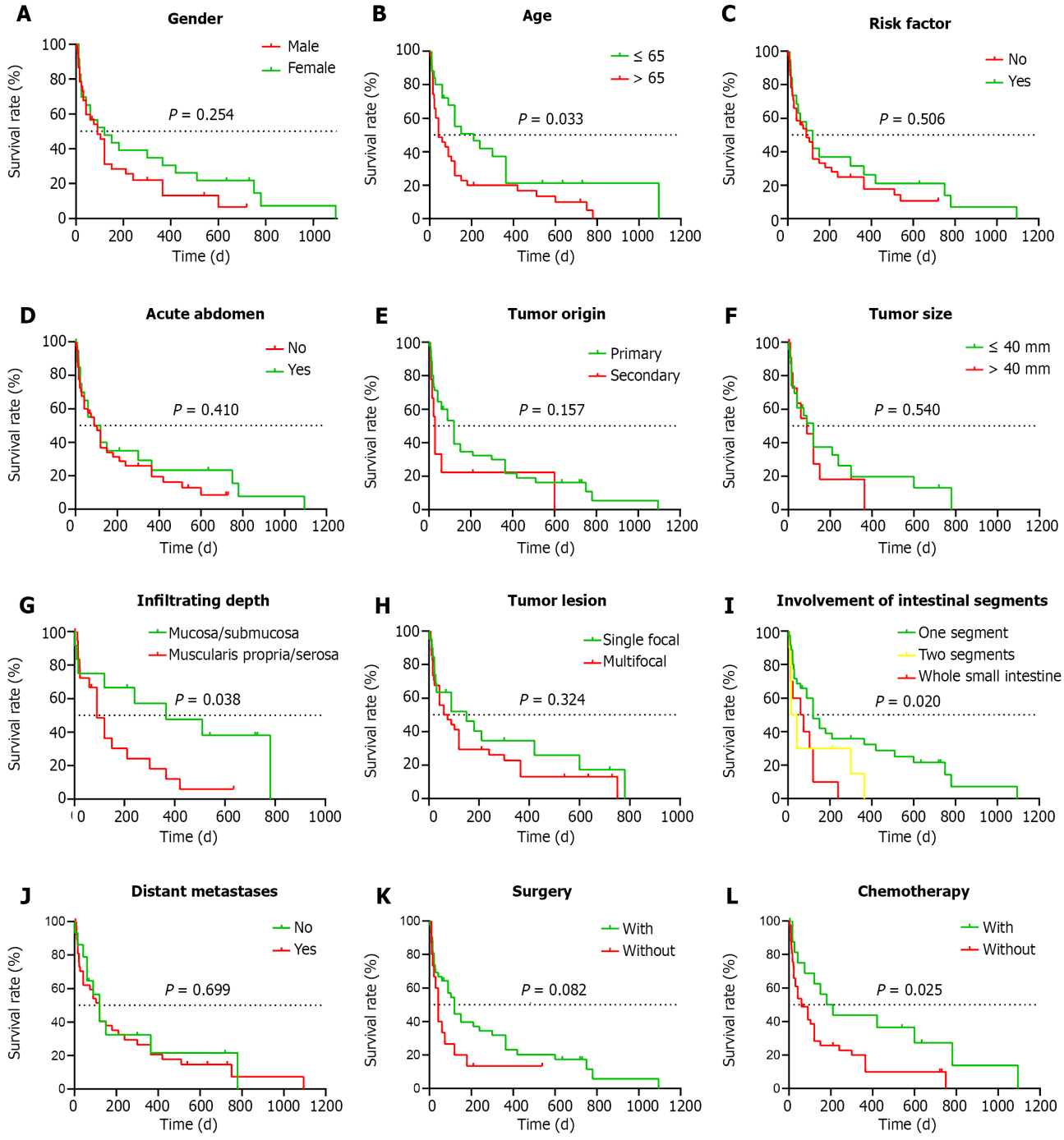
**Figure 6 Age distribution of male and female patients.**

clinical trials mostly focused on cutaneous angiosarcoma, and relevant research on small intestinal angiosarcoma is lacking. Bevacizumab was administered to only one patient with angiosarcoma of the small intestine. However, due to the rapid progression of the patient's disease and failure to take drugs regularly, it was impossible to objectively evaluate its effect[17].

## CONCLUSION

This study reported a case of multiple small intestinal angiosarcomas that resulted in intestinal obstruction with lumbar and bilateral adrenal metastases. Furthermore, we summarized the clinical features, diagnosis, treatment, and prognosis of 82 reported cases of small intestinal angiosarcoma. We found that small intestinal angiosarcoma occurred mainly in older men, and the most common symptom was gastrointestinal bleeding, which mainly manifested as melena. The main treatment methods were surgical resection and chemotherapy, which effectively improved patients' survival. This will help clinicians to understand small intestinal angiosarcomas and guide their clinical diagnosis and treatment. However, statistical bias is inevitable because of the small sample size. In addition, few clinical trials are related to chemotherapy and immunotherapy, and treatment methods are limited. Therefore, we expect that statistical analysis of larger samples and drug clinical trials will improve patients' clinical management and prognosis with small intestinal angiosarcoma.




**DOI:** 10.3748/wjg.v29.i3.561 

**Figure 7 Kaplan-Meier survival analysis.** A: Sex; B: Age; C: Risk factor; D: Acute abdomen; E: Tumor origin; F: Tumor size; G: Infiltrating depth; H: Number of tumor lesions; I: Intestinal segments involvement; J: Distant metastases; K: Surgery; L: Chemotherapy.

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**Author contributions:** Ma XM contributed to data analysis and manuscript writing; Yang BS contributed to conceptualization and methodology; Yang Y contributed to data curation and visualization; Wu GZ contributed to data curation, writing-original draft preparation; Li YW, Yu X, and Ma XL contributed to literature search and screening; Wang YP and Hou XD contributed to revised the manuscript and approved the final version; Guo QH

contributed to supervision and final confirmation.

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LETTER TO THE EDITOR

# Discussion on gemcitabine combined with targeted drugs in the treatment of pancreatic cancer

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## Abstract

Pancreatic cancer is a malignant tumor with poor prognosis. The treatment of pancreatic cancer depends on the tumor stage and type, and includes local treatment (surgery, radiotherapy and ablation intervention) and systemic therapy (chemotherapy, targeted therapy and immunotherapy). We read with great interest the review "Effective combinations of anti-cancer and targeted drugs for pancreatic cancer treatment" published on *World J Gastroenterol* and intended to share some of our perspectives in pancreatic cancer treatment. This review presents the therapeutic effects of the combination of gemcitabine and targeted drugs, which gives us a deeper insight into the combination treatments for pancreatic cancer.

**Key Words:** Pancreatic cancer; Chemotherapy; Targeted therapy; Gemcitabine; Drug; Combination



**Core Tip:** In terms of the choice of chemotherapy regimen for pancreatic cancer, multi-drug chemotherapy is often applied in clinical practice. In general, the combination of chemotherapy and targeted therapy have better efficacy, but whether the combination of the two schemes is more effective than chemotherapy alone requires further investigations.

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## TO THE EDITOR

We have read with great interest the review "Effective combinations of anti-cancer and targeted drugs for pancreatic cancer treatment" published on *World J Gastroenterol*[1]. As is known, gemcitabine alone had limited efficacy in the treatment of pancreatic cancer. This review reported that gemcitabine in combination with targeted agents, like the combination of gemcitabine and Chk1 inhibitor, gemcitabine and KRAS antibody/MEK inhibitor, gemcitabine and autophagy inhibitor, had better efficacy. In addition, the combination of targeted drugs also resulted in better clinical outcome, such as ERK and autophagy inhibitors; ERK, Chk1, and autophagy inhibitors; 2-deoxyglucose and MEK inhibitors; replication stress response and autophagy inhibitors; and immune checkpoint and autophagy inhibitors. It is interesting to note that some natural products, such as cucurbitacin B and glaucarubinone, also had better therapeutic effects in pancreatic cancer when combined with other drugs or with other natural products[1]. We agree with the authors that the combination could improve the therapeutic efficacy in patients with pancreatic cancer. Based on this review and our clinical experience we here share some of perspectives about pancreatic cancer treatment.

According to the National Comprehensive Cancer Network guidelines, there are many methods of chemotherapy used for treating pancreatic cancer, including multi-drug chemotherapy. The current standard first-line treatment regimen for metastatic pancreatic cancer includes gemcitabine and albumin-bound paclitaxel or modified FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin)[2, 3]. A study of 861 untreated patients with metastatic pancreatic cancer has reported a better efficacy of gemcitabine and albumin-bound paclitaxel compared with gemcitabine [median survival, 8.5 *vs* 6.7 mo; hazard ratio = 0.72, 95% confidence interval (CI): 0.620-0.83; $P < 0.001$)[4]. In the current clinical practice, gemcitabine is rarely used alone. This review pointed out that gemcitabine was used for chemotherapy combined with various targeted drugs, but did not mention whether gemcitabine and albumin-bound paclitaxel combined with targeted drugs have better effects, which is important in the treatment of this cancer and needs to be identified. Targeted drugs combined with gemcitabine may have variable efficacy for different stages of pancreatic cancer. The review reported that gemcitabine combined with some targeted drugs yielded better clinical outcome, however, in our opinion, the combination is not always as effective as we expect, which may be worth discussion. The phase III LAP07 trial in 2016 investigated the clinical value of erlotinib combined with gemcitabine in patients with locally advanced pancreatic cancer. The median overall survival of the patients treated with gemcitabine alone was 13.6 mo (95%CI: 12.3-15.3 mo), while the patients receiving gemcitabine combined with erlotinib had a median overall survival of 11.9 mo (95%CI: 10.4-13.5 mo). The combination *vs* gemcitabine alone, despite good adherence, failed to improve survival and was associated with increased grade 3 hematologic, digestive, and skin toxicity[5]. CONKO-006 was a randomized double-blinded phase IIb study designed to evaluate the efficacy of the combination of gemcitabine and sorafenib compared with gemcitabine and placebo in patients with pancreatic adenocarcinoma with postsurgical R1 residual status. The results indicated that there were no differences in recurrence-free survival nor overall survival between the two groups[6]. The exact mechanism by which the combination of drugs could be less effective than gemcitabine alone is difficult to explain and may be related to the greater toxicity of combination drugs. An open-label, multicenter, randomized phase II trial evaluated gemcitabine plus afatinib *vs* gemcitabine alone for metastatic pancreatic cancer. Median overall survival was 7.3 mo with gemcitabine plus afatinib *vs* 7.4 mo with gemcitabine alone. Adverse events like diarrhea and rash were more frequent with gemcitabine plus afatinib[7]. In brief, these studies remind us that different combinations of chemotherapeutic drugs and targeted drugs may have different effects for various stages of pancreatic cancer. In conclusion, this review has led us to focus on new options of pancreatic cancer treatment, which is significant in guiding the clinical pancreatic cancer treatment and pointing out the direction for future research.

## FOOTNOTES

**Author contributions:** Huang JH and Guo W wrote the manuscript; Liu Z edited the manuscript; and all authors have read and approved the final version.

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