ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2021 December 14; 27(46): 7866-8039

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

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Weekly Volume 27 Number 46 December 14, 2021

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The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2021 edition of Journal Citation Report® cites the 2020 impact factor (IF) for *WJG* as 5.742; Journal Citation Indicator: 0.79; IF without journal self cites: 5.590; 5-year IF: 5.044; Ranking: 28 among 92 journals in gastroenterology and hepatology; and Quartile category: Q2. The *WJG*'s CiteScore for 2020 is 6.9 and Scopus CiteScore rank 2020: Gastroenterology is 19/136.

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NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Andrzej S Tarnawski, Subrata Ghosh

EDITORIAL BOARD MEMBERS
http://www.wjgnet.com/1007-9327/editorialboard.htm

PUBLICATION DATE
December 14, 2021

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World J Gastroenterol 2021 December 14; 27(46): 7866-7893

DOI: 10.3748/wjg.v27.i46.7866

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

FRONTIER

Imaging of the chemotherapy-induced hepatic damage: Yellow liver, blue liver, and pseudocirrhosis

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Author contributions: Calistri L, Rastrelli V and Colagrande S contributed equally to this work, in terms of conceptualization, writing the original draft, reviewing and editing; Maraghelli D, Vidali S, Pietragalla M contributed in figures and references research; and all the authors have read and approved the final manuscript.

Conflict-of-interest statement: There are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

Country/Territory of origin: Italy

Specialty type: Radiology, Nuclear Medicine and Medical Imaging

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

Peer-review model: Single blind

Peer-review report's scientific

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Abstract

The liver is the major drug-metabolizing and drug-detoxifying organ. Many drugs can cause liver damage through various mechanisms; however, the liver response to injury includes a relatively narrow spectrum of alterations that, regardless of the cause, are represented by phlogosis, oxidative stress and necrosis. The combination of these alterations mainly results in three radiological findings: vascular alterations, structural changes and metabolic function reduction. Chemotherapy has changed in recent decades in terms of the drugs, protocols and duration, allowing patients a longer life expectancy. As a consequence, we are currently observing an increase in chemotherapy-associated liver injury patterns once considered unusual. Recognizing this form of damage in an early stage is crucial for reconsidering the therapy regimen and thus avoiding severe complications. In this frontier article, we analyze the role of imaging in detecting some of these pathological patterns, such as pseudocirrhosis, "yellow liver" due to chemotherapy-associated steatosis-steatohepatitis, and "blue liver", including sinusoidal obstruction syndrome, veno-occlusive disease and peliosis.

Key Words: Hepatic damage; Yellow liver; Chemotherapy-associated steatohepatitis; Blue liver; Sinusoidal obstruction syndrome; Veno-occlusive disease; Peliosis; Pseudocirrhosis

Core Tip: Chemotherapy-induced hepatic damage represents an increasingly frequent condition observed in oncology patients: recent pharmacological innovations and specific and longer therapies have led to longer life expectancy and, inevitably, to an

quality classification
Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: April 19, 2021
Peer-review started: April 19, 2021
First decision: June 3, 2021
Revised: June 15, 2021
Accepted: November 29, 2021
Article in press: November 29, 2021
Published online: December 14, 2021

P-Reviewer: Chen LW, Pan W
S-Editor: Wang JL
L-Editor: A
P-Editor: Wang JL

increase in systemic side effects and organ damage, primarily in the liver because of its detoxifying function. Even for experienced radiologists, the assessment of radiological patterns associated with liver injury derived from chemotherapy can sometimes be challenging. Our aim is to summarize useful ways to recognize, understand and monitor the evolution of these forms of hepatic damage to support clinicians in decision making.

Citation: Calistri L, Rastrelli V, Nardi C, Maraghelli D, Vidali S, Pietragalla M, Colagrande S. Imaging of the chemotherapy-induced hepatic damage: Yellow liver, blue liver, and pseudocirrhosis. *World J Gastroenterol* 2021; 27(46): 7866-7893
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7866.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7866

INTRODUCTION

The liver plays key roles in the metabolism and detoxification of many commonly used drugs, predisposing hepatocytes to xenobiotic- and toxin-induced injury[1]. Chemotherapy has recently evolved, from the use of cytotoxic drugs to new biological drugs acting on specific molecules critical for cell growth, differentiation, and nutrient supply. The advent of these new treatments, as well as the frequent use of multidrug regimens and the longer duration of systemic therapies due to longer survival, have increased the potential for liver parenchymal damage, collectively referred to as chemotherapy-associated liver injury (CALI)[2].

The first case of CALI was reported in the early 1950s in reference to clinical and laboratory signs of hepatic fibrosis presented by 5 children with acute leukemia during folic acid antagonist treatment[3]. Recently, efforts to identify imaging features and standardize the management of CALI have been made[4,5]. However, with the advent of newer molecular targeted oncological therapies and, more recently, immune checkpoint inhibitors, the evaluation and treatment of liver toxicity associated with these drugs are still evolving[6-9].

Regardless of the cause of injury, CALI can manifest as nonspecific symptoms and signs of abdominal discomfort, evidence of hepatomegaly, and/or elevated liver function tests, often representing a diagnostic dilemma for the oncologist, as the same symptoms and signs of liver injury may also be unrelated to chemotherapy[4,10].

Moreover, many chemotherapy-induced hepatic parenchymal effects can impair the detection of liver metastases. As patients with metastatic cancer increasingly undergo chemotherapy with curative intent, it is mandatory that radiologists understand the pathophysiology of these therapy-induced liver changes and become familiar with their imaging features[11]. Finally, the early recognition of certain adverse reactions is essential for cancer patients to prevent dangerous complications such as acute hepatitis, liver cirrhosis, and even liver failure. Often, patients can be managed with supportive therapies, and the liver toxicity may resolve after discontinuation of chemotherapy.

After a summary of the main forms of liver damage, including drug-induced liver injury (DILI), we analyze the role of imaging in detecting certain pathological patterns of CALI that may appear during oncologic follow-up, such as so-called "yellow liver", "blue liver" and "pseudocirrhosis".

HEPATIC DAMAGE

Various forms of hepatocyte injury are known: infectious (viral hepatitis), autoimmune hepatitis, toxicity/drug-induced injury, metabolic injury (nonalcoholic fatty liver disease), and intracellular depositions (hemochromatosis, alpha-1-antitrypsin, Wilson disease, and metabolic diseases such as glycogen storage disorders)[12]. Other types of damage involve biliary stasis-induced injury ("long standing obstruction of the bile duct"), injury to the hepatic artery that affects circulation, and damage from physical/chemical agents[13-16]. Vascular alterations, inflammation and oxidative stress represent the pathogenesis of various forms of liver damage: in liver ischemia–reperfusion injury (during the liver transplantation process), the damage

involves all parenchymal cells (hepatocytes, endothelial cells and cholangiocytes)[17]. The lack of substrates and oxygen during the ischemic phase of injury results in the mitochondrial production of reactive oxygen species (ROS); in the reperfusion phase, the availability of oxygen further accentuates the oxidative stress, increasing damage to the donor liver; moreover, there is a concomitant release of inflammatory cytokines and an influx of inflammatory cells that amplify tissue injury[18]. Considering a more general definition of damage as a reversible or irreversible modification of cellular and/or tissue function in response to a stressful stimulus, the liver response to injury includes a relatively narrow spectrum of morphologic changes, and accordingly, there are only a few pathologic patterns that can be recognized microscopically[19].

Therefore, dividing liver injury patterns by the cell type being destroyed, regardless of the cause, we can categorize injuries into cell-indiscriminate (most frequently in response to mechanical injury, ischemia, and liver resection), cholestatic (typically in response to mechanical and presumed autoimmune biliary injury), and hepatocyte-associated injuries[20]. Hepatocyte-related injury includes cell death (apoptosis, necrosis, necroptosis, and autophagy) and degenerative and/or intracellular accumulation (*i.e.*, ballooning degeneration, steatosis and iron or copper accumulation)[20,21].

Clinical data and animal models suggest that hepatocyte death is the key trigger of liver disease progression, manifested by the subsequent development of inflammation due to an influx of acute or chronic inflammatory cells involving the lobular parenchyma (diffuse inflammation), foci inside lobules or limited to the portal tracts (focal inflammation). If the damage is severe enough, and if the blood flow is adequate, then hepatic regeneration can restore a functional liver mass. If the damage is chronic, liver fibrosis/cirrhosis may develop[19,22].

LIVER BLOOD FLOW

Knowing the peculiarities of hepatic vascularity helps with understanding the imaging features of liver damage. Hepatic feeding is guaranteed for a 70%-75% by portal flow, with a low oxygen content and high metabolite content, and for a 20%-25% by arterial flow with a high oxygen content and a low metabolite content. The two systems are interconnected through transvasal, transsinusoidal and peribiliary communications that allow the arterial supply to compensate for any small reduction in portal inflow, according to a mechanism regulated by humoral mediators (adenosine, histamine, vasopressin, and prostacyclin) and by the autonomic nervous system, activated by hepatocyte demand for oxygen and metabolites[13,23]. This condition appears on computed tomography (CT)/magnetic resonance (MR) images as hyperdensity/hyperintensity of the involved parenchyma during the arterial phase, also called transient hepatic parenchymal enhancement (THPE)[24]. Depending on the level of the obstacle and the predominance of the shunt involved, different THPE, either localized or diffuse, can be seen on images. There are three diffuse types: (1) If the obstacle diffusely compromises the intralobular vein or the structures downstream (*e.g.*, in Budd-Chiari or right-sided heart failure), the prevailing plexus is the trans-sinusoidal plexus, and the resulting THPE is of the "mosaic" type (Figure 1A); (2) If the obstacle is at the level of the portal axis or upstream from the intralobular vein (as happens in portal thrombosis or cirrhosis, respectively), the prevailing shunt is peribiliary, and the resulting THPE is of the "central-peripheral" type (Figure 1B); and (3) In contrast, if the peribiliary plexus is blocked, as occurs in bile duct dilatation or sometimes in cholangitis, arterialization is "peribiliary"[24] (Figure 1C).

In addition to the major vascular systems, a third type of vasculature contributes no more than 2%–3% of hepatic blood flow, establishing communication between the systemic venous system and the portal system, and it includes capsular veins, Sappey's paraumbilical veins, epiploic and hilar veins, suspensory ligament and diaphragmatic veins, and accessory cystic veins[24]. These components may act according to the pressure gradient through an anomalous blood supply or drainage from vessels to certain areas of the parenchyma, mainly located in segments I–IV. Normally, the third inflow is "afferent" to the liver, but its flow direction can be reversed. Therefore, during portal hypertension or under other stress conditions, the intraportal pressure becomes higher than that of the systemic veins, and the "third" hepatic system becomes efferent, allowing a preferential outflow that can cause a localized reduction in portal inflow, sometimes resulting in a compensatory "arterial buffer response" and correlated sequelae[24]. The same diversion of the third inflow explains both the development of shunting systems in cirrhotic liver and the appearance of pseudonodular lesions in noncirrhotic liver, especially after che-

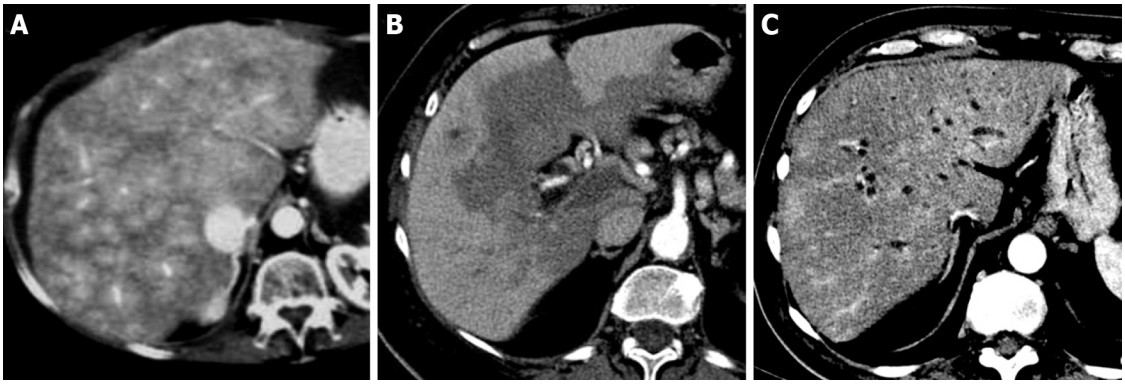

Figure 1 Transient hepatic parenchymal enhancement. A: Contrast-enhanced computed tomography axial scan in the arterial phase shows a "mosaic" pattern of enhancement in patients with Budd-Chiari syndrome; B: "Central-peripheral" pattern in patients with portal thrombosis; C: "Peribiliary" pattern in patients with cholangitis.

motherapy, which can damage minor portal vessels and facilitate blood inflow through the third inflow system[24].

In general, if the cause of arterial rebound persists, hepatocyte can be injured as in normal conditions they need low oxygen tension and high nutrient levels typical of those supplied by portal inflow. Therefore, persistent hemodynamic changes can determine focal metabolic alterations that result in focal sparing in fatty liver or nodular fat accumulation in normal liver, which are typically found in the subdiaphragmatic aspect of the right lobe, the posterior aspect of the left lobe, the periportal aspect of segment IV, around the falciform ligament and around the gallbladder bed [25,26] (Figure 2). Finally, if the obstacle remains, then the insufficient blood supply to much of the liver leads to metabolic infarction, fibrosis and atrophy of the liver (especially in segments V, VI and VII, where the third inflow is lacking or poorly represented) (Figure 3), along with compensatory nodular regenerative hyperplasia (NRH) and large regenerative nodules in areas of hepatic parenchyma that maintain an adequate portal and arterial blood supply (especially the left lobe and segment VIII, with higher third inflow)[24,27-29].

DILI

DILI is a current hot topic, as seen by the increasing number of publications in recent years[30-32]. It is a challenging clinical problem with respect to both diagnosis and management, with an estimated incidence of 14 to 19 cases per 100000 persons[30]. Iproniazid, cinchophen, and sulfonamides were the first prototypical hepatotoxins to be identified[31,33]. By the mid-1980s, close to 1000 drugs were linked to hepatic injury[34]. Clinically, DILI ranges from asymptomatic hypertrasaminasemia and hepatitis to acute or fulminant hepatic failure[35]. Although severe DILI is rare, drugs have become the overall leading cause of acute liver failure in the United States and other Western countries[31]: acetaminophen (paracetamol) is the responsible drug in 40%-50% of these cases, with another 11%-12% of cases caused by herbal compounds and dietary supplements, equaling the frequency of cases due to acute viral hepatitis [36,37].

On the basis of liver function tests, DILI may be defined as predominantly hepatic, distinguished by disproportionate elevations in serum aminotransferases compared with the level of alkaline phosphatase, or cholestasis, distinguished by inverted, disproportionate and mixed patterns[36].

Considering the histopathology, DILI is particularly complex. The United States DILI Network recognizes 18 distinct categories of DILI: acute and chronic hepatitis, acute and chronic cholestasis, cholestasis-hepatitis, granulomatous, macro- and microvesicular steatosis, steatohepatitis, zonal and nonzonal necrosis, vascular injury, hepatocellular alterations, nodular regenerative hyperplasia, mixed or unclassified injury, minimal nonspecific changes, absolutely normal, and massive necrosis[38].

Currently, DILI is classified as either idiosyncratic (injury unpredictable, not dose-dependent, and caused by agents that have little or no intrinsic liver toxicity) or direct (injury predictable, dose-dependent, and caused by agents that are intrinsically toxic to the liver), but indirect injury is now accepted as a third type (caused by the action of

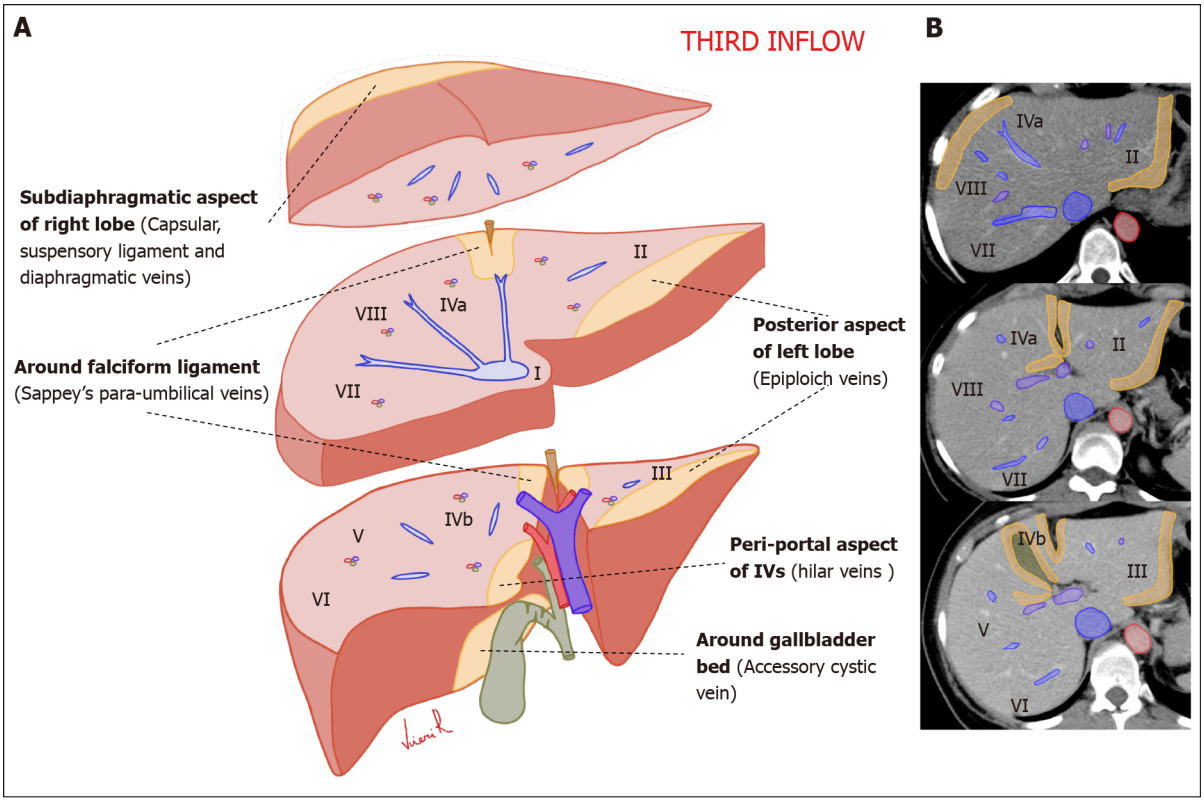

Figure 2 Schematic representation of the anatomical sites of the liver "Third inflow" in hepatic sections. Yellow areas show the typical sites of focal sparing in fatty liver or nodular fat accumulation in the normal liver. A: Volumetric representation; B: Computed tomography axial scan.

the drug not by its toxic or idiosyncratic properties, such as the induction of immune-mediated hepatitis or the worsening of pre-existing hepatitis or fatty liver disease)[30].

The most common forms of DILI involve idiosyncratic hepatotoxicity, including acute and chronic hepatitis (most often associated with isoniazid, nitrofurantoin, and diclofenac and with methyldopa, minocycline, and statins, respectively), acute and chronic cholestasis (correlated with estrogens, androgenic steroids and flurixidine), and mixed hepatitis-cholestasis (due to amoxicillin-clavulanate and fluoroquinolones) patterns[30,32,39].

Many antineoplastic agents can cause acute hepatic necrosis due to direct hepatoxicity, as well as sinusoidal obstructive syndrome (SOS) (myeloablative agents, alkylating agents and monoclonal antibody-cytotoxic conjugates such as gemtuzumab and ozogamicin) or NRH (azathioprine, mercaptopurine and thioguanine)[30,32,39].

Finally, an increasing form of indirect injury is immune-mediated liver injury due to various immunomodulatory agents, tumor necrosis factor antagonists, and, most important, antineoplastic checkpoint inhibitors[40-42]. There are several reports of the reactivation of both hepatitis B and hepatitis C in patients treated with agents such as rituximab, cyclophosphamide, doxorubicin, vincristine, or prednisolone for lymphoma [2,43].

CALI

Among the various forms of DILI, CALI is often reported in the literature, mainly in association with patients with colorectal liver metastases[44-46]. CALI appears to be regimen-specific, generally including two main types of liver injury, vascular changes and fatty changes, which are primarily associated with the development of ROS that lead to cellular damage and activate apoptosis pathways[5]. The prevalence of CALI increases with the duration of chemotherapy, and currently, no convincing data on the reversibility of CALI are available[46].

Various studies support the important clinical impact of CALI. Karoui *et al*[47] demonstrated that CALIs increased the risk of postoperative liver failure by 11%, with others such as Vauthey *et al*[48] demonstrating increased 90-d postoperative mortality in patients with steatohepatitis (14.7% *vs* 1.6%). Nevertheless, it remains unclear

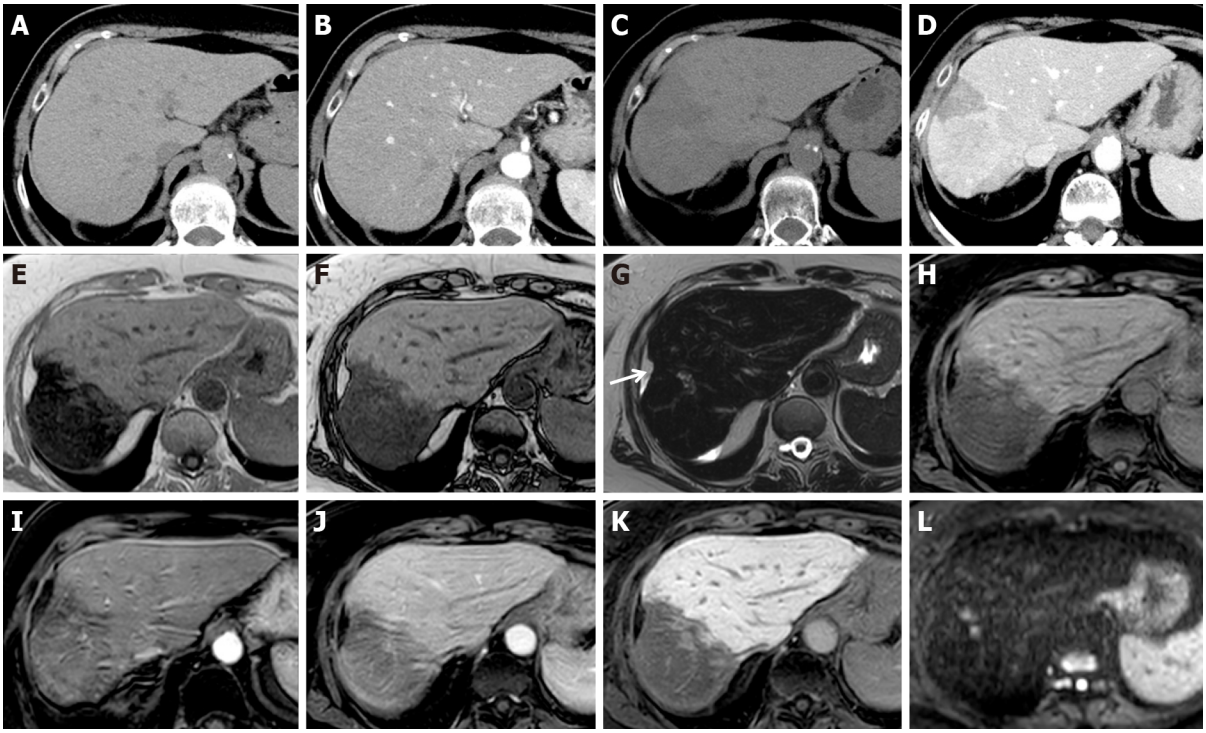

Figure 3 Liver metabolic infarction areas in patients with breast cancer. A, B: Unenhanced (A) and arterial phase (B) computed tomography (CT) axial scan before therapy show normal liver; C, D: Unenhanced (C) and late arterial phase (D) CT axial scans after 3 mo of therapy show early steatotic changes of the parenchyma and inhomogeneous enhancement in segments VII-VIII; E-L: Magnetic resonance after 6 mo of therapy shows progression of parenchymal involution and atrophy in gradient echo (GE) T1w in-phase (E), GE T1w out-of-phase (F), fat sat GE 3D T1w unenhanced (H), arterial (I), portal (J) and hepatobiliary phase (K). Capsular retraction is seen (white arrow). On T2w (G), and high b-value diffusion-weighted (L) images, no signal alteration was detectable.

whether CALI influences survival. Although Tamandl *et al*[49] reported lower survival in patients with SOS, other studies demonstrated that SOS is associated with a lesser degree of regression of liver metastases, and this regression, not SOS, impacts prognosis[50,51]. Controversial data have also been reported for steatosis[50,52]. Moreover, postoperative morbidity and mortality due to liver failure are often related to inadequate function of the residual liver[53]. Therefore, the improved detection of CALI during the preoperative assessment of the future liver remnant is an important clinical issue.

More frequently, oxaliplatin treatment is associated with SOS, which occurs in 19%–52% of patients and is linked to an increased occurrence of NRH[54]. Irinotecan-based treatments are related to the appearance of steatohepatitis, with a rate of 20.2%, and its effects are exacerbated by baseline obesity and/or metabolic syndrome[51,55]. Furthermore, the development of steatosis was observed in 30 to 47% of patients who received 5-fluorouracil therapy, which remains a cornerstone of modern chemotherapy[10,56].

More unusual forms of CALI include pseudocirrhosis, which is mostly observed in patients undergoing chemotherapy for breast cancer, and chemotherapy-induced sclerosing cholangitis (CISC)[57]. CISC is a form of secondary sclerosing cholangitis, occasionally resulting from ischemic injury to the bile ducts associated with hepatic artery infusion with fluoropyrimidines (incidence of 8%-55%). Since biliary endothelial cells, in contrast to hepatocytes, derive their vascular supply almost exclusively from the branches of hepatic arteries[58], arterial occlusion may cause bile duct ischemia and fibrosis without parenchymal infarction. The main finding was segmental or diffuse narrowing of the cystic, biliary-shared, left and right hepatic ducts, with sparing of the common intrapancreatic bile duct, which is usually supplied by branches of the gastroduodenal artery[58]. Reports of CISC triggered by systemic chemotherapy (taxanes, bevacizumab, paclitaxel, or cisplatin) are even more rare[59,60]. Although CISC should be considered rare, it is clinically important, requiring frequent endoscopic intervention to maintain biliary drainage[58].

Finally, over the past two decades, molecular targeted agents, including small-molecule protein kinase inhibitors, monoclonal antibodies, and immune checkpoint inhibitors, have become promising for use in the treatment of various malignant neoplasms (especially malignant melanoma, non-small cell lung cancer and renal cell

carcinoma)[61]. Protein kinase inhibitors are reported to induce a low-grade elevation in serum transaminases in approximately 30% of patients and high-grade elevation in 2% of patients[62]. Liver injury due to immune checkpoint inhibitors most often presents with a hepatocellular biochemical pattern, occurring in 2%-30% of patients, with increasing risk when multiple immune checkpoint inhibitors are administered and in patients who develop other immune-related adverse events, although severe cases remain very rare[63].

Overall, radiologists should know that chemotherapy frequently modifies not only the radiological appearances of liver tumors but also the imaging features of the non-tumor-bearing liver. Excluding CISC, due to its rarity, and acute hepatitis, due to its nonspecific imaging features (hepatosplenomegaly, collapsed gallbladder with wall thickening, decreased liver enhancement, ascites and widening of the periportal space due to edema)[2], we analyze the role of imaging in the identification of more typical features of CALI, namely, yellow liver, blue liver and pseudocirrhosis.

YELLOW LIVER

The term "yellow liver" refers to a macroscopic feature of the liver that can be observed upon histopathologic examination and is determined by a general increase in the parenchymal lipidic content, compared to physiological normal texture[5]. Different pathological conditions fall under the generic definition of yellow liver. Hepatic steatosis is identified by pathological deposition of lipid vesicles in hepatocytes, usually associated with metabolic syndrome, obesity, diabetes, insulin resistance or alcohol[64]. Hepatic steatosis must be differentiated by steatohepatitis, a more serious histologic complication where fat deposition is associated with an inflammatory response, consisting of ballooning of hepatocytes, lobular inflammation, hepatocyte degeneration and thus fibrosis of different grades, including liver cirrhosis [48]. CASH is a form of steatohepatitis that can sometimes develop as a consequence of therapies with chemotherapeutic agents (CTAs) that produce side effects critical for hepatocyte[65,66].

Among the forms of CALI, other than the aforementioned association with 5-fluorouracil-based treatment[57], steatosis is seen in 14.6% and 41.1% of patients with estrogen-receptor-positive breast cancer treated with tamoxifen and anastrozole, respectively[10,67]. Additionally, cases of steatosis in patients receiving pazopanib and bevacizumab, alone and in combination with paclitaxel, have been reported[6]. Similarly, an increased incidence of CASH in recent decades has been reported[56,68,69]. Although the true frequency of these pathologies is not easily determined[65,70], as mentioned above, the prevalence of CASH is increasing in patients undergoing treatment with 5-fluorouracil and irinotecan[71], especially when the drugs are coadministered[48]. Additionally, platinum derivatives (oxaliplatin), taxanes and methotrexate have been linked to this condition, and with a lower frequency, L-asparaginase, dactinomycin, mitomycin C and bleomycin sulfate[72,73].

Interestingly, steatosis and CASH occur not only during treatment for liver metastases but also in the course of systemic chemotherapy for nonmetastatic cancer [71]. Considering the pathogenesis of steatosis, as discussed above (see "Liver blood flow"), persistent hemodynamic changes determining focal metabolic alterations and the third hepatic inflow system are involved. The pathogenesis of CASH remains under discussion, and different mechanisms have been proposed. First, CTAs can be responsible for decreasing fatty acid oxidation, thus generating oxidative stress with hepatocyte dysfunction[74]. According to You *et al*[11], CTAs have been reported to produce abundant ROS, damaging not only cancer cells but also normal cells. This damage promotes both the deposition of lipid vesicles into hepatocytes and inflammation[65,75]. Robinson *et al*[57] described a "two hits" model, in which patients with underlying hepatic steatosis, undergo a "second hit" to the parenchyma, represented by chemotherapy-induced oxidative stress or mitochondrial dysfunction, creating the conditions of inflammation and giving rise to CASH. Finally, minor portal vessels can be increasingly susceptible to the direct damage caused by CTAs, facilitating the reversion of the "third inflow" system and triggering consequent arterial compensation. Therefore, the resultant inadequate perfusion, together with direct damage to hepatocytes, can contribute to the metabolic dysfunction critical for CASH development[24].

Fatty infiltration leading to yellow liver requires a relatively short development time (generally only a few weeks after the beginning of chemotherapy)[57]. Hepatic steatosis and steatohepatitis are typically asymptomatic even when liver function tests

are abnormal, making an early diagnosis a difficult goal[4]. When chemotherapy is withdrawn, there is often a regression of steatosis, suggesting that most of the changes caused by chemotherapy are at least partially reversible[6,57]. In other cases, especially when the diagnosis is delayed or under-evaluated, the parenchymal inflammatory response can lead to more serious and irreversible changes, including fibrosis and atrophy. Finally, the regenerative phenomenon of liver parenchyma, such as in NRH, is a possible compensatory response to injury, as long as adequate perfusion is maintained[28,56,76]. As expected, when steatosis is present prior to the therapy, liver is thought to be more susceptible to CALI due to its impaired regenerative capability and abnormal innate immunity[10]. Diffuse forms of hepatic steatosis and steatohepatitis can be obstacles to surgical planning, *e.g.*, not allowing large liver resection due to presence of metastases[69]. Patients with steatosis who undergo major hepatectomy have increased blood loss, more postoperative complications, and a longer stay in the intensive care unit than patients with healthy livers[19]. Finally, patients' postoperative morbidity and mortality can be increased since both steatosis and steatohepatitis impair liver function[48].

Imaging

Clinicians increasingly demand the quantification of liver fat to grade the level of hepatic damage, not only in living donors for liver transplantation and for patients who must undergo liver resections or bariatric surgery but also in patients receiving potentially hepatotoxic therapies. The ability of MR-based methods to detect and quantify steatosis has been investigated in the past 30 years, and substantial correlations between pathologically and radiologically determined fat fractions have been demonstrated[77-79].

However, most often, in daily practice, steatosis and steatohepatitis are evaluated qualitatively. From a radiological point of view, it is impossible to differentiate between these two forms of liver disease, as they can be distinguished only by histologic alterations[71]. The distribution of CASH, as well as hepatic steatosis, can vary from diffuse to focal infiltration. Ultrasound allows a subjective estimation of the degree of diffuse fatty infiltration using some features that include liver brightness and contrast between the liver and the kidney[80]. On an unenhanced CT scan, diffuse steatosis can be diagnosed when attenuation of the liver is at least 10 Hounsfield units less than that of the spleen, the hepatic-to-splenic attenuation ratio is less than 1, or the liver attenuation is less than 40 Hounsfield units (Figure 4A). In more severe cases, intrahepatic vessels may appear hyperdense relative to fat-containing liver tissue[81]. With MRI, chemical shift gradient-echo imaging with in-phase and out-of-phase acquisitions is the most widely used technique for the assessment of fatty liver (Figure 4E and F). These scans show signal intensity loss on out-of-phase images in comparison with in-phase images, whereas the application of chemical fat saturation sequences is less sensitive[82,83].

While diffuse forms of steatosis are not difficult to recognize, focal fat deposition or fatty sparing can sometimes mimic a hepatic mass or single and multimetastatic disease[84]. However, MRI often serves as a problem-solving tool because signal loss on out-of-phase T1-weighted images cannot be seen in metastasis[85]. Additionally, focal fatty deposition or sparing can be recognized by the characteristic location (in the anatomic sites of the third inflow system), geographic pattern rather than round or oval shapes, absence of a mass effect on the vasculature, poorly delineated margins, and contrast enhancement that is similar to or less than that of the normal liver parenchyma[9,86] (Figure 5). These features usually allow for the differentiation of focal or multifocal fat accumulations from hepatocellular carcinoma, hepatic adenoma, focal nodular hyperplasia (FNH), FNH-like nodules and hypervascular metastases that show mass effects, marked or heterogeneous enhancement, and sometimes necrotic and hemorrhagic areas; however, hepatocellular carcinomas, hepatic adenomas and, more rarely, FNH, may involve microscopic fat content[87]. Clinical manifestations and diffusion-weighted images can help in the more complex differentiation of ischemic or mucinous metastases, abscesses, lymphoma and hypovascular metastases[87-89] (Figure 4K). The differential diagnosis between large areas of focal steatosis with infiltrative hepatocellular carcinoma may be more difficult; irregular liver contours, mild mosaic pattern enhancement and the presence of portal vein thrombosis are suggestive of the latter[87]. Shape, location and MR chemical shift imaging allow us to distinguish periportal fat deposition from other periportal abnormalities (edema, inflammation, hemorrhage, and lymphatic dilatation) and focal sparing in the liver with diffuse steatosis mimicking hypervascular lesions (such as hemangiomas or arterioportal shunts)[87]. Interestingly, when steatosis develops during chemotherapy, in the presence of hepatic metastases, the parenchyma

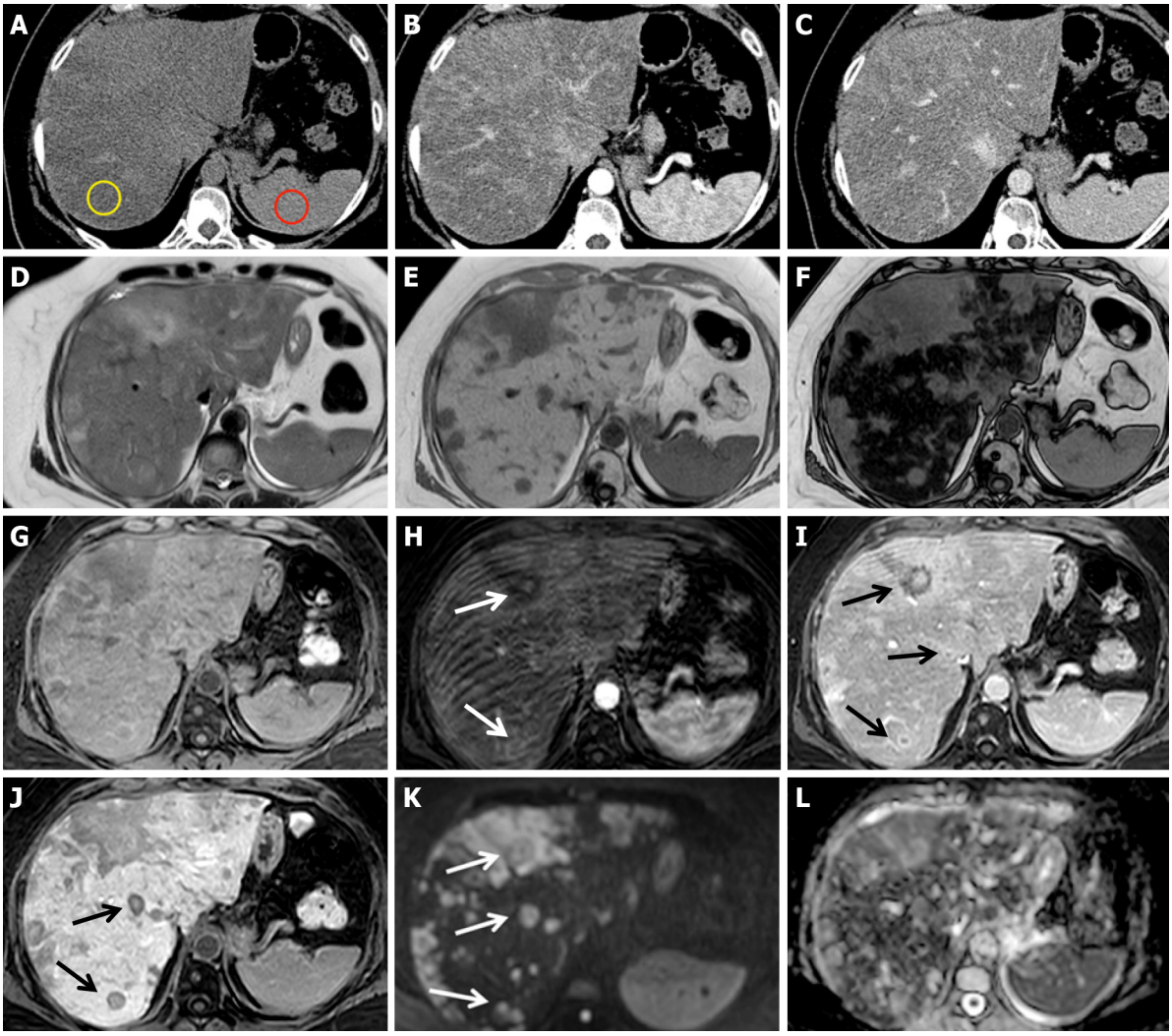

Figure 4 Chemotherapy-associated diffuse steatosis in patients with liver metastatic colorectal cancer 6 mo after the beginning of chemotherapy with 5-fluorouracil + folinic acid + irinotecan. A-C: Unenhanced computed tomography axial scan (A), arterial (B) and portal phase (C) show severe inhomogeneous hepatic steatosis. Liver attenuation in steatotic areas is 8 HU (yellow ROI), less than that of the spleen (43 HU, red ROI), and the hepatic/splenic attenuation ratio is << 1. No nodules are visible; D-L: On magnetic resonance performed during the following week, gradient echo (GE) T1w in-phase (E) and out-of-phase (F) images confirm severe hepatic steatosis. On unenhanced fat sat GE 3D T1w images (G), arterial (H), portal (I), hepatobiliary (J) phases and high b-value diffusion-weighted images (K), multiple metastases are evident, some of which are characterized by rim enhancement (arrows). The T2-weighted image (D) and apparent diffusion coefficient map (L) are also shown.

surrounding the lesion can be spared from steatosis[90]. Once again, this outcome may be attributed to a modification of parenchymal perfusion, and in particular, a reduction of portal inflow in the peritumoral area caused by direct compression of the adjacent parenchyma, the presence of tumor emboli in the portal vein branches[90] and/or the neoangiogenesis that accompanies tumoral growth, increasing arterial perfusion[91,92].

Morphological changes such as increased craniocaudal liver diameter and an increased caudate-to-right lobe ratio are more characteristic of steatohepatitis[93]. Finally, when CASH is diagnosed in advanced stages and, in particular, when it progresses toward cirrhosis, typical morphological changes of the latter can be observed[94].

BLUE LIVER

Blue liver refers to parenchymal venous congestion resulting from blockage of blood outflow, macroscopically characterized by an intraoperative subcapsular livid appearance and a similar "marble" bluish-red discoloration on the cut surface[95]. Budd-Chiari syndrome is a typical postsinusoidal form of blue liver. In this case, the physical obstacle to hepatic outflow, represented by stenosis or a thrombus, is located

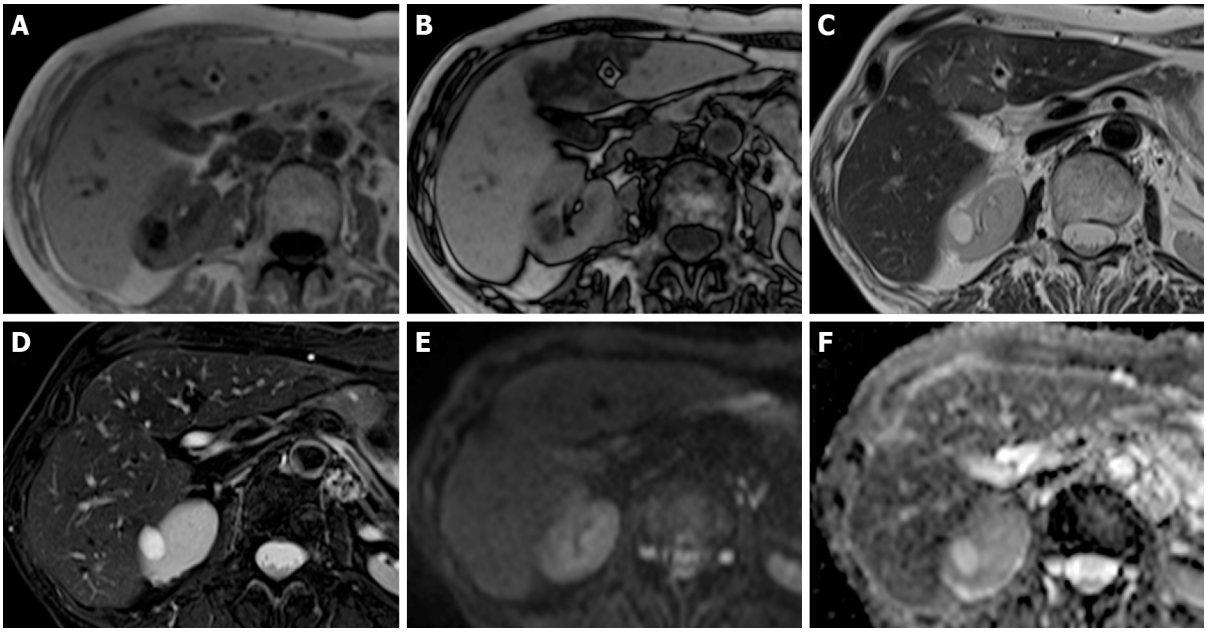

Figure 5 Chemotherapy-associated focal steatosis in patients with lung cancer 3 mo after the beginning of immunotherapy. A-F: On magnetic resonance, focal geographic fatty deposition, poorly delineated, is seen as signal hypointensity on gradient echo (GE) T1w out-of-phase (B) in the periportal aspect of segment IV and around the falciform ligament. On T2w images (C) it is weakly hyperintense. No signal alterations are seen on the GE T1w in-phase images (A), T2w fat saturation (D), diffusion-weighted (E) images, or apparent diffusion coefficient map (F).

in the hepatic veins or in the inferior vena cava, and therefore, considering the blood flow, after the hepatic sinusoids[24]. However, different causes of blue liver are possible. Other postsinusoidal forms include increased blood pressure in the right atrium (*e.g.*, congestive heart failure, constrictive pericarditis, and mitral stenosis)[96]. Moreover, injuries to the sinusoidal endothelium itself can cause a sinusoidal form of blue liver that is mainly known as SOS, but that includes a full spectrum of histologic features involving restrictive (nonthrombotic sinusoidal obstruction and perisinusoidal fibrosis), dilating (hepatic sinusoid dilation and peliosis) and regenerative (NRH) aspects of the disease[97].

Considering CALI, sinusoidal endothelial injury can be defined as nontumor-bearing hepatic parenchymal damage resulting from chemotherapy itself, not associated with the presence or infiltration of hepatic metastasis[11,98]. Therefore, the injury primarily originates at the level of the sinusoidal endothelium, eventually extending distally to the centrilobular veins or proximally to the portal branches. Its pathogenesis remains under discussion, but some consistent data have been reported. Namely, oxaliplatin induces depolymerization of F-actin in sinusoidal endothelial cells and activates matrix metallopeptidase (MMP-9 and MMP-2)[57,99,100]. This activation results in cytokine production, which induces sinusoidal endothelial damage and swelling[101]. Depletion of antioxidants such as glutathione and nitric oxide from endothelial cells can increase the damage[102]. Therefore, floating red blood cells enter the space of Disse through the gaps formed in the sinusoidal endothelium (the basis of peliosis), and collagen fibers are deposited in the extravascular space (perisinusoidal fibrosis). A combination of these factors, in addition to clogging of necrotic sinusoidal endothelial cells in sinusoids, results in sinusoidal narrowing, thus causing obstruction and increased pressure in the sinusoids. When sinusoidal outflow blockage is sustained, sinusoids upstream of the obstruction undergo dilation and disruption, resulting in pseudocystic blood-filled lacunae typical of peliosis. Concurrently, the damage from altered hepatic blood flow induces the regeneration of residual hepatocytes to replace the parenchymal damaged cells, giving rise to NRH[103].

As a consequence, the histology of sinusoidal injury involved in blue liver is heterogeneous. In the early phase, vascular alterations are predominant, including sinusoidal dilatation and congestion, perisinusoidal hemorrhage and peliosis. With the progression of the damage, fibrosis of different grades (which can be localized in the perisinusoidal space around the centrilobular vein or portal vein) is dominant, and hepatocyte disruption and NRH are evident. Although primarily originating from impaired hepatic perfusion, blue liver is characterized by parenchymal nodularity without fibrous septa, with a benign aspect similar to that of FNH, resulting from the

regeneration of hepatocytes replacing the parenchymal damaged cells[57]. Oxaliplatin is a well-known drug implicated in the development of NRH, vascular injury, such as sinusoidal ballooning, microvascular injury, and long-term fibrosis. Furthermore, paclitaxel, capecitabine, doxorubicin, and trastuzumab are also known to be causative CTAs[104].

In cases of severe progression, the chronic presentation of blue liver can be similar to that observed in cirrhosis[105]. However, in the advanced stage of chemotherapy-induced sinusoidal injury, fibrosis develops primarily between the centrilobular veins; in contrast, in primary liver disease, such as cirrhosis, bridging fibrosis, promoted by inflammation, usually develops between portal spaces. Therefore, the histologic pattern that occurs in certain forms of blue liver has been variably defined as "cardiac cirrhosis", "reversed lobulation" or "centrilobular cirrhosis"[96].

Veno-occlusive disease and SOS

Veno-occlusive disease (VOD) is a well-established condition historically associated with myelosuppressive therapy in hematologic malignancies, and it is characterized by the obliteration of small hepatic venules and centrilobular fibrosis without macroscopic signs of obstruction[97]. This pathologic alteration was first reported in 1920 by Willmot *et al*[106] and was caused by lethal intoxication by pyrrolizidine alkaloids, which are present in certain herbal remedies. The association between chemotherapy and VOD became clear in the 1950s[107]. Finally, in 1999, when DeLeve *et al*[98] recognized that the disease originated primarily in the hepatic sinusoid and did not necessarily involve the centrilobular vein, the disease was renamed SOS. These two forms therefore indicate a non-thrombotic obstruction of hepatic sinusoids with (VOD) or without (SOS) involvement of the centrilobular veins, whereas large hepatic veins remain patent[98].

More often, these pathological conditions diffusely involve the nontumor-bearing hepatic parenchyma. However, rare cases of focal SOS have been reported. The true incidence of sinusoidal focal injury remains unknown, but radiologists should be aware of it since, similar to focal steatosis, it can mimic hepatic metastasis[11,108].

Several CTAs are critical for the sinusoidal type of CALI. In particular, cyclophosphamide has been associated with the development of a rapidly progressive form of VOD[109]. Even 5-fluorouracil, mercaptopurine, dacarbazine, and vincristine have been associated with it[110], with an onset of damage ranging from 1 to 3 wk after initiation of therapy[4].

However, oxaliplatin, more than other CTAs, seems to be particularly involved in the development of SOS. According to Rubbia-Brandt *et al*[103], 51%–79% of patients who underwent oxaliplatin-based therapy developed SOS, compared with only 21%–30% of the patients who received different regimens[75,111]. Nonetheless, the incidence of sinusoidal injury is significantly higher in patients who receive more than 6 cycles of chemotherapy, while adding the anti-vascular endothelial growth factor antibody bevacizumab seems to have a protective effect[112].

Most patients with diffuse SOS are asymptomatic, in contrast to patients with VOD [97]. Clinical presentation in the acute phase may include abdominal pain and ascites. In the subacute setting, patients present with recurrent ascites and hepatosplenomegaly resulting from portal hypertension, which can be confirmed with a transjugular biopsy (pressure gradient > 10 mmHg), while the chronic presentation is similar to that of cirrhosis. Hematic tests may show nonspecifically increased bilirubin and hepatic enzyme levels[105].

Concerning prognosis, diffuse forms of SOS are associated with poor outcomes and a higher complication rate after major hepatectomy. Especially since the liver parenchyma tends to become soft and brittle, patients who undergo hepatectomy show an increased risk of perioperative morbidity (approximately 30%)[97,99] and postoperative complications[113]. Indeed, the presence of SOS is associated with a reduced liver functional reserve[108].

Imaging

Since the disease was renamed, imaging findings of VOD have been limited to case reports[114], whereas data regarding SOS are more consistent.

Concerning oxaliplatin-based chemotherapy, the radiological features reach a maximal severity approximately 4 mo after the beginning of therapy, and they show radiologic remission approximately 3 mo after discontinuation[115]. The cessation of chemotherapy is often followed by a reduction in these abnormalities, suggesting that SOS, at least for mild-to-moderate forms, both diffuse and focal, is potentially reversible[108].

Morphological alterations such as hepatosplenomegaly, periportal edema, edematous wall thickening of the gallbladder and ascites can be seen[116]. With the detection of ascites, it is important to confirm the diagnosis of SOS and distinguish it from the malignant ascites associated with peritoneal spread or metastasis[117]. US may show a decrease or reversal of blood flow in the portal veins, but its usefulness is debated[105,108]. Contrast-enhanced CT imaging features include arterial-portal heterogeneous parenchymal enhancement, characterized by a "mosaic pattern" or diffuse linear hypoattenuation lesions resulting from hepatic congestion, which tend to be homogenous in the late phase. These alterations are predominantly located in the peripheral area (67.1%) and right hepatic lobe (62.4%), with an irregular distribution of abnormal areas intermingled with intact lobules[11,118]. A reduced caliber of suprahepatic veins has also been reported[4]. Eventually, complications of portal hypertension, such as the presence of periesophageal varices, form[116].

Similarly, MR images show a heterogeneous reticular or linear pattern in the nontumor-bearing parenchyma characterized by hypointensity on T1-weighted images and hyperintensity on T2-weighted images. Using liver-specific contrast agent (CA), reticular hypointensity of background liver tissue on hepatobiliary phase images with a high prevalence in peripheral areas of the liver is highly specific for SOS, occurring in 69% of cases[11,119]. This radiological finding is probably due to reduced uptake of liver-specific CA resulting from dysfunctional damaged hepatocytes and reduced portal flow[119] (Figure 6I, J and K). In focal SOS, the presence of ill-defined margins, especially on hepatobiliary phase images, and the absence of restricted diffusion on diffusion-weighted images, can help differentiate focal hepatic toxicity from metastasis[118].

Severe forms of SOS can also progress after discontinuation of therapy, leading to the appearance of regenerative phenomena including cirrhotic alterations[10]. In addition, in oxaliplatin-treated patients, portal hypertension and histological changes in NRH can arise during long-term therapy with 6-thioguanine for acute lymphatic leukemia[120]. The nodularity of NRH is usually microscopic and thus is not detectable on images. Larger nodules can show hyper/hypointensity on T1/T2-weighted images and increased vascularity (hyperintensity in the arterial phase, followed by iso- or slight hyperintensity in the portal and equilibrium phases), but their benign nature can be confirmed by their normal uptake of liver-specific MR CAs [57].

Finally, FNH-like lesions have been described to occur in these patients many years after the discontinuation of chemotherapy[108] (Figure 7).

Hepatic sinusoid dilatation and peliosis hepatis

Hepatic sinusoid dilatation (HSD) is a rare hepatic vascular lesion characterized by diffuse dilatation of hepatic capillaries with or without venous outflow obstruction. Causes of HSD with hepatic outflow obstruction include Budd Chiari syndrome, pericardial disease or right heart failure, and sinusoidal occlusion secondary to endothelial sinusoidal damage itself, as in SOS. It can be classified according to the affected zone of the hepatic lobule as centrilobular, periportal, or irregular[108]. On the other hand, forms of HSD without venous outflow obstruction are caused by extrahepatic acute inflammatory conditions (pyelonephritis, cholecystitis, pneumonia, pancreatitis, and inflammatory bowel diseases), use of oral contraceptives (still debated as a possible cause) and chronic conditions, such as congenital or idiopathic vascular diseases, neoplasms with or without secondary liver involvement, inflammatory or infectious diseases, the use of hormones and drugs.

HSD can be distinguished from peliosis, since the latter shows evidence of rupture of the reticulin fibers that support hepatocytes and sinusoids[121]. More precisely, peliosis hepatis is characterized by multiple blood-filled cystic lesions at the level of the sinusoids, with dimensions ranging from 1 mm to several centimeters, randomly distributed throughout the lobule, with loss of the endothelium[120]. Peliosis hepatitis was first described in 1861 on the basis of the Greek word "pelios", meaning "reddish" or "bluish", referring to the parenchymal color[122,123]. It is often a primary idiopathic condition (Figure 8). However, different etiologies have been proposed for secondary forms, including toxins (arsenic and polyvinyl chloride) and certain drugs, such as steroids, oral contraceptives, tamoxifen, 6-thioguanine, 6-mercaptopurine and methotrexate. Chronic wasting diseases have been proposed as another possible causes (diabetes mellitus, malignancy, acquired immunodeficiency syndrome, pregnancy, and infectious diseases, such as tuberculosis, leprosy, Bartonella and adenovirus)[105,120,124].

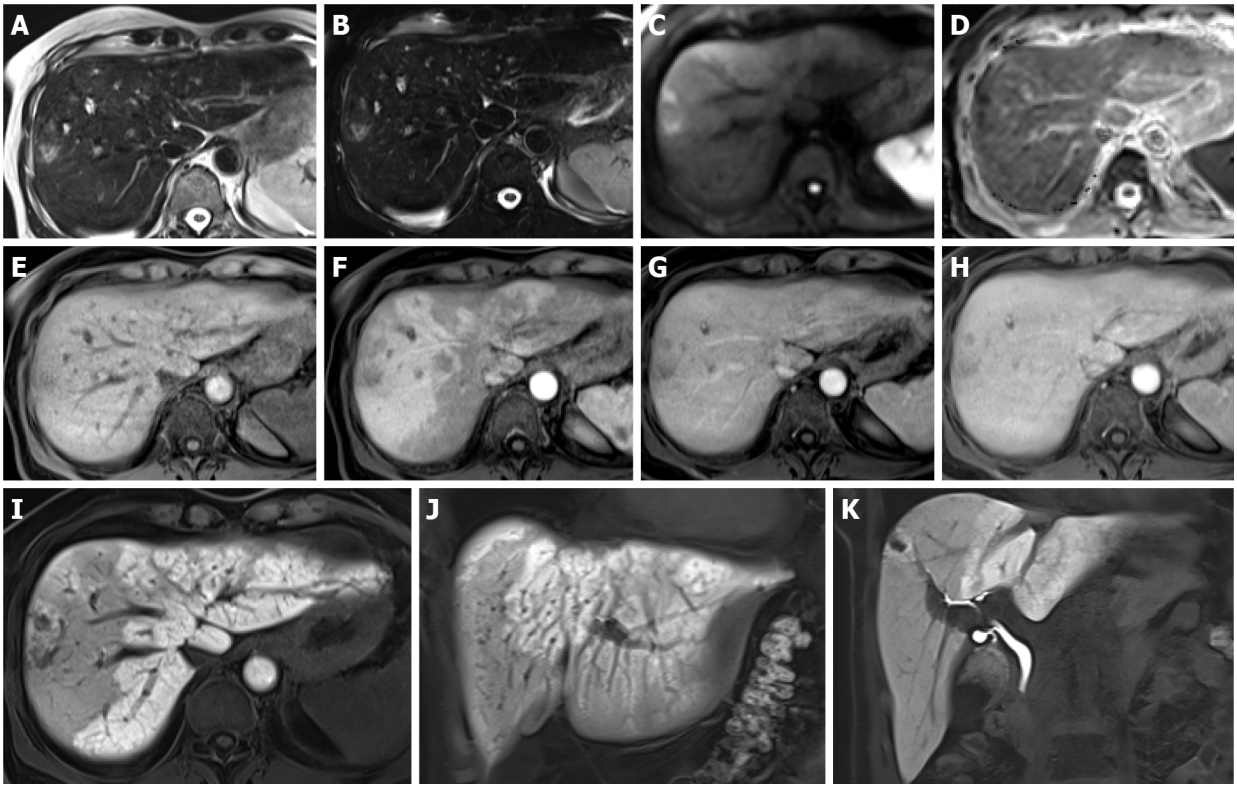

Figure 6 Association between sinusoidal obstructive syndrome and peliosis in patients without a history of hepatopathy, with lung cancer treated with 3 cycles of cisplatin and etoposide and 12 subsequent cycles of immunotherapy. A-K: After 1 year of therapy, magnetic resonance was performed for abdominal pain and an increase in liver enzymes. Hyperintense nodules on T2w (A), T2w fat sat (B) and high b-value diffusion-weighted images (C) are seen. On the apparent diffusion coefficient map (D), they are hypointense. On dynamic imaging, weak enhancement is seen (E: Unenhanced image; F, G, H: Arterial, portal and equilibrium phases, respectively). In the hepatobiliary phase, they appear predominantly hypointense (I-K). A transcutaneous biopsy was performed, resulting in peliosis nodules. Mosaic pattern enhancement of the liver parenchyma in the arterial phase (F) and reticular aspects in the hepatobiliary phase (I-J) were consistent with sinusoidal obstructive syndrome.

Its pathogenesis is not completely clear, except for SOS-dependent peliosis, and regardless of the cause, sinusoidal damage has been generally proposed as being critical to outflow blockage and dilation of the sinusoids/central vein of the hepatic lobule[120,125]. Moreover, hepatocellular necrosis may represent another possible mechanism with the subsequent formation of blood-filled lacunae[126,127]. Two different histological forms of peliosis can be identified: the parenchymal type, usually associated with hemorrhagic parenchymal necrosis and characterized by a lack of endothelial lining within the blood-filled lacunae, and the phlebectatic type, with a dilated central vein, showing an endothelial lining within the cystic spaces[128]. However, these seem to represent different temporal phases of the same condition, with the endothelial lining in the blood-filled lacunae continuously being disrupted and rapidly reconstituted[129].

The distribution of the lesions can vary considerably, from focal areas of peliosis within the liver parenchyma to widespread forms occupying most of the liver parenchyma[130,131]. Peliosis hepatis is usually an asymptomatic condition and therefore is often incidentally diagnosed. However, patients can present with hepatomegaly, portal hypertension, hepatic failure and ascites. Severe abdominal pain is a possible complication associated with minor trauma, resulting in hepatic rupture and hemoperitoneum[132]. The evolution of peliosis is variable and unpredictable. Peliosis sometimes worsens in terms of extension, thus remaining asymptomatic[133,134]. In the presence of underlying conditions such as HCV-related cirrhosis, it can promote the risk of liver failure[130,134]. In some cases, especially in young patients, this alteration can cause compression and stenosis of the vena cava[135]. However, regression is also possible once the etiologic agent causing secondary peliosis is identified and treated[130,134], and idiopathic forms can undergo spontaneous regression[136].

Imaging

On contrast-enhanced CT and MR images, HSD is associated with the typical features

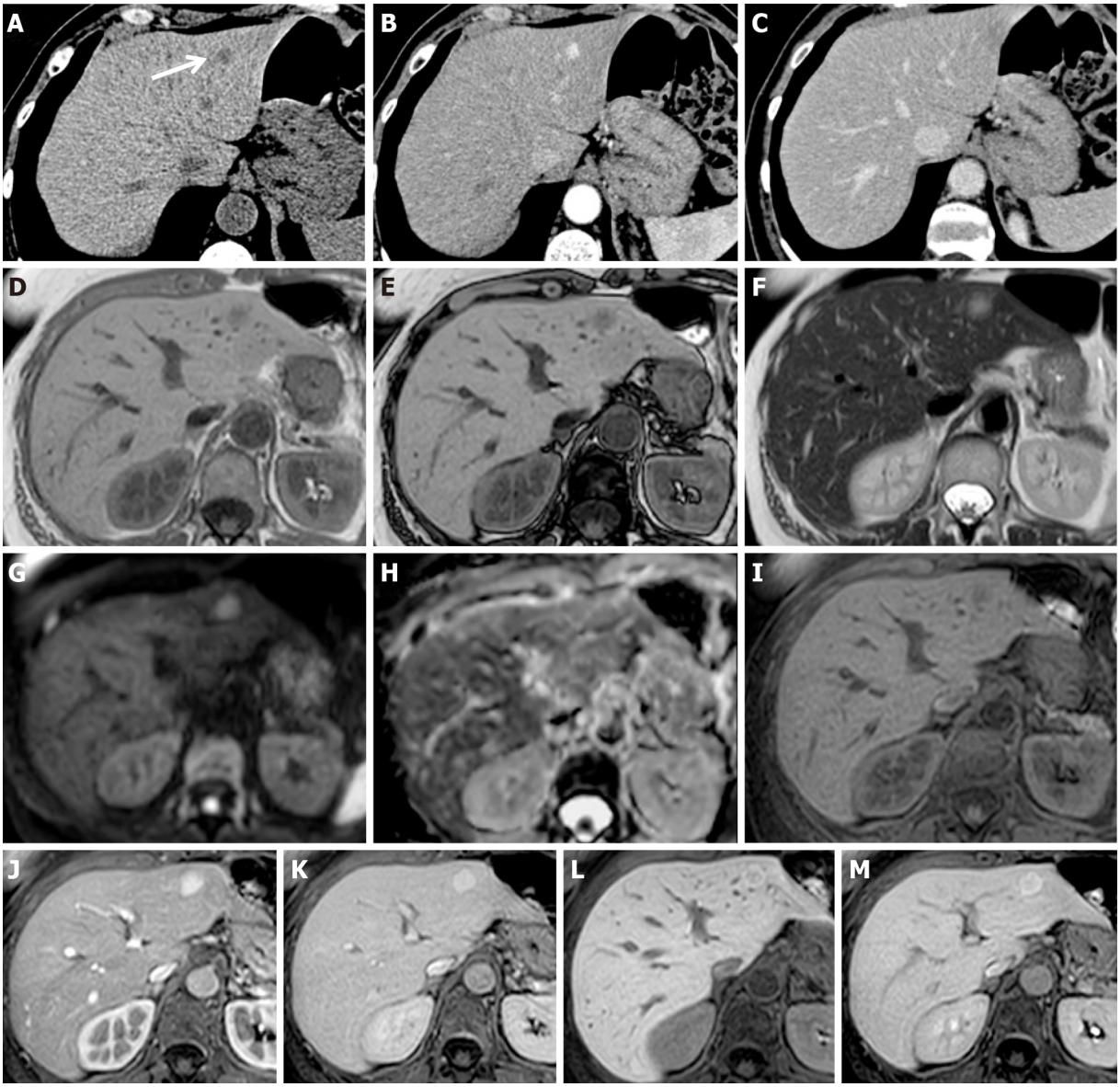

Figure 7 Focal nodular hyperplasia-like nodules in patients with colorectal cancer treated with surgery and adjuvant chemotherapy. A-C: Six months after adjuvant chemotherapy discontinuation, contrast-enhanced computed tomography (CT) showed a newly appeared nodule in segment II (white arrow), hypodense on unenhanced scan (A), with contrast enhancement on arterial phase (B) without washout in portal phase (C); D-M: Magnetic resonance performed 3 mo after CT showed a volumetric increase in the nodule, characterized by signal hypointensity in gradient echo T1w in-phase (D) and out-of-phase (E) and weak hyperintensity in T2w images (F), without diffusivity restriction on diffusion-weighted imaging (G-H). After liver-specific contrast agent administration, it presented homogeneous wash-in on the arterial phase (J) compared to the unenhanced image (I), no wash-out (K), and weak central signal hypointensity on equilibrium (L) and hepatobiliary (M) phases. A transcutaneous biopsy was performed, resulting in focal nodular hyperplasia-like nodules.

described for SOS, with mosaic pattern enhancement in the arterial-portal phase and reticular hypointense appearance in the MR hepatobiliary phase. On T2-weighted images, the affected areas may show slightly increased and heterogeneous signal intensity[137] (Figure 9).

The imaging features of peliosis depend on its extension, pathologic type and stage of blood components. In a few cases, the number or size of the peliotic lesions can increase in a short period and disseminate throughout the liver, resembling the progression of liver carcinoma or metastases[138].

On US, peliotic lesions appear homogeneous and hyperechoic, associated with pseudocyst formation, which may correspond to venous lacunae in the parenchyma [124], whereas in fatty liver, they will appear as hypoechoic lesions. In addition, when hemorrhage is present, US shows heterogeneously hypoechoic lesions. Unenhanced CT generally shows hypodense lesions, eventually associated with hyperdense foci, secondary to hemorrhage or calcifications (Figure 10). In dynamic phases, the pattern is variable; usually, in the arterial phase, the lesions show vessel-like enhancement at the center (target sign), with centrifugal enhancement during the venous phase;

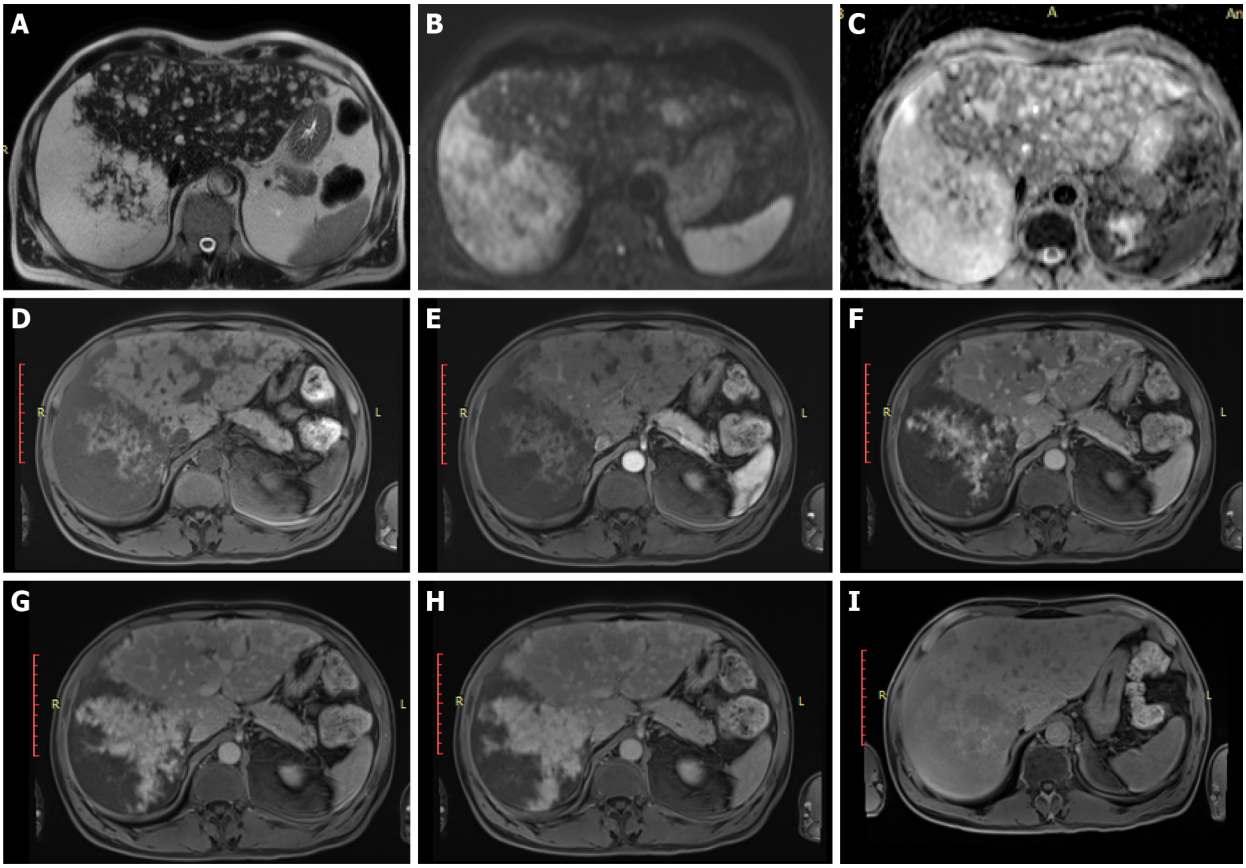

Figure 8 Primary idiopathic diffuse peliosis in patients without a cancer history. A-C: On magnetic resonance T2w images (A) and on diffusion-weighted imaging (B: High b value; C: Apparent diffusion coefficient map), numerous hemangioma-like lesions are visible, the largest in segments VII-VIII; D-I: After interstitial contrast agent administration, progressive centrifugal enhancement of the lesions was observed (D: Fat sat gradient echo 3D T1w unenhanced image; E, F, G: Arterial, portal and equilibrium phases; H-I: 5 and 30 min after contrast agent administration).

however, a centripetal enhancement pattern is also possible, which may be confused with that of a hemangioma[139]. Lesions tend to acquire diffuse homogeneous enhancement in the delayed phase[120,128]. In the presence of thrombosed cavities, these latter may show no enhancement. MR examination is the gold standard for radiologic diagnosis, presenting high specificity and sensitivity in the detection of the features of peliosis[134,136]. In MRI, on T2-weighted sequences, peliotic lesions are usually hyperintense compared to liver parenchyma with foci with a higher signal, which is likely attributable to hemorrhagic necrosis. On T1-weighted sequences, the lesions are hypointense, although isointense and hyperintense foci have also been described, depending on the age and the status of the blood components[120,140]. Exophytic extension of the peliotic nodules and fluid-fluid level, probably due to old and new blood products in the lesions, are rarely reported[140]. The dynamic behavior after CA administration is similar to that seen on CT scan, typically centrifugal, and more rarely centripetal[120]. In the hepatobiliary phase, a "branching" appearance caused by the direct demonstration of the vascular component within the lesion has been reported[134]. Although peliosis hepatis is a benign condition, the apparent diffusion coefficient values are lower than those of a normal-appearing liver, probably due to its content, including thrombi and hemorrhaged areas[140] (Figure 6). If the clinical and radiological findings are suggestive of peliosis, percutaneous liver biopsy should be avoided because of the significant risk of severe bleeding[141].

A summary of the liver mosaic appearance enhancement in blue liver and a classification of hepatic peliosis types are shown in Figure 11 and Figure 12, respectively.

PSEUDOCIRRHOSIS

Pseudocirrhosis is a pathological condition characterized by morphological changes of the liver mimicking macronodular cirrhosis without histopathological confirmation [104,142]. The ''pseudo'' prefix can also lead to confusion, indicating a more benign

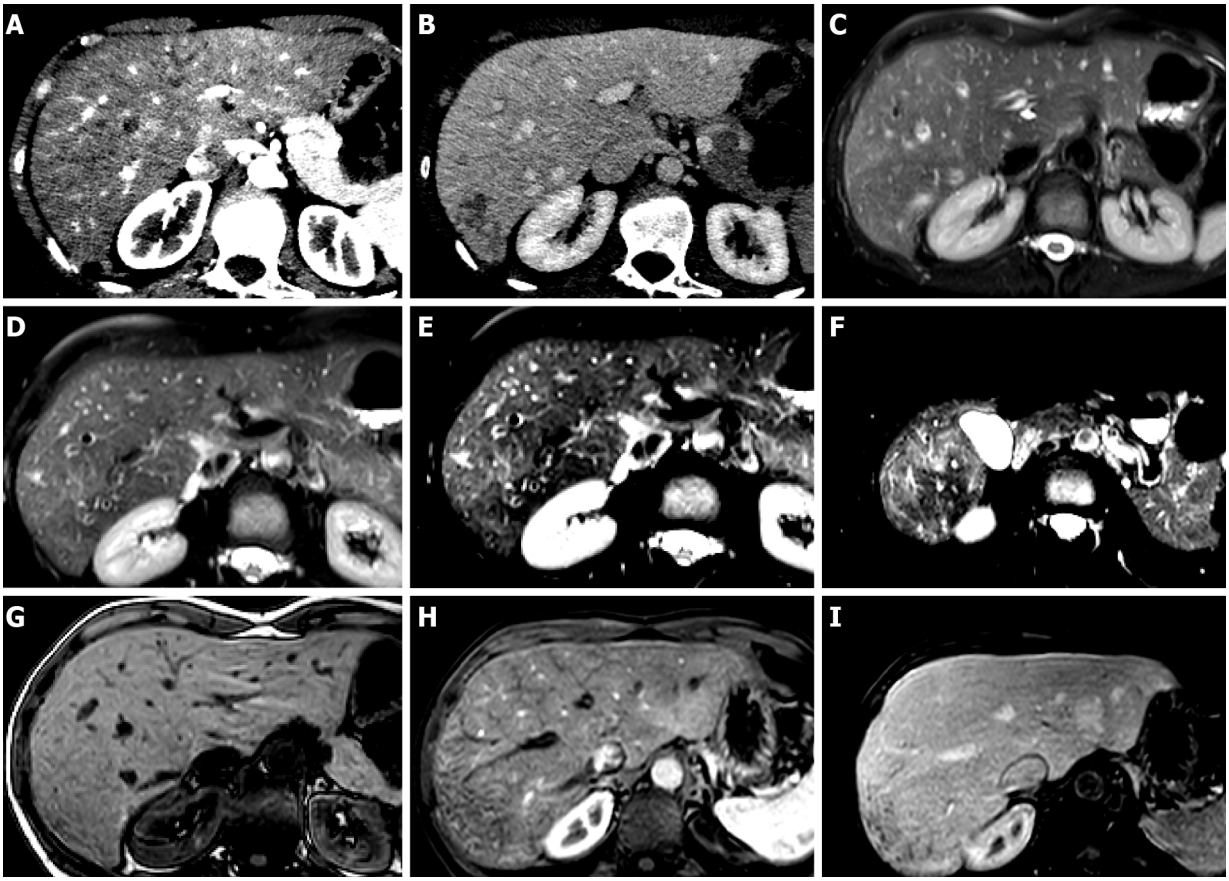

Figure 9 Hepatic sinusoid dilatation in patients with breast cancer during hormone therapy. A, B: Arterial (A) and portal (B) computed tomography axial scans show a liver mosaic pattern of arterial enhancement, with reticular aspects on the subcapsular parenchyma of segment VII in the portal phase; C-F: Magnetic resonance (MR) T2w images show mild signal hyperintensity on different liver sections and different echo times; G-I: MR unenhanced (G), arterial (H) and portal phases (I) confirm the mosaic pattern mostly subcapsular of the liver parenchyma.

condition than cirrhosis; indeed, even for patients who are asymptomatic and pseudocirrhosisis identified only incidentally during oncological follow-up, most patients can develop serious systemic complications, sometimes life-threatening, including portal hypertension, ascites and splenomegaly[143]. Abdominal distension, ascites and splenomegaly are the most common initial presentations in patients. Therefore, early recognition is important.

Breast cancer liver metastasis treated with chemotherapy is the most commonly reported cause of pseudocirrhosis[143,144]. However, it has also been linked to other metastatic diseases, including gastroenteric (pancreatic, esophageal, and colon), small-cell lung and thyroid cancers[144,145]. Vuppalanchi *et al*[146] estimated a prevalence of up to 50% in patients with metastatic breast cancer. Qayyum *et al*[142] said that approximately 75% of patients with liver metastatic breast cancer receiving chemotherapy demonstrated various degrees of hepatic contour abnormalities, from limited retraction to diffuse nodularity, and that approximately 9% of these patients developed portal hypertension. Morphological changes were seen after a median follow-up interval of 15 mo[142]. Indeed, the real prevalence of pseudocirrhosis has not yet been defined[147]. Interestingly, it is often observed in patients with a major morphologic response to chemotherapy[145]. Among the various CTAs, most cases of pseudocirrhotic changes are described after patients receive regimens including gemcitabine, 5-flurouracil, oxaliplatin[2] and trastuzumab[4,104]. More recently, Vuppalanchi *et al*[146] described two cases of pseudocirrhosis in patients after they had received the latest target therapy.

The pathophysiology of postchemotherapy pseudocirrhosis is still unknown, but it is proposed to be multifactorial and represent a mechanism of both cancer regression as a response of hepatic metastasis to CTAs and a consequence of the hepatotoxic effect of the treatment itself and cancer progression, with fibrosis surrounding the infiltrating hepatic tumor[147,148]. Tumor shrinkage in response to chemotherapy causes hepatic capsular retraction and scar formation around metastatic lesions, thus resulting in macronodular cirrhosis[149,150]. The regenerative response of hepatocytes

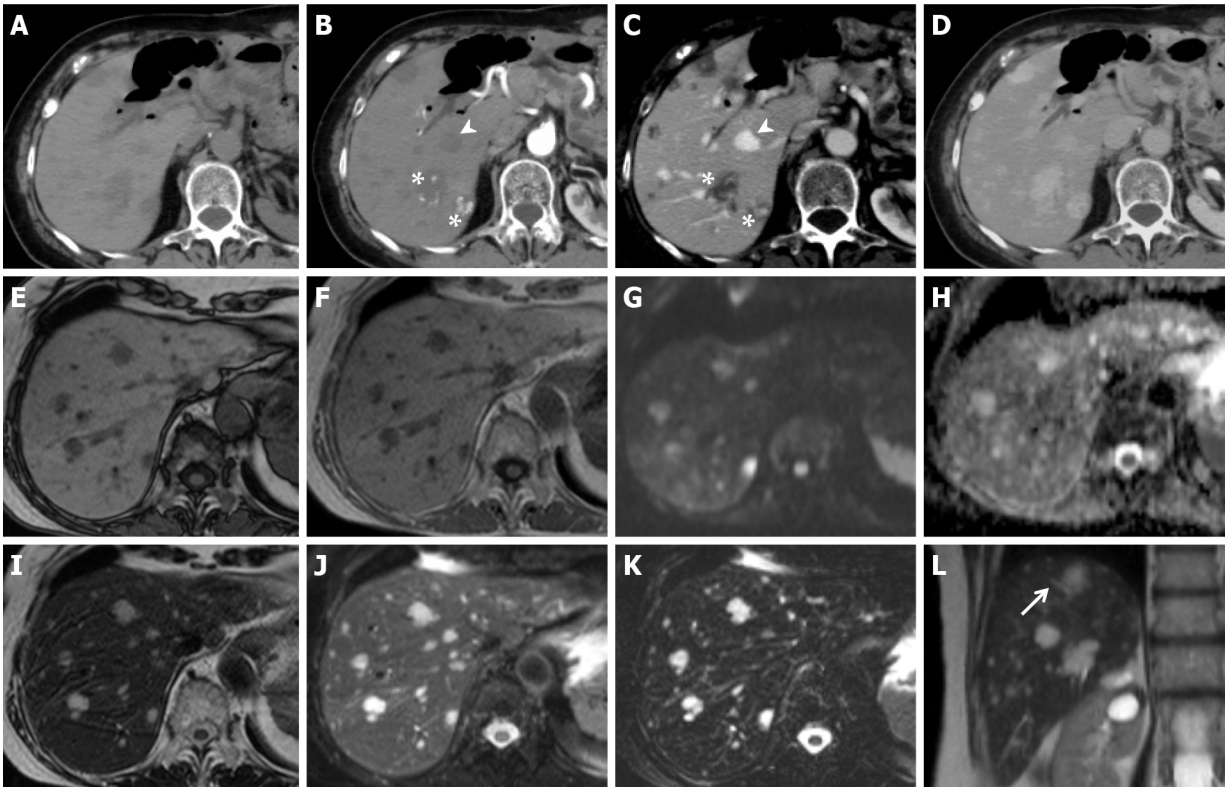

Figure 10 Secondary idiopathic multiple peliotic lesions in patients with a history of 6-mercaptopurine treatment for leukemia. A-D: Contrast-enhanced computed tomography shows multiple lesions, hypodense on unenhanced scan (A) with dystrophic calcifications and hyperdense foci, probably secondary to hemorrhage. On dynamic imaging (B, axial arterial phase; C, axial portal phase), the lesions present centripetal (arrowhead) or centrifugal (asterisk) globular contrast enhancement without signs of washout. In the delayed phase (D), they appear isodense compared with the hepatic parenchyma; E-L: Magnetic resonance confirming the presence of hypointense lesions on T1w images (E-F) and hyperintense lesions on T2w images (I, J and L, arrow), which maintain high signal in long echoes echo time 320 ms (K). No signs of altered diffusion (G-H) or mass effects are shown. These characteristics were consistent with multiple peliotic lesions.

to ischemia following chemotherapy-induced injury has been proposed as another mechanism; in this case, the development of NRH is thought to be critical to compression of the surrounding parenchyma, resulting in atrophy[151]. Finally, sinusoidal obstruction may contribute to pseudocirrhosis[146,149]. This effect may be secondary to both chemotherapy-induced sinusoidal damage and mechanical compression resulting from metastases, leading to rebound arterialization and portal flow reduction, which helps to explain the atrophy of the parenchyma and the cirrhotic appearance of the liver[152]. Interestingly, the mechanism is quite similar to that proposed by Breen for hepatic changes during cirrhotic progression[153]. A general rule of progression is proposed as follows: less portal inflow, an arterial phenomenon, metabolic infarction and fatty changes, fibrosis and atrophy[24,154]. Importantly, in this setting, in contrast to liver cirrhosis, histologic examination is consistent with NRH without bridging fibrosis[151]. In chemotherapy-naïve patients, however, pseudocirrhosis seems to occur only rarely[155] (Figure 13). This second type of pseudocirrhosis is linked to cancer progression and may be related to tumor size, with extensive fibrosis corresponding to a desmoplastic reaction surrounding the infiltrating tumors[155,156]. The pressure generated by fibrosis determines parenchymal portal flow lessening, with a consequent arterial reaction[24]. Therefore, in chemotherapy-naïve patients, the pathogenesis may also be similar to that after chemotherapy. Histologic examination of this second setting of pseudocirrhosis shows extensive fibrosis resulting from a desmoplastic reaction determined by the infiltrating lesion[149].

Imaging

The diagnosis of pseudocirrhosis is radiological and is defined by features typical of cirrhosis[104,142]. Because it progresses rapidly compared with 'true' liver cirrhosis, it can be easy to detect serial changes in liver morphology on imaging studies.

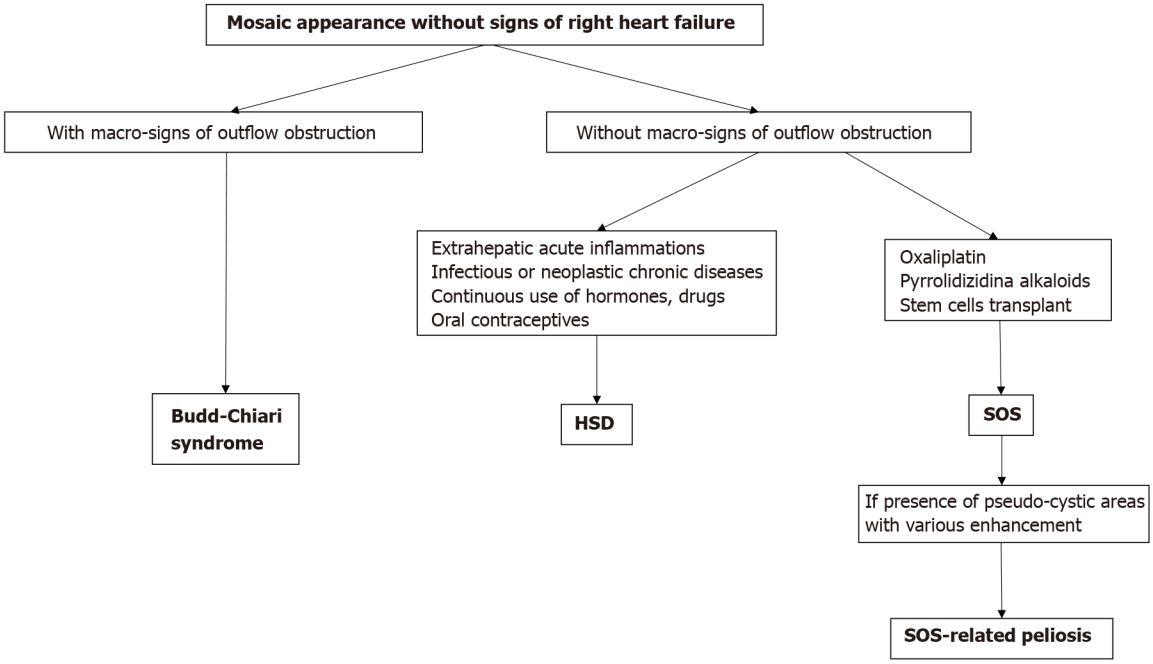

Figure 11 Diagram of different forms of mosaic pattern enhancement in blue liver syndrome. HSD: Hepatic sinusoid dilatation; SOS: Sinusoidal obstruction syndrome.

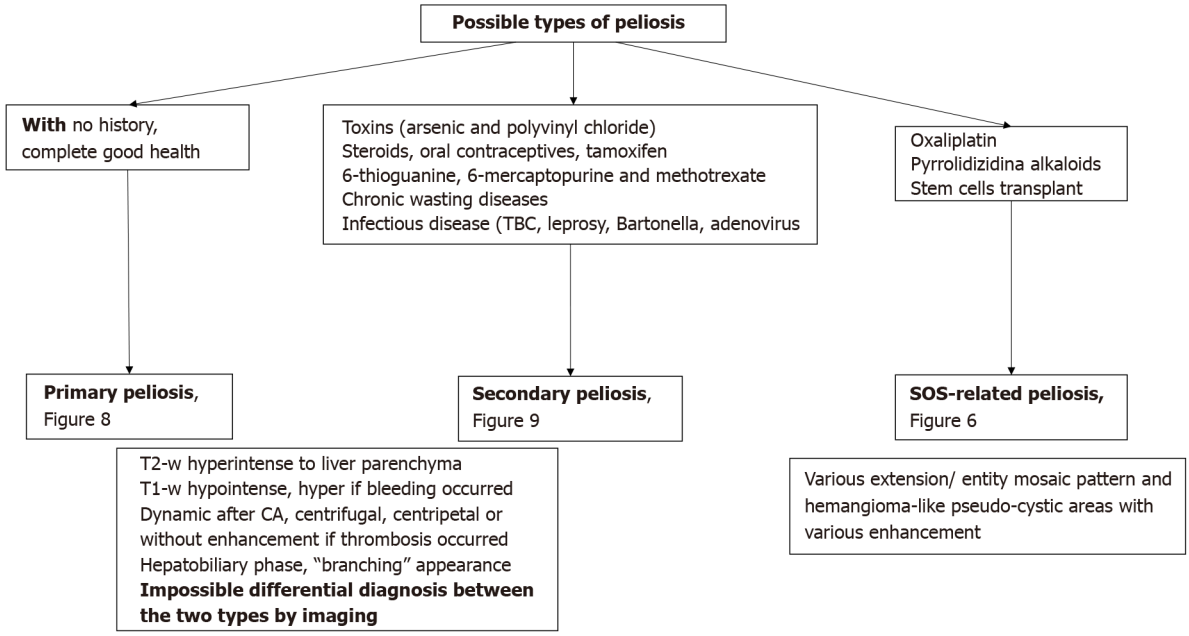

Figure 12 Diagram showing the classification of hepatic peliosis. SOS: Sinusoidal obstruction syndrome; CA: Contrast agent.

On CT or MR, hepatomegaly and diffuse fatty changes of the liver parenchyma were initially seen, with smooth hepatic surfaces and metastases that focally bulge out. These are followed by a reduction in the hepatic volume along with capsular retraction [104] (Figure 14). With time, fibrosis becomes prominent, confluent low-attenuation nodularity with irregular enhancement can be seen, and parenchymal atrophy of the right lobe associated with relative hypertrophy of the caudate and left lobe becomes more evident[104]. Moreover, other findings complicating cirrhotic changes include signs of portal hypertension such as splenomegaly, ascites and portosystemic varices [143]. Liver-specific gadolinium-enhanced MR can confirm the same morphological alterations, allowing for more accuracy in the characterization of any metastases. These lesions may appear as several focal lesions with high signal intensity on T2-weighted images and low signal intensity on T1-weighted images, with rim

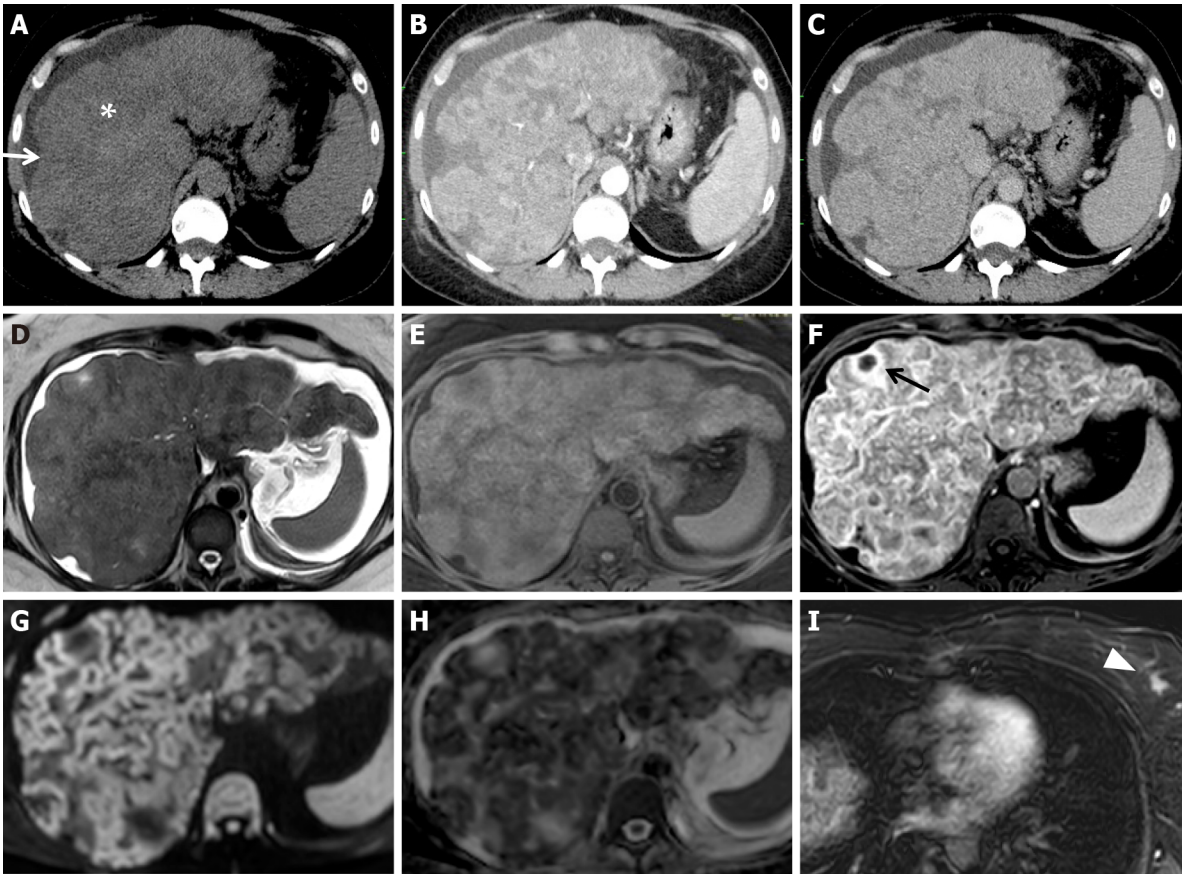

Figure 13 Pathologically proven pseudocirrhosis due to a small breast cancer in a chemotherapy "naïve patient", having received no chemotherapy. A-C: On unenhanced (A) computed tomography (CT) axial scans, a lobulated liver contour with retraction of the capsular surface (white arrow), low-attenuation parenchymal areas, and ascites (white asterisk) are seen. On arterial (B) and portal (C) CT axial scans, architectural disorder and heterogeneous contrast enhancement are detectable; D-I: On magnetic resonance, the presence of ascites is confirmed on T2w images (D). Profound structural and architectural changes due to the presence of coarse nodules separated by areas of fibrosis in an unenhanced fat sat gradient echo 3D T1w image (E) and a contrast-enhanced phase T1w image at equilibrium (F) are visible; various confluent nodules with irregular hyperintense rims on high b-value diffusion-weighted images (G) and low signal intensity in apparent diffusion coefficient map value (H) were observed. A small necrotic area inside a nodule is indicated in F (black arrow). One small left breast cancer nodule (white arrowhead) on a contrast-enhanced T1w image is visible in the arterial phase (I).

enhancement after CA administration[157] (Figure 15). Tumor markers do not increase during the period of pseudocirrhosis, indicating that progression of metastasis is unlikely[104]. Furthermore, nonspecific radiological findings may lead to a misinterpretation of the cancer response[147,158]. In addition, noncirrhotic causes of diffuse liver surface nodularity vary, and the clinical presentations are quite similar. In some of these causes, such as chronic Budd-Chiari syndrome, chronic portal vein thrombosis and pseudomyxoma peritonei, hepatic contour changes are easily distinguishable from cirrhosis because of their characteristic features. The latter shows coarse and lobulated contours, while nodularity associated with cirrhosis is typically relatively fine and diffuse. However, noncirrhotic causes of fine, diffuse nodularity are occasionally shown not only in pseudocirrhosis but also in hepatic failure and sarcoidosis[143]. Fulminant hepatic failure can present with diffuse surface nodularity due to a combination of alternating foci of confluent regenerative nodules and necrosis[159]. Sarcoidosis of the liver is rarely observable on imaging because noncaseating granulomas are usually microscopic. However, it can sometimes be visible as diffuse granular heterogeneity with or without fine nodularity of the hepatic surface[160,161].

Moreover, once pseudocirrhosis has been properly assessed, careful monitoring and appropriate management of complications are necessary to avoid progression toward life-threatening complications, such as hepatic failure, encephalopathy, and esophageal/gastric variceal bleeding, similar to those seen in classic severe cirrhosis [144,147]. Therapy should be modified and sometimes interrupted[154] because imaging features of pseudocirrhosis have been shown to completely resolve in some patients[154,158].

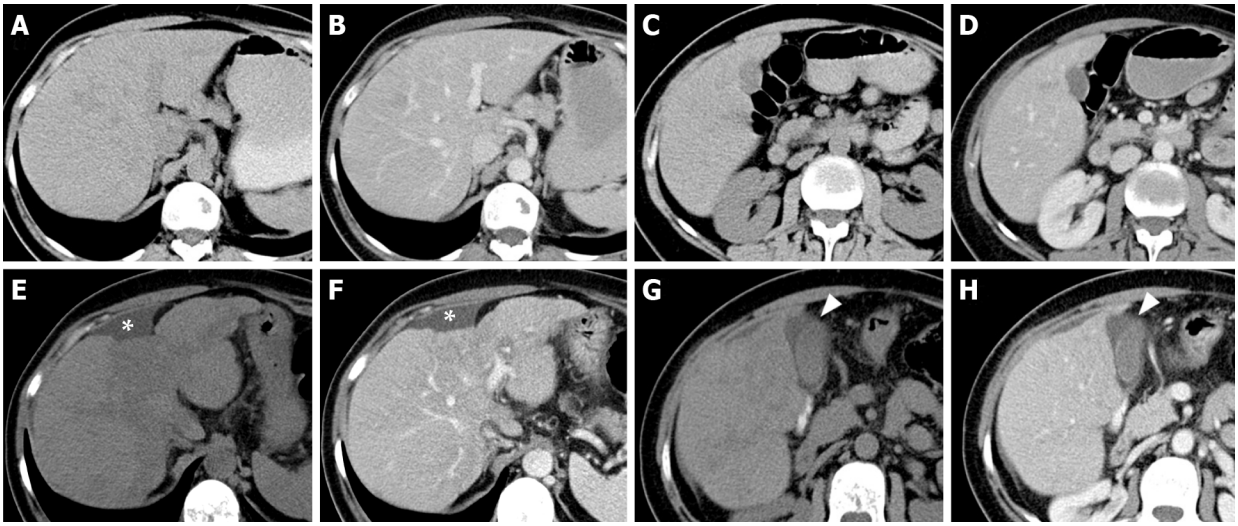

Figure 14 Early features of pseudocirrhosis in patients with metastatic breast cancer treated with gemcitabine for 12 mo. A-D: On axial unenhanced (A, C) and contrast-enhanced portal (B, D) computed tomography (CT) scan images, executed prior chemotherapy, the liver presents a regular volume, morphology and a smooth surface. No signs of ascites are present; E-H: On CT exam after chemotherapy (12 mo) at the same levels, in the same phases, fatty changes of the liver parenchyma, reduction of the hepatic volume with relative hypertrophy of the left lobe, irregular margins and capsular retraction corresponding to the IV segment (asterisk) were detectable. Peri-hepatic and pericholecystic effusion occurred (arrowhead).

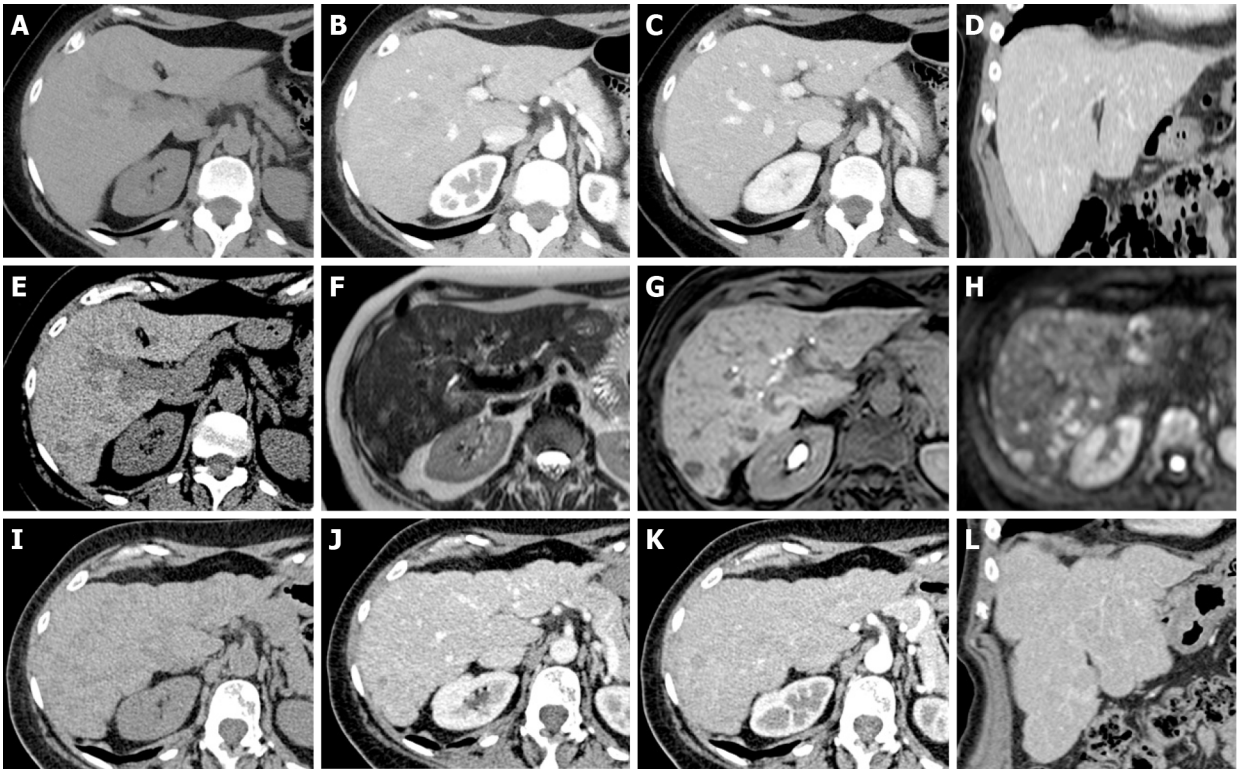

Figure 15 Pseudocirrhosis in patients with breast cancer treated with surgery and 6 mo of chemotherapy (capecitabine and monoclonal antibodies). A-D: Unenhanced (A: Axial) and contrast-enhanced computed tomography (CT) (B: Axial arterial phase; C: Axial portal phase; D: Coronal portal phase) was performed at staging. The liver shows regular volume, morphology and a smooth surface. No focal lesions were found; thus, no chemotherapy was undertaken; E: At the 1-year follow-up, unenhanced CT demonstrated the appearance of a hypodense focal lesion (arrowhead); F-H: A complete magnetic resonance study with liver-specific contrast agent confirmed the presence of new focal lesions consistent with metastases. Mild hyperintensity in the T2w sequence (F), clear hypointensity in the fat sat gradient echo 3D T1w hepatobiliary phase (G) and high signal in diffusion-weighted images (H) are shown. Chemotherapy was started. I-L: A 6-mo follow-up unenhanced (I: Axial) and contrast-enhanced CT (J: Axial arterial phase; K: Axial portal phase; L: Coronal portal phase) shows typical signs of liver pseudocirrhotic changes: parenchymal volume reduction, irregular macrocyclic margins, right lobe atrophy and caudate lobe hypertrophy.

CONCLUSION

In conclusion, many drugs can cause liver damage through various mechanisms in

oncologic patients. As a consequence of the longer life expectancy of these patients, chemotherapy-associated liver injury is becoming increasingly frequent. Radiologists need to be aware of and know the imaging patterns of chemotherapy injury, supporting clinicians in therapeutic decisions and thus preventing severe complications for patients.

REFERENCES

1 **Field KM**, Dow C, Michael M. Part I: Liver function in oncology: biochemistry and beyond. *Lancet Oncol* 2008; **9**: 1092-1101 [PMID: 19012858 DOI: 10.1016/S1470-2045(08)70279-1]
2 **Sharma A**, Houshyar R, Bhosale P, Choi JI, Gulati R, Lall C. Chemotherapy induced liver abnormalities: an imaging perspective. *Clin Mol Hepatol* 2014; **20**: 317-326 [PMID: 25320738 DOI: 10.3350/cmh.2014.20.3.317]
3 **Colsky J**, Greenspan EM, Warren TN. Hepatic fibrosis in children with acute leukemia after therapy with folic acid antagonists. *AMA Arch Pathol* 1955; **59**: 198-206 [PMID: 13227717]
4 **Ngo D**, Jia JB, Green CS, Gulati AT, Lall C. Cancer therapy related complications in the liver, pancreas, and biliary system: an imaging perspective. *Insights Imaging* 2015; **6**: 665-677 [PMID: 26443452 DOI: 10.1007/s13244-015-0436-7]
5 **White MA**, Fong Y, Singh G. Chemotherapy-Associated Hepatotoxicities. *Surg Clin North Am* 2016; **96**: 207-217 [PMID: 27017860 DOI: 10.1016/j.suc.2015.11.005]
6 **Howard SA**, Krajewski KM, Thornton E, Jagannathan JP, O'Regan K, Cleary J, Ramaiya N. Decade of molecular targeted therapy: abdominal manifestations of drug toxicities--what radiologists should know. *AJR Am J Roentgenol* 2012; **199**: 58-64 [PMID: 22733894 DOI: 10.2214/AJR.11.7432]
7 **Lee KW**, Chan SL. Hepatotoxicity of targeted therapy for cancer. *Expert Opin Drug Metab Toxicol* 2016; **12**: 789-802 [PMID: 27187715 DOI: 10.1080/17425255.2016.1190831]
8 **Michot JM**, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, Berdelou A, Varga A, Bahleda R, Hollebecque A, Massard C, Fuerea A, Ribrag V, Gazzah A, Armand JP, Amellal N, Angevin E, Noel N, Boutros C, Mateus C, Robert C, Soria JC, Marabelle A, Lambotte O. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer* 2016; **54**: 139-148 [PMID: 26765102 DOI: 10.1016/j.ejca.2015.11.016]
9 **Alessandrino F**, Tirumani SH, Krajewski KM, Shinagare AB, Jagannathan JP, Ramaiya NH, Di Salvo DN. Imaging of hepatic toxicity of systemic therapy in a tertiary cancer centre: chemotherapy, haematopoietic stem cell transplantation, molecular targeted therapies, and immune checkpoint inhibitors. *Clin Radiol* 2017; **72**: 521-533 [PMID: 28476244 DOI: 10.1016/j.crad.2017.04.003]
10 **Maor Y**, Malnick S. Liver injury induced by anticancer chemotherapy and radiation therapy. *Int J Hepatol* 2013; **2013**: 815105 [PMID: 23970972 DOI: 10.1155/2013/815105]
11 **You SH**, Park BJ, Kim YH. Hepatic Lesions that Mimic Metastasis on Radiological Imaging during Chemotherapy for Gastrointestinal Malignancy: Recent Updates. *Korean J Radiol* 2017; **18**: 413-426 [PMID: 28458594 DOI: 10.3348/kjr.2017.18.3.413]
12 **Kouroumalis E**, Voumvouraki A, Augoustaki A, Samonakis DN. Autophagy in liver diseases. *World J Hepatol* 2021; **13**: 6-65 [PMID: 33584986 DOI: 10.4254/wjh.v13.i1.6]
13 **Itai Y**, Matsui O. Blood flow and liver imaging. *Radiology* 1997; **202**: 306-314 [PMID: 9015047 DOI: 10.1148/radiology.202.2.9015047]
14 **Strasberg SM**, Helton WS. An analytical review of vasculobiliary injury in laparoscopic and open cholecystectomy. *HPB (Oxford)* 2011; **13**: 1-14 [PMID: 21159098 DOI: 10.1111/j.1477-2574.2010]
15 **Gu X**, Manautou JE. Molecular mechanisms underlying chemical liver injury. *Expert Rev Mol Med* 2012; **14**: e4 [PMID: 22306029 DOI: 10.1017/S1462399411002110]
16 **Kim J**, Jung Y. Radiation-induced liver disease: current understanding and future perspectives. *Exp Mol Med* 2017; **49**: e359 [PMID: 28729640 DOI: 10.1038/emm.2017.85]
17 **Bhogal RH**, Mirza DF, Afford SC, Mergental H. Biomarkers of Liver Injury during Transplantation in an Era of Machine Perfusion. *Int J Mol Sci* 2020; **21** [PMID: 32106626 DOI: 10.3390/ijms21051578]
18 **Konishi T**, Lentsch AB. Hepatic Ischemia/Reperfusion: Mechanisms of Tissue Injury, Repair, and Regeneration. *Gene Expr* 2017; **17**: 277-287 [PMID: 28893351 DOI: 10.3727/105221617X15042750874156]
19 **O'Neil M**, Damjanov I, Taylor RM. Pattern of Liver Injury. In: Liver Pathology for Clinicians. 1st ed. Switzerland: Springer, 2015: 45-89
20 **Tu T**, Calabro SR, Lee A, Maczurek AE, Budzinska MA, Warner FJ, McLennan SV, Shackel NA. Hepatocytes in liver injury: Victim, bystander, or accomplice in progressive fibrosis? *J Gastroenterol Hepatol* 2015; **30**: 1696-1704 [PMID: 26239824 DOI: 10.1111/jgh.13065]
21 **Malhi H**, Guicciardi ME, Gores GJ. Hepatocyte death: a clear and present danger. *Physiol Rev* 2010; **90**: 1165-1194 [PMID: 20664081 DOI: 10.1152/physrev.00061.2009]
22 **Luedde T**, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. *Gastroenterology* 2014; **147**: 765-783.e4 [PMID: 25046161 DOI: 10.1053/j.gastro.2014.07.018]
23 **Colagrande S**, Centi N, Carmignani L, Salvatore Politi L, Villari N. [Meaning and etiopathogenesis of sectorial transient hepatic attenuation differences (THAD)]. *Radiol Med* 2003; **105**: 180-187

[PMID: 12835641]

24 **Colagrande S**, Pradella S, Lucarini S, Marra F. Transient Hepatic Parenchymal Enhancement detected at dynamic imaging: a short instruction manual for the clinician. *Dig Liver Dis* 2012; **44**: 363-368 [PMID: 22153702 DOI: 10.1016/j.dld.2011.10.026]

25 **Colagrande S**, Centi N, Galdiero R, Ragozzino A. Transient hepatic intensity differences: part 2, Those not associated with focal lesions. *AJR Am J Roentgenol* 2007; **188**: 160-166 [PMID: 17179359 DOI: 10.2214/AJR.05.1367]

26 **Yoshimitsu K**, Honda H, Kuroiwa T, Irie H, Aibe H, Shinozaki K, Masuda K. Unusual hemodynamics and pseudolesions of the noncirrhotic liver at CT. *Radiographics* 2001; **21** Spec No: S81-S96 [PMID: 11598250 DOI: 10.1148/radiographics.21.suppl_1.g01oc06s81]

27 **Hann LE**, Getrajdman GI, Brown KT, Bach AM, Teitcher JB, Fong Y, Blumgart LH. Hepatic lobar atrophy: association with ipsilateral portal vein obstruction. *AJR Am J Roentgenol* 1996; **167**: 1017-1021 [PMID: 8819404 DOI: 10.2214/ajr.167.4.8819404]

28 **Brancatelli G**, Federle MP, Grazioli L, Golfieri R, Lencioni R. Benign regenerative nodules in Budd-Chiari syndrome and other vascular disorders of the liver: radiologic-pathologic and clinical correlation. *Radiographics* 2002; **22**: 847-862 [PMID: 12110714 DOI: 10.1148/radiographics.22.4.g02jl17847]

29 **Pinzani M**, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. *J Hepatol* 2005; **42** Suppl: S22-S36 [PMID: 15777570 DOI: 10.1016/j.jhep.2004.12.008]

30 **Hoofnagle JH**, Björnsson ES. Drug-Induced Liver Injury - Types and Phenotypes. *N Engl J Med* 2019; **381**: 264-273 [PMID: 31314970 DOI: 10.1056/NEJMra1816149]

31 **Lewis JH**. The Art and Science of Diagnosing and Managing Drug-induced Liver Injury in 2015 and Beyond. *Clin Gastroenterol Hepatol* 2015; **13**: 2173-89.e8 [PMID: 26116527 DOI: 10.1016/j.cgh.2015.06.017]

32 **Fisher K**, Vuppalanchi R, Saxena R. Drug-Induced Liver Injury. *Arch Pathol Lab Med* 2015; **139**: 876-887 [PMID: 26125428 DOI: 10.5858/arpa.2014-0214-RA]

33 **Zimmerman HJ**. Classification of Hepatotoxins and Mechanism of Toxicity. In: Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 2nd ed. Philadelphia: Lippincott, William & Wilkins, 1999: 111-146

34 **Sarges P**, Steinberg JM, Lewis JH. Drug-Induced Liver Injury: Highlights from a Review of the 2015 Literature. *Drug Saf* 2016; **39**: 801-821 [PMID: 27142208 DOI: 10.1007/s40264-016-0427-8]

35 **Alempijevic T**, Zec S, Milosavljevic T. Drug-induced liver injury: Do we know everything? *World J Hepatol* 2017; **9**: 491-502 [PMID: 28443154 DOI: 10.4254/wjh.v9.i10.491]

36 **Björnsson E**. Drug-induced liver injury: Hy's rule revisited. *Clin Pharmacol Ther* 2006; **79**: 521-528 [PMID: 16765139 DOI: 10.1016/j.clpt.2006.02.012]

37 **Reuben A**, Koch DG, Lee WM; Acute Liver Failure Study Group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. *Hepatology* 2010; **52**: 2065-2076 [PMID: 20949552 DOI: 10.1002/hep.23937]

38 **Kleiner DE**, Chalasani NP, Lee WM, Fontana RJ, Bonkovsky HL, Watkins PB, Hayashi PH, Davern TJ, Navarro V, Reddy R, Talwalkar JA, Stolz A, Gu J, Barnhart H, Hoofnagle JH; Drug-Induced Liver Injury Network (DILIN). Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations. *Hepatology* 2014; **59**: 661-670 [PMID: 24037963 DOI: 10.1002/hep.26709]

39 **Kleiner DE**. Drug-induced Liver Injury: The Hepatic Pathologist's Approach. *Gastroenterol Clin North Am* 2017; **46**: 273-296 [PMID: 28506365 DOI: 10.1016/j.gtc.2017.01.004]

40 **Aliberti S**, Grignani G, Allione P, Fizzotti M, Galatola G, Pisacane A, Aglietta M. An acute hepatitis resembling autoimmune hepatitis occurring during imatinib therapy in a gastrointestinal stromal tumor patient. *Am J Clin Oncol* 2009; **32**: 640-641 [PMID: 19955903 DOI: 10.1097/COC.0b013e31802b4ef7]

41 **Ghabril M**, Bonkovsky HL, Kum C, Davern T, Hayashi PH, Kleiner DE, Serrano J, Rochon J, Fontana RJ, Bonacini M; US Drug-Induced Liver Injury Network. Liver injury from tumor necrosis factor-α antagonists: analysis of thirty-four cases. *Clin Gastroenterol Hepatol* 2013; **11**: 558-564.e3 [PMID: 23333219 DOI: 10.1016/j.cgh.2012.12.025]

42 **Kleiner DE**, Berman D. Pathologic changes in ipilimumab-related hepatitis in patients with metastatic melanoma. *Dig Dis Sci* 2012; **57**: 2233-2240 [PMID: 22434096 DOI: 10.1007/s10620-012-2140-5]

43 **Faggioli P**, De Paschale M, Tocci A, Luoni M, Fava S, De Paoli A, Tosi A, Cassi E. Acute hepatic toxicity during cyclic chemotherapy in non Hodgkin's lymphoma. *Haematologica* 1997; **82**: 38-42 [PMID: 9107080]

44 **Parmar KL**, O'Reilly D, Valle JW, Braun M, Naish JH, Williams SR, Lloyd WK, Malcomson L, Cresswell K, Bamford C, Renehan AG. Prospective study of change in liver function and fat in patients with colorectal liver metastases undergoing preoperative chemotherapy: protocol for the CLiFF Study. *BMJ Open* 2020; **10**: e027630 [PMID: 32967864 DOI: 10.1136/bmjopen-2018-027630]

45 **Reginelli A**, Vacca G, Zanaletti N, Troiani T, Natella R, Maggialetti N, Palumbo P, Giovagnoni A, Ciardiello F, Cappabianca S. Diagnostic value/performance of radiological liver imaging during chemoterapy for gastrointestinal malignancy: a critical review. *Acta Biomed* 2019; **90**: 51-61 [PMID: 31085973 DOI: 10.23750/abm.v90i5-S.8346]

46 **Vigano L**, De Rosa G, Toso C, Andres A, Ferrero A, Roth A, Sperti E, Majno P, Rubbia-Brandt L. Reversibility of chemotherapy-related liver injury. *J Hepatol* 2017; **67**: 84-91 [PMID: 28284915 DOI: 10.1016/j.jhep.2017.02.031]

47 **Karoui M**, Penna C, Amin-Hashem M, Mitry E, Benoist S, Franc B, Rougier P, Nordlinger B. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. *Ann Surg* 2006; **243**: 1-7 [PMID: 16371728 DOI: 10.1097/01.sla.0000193603.26265.c3]

48 **Vauthey JN**, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, Xiong HQ, Eng C, Lauwers GY, Mino-Kenudson M, Risio M, Muratore A, Capussotti L, Curley SA, Abdalla EK. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**: 2065-2072 [PMID: 16648507 DOI: 10.1200/JCO.2005.05.3074]

49 **Tamandl D**, Klinger M, Eipeldauer S, Herberger B, Kaczirek K, Gruenberger B, Gruenberger T. Sinusoidal obstruction syndrome impairs long-term outcome of colorectal liver metastases treated with resection after neoadjuvant chemotherapy. *Ann Surg Oncol* 2011; **18**: 421-430 [PMID: 20844968 DOI: 10.1245/s10434-010-1317-4]

50 **Viganò L**, Capussotti L, De Rosa G, De Saussure WO, Mentha G, Rubbia-Brandt L. Liver resection for colorectal metastases after chemotherapy: impact of chemotherapy-related liver injuries, pathological tumor response, and micrometastases on long-term survival. *Ann Surg* 2013; **258**: 731-40; discussion 741 [PMID: 24045448 DOI: 10.1097/SLA.0b013e3182a6183e]

51 **Zhao J**, Sawo P, Rensen SS, Rouflart MMJ, Winstanley A, Vreuls CPH, Verheij J, van Mierlo KMC, Lodewick TM, van Woerden V, van Tiel FH, van Dam RM, Dejong CHC, Olde Damink SWM. Impact of chemotherapy-associated liver injury on tumour regression grade and survival in patients with colorectal liver metastases. *HPB (Oxford)* 2018; **20**: 147-154 [PMID: 28969959 DOI: 10.1016/j.hpb.2017.08.030]

52 **Parkin E**, O'Reilly DA, Adam R, Kaiser GM, Laurent C, Elias D, Capussotti L, Renehan AG; LiverMetSurvey Centres. Equivalent survival in patients with and without steatosis undergoing resection for colorectal liver metastases following pre-operative chemotherapy. *Eur J Surg Oncol* 2014; **40**: 1436-1444 [PMID: 25189474 DOI: 10.1016/j.ejso.2014.07.040]

53 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Extended hepatic resection for hepatocellular carcinoma in patients with cirrhosis: is it justified? *Ann Surg* 2002; **236**: 602-611 [PMID: 12409666 DOI: 10.1097/00000658-200211000-00010]

54 **Cayet S**, Pasco J, Dujardin F, Besson M, Orain I, De Muret A, Miquelestorena-Standley E, Thiery J, Genet T, Le Bayon AG. Diagnostic performance of contrast-enhanced CT-scan in sinusoidal obstruction syndrome induced by chemotherapy of colorectal liver metastases: Radio-pathological correlation. *Eur J Radiol* 2017; **94**: 180-190 [PMID: 28712693 DOI: 10.1016/j.ejrad.2017.06.025]

55 **Gentilucci UV**, Santini D, Vincenzi B, Fiori E, Picardi A, Tonini G. Chemotherapy-induced steatohepatitis in colorectal cancer patients. *J Clin Oncol* 2006; **24**: 5467; author reply 5467-5467; author reply 5468 [PMID: 17135651 DOI: 10.1200/JCO.2006.08.1828]

56 **Peppercorn PD**, Reznek RH, Wilson P, Slevin ML, Gupta RK. Demonstration of hepatic steatosis by computerized tomography in patients receiving 5-fluorouracil-based therapy for advanced colorectal cancer. *Br J Cancer* 1998; **77**: 2008-2011 [PMID: 9667683 DOI: 10.1038/bjc.1998.333]

57 **Robinson PJ**. The effects of cancer chemotherapy on liver imaging. *Eur Radiol* 2009; **19**: 1752-1762 [PMID: 19238392 DOI: 10.1007/s00330-009-1333-6]

58 **Sandrasegaran K**, Alazmi WM, Tann M, Fogel EL, McHenry L, Lehman GA. Chemotherapy-induced sclerosing cholangitis. *Clin Radiol* 2006; **61**: 670-678 [PMID: 16843750 DOI: 10.1016/j.crad.2006.02.013]

59 **Delis S**, Triantopoulou C, Bakoyiannis A, Tassopoulos N, Athanasiou K, Dervenis C. Sclerosing cholangitis in the era of target chemotherapy: a possible anti-VEGF effect. *Dig Liver Dis* 2009; **41**: 72-77 [PMID: 18294938 DOI: 10.1016/j.dld.2007.11.016]

60 **Kusakabe A**, Ohkawa K, Fukutake N, Sakakibara M, Imai T, Abe Y, Takada R, Ikezawa K, Nawa T, Ashida R, Kimura T, Nagata S, Katayama K. Chemotherapy-Induced Sclerosing Cholangitis Caused by Systemic Chemotherapy. *ACG Case Rep J* 2019; **6**: e00136 [PMID: 31620533 DOI: 10.14309/crj.0000000000000136]

61 **Zen Y**, Yeh MM. Hepatotoxicity of immune checkpoint inhibitors: a histology study of seven cases in comparison with autoimmune hepatitis and idiosyncratic drug-induced liver injury. *Mod Pathol* 2018; **31**: 965-973 [PMID: 29403081 DOI: 10.1038/s41379-018-0013-y]

62 **Shah RR**, Morganroth J, Shah DR. Hepatotoxicity of tyrosine kinase inhibitors: clinical and regulatory perspectives. *Drug Saf* 2013; **36**: 491-503 [PMID: 23620168 DOI: 10.1007/s40264-013-0048-4]

63 **Jennings JJ**, Mandaliya R, Nakshabandi A, Lewis JH. Hepatotoxicity induced by immune checkpoint inhibitors: a comprehensive review including current and alternative management strategies. *Expert Opin Drug Metab Toxicol* 2019; **15**: 231-244 [PMID: 30677306 DOI: 10.1080/17425255.2019.1574744]

64 **Aguilera-Méndez A**. Nonalcoholic hepatic steatosis: a silent disease Rev Med Inst Mex Seguro Soc 2019; 56: 544-549 [PMID: 30889343]

65 **Khan AZ**, Morris-Stiff G, Makuuchi M. Patterns of chemotherapy-induced hepatic injury and their implications for patients undergoing liver resection for colorectal liver metastases. *J Hepatobiliary Pancreat Surg* 2009; **16**: 137-144 [PMID: 19093069 DOI: 10.1007/s00534-008-0016-z]

66 **Viswanathan C**, Truong MT, Sagebiel TL, Bronstein Y, Vikram R, Patnana M, Silverman PM,

Bhosale PR. Abdominal and pelvic complications of nonoperative oncologic therapy. *Radiographics* 2014; **34**: 941-961 [PMID: 25019433 DOI: 10.1148/rg.344140082]

67 **Ushijima K**, Yahata H, Yoshikawa H, Konishi I, Yasugi T, Saito T, Nakanishi T, Sasaki H, Saji F, Iwasaka T, Hatae M, Kodama S, Terakawa N, Yaegashi N, Hiura M, Sakamoto A, Tsuda H, Fukunaga M, Kamura T. Multicenter phase II study of fertility-sparing treatment with medroxyprogesterone acetate for endometrial carcinoma and atypical hyperplasia in young women. *J Clin Oncol* 2007; **25**: 2798-2803 [PMID: 17602085 DOI: 10.1200/JCO.2006.08.8344]

68 **Kooby DA**, Fong Y, Suriawinata A, Gonen M, Allen PJ, Klimstra DS, DeMatteo RP, D'Angelica M, Blumgart LH, Jarnagin WR. Impact of steatosis on perioperative outcome following hepatic resection. *J Gastrointest Surg* 2003; **7**: 1034-1044 [PMID: 14675713 DOI: 10.1016/j.gassur.2003.09.012]

69 **Fernandez FG**, Ritter J, Goodwin JW, Linehan DC, Hawkins WG, Strasberg SM. Effect of steatohepatitis associated with irinotecan or oxaliplatin pretreatment on resectability of hepatic colorectal metastases. *J Am Coll Surg* 2005; **200**: 845-853 [PMID: 15922194 DOI: 10.1016/j.jamcollsurg.2005.01.024]

70 **Zorzi D**, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. *Br J Surg* 2007; **94**: 274-286 [PMID: 17315288 DOI: 10.1002/bjs.5719]

71 **Birch JC**, Khatri G, Watumull LM, Arriaga YE, Leyendecker JR. Unintended Consequences of Systemic and Ablative Oncologic Therapy in the Abdomen and Pelvis. *Radiographics* 2018; **38**: 1158-1179 [PMID: 29995613 DOI: 10.1148/rg.2018170137]

72 **McDonald GB**, Tirumali N. Intestinal and liver toxicity of antineoplastic drugs. *West J Med* 1984; **140**: 250-259 [PMID: 6375139]

73 **Bethke A**, Kühne K, Platzek I, Stroszczynski C. Neoadjuvant treatment of colorectal liver metastases is associated with altered contrast enhancement on computed tomography. *Cancer Imaging* 2011; **11**: 91-99 [PMID: 21771709 DOI: 10.1102/1470-7330.2011.0015]

74 **Meunier L**, Larrey D. Chemotherapy-associated steatohepatitis. *Ann Hepatol* 2020; **19**: 597-601 [PMID: 32061473 DOI: 10.1016/j.aohep.2019.11.012]

75 **Mehta NN**, Ravikumar R, Coldham CA, Buckels JA, Hubscher SG, Bramhall SR, Wigmore SJ, Mayer AD, Mirza DF. Effect of preoperative chemotherapy on liver resection for colorectal liver metastases. *Eur J Surg Oncol* 2008; **34**: 782-786 [PMID: 18160247 DOI: 10.1016/j.ejso.2007.09.007]

76 **Morris-Stiff G**, White AD, Gomez D, Cameron IC, Farid S, Toogood GJ, Lodge JP, Prasad KR. Nodular regenerative hyperplasia (NRH) complicating oxaliplatin chemotherapy in patients undergoing resection of colorectal liver metastases. *Eur J Surg Oncol* 2014; **40**: 1016-1020 [PMID: 24370284 DOI: 10.1016/j.ejso.2013.09.015]

77 **Qayyum A**, Nystrom M, Noworolski SM, Chu P, Mohanty A, Merriman R. MRI steatosis grading: development and initial validation of a color mapping system. *AJR Am J Roentgenol* 2012; **198**: 582-588 [PMID: 22357996 DOI: 10.2214/AJR.11.6729]

78 **Wu CH**, Ho MC, Jeng YM, Hsu CY, Liang PC, Hu RH, Lai HS, Shih TT. Quantification of hepatic steatosis: a comparison of the accuracy among multiple magnetic resonance techniques. *J Gastroenterol Hepatol* 2014; **29**: 807-813 [PMID: 24224538 DOI: 10.1111/jgh.12451]

79 **Corrias G**, Krebs S, Eskreis-Winkler S, Ryan D, Zheng J, Capanu M, Saba L, Monti S, Fung M, Reeder S, Mannelli L. MRI liver fat quantification in an oncologic population: the added value of complex chemical shift-encoded MRI. *Clin Imaging* 2018; **52**: 193-199 [PMID: 30103108 DOI: 10.1016/j.clinimag.2018.08.002]

80 **Ferraioli G**, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. *World J Gastroenterol* 2019; **25**: 6053-6062 [PMID: 31686762 DOI: 10.3748/wjg.v25.i40.6053]

81 **Ricci C**, Longo R, Gioulis E, Bosco M, Pollesello P, Masutti F, Crocè LS, Paoletti S, de Bernard B, Tiribelli C, Dalla Palma L. Noninvasive *in vivo* quantitative assessment of fat content in human liver. *J Hepatol* 1997; **27**: 108-113 [PMID: 9252082 DOI: 10.1016/s0168-8278(97)80288-7]

82 **Venkataraman S**, Braga L, Semelka RC. Imaging the fatty liver. *Magn Reson Imaging Clin N Am* 2002; **10**: 93-103 [PMID: 11998577 DOI: 10.1016/s1064-9689(03)00051-5]

83 **Rinella ME**, McCarthy R, Thakrar K, Finn JP, Rao SM, Koffron AJ, Abecassis M, Blei AT. Dual-echo, chemical shift gradient-echo magnetic resonance imaging to quantify hepatic steatosis: Implications for living liver donation. *Liver Transpl* 2003; **9**: 851-856 [PMID: 12884199 DOI: 10.1053/jlts.2003.50153]

84 **Choi JS**, Kim MJ. Education and imaging: hepatobiliary and pancreatic: focal steatohepatitis mimicking a metastasis. *J Gastroenterol Hepatol* 2011; **26**: 415 [PMID: 21261737 DOI: 10.1111/j.1440-1746.2010.06614.x]

85 **Boll DT**, Merkle EM. Diffuse liver disease: strategies for hepatic CT and MR imaging. *Radiographics* 2009; **29**: 1591-1614 [PMID: 19959510 DOI: 10.1148/rg.296095513]

86 **Itai Y**, Matsui O. 'Nonportal' splanchnic venous supply to the liver: abnormal findings on CT, US and MRI. *Eur Radiol* 1999; **9**: 237-243 [PMID: 10101644 DOI: 10.1007/s003300050661]

87 **Hamer OW**, Aguirre DA, Casola G, Lavine JE, Woenckhaus M, Sirlin CB. Fatty liver: imaging patterns and pitfalls. *Radiographics* 2006; **26**: 1637-1653 [PMID: 17102041 DOI: 10.1148/rg.266065004]

88 **Colagrande S**, Castellani A, Nardi C, Lorini C, Calistri L, Filippone A. The role of diffusion-weighted imaging in the detection of hepatic metastases from colorectal cancer: A comparison with

unenhanced and Gd-EOB-DTPA enhanced MRI. *Eur J Radiol* 2016; **85**: 1027-1034 [PMID: 27130067 DOI: 10.1016/j.ejrad.2016.02.011]

89 **Colagrande S**, Calistri L, Grazzini G, Nardi C, Busoni S, Morana G, Grazioli L. MRI features of primary hepatic lymphoma. *Abdom Radiol (NY)* 2018; **43**: 2277-2287 [PMID: 29460044 DOI: 10.1007/s00261-018-1476-5]

90 **Itai Y**. Peritumoral sparing of fatty liver: another important instance of focal sparing caused by a hepatic tumor. *AJR Am J Roentgenol* 2000; **174**: 868-870 [PMID: 10701644 DOI: 10.2214/ajr.174.3.1740868b]

91 **Haugeberg G**, Strohmeyer T, Lierse W, Böcker W. The vascularization of liver metastases. Histological investigation of gelatine-injected liver specimens with special regard to the vascularization of micrometastases. *J Cancer Res Clin Oncol* 1988; **114**: 415-419 [PMID: 2457592 DOI: 10.1007/BF02128188]

92 **Miles KA**. Tumour angiogenesis and its relation to contrast enhancement on computed tomography: a review. *Eur J Radiol* 1999; **30**: 198-205 [PMID: 10452718 DOI: 10.1016/s0720-048x(99)00012-1]

93 **Oliva MR**, Mortele KJ, Segatto E, Glickman JN, Erturk SM, Ros PR, Silverman SG. Computed tomography features of nonalcoholic steatohepatitis with histopathologic correlation. *J Comput Assist Tomogr* 2006; **30**: 37-43 [PMID: 16365570 DOI: 10.1097/01.rct.0000193818.31749.84]

94 **Kudo M**, Zheng RQ, Kim SR, Okabe Y, Osaki Y, Iijima H, Itani T, Kasugai H, Kanematsu M, Ito K, Usuki N, Shimamatsu K, Kage M, Kojiro M. Diagnostic accuracy of imaging for liver cirrhosis compared to histologically proven liver cirrhosis. A multicenter collaborative study. *Intervirology* 2008; **51** Suppl 1: 17-26 [PMID: 18544944 DOI: 10.1159/000122595]

95 **Al-Qudah G**, Ghanem M, Blebea J, Shaheen S. Blue Liver: Case Report of Blue Liver. *Am J Case Rep* 2020; **21**: e923553 [PMID: 32738134 DOI: 10.12659/AJCR.923553]

96 **Fortea JI**, Puente Á, Cuadrado A, Huelin P, Pellón R, González Sánchez FJ, Mayorga M, Cagigal ML, García Carrera I, Cobreros M, Crespo J, Fábrega E. Congestive Hepatopathy. *Int J Mol Sci* 2020; **21** [PMID: 33321947 DOI: 10.3390/ijms21249420]

97 **Ward J**, Guthrie JA, Sheridan MB, Boyes S, Smith JT, Wilson D, Wyatt JI, Treanor D, Robinson PJ. Sinusoidal obstructive syndrome diagnosed with superparamagnetic iron oxide-enhanced magnetic resonance imaging in patients with chemotherapy-treated colorectal liver metastases. *J Clin Oncol* 2008; **26**: 4304-4310 [PMID: 18779617 DOI: 10.1200/JCO.2008.16.1893]

98 **DeLeve LD**, McCuskey RS, Wang X, Hu L, McCuskey MK, Epstein RB, Kanel GC. Characterization of a reproducible rat model of hepatic veno-occlusive disease. *Hepatology* 1999; **29**: 1779-1791 [PMID: 10347121 DOI: 10.1002/hep.510290615]

99 **Aloia T**, Sebagh M, Plasse M, Karam V, Lévi F, Giacchetti S, Azoulay D, Bismuth H, Castaing D, Adam R. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. *J Clin Oncol* 2006; **24**: 4983-4990 [PMID: 17075116 DOI: 10.1200/JCO.2006.05.8156]

100 **Arakawa Y**, Shimada M, Utsunomya T, Imura S, Morine Y, Ikemoto T, Hanaoka J, Sugimoto K, Bando Y. Oxaliplatin-related sinusoidal obstruction syndrome mimicking metastatic liver tumors. *Hepatol Res* 2013; **43**: 685-689 [PMID: 23730707 DOI: 10.1111/j.1872-034X.2012.01114.x]

101 **Sakumura M**, Tajiri K, Miwa S, Nagata K, Kawai K, Miyazono T, Arita K, Wada A, Murakami J, Sugiyama T. Hepatic Sinusoidal Obstruction Syndrome Induced by Non-transplant Chemotherapy for Non-Hodgkin Lymphoma. *Intern Med* 2017; **56**: 395-400 [PMID: 28202860 DOI: 10.2169/internalmedicine.56.7669]

102 **King PD**, Perry MC. Hepatotoxicity of chemotherapy. *Oncologist* 2001; **6**: 162-176 [PMID: 11306728 DOI: 10.1634/theoncologist.6-2-162]

103 **Rubbia-Brandt L**, Lauwers GY, Wang H, Majno PE, Tanabe K, Zhu AX, Brezault C, Soubrane O, Abdalla EK, Vauthey JN, Mentha G, Terris B. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. *Histopathology* 2010; **56**: 430-439 [PMID: 20459550 DOI: 10.1111/j.1365-2559.2010.03511.x]

104 **Jeong WK**, Choi SY, Kim J. Pseudocirrhosis as a complication after chemotherapy for hepatic metastasis from breast cancer. *Clin Mol Hepatol* 2013; **19**: 190-194 [PMID: 23837145 DOI: 10.3350/cmh.2013.19.2.190]

105 **Elsayes KM**, Shaaban AM, Rothan SM, Javadi S, Madrazo BL, Castillo RP, Casillas VJ, Menias CO. A Comprehensive Approach to Hepatic Vascular Disease. *Radiographics* 2017; **37**: 813-836 [PMID: 28430541 DOI: 10.1148/rg.2017160161]

106 **Willmot FC**, Robertson GW. Senecio disease, or cirrhosis of the liver due to senecio poisoning. *Lancet* 1920; **196**: 848-849 [DOI: 10.1016/s0140-6736(01)00020-4]

107 **Selzer G**, Parker RG. Senecio poisoning exhibiting as Chiari's syndrome; a report on twelve cases. *Am J Pathol* 1951; **27**: 885-907 [PMID: 14868767]

108 **Brancatelli G**, Furlan A, Calandra A, Dioguardi Burgio M. Hepatic sinusoidal dilatation. *Abdom Radiol (NY)* 2018; **43**: 2011-2022 [PMID: 29392360 DOI: 10.1007/s00261-018-1465-8]

109 **Lee WM**. Drug-induced hepatotoxicity. *N Engl J Med* 1995; **333**: 1118-1127 [PMID: 7565951 DOI: 10.1056/NEJM199510263331706]

110 **Stoneham S**, Lennard L, Coen P, Lilleyman J, Saha V. Veno-occlusive disease in patients receiving thiopurines during maintenance therapy for childhood acute lymphoblastic leukaemia. *Br J Haematol* 2003; **123**: 100-102 [PMID: 14510948 DOI: 10.1046/j.1365-2141.2003.04578.x]

111 **Nakano H**, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu MP, Dufour P, Bachellier P, Jaeck

D. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**: 118-124 [PMID: 18156931 DOI: 10.1097/SLA.0b013e31815774de]

112 **Ribero D**, Wang H, Donadon M, Zorzi D, Thomas MB, Eng C, Chang DZ, Curley SA, Abdalla EK, Ellis LM, Vauthey JN. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. *Cancer* 2007; **110**: 2761-2767 [PMID: 17960603 DOI: 10.1002/cncr.23099]

113 **Fan CQ**, Crawford JM. Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). *J Clin Exp Hepatol* 2014; **4**: 332-346 [PMID: 25755580 DOI: 10.1016/j.jceh.2014.10.002]

114 **van den Bosch MA**, van Hoe L. MR imaging findings in two patients with hepatic veno-occlusive disease following bone marrow transplantation. *Eur Radiol* 2000; **10**: 1290-1293 [PMID: 10939493 DOI: 10.1007/s003300000330]

115 **Han NY**, Park BJ, Kim MJ, Sung DJ, Cho SB. Hepatic Parenchymal Heterogeneity on Contrast-enhanced CT Scans Following Oxaliplatin-based Chemotherapy: Natural History and Association with Clinical Evidence of Sinusoidal Obstruction Syndrome. *Radiology* 2015; **276**: 766-774 [PMID: 25822471 DOI: 10.1148/radiol.2015141749]

116 **Erturk SM**, Mortelé KJ, Binkert CA, Glickman JN, Oliva MR, Ros PR, Silverman SG. CT features of hepatic venoocclusive disease and hepatic graft-versus-host disease in patients after hematopoietic stem cell transplantation. *AJR Am J Roentgenol* 2006; **186**: 1497-1501 [PMID: 16714636 DOI: 10.2214/AJR.05.0539]

117 **Sangisetty SL**, Miner TJ. Malignant ascites: A review of prognostic factors, pathophysiology and therapeutic measures. *World J Gastrointest Surg* 2012; **4**: 87-95 [PMID: 22590662 DOI: 10.4240/wjgs.v4.i4.87]

118 **Han NY**, Park BJ, Sung DJ, Kim MJ, Cho SB, Lee CH, Jang YJ, Kim SY, Kim DS, Um SH, Won NH, Yang KS. Chemotherapy-induced focal hepatopathy in patients with gastrointestinal malignancy: gadoxetic acid--enhanced and diffusion-weighted MR imaging with clinical-pathologic correlation. *Radiology* 2014; **271**: 416-425 [PMID: 24475862 DOI: 10.1148/radiol.13131810]

119 **Shin NY**, Kim MJ, Lim JS, Park MS, Chung YE, Choi JY, Kim KW, Park YN. Accuracy of gadoxetic acid-enhanced magnetic resonance imaging for the diagnosis of sinusoidal obstruction syndrome in patients with chemotherapy-treated colorectal liver metastases. *Eur Radiol* 2012; **22**: 864-871 [PMID: 22108766 DOI: 10.1007/s00330-011-2333-x]

120 **Iannaccone R**, Federle MP, Brancatelli G, Matsui O, Fishman EK, Narra VR, Grazioli L, McCarthy SM, Piacentini F, Maruzzelli L, Passariello R, Vilgrain V. Peliosis hepatis: spectrum of imaging findings. *AJR Am J Roentgenol* 2006; **187**: W43-W52 [PMID: 16794138 DOI: 10.2214/AJR.05.0167]

121 **Wanless IR**, Huang WY. Vascular disorders. In: Burt A, Portmann B, Ferrell L, editors. MacSween's pathology of the liver. 6th ed. New York: Elsevier, 2012: 618-619.

122 **Xiong WJ**, Hu LJ, Jian YC, He Y, Zhou W, Guo XL, Zheng YX. Focal peliosis hepatis in a colon cancer patient resembling metastatic liver tumor. *World J Gastroenterol* 2012; **18**: 5999-6002 [PMID: 23139621 DOI: 10.3748/wjg.v18.i41.5999]

123 **Yu CY**, Chang LC, Chen LW, Lee TS, Chien RN, Hsieh MF, Chiang KC. Peliosis hepatis complicated by portal hypertension following renal transplantation. *World J Gastroenterol* 2014; **20**: 2420-2425 [PMID: 24605041 DOI: 10.3748/wjg.v20.i9.2420]

124 **Crocetti D**, Palmieri A, Pedullà G, Pasta V, D'Orazi V, Grazi GL. Peliosis hepatis: Personal experience and literature review. *World J Gastroenterol* 2015; **21**: 13188-13194 [PMID: 26675327 DOI: 10.3748/wjg.v21.i46.13188]

125 **Colagrande S**, Tonarelli A, Leone F. [Peliosis hepatis: semiology with ultrasonography, computerized tomography, and magnetic resonance. Report of 2 cases]. *Radiol Med* 1995; **89**: 897-900 [PMID: 7644754]

126 **Brancatelli G**, Vilgrain V, Federle MP, Hakime A, Lagalla R, Iannaccone R, Valla D. Budd-Chiari syndrome: spectrum of imaging findings. *AJR Am J Roentgenol* 2007; **188**: W168-W176 [PMID: 17242224 DOI: 10.2214/AJR.05.0168]

127 **Mungan Z**, Pinarbasi B, Bakir B, Gulluoglu M, Baran B, Akyuz F, Demir K, Kaymakoglu S. Congenital portal vein aneurysm associated with peliosis hepatis and intestinal lymphangiectasia. *Gastroenterol Res Pract* 2009; **2009**: 479264 [PMID: 20368998 DOI: 10.1155/2009/479264]

128 **Gouya H**, Vignaux O, Legmann P, de Pigneux G, Bonnin A. Peliosis hepatis: triphasic helical CT and dynamic MRI findings. *Abdom Imaging* 2001; **26**: 507-509 [PMID: 11503089 DOI: 10.1007/s00261-001-0023-x]

129 **Buelow B**, Otjen J, Sabath AP, Harruff RC. Peliosis hepatis presenting as liver rupture in a vulnerable adult: a case report. *Am J Forensic Med Pathol* 2012; **33**: 307-310 [PMID: 22104329 DOI: 10.1097/PAF.0b013e31823a8b38]

130 **Samyn M**, Hadzic N, Davenport M, Verma A, Karani J, Portmann B, Mieli-Vergani G. Peliosis hepatis in childhood: case report and review of the literature. *J Pediatr Gastroenterol Nutr* 2004; **39**: 431-434 [PMID: 15448437 DOI: 10.1097/00005176-200410000-00024]

131 **Ben Hassen W**, Wagner M, Lucidarme O. Unusual hepatic lesion in a patient with a lung tumor. *Gastroenterology* 2014; **147**: e7-e8 [PMID: 24880008 DOI: 10.1053/j.gastro.2014.02.050]

132 **Cimbanassi S**, Aseni P, Mariani A, Sammartano F, Bonacina E, Chiara O. Spontaneous hepatic rupture during pregnancy in a patient with peliosis hepatis. *Ann Hepatol* 2015; **14**: 553-558 [PMID: 26019044]

133 **Choi SK**, Jin JS, Cho SG, Choi SJ, Kim CS, Choe YM, Lee KY. Spontaneous liver rupture in a patient with peliosis hepatis: a case report. *World J Gastroenterol* 2009; **15**: 5493-5497 [PMID: 19916182 DOI: 10.3748/wjg.15.5493]

134 **Ferrozzi F**, Tognini G, Zuccoli G, Cademartiri F, Pavone P. Peliosis hepatis with pseudotumoral and hemorrhagic evolution: CT and MR findings. *Abdom Imaging* 2001; **26**: 197-199 [PMID: 11178700 DOI: 10.1007/s002610000131]

135 **Hiorns MP**, Rossi UG, Roebuck DJ. Peliosis hepatis causing inferior vena cava compression in a 3-year-old child. *Pediatr Radiol* 2005; **35**: 209-211 [PMID: 15448948 DOI: 10.1007/s00247-004-1311-8]

136 **Savastano S**, San Bortolo O, Velo E, Rettore C, Altavilla G. Pseudotumoral appearance of peliosis hepatis. *AJR Am J Roentgenol* 2005; **185**: 558-559 [PMID: 16037541 DOI: 10.2214/ajr.185.2.01850558]

137 **Furlan A**, Minervini MI, Borhani AA, Dioguardi Burgio M, Tublin ME, Brancatelli G. Hepatic Sinusoidal Dilatation: A Review of Causes With Imaging-Pathologic Correlation. *Semin Ultrasound CT MR* 2016; **37**: 525-532 [PMID: 27986171 DOI: 10.1053/j.sult.2016.08.007]

138 **Terlizzi JP**, Azizi R, Chow MD, Underberg-Davis S, Nosher JL, Stafford PW, Pierre J. Peliosis hepatis in a child with myotubular myopathy: successful treatment using hepatic artery embolization. *J Pediatr Surg* 2013; **48**: e9-e12 [PMID: 23932635 DOI: 10.1016/j.jpedsurg.2013.06.014]

139 **Steinke K**, Terraciano L, Wiesner W. Unusual cross-sectional imaging findings in hepatic peliosis. *Eur Radiol* 2003; **13**: 1916-1919 [PMID: 12942295 DOI: 10.1007/s00330-002-1675-9]

140 **Battal B**, Kocaoglu M, Atay AA, Bulakbasi N. Multifocal peliosis hepatis: MR and diffusion-weighted MR-imaging findings of an atypical case. *Ups J Med Sci* 2010; **115**: 153-156 [PMID: 20095924 DOI: 10.1080/03009730903262118]

141 **Vignaux O**, Legmann P, de Pinieux G, Chaussade S, Spaulding C, Couturier D, Bonnin A. Hemorrhagic necrosis due to peliosis hepatis: imaging findings and pathological correlation. *Eur Radiol* 1999; **9**: 454-456 [PMID: 10087115 DOI: 10.1007/s003300050691]

142 **Qayyum A**, Lee GK, Yeh BM, Allen JN, Venook AP, Coakley FV. Frequency of hepatic contour abnormalities and signs of portal hypertension at CT in patients receiving chemotherapy for breast cancer metastatic to the liver. *Clin Imaging* 2007; **31**: 6-10 [PMID: 17189839 DOI: 10.1016/j.clinimag.2006.09.028]

143 **Jha P**, Poder L, Wang ZJ, Westphalen AC, Yeh BM, Coakley FV. Radiologic mimics of cirrhosis. *AJR Am J Roentgenol* 2010; **194**: 993-999 [PMID: 20308502 DOI: 10.2214/AJR.09.3409]

144 **Adike A**, Karlin N, Menias C, Carey EJ. Pseudocirrhosis: A Case Series and Literature Review. *Case Rep Gastroenterol* 2016; **10**: 381-391 [PMID: 27721722 DOI: 10.1159/000448066]

145 **Battisti S**, Guida FM, Pagliara E, Tonini G, Zobel BB, Santini D. Pseudocirrhosis after anti-EGFR-based neoadjuvant therapy for hepatic metastasis from colon cancer: a different point of view. *Clin Colorectal Cancer* 2014; **13**: e13-e15 [PMID: 25085312 DOI: 10.1016/j.clcc.2014.06.003]

146 **Vuppalanchi R**, Saxena R, Storniolo AMV, Chalasani N. Pseudocirrhosis and liver failure in patients with metastatic breast cancer after treatment with palbociclib. *Hepatology* 2017; **65**: 1762-1764 [PMID: 27397671 DOI: 10.1002/hep.28720]

147 **Lee SL**, Chang ED, Na SJ, Kim JS, An HJ, Ko YH, Won HS. Pseudocirrhosis of breast cancer metastases to the liver treated by chemotherapy. *Cancer Res Treat* 2014; **46**: 98-103 [PMID: 24520229 DOI: 10.4143/crt.2014.46.1.98]

148 **Fennessy FM**, Mortele KJ, Kluckert T, Gogate A, Ondategui-Parra S, Ros P, Silverman SG. Hepatic capsular retraction in metastatic carcinoma of the breast occurring with increase or decrease in size of subjacent metastasis. *AJR Am J Roentgenol* 2004; **182**: 651-655 [PMID: 14975965 DOI: 10.2214/ajr.182.3.1820651]

149 **Sass DA**, Clark K, Grzybicki D, Rabinovitz M, Shaw-Stiffel TA. Diffuse desmoplastic metastatic breast cancer simulating cirrhosis with severe portal hypertension: a case of "pseudocirrhosis". *Dig Dis Sci* 2007; **52**: 749-752 [PMID: 17265127 DOI: 10.1007/s10620-006-9332-9]

150 **Kang SP**, Taddei T, McLennan B, Lacy J. Pseudocirrhosis in a pancreatic cancer patient with liver metastases: a case report of complete resolution of pseudocirrhosis with an early recognition and management. *World J Gastroenterol* 2008; **14**: 1622-1624 [PMID: 18330959 DOI: 10.3748/wjg.14.1622]

151 **Young ST**, Paulson EK, Washington K, Gulliver DJ, Vredenburgh JJ, Baker ME. CT of the liver in patients with metastatic breast carcinoma treated by chemotherapy: findings simulating cirrhosis. *AJR Am J Roentgenol* 1994; **163**: 1385-1388 [PMID: 7992734 DOI: 10.2214/ajr.163.6.7992734]

152 **Gülberg V**, Haag K, Rössle M, Gerbes AL. Hepatic arterial buffer response in patients with advanced cirrhosis. *Hepatology* 2002; **35**: 630-634 [PMID: 11870377 DOI: 10.1053/jhep.2002.31722]

153 **Breen DJ**, Rutherford EE, Stedman B, Lee-Elliott C, Hacking CN. Intrahepatic arterioportal shunting and anomalous venous drainage: understanding the CT features in the liver. *Eur Radiol* 2004; **14**: 2249-2260 [PMID: 15197479 DOI: 10.1007/s00330-004-2334-0]

154 **Golfieri R**, Gavelli G. [Liver hemodynamic changes, pseudolesions and benign nodular flow-dependent lesions. Their pathogenesis and spiral computed tomographic radiological aspects]. *Radiol Med* 2000; **99**: 233-249 [PMID: 10884824]

155 **Nascimento AB**, Mitchell DG, Rubin R, Weaver E. Diffuse desmoplastic breast carcinoma metastases to the liver simulating cirrhosis at MR imaging: report of two cases. *Radiology* 2001; **221**: 117-121 [PMID: 11568328 DOI: 10.1148/radiol.2211001754]

156 **Sonnenblick A**, Appelbaum L, Peretz T. Liver failure on the background of pseudocirrhosis in patients with liver metastasis from breast cancer, who responded to treatment. *Onkologie* 2011; **34**: 199-201 [PMID: 21447980 DOI: 10.1159/000327010]

157 **Burkill GJ**, King LJ, Scurr E, Healy JC. Breast carcinoma metastases to the liver simulating cirrhosis. *Radiology* 2002; **225**: 917; author reply 917-917; author reply 918 [PMID: 12461279 DOI: 10.1148/radiol.2253020336]

158 **Vilgrain V**, Lagadec M, Ronot M. Pitfalls in Liver Imaging. *Radiology* 2016; **278**: 34-51 [PMID: 26690991 DOI: 10.1148/radiol.2015142576]

159 **Romero M**, Palmer SL, Kahn JA, Ihde L, Lin LM, Kosco A, Shinar R, Ghandforoush A, Chan LS, Petrovic LM, Sher LS, Fong TL. Imaging appearance in acute liver failure: correlation with clinical and pathology findings. *Dig Dis Sci* 2014; **59**: 1987-1995 [PMID: 24691628 DOI: 10.1007/s10620-014-3106-6]

160 **Gezer NS**, Başara I, Altay C, Harman M, Rocher L, Karabulut N, Seçil M. Abdominal sarcoidosis: cross-sectional imaging findings. *Diagn Interv Radiol* 2015; **21**: 111-117 [PMID: 25512071 DOI: 10.5152/dir.2014.14210]

161 **Masuda K**, Takenaga S, Morikawa K, Kano A, Ojiri H. Hepatic sarcoidosis with atypical radiological manifestations: A case report. *Radiol Case Rep* 2018; **13**: 936-939 [PMID: 30105085 DOI: 10.1016/j.radcr.2018.06.013]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2021 December 14; 27(46): 7894-7908

DOI: 10.3748/wjg.v27.i46.7894

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

FRONTIER

Hepatic pseudolesions caused by alterations in intrahepatic hemodynamics

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Author contributions: Kobayashi S designed and wrote the manuscript, and collected relevant data and approve the final manuscript.

Conflict-of-interest statement: The author declares that he has no conflict of interest.

Country/Territory of origin: Japan

Specialty type: Anatomy and morphology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

Hepatic pseudolesion may occur in contrast-enhanced computed tomography and magnetic resonance imaging due to the unique haemodynamic characteristics of the liver. The concept of hepatic arterial buffer response (HABR) has become mainstream for the understanding of the mechanism of the reciprocal effect between hepatic arterial and portal venous flow. And HABR is thought to be significantly related to the occurrence of the abnormal imaging findings on arterial phase of contrast enhanced images, such as hepatic arterial-portal vein shunt and transient hepatic attenuation difference, which mimic hypervascular tumor and may cause clinical problems. Third inflow to the liver also cause hepatic pseudolesion, and some of the cases may show histopathologic change such as focal hyperplasia, focal fatty liver, and focal sparing of fatty liver, and called pseudotumor. To understand these phenomena might be valuable for interpreting the liver imaging findings.

Key Words: Pseudolesion; Focal sparing of fatty liver; Computed tomography; Hepatic blood flow; Hepatic hemodynamics; Hyperplastic change

Core Tip: Understanding the characteristics of hepatic blood flow and the pathophysiology of pseudolesions caused by alterations in intrahepatic hemodynamics is important for diagnostic imaging of liver lesions. The concept of hepatic arterial buffer response, a unique mechanism for regulating hepatic blood flow, might be essential for elucidating the pathogenesis of hepatic arterial-portal vein shunting and transient hepatic attenuation difference on dynamic contrast-enhanced imaging of the liver. In addition, some pseudolesions are associated with histopathologic changes such as focal hyperplasia, focal fatty liver, and focal sparing of fatty liver. Understanding these

Received: April 19, 2021
Peer-review started: April 19, 2021
First decision: June 23, 2021
Revised: June 23, 2021
Accepted: November 25, 2021
Article in press: November 25, 2021
Published online: December 14, 2021

P-Reviewer: Chen Q
S-Editor: Gong ZM
L-Editor: A
P-Editor: Gong ZM

phenomena may aid in interpreting liver imaging findings.

Citation: Kobayashi S. Hepatic pseudolesions caused by alterations in intrahepatic hemodynamics. *World J Gastroenterol* 2021; 27(46): 7894-7908
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7894.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7894

INTRODUCTION

Hepatocellular carcinoma (HCC) is among the leading causes of cancer-related death, and colorectal carcinoma is prone to induce hepatic metastasis. Thus, there is a growing need to develop diagnostic imaging techniques that can properly identify localized malignancies in the liver.

Ultrasound is widely available, making it helpful for screening patients with hepatic mass lesions. However, given its lack of objectivity, computed tomography (CT) and magnetic resonance (MR) imaging are mainly utilized for closer examination of potentially malignant lesions.

The liver is an organ with a unique blood supply involving two types of inflow vessels: the hepatic artery and portal vein. The differential diagnosis of hepatic masses is made using imaging findings observed in dynamic contrast-enhanced studies, including hepatic arterial phase, portal venous phase, and equilibrium phase images. However, unique hemodynamic characteristics of the liver may lead to the occurrence of pseudolesions on contrast-enhanced images[1].

Radiologically, a pseudolesion is defined as a focal mass-like finding observed only on diagnostic imaging, without any actual histopathological abnormality[1]. Hepatic pseudolesions represent an important imaging challenge because they sometimes present findings similar to those of hepatic malignancy. In addition, some pseudolesions may cause focal parenchymal changes due to localized impairments in blood flow compared to the surrounding hepatic parenchyma, and such pseudolesions are referred to as pseudotumors[2].

Given their importance in the diagnostic imaging of liver lesions, we first introduce the characteristics of hepatic blood flow, following which we describe the mechanisms by which alterations in intrahepatic hemodynamics can lead to pseudolesions. Finally, we review hepatic parenchymal changes that occur in the region containing the intrahepatic hemodynamic abnormality.

OVERVIEW OF HEPATIC BLOOD FLOW

The liver is the largest parenchymal organ in the abdomen. It differs from other abdominal parenchymal organs in that there are two types of inflow vessels: the hepatic artery and the portal vein. Total hepatic blood flow is approximately 800-1200 mL/min (approximately 100 mL/min per 100 g liver wet weight). The portal vein supplies 75%-80% of the hepatic blood flow, while the hepatic artery supplies the remaining percentage. Hepatic blood volume is approximately 25-30 mL/100 g liver weight, representing roughly 10%-15% of the total blood volume. The average pressure in the hepatic artery is almost the same as aortic pressure; in contrast, portal vein pressure is approximately 6-10 mmHg in humans, while hepatic venous pressure is approximately 2-4 mmHg[3].

The portal vein collects blood from the splenic, gastric, superior mesenteric, and inferior mesenteric veins and flows into the liver through the hepatic hilum. Portal blood is mostly composed of blood from the gastrointestinal tract, and portal blood flow varies greatly depending on the feeding state. That is, portal blood flow increases after ingestion and decreases during fasting.

Hepatic arterial blood is rich in oxygen, and the peripheral hepatic arterial branches — either directly or after forming a capillary plexus around the bile duct and nourishing the bile duct — flow into sinusoids to supply oxygen to the hepatocytes and other structures.

The ratio of portal to hepatic arterial blood inflow to the liver is approximately 3:1, and the oxygen supply is mainly bestowed by hepatic arteries. Researchers have assumed that there is a complementary interaction between portal blood flow and hepatic arterial blood flow, meaning that hepatic arterial blood flow increases when portal blood flow decreases and that an increase in portal blood flow compensates for a decrease in hepatic arterial blood flow[2].

Several mutual routes of communication connect the hepatic artery and portal vein within the liver, including the trans-sinusoidal route, tumor thrombus-induced transvasal route, transtumoral shunt, transplexal route (peribiliary route), and arterio-portal fistula[4,5]. Among these connecting routes, the trans-sinusoidal route may represent the main complementary interaction between portal and hepatic arterial blood flow.

Recent studies have proposed the concept of hepatic arterial buffer response (HABR) for understanding the mechanism underlying the reciprocal effect between hepatic arterial and portal venous flow. As portal blood flow increases or decreases depending on the activity of the gastrointestinal tract, the liver has no control over portal blood flow. Therefore, when portal blood flow is reduced, hepatic arterial blood flow is controlled to maintain hepatic blood flow (*i.e.*, the oxygen supply to the liver) [6].

To elaborate, the space of Mall, which surrounds the terminal branches of the portal vein and hepatic artery before they drain into the hepatic sinusoid, constantly secretes adenosine, a vasoactive substance that serves to dilate the hepatic artery. When the normal portal blood flow is abundant, adenosine in the space of Mall is washed away by the influence of portal blood flow and does not dilate the hepatic arteries. However, when portal blood flow decreases, adenosine remains in the space of Mall, dilates the hepatic artery, and increases hepatic arterial flow to compensate for the decrease in portal blood flow to maintain hepatic sinusoidal blood flow. This is called the adenosine wash-out theory[7-9]. This mechanism of hepatic artery dilatation takes place in the hepatic arteriole, the distal part of the intrahepatic hepatic artery within the portal tract. HABR is thought to be significantly related to abnormal imaging findings observed on contrast-enhanced arterial phase images, such as hepatic arterial-portal vein shunting (AP shunting) and transient hepatic attenuation difference (THAD), as described below (Figure 1).

DEFINITIONS OF AND IMAGING FINDINGS ASSOCIATED WITH AP SHUNTING AND THAD

Choi *et al*[4] defined AP shunting as an organic or functional communication between the hepatic arterial branch and the portal venous system, resulting in the redistribution of arterial flow into a focal region of the portal venous flow. When blood flow through the portal vein is diminished or absent, the hepatic artery takes over perfusion of the liver through the AP shunt[4]. When portal vein obstruction occurs, increased hepatic arterial blood flow occurs mainly through the peribiliary plexus[10]. Namely, AP shunting is a consequence of the HABR mechanism.

On dynamic contrast-enhanced images, AP shunting is associated with (1) early enhancement of peripheral portal vein branches before the central portal vein is enhanced; and (2) THAD[4] (Figure 1).

THAD refers to transient, peripheral, wedge-shaped hepatic parenchymal enhancement (usually with a straight margin) that occurs during the hepatic arterial phase of contrast-enhanced imaging[10]. This phenomenon arises because increased arterial flow compensates for decreased portal venous flow and because the inflow of contrast material from a high-pressure arterial blood system into a low-pressure portal branch opacifies the focal area of the liver, while contrast material in the adjacent parenchyma is diluted by the unenhanced portal venous flow[4]. On portal venous phase images, the involved site returns to normal or nearly normal attenuation. Normal vessels pass through the area of THAD, and this finding can aid in differentiating THAD from hypervascular liver tumors such as HCC on contrast-enhanced imaging.

CAUSES OF LOCALIZED INTRAHEPATIC HEMODYNAMIC ALTERATIONS

The ratio of portal blood flow to hepatic artery blood flow is usually considered to be

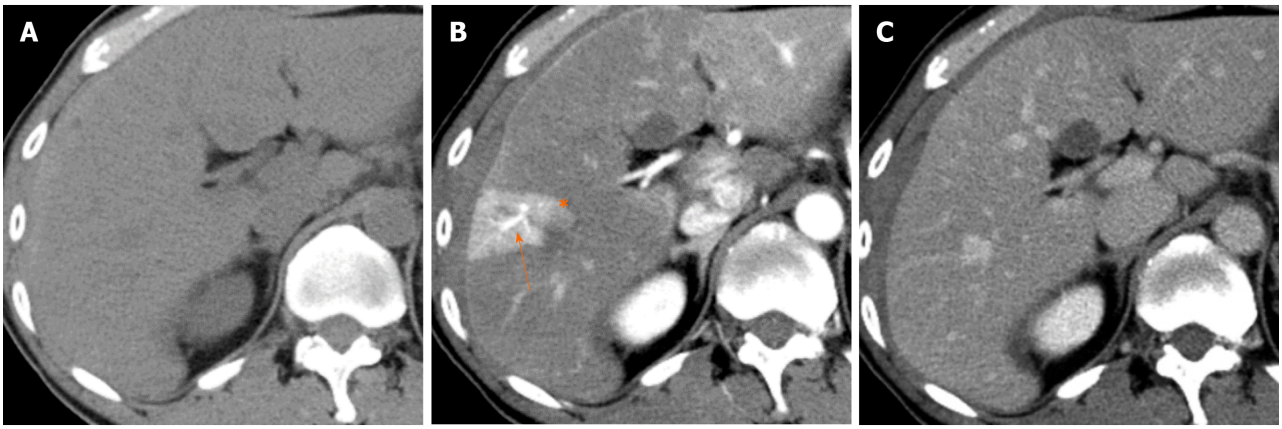

Figure 1 Arterial-portal vein shunt of 40th male. A: Pre contrast enhanced computed tomography (CT) of the liver shows no definite focal liver mass in segment V of the right lobe. B: On arterial phase contrast enhanced CT image, wedge shaped focal enhanced area is observed in peripheral part of segment V of the liver (*). Well opacified portal vein branch is observed within the focal enhanced area (arrow). C: On equilibrium phase of contrast enhanced CT, there are no attenuation differences in the peripheral part of segment V of the liver. Therefore, focal enhanced area observed in arterial phase contrast enhanced CT image is diagnosed as transient hepatic attenuation difference.

approximately 3:1 within the liver. Local disruption of this ratio leads to focal changes in blood flow on contrast-enhanced CT and MR images. The causes of intrahepatic hemodynamic changes include increased or decreased hepatic arterial blood flow, increased or decreased portal vein blood flow, and decreased hepatic venous blood flow. Anatomical variations can also cause intrahepatic hemodynamic changes.

Increased or decreased flow in regular liver vessels

Causes of increased hepatic arterial blood flow include HABR due to decreased portal blood flow and the presence of congenital or acquired shunt pathways [*e.g.*, hepatic AP shunts), hepatic arterial–hepatic venous shunts (AV shunts)], hereditary hemorrhagic telangiectasia (Figure 2), hepatic trauma, and others[11].

Physiological causes of increased portal blood flow include diurnal variations that occur with food intake, while pathological causes include "small-for-size grafts" at liver transplantation.

Causes of reduced portal blood flow include extrahepatic portal obstruction (Figure 3), portal vein thrombosis, portal vein tumor thrombus (Figure 4), agenesis of the portal vein (congenital porto-systemic shunt), patent ductus venosus, and porto-sinusoidal vascular disease (formerly known as idiopathic portal hypertension). In addition, blood flow from the portal vein to hepatic sinusoids is reduced in patients with liver cirrhosis exhibiting porto-systemic shunting.

Causes of decreased hepatic venous blood flow include Budd-Chiari syndrome (BCS) (Figure 5), sinusoidal obstruction syndrome (SOS) (Figure 6), hepatic vein thrombosis, and hepatic vein tumor thrombus. Secondary to these lesions, the liver exhibits a state of hepatic congestion. Increased hepatic venous pressure leading to decreased hepatic venous blood flow may also occur in patients with congestive heart failure and those who have undergone the Fontan procedure.

Localized portal hypoperfusion and the associated focal increases in hepatic arterial blood flow due to HABR are associated with THAD and the presence of hypervascularized pseudolesions on contrast-enhanced images due to increased inflow of contrast medium into the sinusoids during the arterial phase[12,13] (Figures 1-4). In contrast, when hepatic venous blood flow is decreased due to obstruction of hepatic venous outflow, blood drainage from the sinusoids toward the inferior vena cava is stagnant, and the sinusoidal pressure increases. This results in a decrease in the inflow of low-pressure portal blood into the sinusoids and an increase in the inflow of hepatic arterial blood, which causes reticular heterogeneous staining on arterial and portal phase contrast-enhanced images (Figures 5 and 6). This type of contrast-enhanced imaging finding is usually observed in patients with congestive liver, which occurs secondary to congestive heart failure, BCS, and SOS. Thus, when arterial phase contrast-enhanced images show THAD or reticular staining of the liver parenchyma, the presence of intrahepatic hemodynamic abnormalities can be inferred.

In addition to focal alterations in intrahepatic hemodynamics, anatomical variations in the portal venous system and other characteristic anatomical features of the vessel surrounding the liver can cause focal hemodynamic changes in several specific

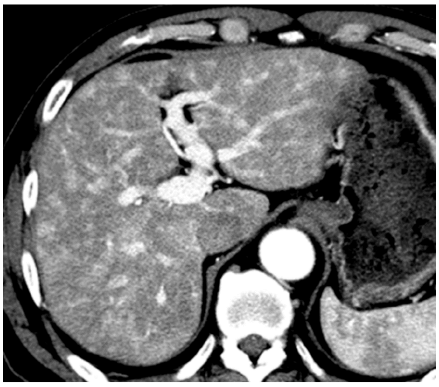

Figure 2 Hereditary hemorrhagic telangiectasia of 70th male. On arterial phase contrast enhanced computed tomography of the liver, there are multiple pathy attenuated areas throughout the liver. Which are multiple transient hepatic attenuation difference caused by multiple arterial-portal venous shunts in hereditary hemorrhagic telangiectasia.

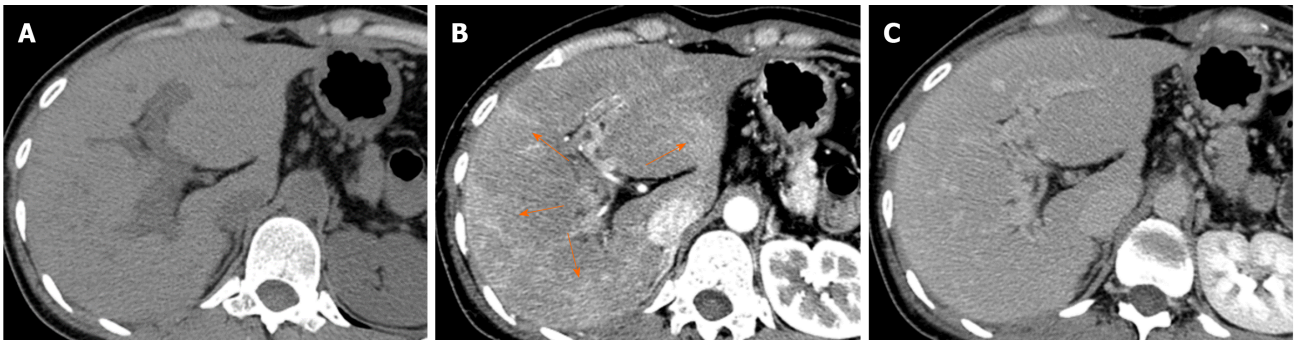

Figure 3 Extrahepatic portal obstruction of 30th female. A: Pre contrast enhanced computed tomography (CT) of the liver shows no definite focal liver lesion. B: On arterial phase contrast enhanced CT of the liver, there are multiple pathy attenuated areas in the subcapsular peripheral portion of the liver (arrow). C: On equilibrium phase contrast enhanced CT of the liver, there are no attenuation differences in the peripheral part of the liver.

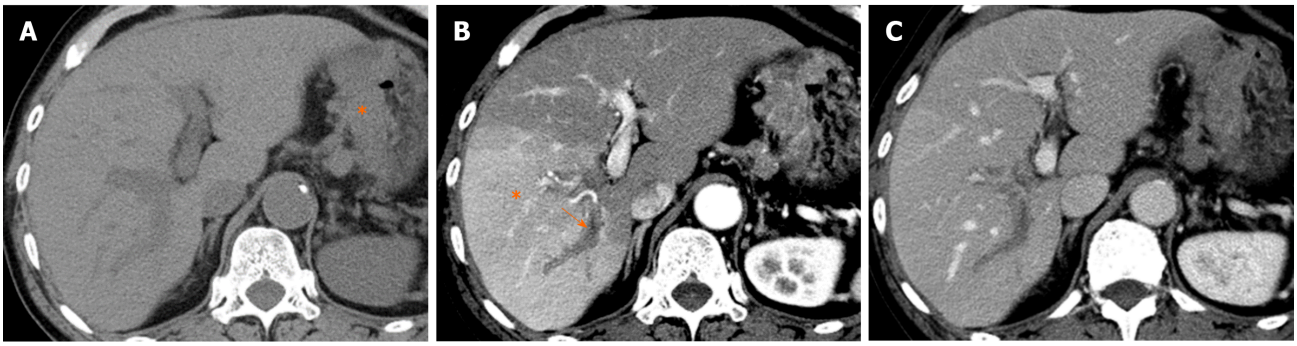

Figure 4 Portal vein tumor thrombus of gastric cancer in 70th male. A: Pre contrast enhanced computed tomography (CT) of the liver shows no definite focal liver lesion. Wall thickening on the lesser curvature side of the stomach caused by gastric cancer is observed (*). B: On arterial phase contrast enhanced CT image, focal segmental enhanced area is observed in the right lobe of the liver (*). There is hypovascular tumor thrombus within the right portal vein (arrow). C: On equilibrium phase contrast enhanced CT of the liver, there are no attenuation differences in the liver.

portions of the liver[14].

Third inflow

The parabiliary venous system, epigastric–paraumbilical venous system, and cholecystic vein directly enter the liver independently of the portal venous system. These vessels are called the "third inflow," referring to the third hepatofugal flow after the hepatic arterial and portal vein systems[15].

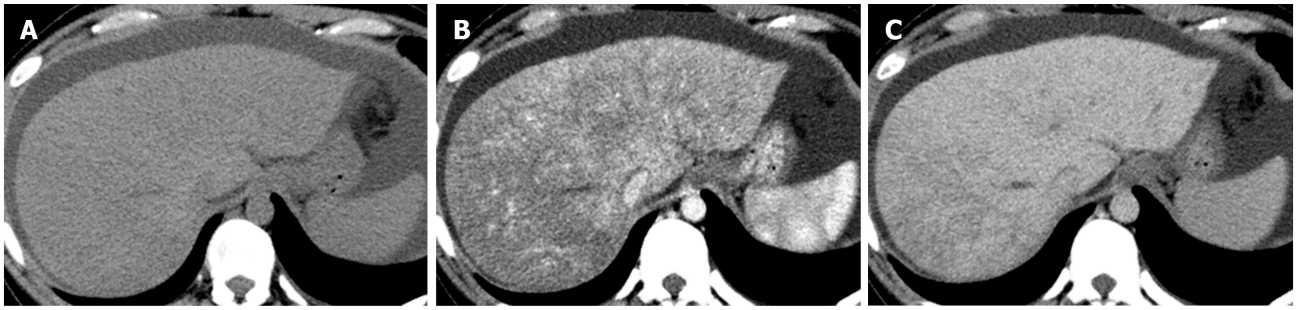

Figure 5 Acute Budd-Chiari syndrome of 20th female. A: Pre contrast enhanced computed tomography (CT) of the liver shows no definite focal liver lesion in the liver. B: On portal phase contrast enhanced CT image, Irregular reticular hypo-enhancement is observed in the liver, which is caused by congestive change induced by hepatic outflow obstruction of Budd-Chiari syndrome. C: On equilibrium phase contrast enhanced CT of the liver, although intrahepatic parenchymal attenuation differences of the liver in the left lobe of the liver have disappeared, minimal attenuation differences are still observed in the right lobe of the liver.

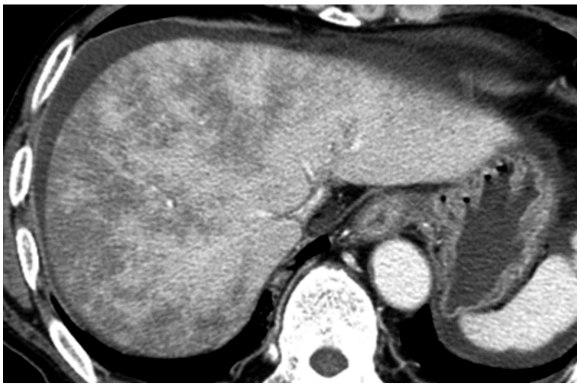

Figure 6 Sinusoidal obstruction syndrome after umbilical cord blood transplantation to acute myelocytic leukemia in 60th male. Portal phase image of contrast enhanced computed tomography shows irregular reticular hypodensity which are caused by hepatic congestion caused by sinusoidal portal flow disturbance.

Parabiliary venous system

The parabiliary system, termed the pancreatico-pyloro-duodenal vein, collects venous blood from the pancreatic head, stomach, and duodenum and usually joins the main portal vein outside the liver before flowing into the liver. However, in some cases, the pancreatico-pyloro-duodenal vein does not connect to the main portal vein before it enters the liver, instead directly entering the liver and perfusing the hepatic sinusoids at the posterior aspect of segment IV without fusion to the main portal vein. As this venous system includes the right gastric vein, this anatomical variation is sometimes referred to as "aberrant right gastric vein"[17]. The incident of aberrant right gastric vein varies from 1.5% to 49%[16-18], while the incidence of aberrant left gastric vein varies from 0.8% to 4%[18,19].

Although most aberrant gastric veins enter the liver and perfuse the hepatic sinusoids at the posterior aspect of segment IV (Figure 7), some may enter the liver and perfuse the liver parenchyma at the posterior edge of segment II or III[20] (Figure 8).

Epigastric–paraumbilical venous system

The paraumbilical vein is divided into the vein of Burow, superior vein of Sappey, and inferior vein of Sappey[21]. Among them, under conditions of portal hypertension, the inferior vein of Sappey is often dilated and forms a porto-systemic collateral pathway connected with the portal system in the anterolateral part of segment IV adjacent to the falciform ligament and epigastric veins.

When superior vena cava obstruction occurs, hyperenhancement of segment IV (*i.e.*, quadrate lobe hot-spot sign) can be observed on contrast-enhanced CT/MR images[22] (Figure 9). This phenomenon is the result of the inferior vein of Sappey acting as the hepatopetal collateral route.

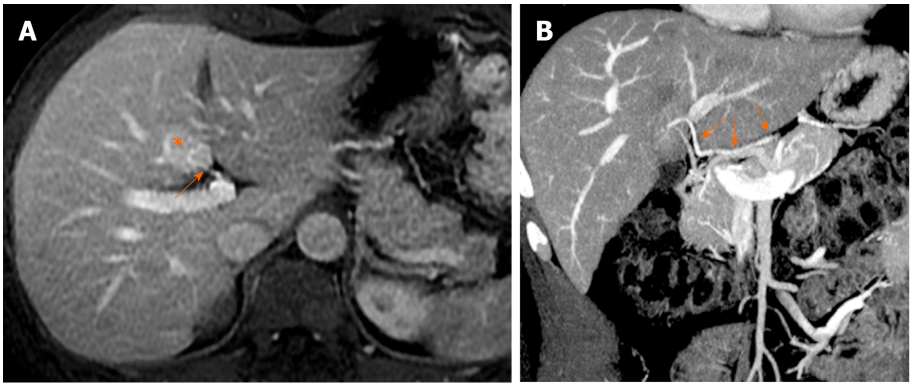

Figure 7 Hypervascular pseudolesion observed in posterior aspect of segment IV (50th male). A: On portal phase image of contrast enhanced magnetic resonance imaging with Gadolinium based contrast agent, focal hyper-attenuation area is observed in posterior aspect of segment IV of the liver (*). Tiny vascular branch is directly entering the area at hepatic hilum, which is an aberrant right gastric vein directly entering to the liver (arrow). B: On coronal reconstruction of portal phase image of contrast enhanced computed tomography, aberrant right gastric vein directly enters the posterior aspect of segment IV of the liver without fusion to the main portal vein (arrows).

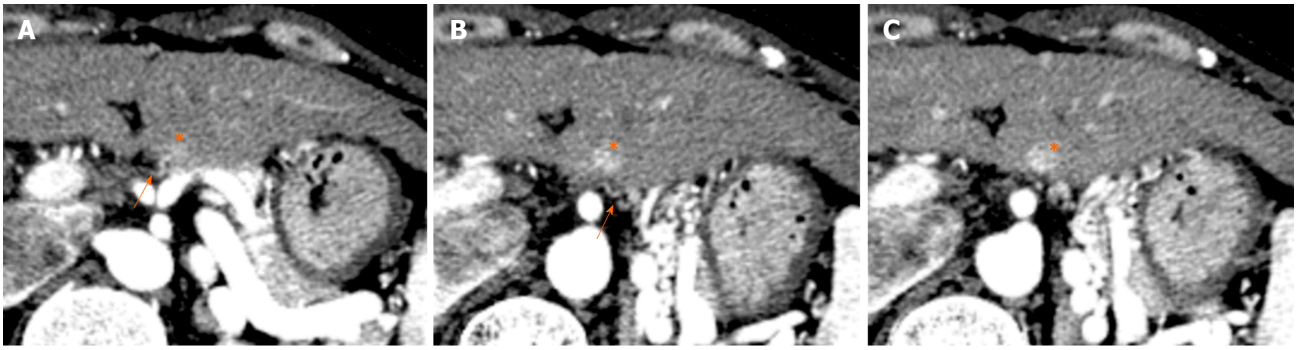

Figure 8 Hypervascular pseudolesion observed in posterior aspect of segment II (40th female). A-C: On arterial phase images of contrast enhanced computed tomography, tiny focal hyper-attenuation area is observed in posterior aspect of segment II of the liver (*). Tiny vascular branch is directly entering the area from outside of the liver, which is an aberrant left gastric vein directly entering to the liver (arrows).

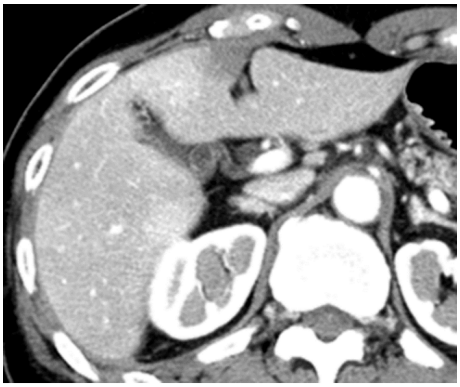

Figure 9 Hypovascular pseudolesion in the drainage area of the vein of Sappey (70th female). On arterial phase contrast enhanced computed tomography (CT) image, focal hypoattenuation area is observed in anterior portion of segment IV of the liver adjacent to the falciform ligament, which is not detected on both pre-contrast CT and equilibrium phase contrast enhanced CT (images are not shown). This is hypovascular pseudolesion in the drainage area of the vein of Sappey.

Cholecystic veins

The blood supply and drainage of the gallbladder are also related to the occurrence of focal hepatic hemodynamic changes. Arterial supply of the gallbladder is provided by the hepatic artery (mainly the right hepatic artery[23]), and the cholecystic vein drains into the liver sinusoids surrounding the gallbladder usually after being connected to the peripheral branch of the intrahepatic portal vein. In detail, cholecystic venous

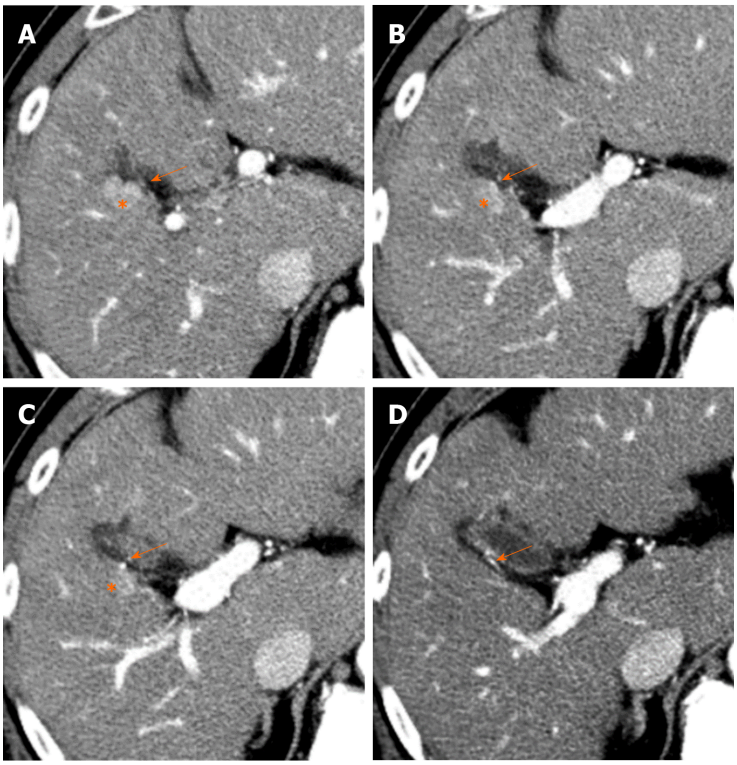

Figure 10 Hypervascular pseudolesion in cholecystic venous drainage area (50th male). A-D: On sequential images of arterial phase contrast enhanced computed tomography, round hyper-attenuation area is observed in segment V of the liver adjacent to the gallbladder (*). Tiny enhanced vessel is directly entered to the enhanced liver area from the gallbladder wall (arrows), which is cholecystic venous drainage to the liver and hypervascular pseudolesion in cholecystic venous drainage area.

blood most frequently enters the peripheral portal branches of liver segments V (96%) and IV (93%)[23]. As the cholecystic venous blood originates from the hepatic artery, the concentration of nutrients and humoral factors such as hormones, which flow into the hepatic sinusoids of the cholecystic venous drainage area, differs from that in other hepatic sinusoids into which the portal venous blood flows. Such differences in the composition of influx blood between the cholecystic venous drainage area and the rest of the liver can cause focal differences in contrast-enhanced imaging and histopathological findings (Figure 10).

FOCAL PARENCHYMAL CHANGES IN THE LIVER DUE TO HEMODYNAMIC ALTERATIONS

Focal alterations in intrahepatic hemodynamics may not only present as pseudolesions on contrast-enhanced images but may also result in histological changes of the liver parenchyma at the site of the blood flow change. There are three major patterns of such histological changes in the liver parenchyma.

Focal hyperplasia

Focal hyperplasia of the liver is observed in patients with cirrhotic liver and may be related to the presence of anomalous portal flow such as aberrant gastric venous drainage. Matsui *et al*[24] reported that in patients with cirrhotic liver with aberrant gastric venous drainage, 22%-50% of cases are associated with focal hyperplastic changes at the posterior aspect of segment IV, where the aberrant gastric venous drainage is present[24] (Figure 11). Focal hyperplastic changes such as anomalous portal venous drainage in the caudate lobe have also been reported in cases of cirrhotic liver, with the authors surmising that the etiology of such hyperplastic changes is intimately related to the anomalous portal flow[25,26]. Similarly, focal hyperplasia with anomalous portal flow in the caudate lobe has also been reported in a patient without cirrhosis[27].

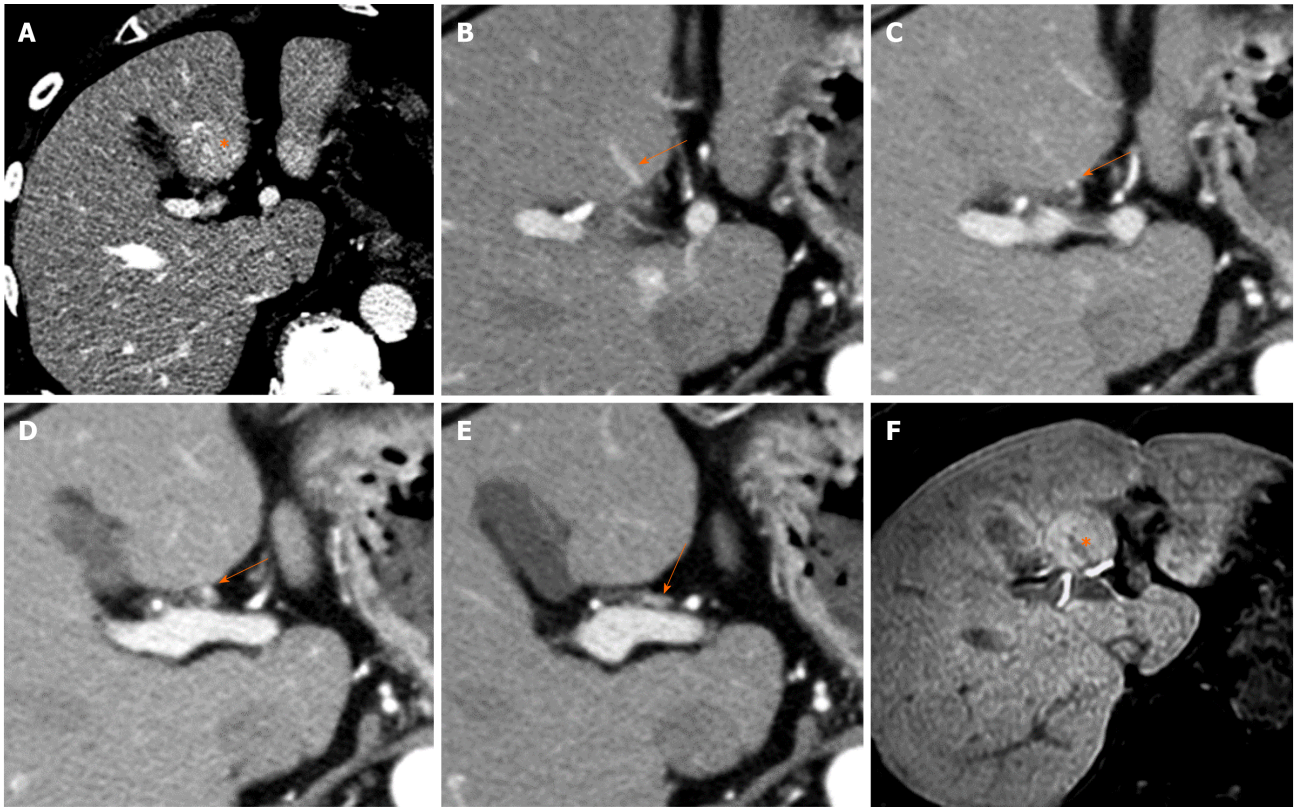

Figure 11 Focal hyperplastic change in posterior aspect of segment IV (70th male). A: On the portal phase contrast enhanced computed tomography (CT) image, focal hyper-attenuation area is observed in the posterior aspect of segment IV of the liver (*), which is not detected on both pre-contrast CT and equilibrium phase contrast enhanced CT (images are not shown). This is hypervascular pseudolesion observed in the area of aberrant right gastric venous drainage to the liver. B-E: On sequential images of arterial phase contrast enhanced CT, aberrant right gastric vein directly entering to the posterior aspect of segment IV of the liver is well opacified (arrows). F: On hepatobiliary phase of Gd-EOB-DTPA enhanced magnetic resonance imaging, slightly hyper-intensity area is observed in the same place of focal hyper-attenuation area observed in the portal phase contrast enhanced CT image (*), which represents focal hyperplasetic change of the liver in aberrant right gastric venous drainage area in the posterior aspect of segment IV.

Researchers have examined the etiology of liver hyperplasia occurring after hepatectomy or portal vein embolization. At present, it is believed that blood flow, shear stress, and adenosine are involved in the development of hyperplasia in the liver [28]. Studies on liver regeneration after hepatectomy or portal vein embolization have also suggested that hyperplasia and atrophy of the liver occur when the liver is unable to compensate for changes in blood flow caused by surgery or portal vein embolization. Several studies support the hypothesis that after portal vein embolization, portal blood flow increases in the unembolized liver lobe, which causes acute portal hypertension. This change leads to increased shear stress and nitric oxide production, which in turn triggers liver regeneration[29-31]. In contrast, decreased intrahepatic shear stress is thought to induce liver atrophy[31]. This mechanism is thought to maintain the ratio between liver mass and blood flow, most likely to ensure maintenance of adequate clearance function[31].

In small-for-size syndrome after liver transplantation, HABR reduces hepatic arterial flow due to excessive portal flow, which leads to decreased oxygen delivery to the liver parenchyma. The lack of adequate oxygen for liver regeneration increases the risk of liver failure. However, normalization of portal pressure and portal blood flow is believed to improve liver regeneration[7]. Thus, the degree of hepatic blood flow, especially portal blood flow, plays a major role in liver regeneration and atrophy.

Ethanol consumption is also involved in liver regeneration and atrophy. Gluud *et al* [32] observed that the frequency of hyperplastic nodules decreased with higher ethanol intake in patients with cirrhosis, indicating that ethanol consumption may inhibit liver regeneration[32]. Histopathologically, ethanol intake causes damage to the hepatic veins, especially perivenular fibrosis[33]. Impaired hepatic veins lead to decreased outflow of sinusoidal blood, resulting in stasis of blood flow and hepatic congestion, thereby inducing liver atrophy.

Focal hyperplastic changes are observed in the aberrant right gastric venous drainage area in patients with alcoholic cirrhotic liver[24]. One possible explanation

for this is the difference in blood ethanol concentrations of the main portal and aberrant right gastric vein. In humans, 20% of ingested ethanol is absorbed through the stomach, while 80% is absorbed through the small intestine[34]. This means that the ethanol concentration in the venous blood of the small intestine is higher than that in the venous blood of the stomach. Normally, both the venous blood from the stomach and small intestine join and form the main portal vein before flowing into the liver, so there is no difference in ethanol concentration in the portal venous blood within the liver. However, in the presence of aberrant right gastric venous drainage, hepatic venous injury is less likely to occur at the drainage area because the ethanol concentration is lower in the gastric vein. In contrast, in the area perfused by the portal flow, hepatic venous injury is more likely to occur as the blood flow of the main portal vein collects the venous flow of the small intestine, which contains higher ethanol concentration.

As a result, most of the liver areas that receive blood flow from the main portal vein exhibit congestion due to hepatic vein injury, while the area supplied by the aberrant right gastric venous drainage exhibits less extensive hepatic vein injury and less severe congestion than the rest of the liver. Hepatic atrophy occurs in areas with severe hepatic congestion, while areas with less hepatic congestion are relatively hyperplastic. This may be why focal hyperplasia occurs in the aberrant right gastric venous drainage area in patients with alcoholic cirrhosis.

Similarly, hepatic venous injury is less likely to occur at the cholecystic venous drainage area surrounding the gallbladder in segments IV and V of the liver due to the relatively low amount of ethanol in the venous blood compared to the rest of the liver. Therefore, focal hyperplastic changes may also occur in this area in patients with alcoholic cirrhosis (Figure 12).

The contribution of hepatic venous drainage to liver deformity and atrophy has also been noted in patients with conditions other than alcoholic liver disease. Ozaki *et al*[35] demonstrated that, because the diameter of the middle hepatic vein is small and venous blood pressure is high compared to that in the other hepatic veins, the middle hepatic venous drainage area tends to exhibit congestive changes. Such changes lead to selective atrophy of the middle hepatic venous drainage area (*e.g.*, segment IV) as well as relative hyperplastic changes in the right and left hepatic venous drainage area [35].

Other factors that may contribute to focal hyperplastic changes in the third inflow area include different concentrations of bile acid in the main portal vein and the third inflow. Previous studies have reported that enterohepatic circulation is involved in liver regeneration[36], and that decrease in bile acid return to the liver triggers hepatocyte proliferation[37]. Because bile acids circulate in the gut–liver axis, the concentration of bile acids in the third inflow is lower than that in the main portal venous flow. Therefore, differences in the concentration of bile acids in the inflow may contribute to the development of focal hyperplasia of the liver parenchyma in the aberrant venous drainage area[38-40].

Localized hepatocellular hyperplastic changes in the normal liver that mimic liver neoplasms on imaging are referred to as focal nodular hyperplasia (FNH). Researchers have proposed that the pathogenesis of FNH is related to a disturbance of sinusoidal blood outflow[41-43] or to the presence of abnormal anomalous vessels[44]. These studies indicate that localized alterations in intrahepatic hemodynamics play a major role in the pathogenesis of FNH.

In summary, the presence of intrahepatic hemodynamic alterations is essential for the development of focal hyperplastic changes in the liver. Such changes are also influenced by concomitant factors such as differences in the blood concentrations of nutrients, ethanol, hormones, and so on at the site. Further research is required to elucidate the mechanism underlying the development of focal hyperplastic changes in the liver.

Focal fat deposition

Focal fat deposition in the liver is occasionally observed in the anteromedial portion of segment IV (adjacent to the falciform ligament)[45] and in the posterior aspect of segment IV[46] (Figures 13 and 14). Focal fat deposition at the posterior aspect of segment IV is related to the presence of aberrant right gastric venous drainage[47], while that at the anteromedial portion adjacent to the falciform ligament is related to the presence of inferior vein of Sappey drainage[47,48].

Vilgrain *et al*[49] suggest that differences in the concentration of insulin in the blood entering the liver contribute to focal fat deposition in the liver. As an aberrant right gastric vein may collect venous blood from the head of the pancreas and flow into the posterior aspect of segment IV, the concentration of insulin may in turn be higher in

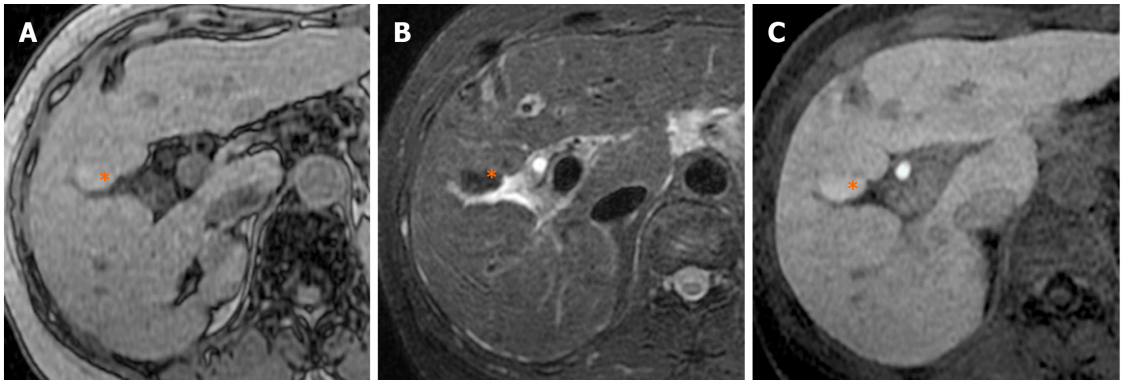

Figure 12 Focal hyperplastic change in cholecystic venous drainage area (50th male). A: On T1 weighted opposed-phase magnetic resonance (MR) image, focal hyperintense lesion is observed at segment IV of the liver adjacent to the gallbladder (*). B: The focal lesion shows hypointensity on fat suppressed T2 weighted MR image (*). C: The focal lesion shows hyperintensity on hepatobiliary phase of Gd-EOB-DTPA enhanced magnetic resonance imaging (*). These findings observed at segment IV of the liver adjacent to the gallbladder represent focal hyperplastic change of the liver in cholecystic venous drainage area.

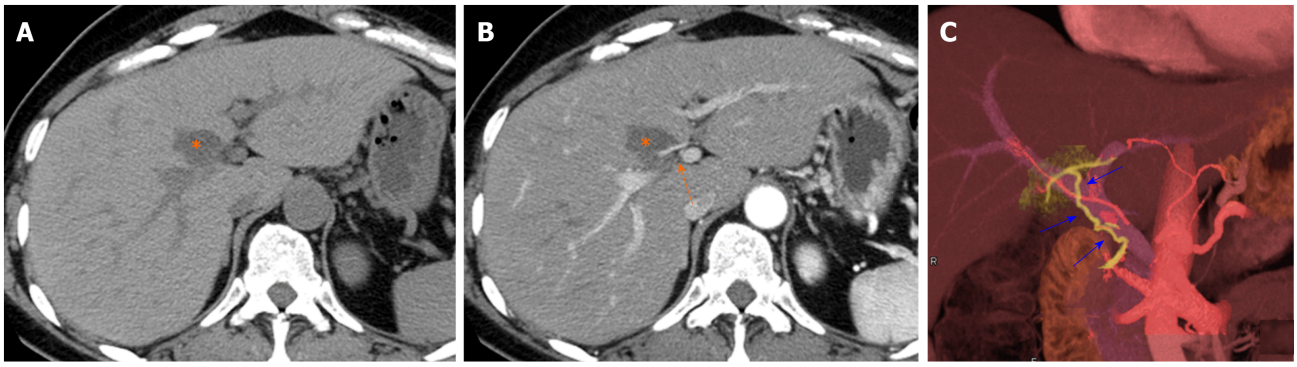

Figure 13 Focal fat deposition in posterior aspect of segment IV (50th female). A: On pre-contrast enhanced computed tomography (CT), focal hypodense lesion is observed at the posterior aspect of segment IV of the liver (*). B: On arterial phase contrast enhanced CT image, the lesion shows hypodense (*) and an enhanced vascular branch is directly entering the area at hepatic hilum (arrow). C: On three-dimensional reconstructed CT image with contrast enhancement, an aberrant right gastric vein directly drains into the posterior aspect of segment IV of the liver without connecting the main portal vein is observed (arrows).

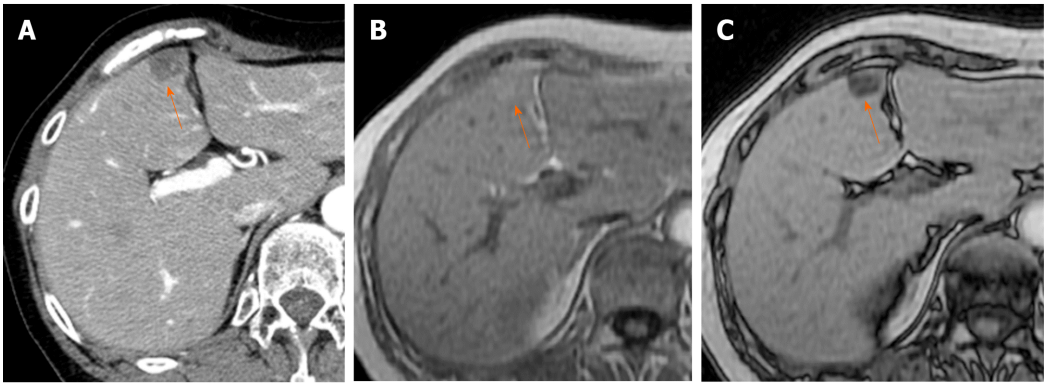

Figure 14 Focal fat deposition in the drainage area of the vein of Sappey (60th female). A: On arterial phase contrast enhanced computed tomography image, focal hypoattenuation area is observed in anterior portion of segment IV of the liver adjacent to the falciform ligament (arrow). B and C: On T1 weighted in-phase and opposed-phase image of the liver, the lesion shows hyperintense on in-phase (B, arrow) and shows hypointense on opposed-phase (C, arrow), which represent focal fat deposition of the liver at the drainage area of inferior vein of Sappey.

the inflow area of the aberrant right gastric vein than in other areas. This may lead to focal fat deposition in the posterior aspect of segment IV, where aberrant right gastric venous drainage is present.

Focal fat deposition is also observed in the hepatic parenchyma surrounding the metastasis of pancreas islet cell tumors, which produce insulin. The etiology of focal fat deposition in such cases may be the same as that described above[49,50].

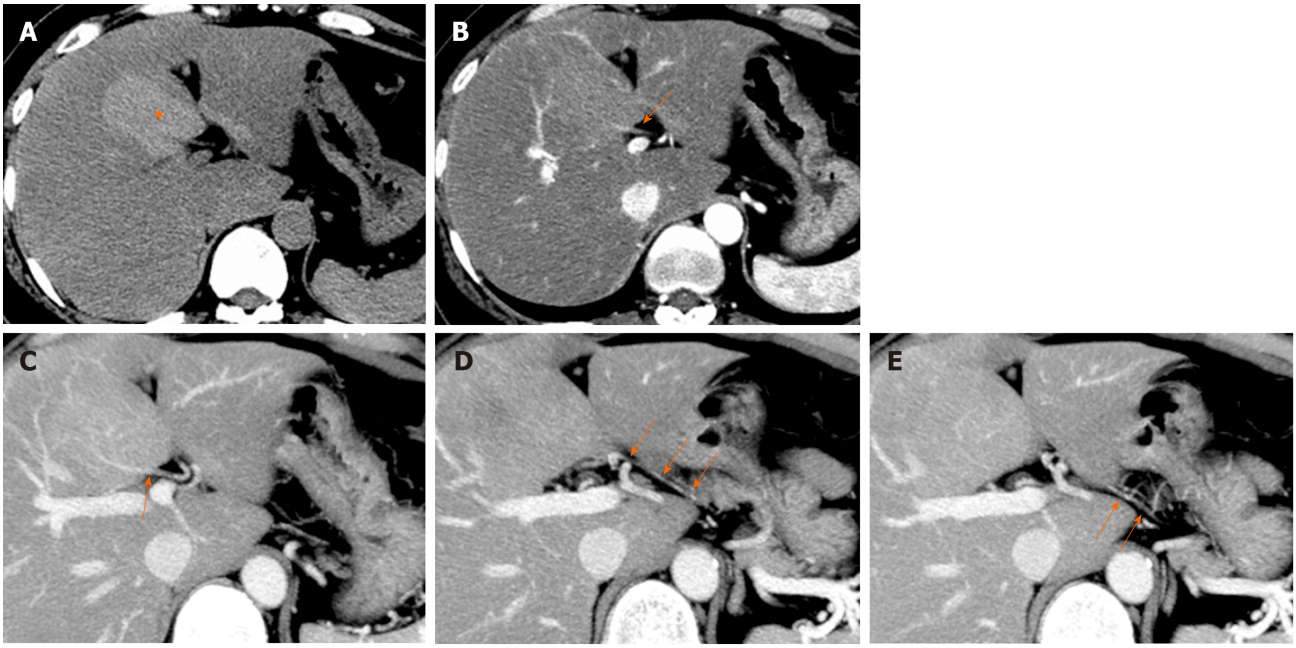

Figure 15 Focal spared area of fatty liver in posterior aspect of segment IV (40th female). A: On pre-contrast enhanced computed tomography (CT), a focal hyperdense lesion compared to the background liver parenchyma is observed in posterior aspect of segment IV of the liver (*). Background liver shows decreased density and suggestive of fatty liver and hyperdese area is diagnosed as focal sparing of fatty liver. B: On arterial phase contrast enhanced CT image, an enhanced vascular branch is directly entering the area at hepatic hilum (arrow). C-E: On sequential images of arterial phase contrast enhanced CT, aberrant right gastric vein directly entering to the posterior aspect of segment IV of the liver is well opacified (arrows). These findings represent focal spared area of the fatty liver in aberrant right gastric venous drainage area of the liver at the posterior aspect of segment IV of the liver.

Focal sparing of fatty liver

Focal sparing of fatty liver refers to the presence of focal areas exhibiting a relative decrease in the degree of fat deposition in cases of fatty liver. This type of focal sparing represents the opposite of focal fat deposition in terms of steatotic liver changes and is intimately related to alterations in intrahepatic hemodynamics. Focal sparing of fatty liver is sometimes observed in the posterior aspect of segment IV (Figure 15) and in the liver parenchyma surrounding the gallbladder in segments IV and V.

Matsui *et al*[51] reported a strong correlation between the focally spared area at the posterior edge of segment IV in fatty liver and aberrant gastric venous drainage directed to segment IV.

Fatty liver is an abnormality of the liver caused by overnutrition. However, when aberrant right gastric venous flow with a low level of nutrients compared to the main portal vein enters the posterior aspect of segment IV, focal sparing of fatty liver is assumed to occur in the third inflow area. Vilgrain *et al*[49] reported that if the insulin concentration of the aberrant gastric venous flow is low in patients with fatty liver, the aberrant venous drainage area will exhibit less fat deposition and focal sparing on liver imaging.

The blood supply of the gallbladder is provided by the cholecystic artery originating from the hepatic arterial branches, in which the blood contains enough oxygen but contains fewer nutrients than the portal venous blood. The cholecystic vein drains into the liver parenchyma surrounding the gallbladder. The venous flow that perfuses the liver area surrounding the gallbladder contains less nutrients than other areas of the liver supplied by the portal vein. For this reason, focal sparing of fatty liver occurs in the liver parenchyma surrounding the gallbladder in segments IV and V. This hypothesis is further supported by the finding that the incidence of focal sparing of fatty liver is significantly lower in patients who have undergone cholecystectomy than in those with intact gallbladders[52].

CONCLUSION

In the present review, we discussed the characteristics of hepatic blood flow and pathophysiology of pseudolesions that can occur due to alterations in intrahepatic hemodynamics. Understanding HABR, a unique mechanism for regulating hepatic

blood flow, might be essential for elucidating the pathogenesis of AP shunting and THAD on dynamic contrast-enhanced imaging of the liver. In addition, some pseudolesions are associated with histopathologic changes such as focal hyperplasia, focal fatty liver, and focal sparing of fatty liver. Understanding these phenomena may aid in interpreting liver imaging findings.

REFERENCES

1 **Kobayashi S**, Gabata T, Matsui O. Radiologic manifestation of hepatic pseudolesions and pseudotumors in the third inflow area. *Imaging Med* 2010; **2**: 519-528 [DOI: 10.2217/IIM.10.50]
2 **Scriven MW**, Shandall A, Fitzgerald EJ, Puntis MC. Hepatic 'pseudotumours': an important diagnostic pitfall. *Ann R Coll Surg Engl* 1993; **75**: 43-45 [PMID: 8422144]
3 **Eipel C**, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol* 2010; **16**: 6046-6057 [PMID: 21182219 DOI: 10.3748/wjg.v16.i48.6046]
4 **Choi BI**, Lee KH, Han JK, Lee JM. Hepatic arterioportal shunts: dynamic CT and MR features. *Korean J Radiol* 2002; **3**: 1-15 [PMID: 11919473 DOI: 10.3348/kjr.2002.3.1.1]
5 **Wang Q**, Koniaris LG, Milgrom DP, Patel A, Hu M, Cui E, Deng Y, Akisik F. CT and MRI imaging and interpretation of hepatic arterioportal shunts. *Transl Gastroenterol Hepatol* 2019; **4**: 34 [PMID: 31231701 DOI: 10.21037/tgh.2019.05.05]
6 **Jha RC**, Khera SS, Kalaria AD. Portal Vein Thrombosis: Imaging the Spectrum of Disease With an Emphasis on MRI Features. *AJR Am J Roentgenol* 2018; **211**: 14-24 [PMID: 29792748 DOI: 10.2214/AJR.18.19548]
7 **Richter S**, Vollmar B, Mücke I, Post S, Menger MD. Hepatic arteriolo-portal venular shunting guarantees maintenance of nutritional microvascular supply in hepatic arterial buffer response of rat livers. *J Physiol* 2001; **531**: 193-201 [PMID: 11179403 DOI: 10.1111/j.1469-7793.2001.0193j.x]
8 **Lautt WW**. Regulatory processes interacting to maintain hepatic blood flow constancy: Vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. *Hepatol Res* 2007; **37**: 891-903 [PMID: 17854463 DOI: 10.1111/j.1872-034X.2007.00148.x]
9 **Rush N**, Sun H, Nakanishi Y, Mneimneh W, Kwo PY, Saxena R. Hepatic arterial buffer response: pathologic evidence in non-cirrhotic human liver with extrahepatic portal vein thrombosis. *Mod Pathol* 2016; **29**: 489-499 [PMID: 26916069 DOI: 10.1038/modpathol.2016.43]
10 **Itai Y**, Matsui O. Blood flow and liver imaging. *Radiology* 1997; **202**: 306-314 [PMID: 9015047 DOI: 10.1148/radiology.202.2.9015047]
11 **Schmalz MJ**, Radhakrishnan K. Vascular anomalies associated with hepatic shunting. *World J Gastroenterol* 2020; **26**: 6582-6598 [PMID: 33268948 DOI: 10.3748/wjg.v26.i42.6582]
12 **Colagrande S**, Centi N, Pradella S, Duranti B, Belli G, Villari N. Transient hepatic attenuation differences and focal liver lesions: sump effect due to primary arterial hyperperfusion. *J Comput Assist Tomogr* 2009; **33**: 259-265 [PMID: 19346856 DOI: 10.1097/RCT.0b013e31818050bc]
13 **Ahn JH**, Yu JS, Hwang SH, Chung JJ, Kim JH, Kim KW. Nontumorous arterioportal shunts in the liver: CT and MRI findings considering mechanisms and fate. *Eur Radiol* 2010; **20**: 385-394 [PMID: 19657644 DOI: 10.1007/s00330-009-1542-z]
14 **Hashimoto M**, Heianna J, Tate E, Nishii T, Iwama T, Ishiyama K. Small veins entering the liver. *Eur Radiol* 2002; **12**: 2000-2005 [PMID: 12136318 DOI: 10.1007/s00330-002-1321-6]
15 **Yoshimitsu K**, Honda H, Kuroiwa T, Irie H, Aibe H, Shinozaki K, Masuda K. Unusual hemodynamics and pseudolesions of the noncirrhotic liver at CT. *Radiographics* 2001; **21** Spec No: S81-S96 [PMID: 11598250 DOI: 10.1148/radiographics.21.suppl_1.g01oc06s81]
16 **Deneve E**, Caty L, Fontaine C, Guillem P. Simultaneous aberrant left and right gastric veins draining directly into the liver. *Ann Anat* 2003; **185**: 263-266 [PMID: 12801091 DOI: 10.1016/S0940-9602(03)80037-7]
17 **Takayasu K**, Aoki K, Ichikawa T, Ohmura T, Sekiguchi R, Terauchi T, Takayama T. Aberrant right gastric vein directly communicating with left portal vein system. Incidence and implications. *Acta Radiol* 1990; **31**: 575-577 [PMID: 2278780]
18 **Seong NJ**, Chung JW, Kim HC, Park JH, Jae HJ, An SB, Cho BH. Right gastric venous drainage: angiographic analysis in 100 patients. *Korean J Radiol* 2012; **13**: 53-60 [PMID: 22247636 DOI: 10.3348/kjr.2012.13.1.53]
19 **Miyaki T**, Yamada M, Kumaki K. Aberrant course of the left gastric vein in the human. Possibility of a persistent left portal vein. *Acta Anat (Basel)* 1987; **130**: 275-279 [PMID: 3434179 DOI: 10.1159/000146456]
20 **Unal E**, Ozmen MN, Akata D, Karcaaltincaba M. Imaging of aberrant left gastric vein and associated pseudolesions of segments II and III of the liver and mimickers. *Diagn Interv Radiol* 2015; **21**: 105-110 [PMID: 25698094 DOI: 10.5152/dir.2014.14360]
21 **Martin BF**, Tudor RG. The umbilical and paraumbilical veins of man. *J Anat* 1980; **130**: 305-322 [PMID: 7400038]
22 **Virmani V**, Lal A, Ahuja CK, Khandelwal N. The CT Quadrate lobe hot spot sign. *Ann Hepatol* 2010; **9**: 296-298 [PMID: 20720272]

23 **Yoshimitsu K**, Honda H, Kaneko K, Kuroiwa T, Irie H, Chijiiwa K, Takenaka K, Masuda K. Anatomy and clinical importance of cholecystic venous drainage: helical CT observations during injection of contrast medium into the cholecystic artery. *AJR Am J Roentgenol* 1997; **169**: 505-510 [PMID: 9242765 DOI: 10.2214/ajr.169.2.9242765]

24 **Matsui O**, Kadoya M, Yoshikawa J, Gabata T, Takahashi S, Ueda K, Kawamori Y, Takashima T, Nakanuma Y. Aberrant gastric venous drainage in cirrhotic livers: imaging findings in focal areas of liver parenchyma. *Radiology* 1995; **197**: 345-349 [PMID: 7480675 DOI: 10.1148/radiology.197.2.7480675]

25 **Gabata T**, Matsui O, Kadoya M, Yoshikawa J, Mitchell DG, Ueda K, Kawamori Y, Takashima T. Giant hyperplasia of the caudate lobe of the cirrhotic liver: correlation with an anomaly of the caudate portal branch. *Abdom Imaging* 1999; **24**: 153-156 [PMID: 10024401 DOI: 10.1007/s002619900465]

26 **Ohtomo K**, Matsuoka Y, Okada M, Amo K, Abe O, Minami M, Kawauchi N, Sasaki Y. Pseudotumorous enlargement of the paracaval portion of the caudate lobe: a report of two cases with CT and MR appearance. *Abdom Imaging* 1997; **22**: 398-400 [PMID: 9157859 DOI: 10.1007/s002619900219]

27 **Kwak HS**, Lee JM, Lee SY, Han YM, Kim CS, Moon WS, Yu HC. Pseudotumorous hyperplasia of the caudate lobe in the non-cirrhotic liver: MR and CT arterial portography appearance. *Hepatogastroenterology* 2000; **47**: 909-911 [PMID: 11020845]

28 **Abshagen K**, Eipel C, Vollmar B. A critical appraisal of the hemodynamic signal driving liver regeneration. *Langenbecks Arch Surg* 2012; **397**: 579-590 [PMID: 22311102 DOI: 10.1007/s00423-012-0913-0]

29 **Sato Y**, Koyama S, Tsukada K, Hatakeyama K. Acute portal hypertension reflecting shear stress as a trigger of liver regeneration following partial hepatectomy. *Surg Today* 1997; **27**: 518-526 [PMID: 9306545 DOI: 10.1007/BF02385805]

30 **Schoen JM**, Wang HH, Minuk GY, Lautt WW. Shear stress-induced nitric oxide release triggers the liver regeneration cascade. *Nitric Oxide* 2001; **5**: 453-464 [PMID: 11587560 DOI: 10.1006/niox.2001.0373]

31 **Gock M**, Eipel C, Linnebacher M, Klar E, Vollmar B. Impact of portal branch ligation on tissue regeneration, microcirculatory response and microarchitecture in portal blood-deprived and undeprived liver tissue. *Microvasc Res* 2011; **81**: 274-280 [PMID: 21397614 DOI: 10.1016/j.mvr.2011.03.005]

32 **Gluud C**, Christoffersen P, Eriksen J, Wantzin P, Knudsen BB. Influence of ethanol on development of hyperplastic nodules in alcoholic men with micronodular cirrhosis. *Gastroenterology* 1987; **93**: 256-260 [PMID: 3596160 DOI: 10.1016/0016-5085(87)91011-0]

33 **Mak KM**, Png CYM. The Hepatic Central Vein: Structure, Fibrosis, and Role in Liver Biology. *Anat Rec (Hoboken)* 2020; **303**: 1747-1767 [PMID: 31581357 DOI: 10.1002/ar.24273]

34 **Marek E**, Kraft WK. Ethanol pharmacokinetics in neonates and infants. *Curr Ther Res Clin Exp* 2014; **76**: 90-97 [PMID: 25379066 DOI: 10.1016/j.curtheres.2014.09.002]

35 **Ozaki K**, Matsui O, Kobayashi S, Sanada J, Koda W, Minami T, Kawai K, Gabata T. Selective atrophy of the middle hepatic venous drainage area in hepatitis C-related cirrhotic liver: morphometric study by using multidetector CT. *Radiology* 2010; **257**: 705-714 [PMID: 20843994 DOI: 10.1148/radiol.10100468]

36 **Leng L**, Ma J, Lv L, Gao D, Li M, Wang Y, Zhu Y. Serum proteome profiling provides a deep understanding of the 'gut-liver axis' in relation to liver injury and regeneration. *Acta Biochim Biophys Sin (Shanghai)* 2021; **53**: 372-380 [PMID: 33511977 DOI: 10.1093/abbs/gmab001]

37 **Naugler WE**. Bile acid flux is necessary for normal liver regeneration. *PLoS One* 2014; **9**: e97426 [PMID: 24841254 DOI: 10.1371/journal.pone.0097426]

38 **Milona A**, Owen BM, van Mil S, Dormann D, Mataki C, Boudjelal M, Cairns W, Schoonjans K, Milligan S, Parker M, White R, Williamson C. The normal mechanisms of pregnancy-induced liver growth are not maintained in mice lacking the bile acid sensor Fxr. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G151-G158 [PMID: 19815629 DOI: 10.1152/ajpgi.00336.2009]

39 **Gadaleta RM**, van Mil SW, Oldenburg B, Siersema PD, Klomp LW, van Erpecum KJ. Bile acids and their nuclear receptor FXR: Relevance for hepatobiliary and gastrointestinal disease. *Biochim Biophys Acta* 2010; **1801**: 683-692 [PMID: 20399894 DOI: 10.1016/j.bbalip.2010.04.006]

40 **Wu W**, Wu Q, Liu X. Chronic activation of FXR-induced liver growth with tissue-specific targeting Cyclin D1. *Cell Cycle* 2019; **18**: 1784-1797 [PMID: 31223053 DOI: 10.1080/15384101.2019.1634955]

41 **Bioulac-Sage P**, Kakar S, Nault JC. Focal nodular hyperplasia of the liver. WHO Classification of Tumours. 5th Edition Digestive System Tumors. Edited by WHO classification of Tumours Editorial Board. Lyon (France), International Agency for Research on Cancer; 2019: 221-223

42 **Bioulac-Sage P**, Laumonier H, Cubel G, Saric J, Balabaud C. Over-expression of glutamine synthase in focal nodular hyperplasia (part 1): early stages in the formation support the hypothesis of a focal hyper-arterialisation with venous (portal and hepatic) and biliary damage. *Comp Hepatol* 2008; **7**: 2 [PMID: 18312631 DOI: 10.1186/1476-5926-7-2]

43 **Bioulac-Sage P**, Laumonier H, Rullier A, Cubel G, Laurent C, Zucman-Rossi J, Balabaud C. Over-expression of glutamine synthetase in focal nodular hyperplasia: a novel easy diagnostic tool in surgical pathology. *Liver Int* 2009; **29**: 459-465 [PMID: 18803590 DOI: 10.1111/j.1478-3231.2008.01849.x]

44 **Kondo F**. Benign nodular hepatocellular lesions caused by abnormal hepatic circulation: etiological

analysis and introduction of a new concept. *J Gastroenterol Hepatol* 2001; **16**: 1319-1328 [PMID: 11851827 DOI: 10.1046/j.1440-1746.2001.02576.x]

45 **Kawamori Y**, Matsui O, Takahashi S, Kadoya M, Takashima T, Miyayama S. Focal hepatic fatty infiltration in the posterior edge of the medial segment associated with aberrant gastric venous drainage: CT, US, and MR findings. *J Comput Assist Tomogr* 1996; **20**: 356-359 [PMID: 8626889 DOI: 10.1097/00004728-199605000-00004]

46 **Fukukura Y**, Fujiyoshi F, Inoue H, Sasaki M, Hokotate H, Baba Y, Nakajo M. Focal fatty infiltration in the posterior aspect of hepatic segment IV: relationship to pancreaticoduodenal venous drainage. *Am J Gastroenterol* 2000; **95**: 3590-3595 [PMID: 11151897 DOI: 10.1111/j.1572-0241.2000.03298.x]

47 **Kobayashi S**, Matsui O, Kadoya M, Yoshikawa J, Gabata T, Kawamori Y, Sanada J, Terayama N. CT arteriographic confirmation of focal hepatic fatty infiltration adjacent to the falciform ligament associated with drainage of inferior vein of Sappey: a case report. *Radiat Med* 2001; **19**: 51-54 [PMID: 11305620]

48 **Genchellac H**, Yilmaz S, Ucar A, Dursun M, Demir MK, Yekeler E. Hepatic pseudolesion around the falciform ligament: prevalence, aberrant venous supply, and fatty infiltration evaluated by multidetector computed tomography and magnetic resonance imaging. *J Comput Assist Tomogr* 2007; **31**: 526-533 [PMID: 17882026 DOI: 10.1097/01.rct.0000284387.68449.ec]

49 **Vilgrain V**, Ronot M, Abdel-Rehim M, Zappa M, d'Assignies G, Bruno O, Vullierme MP. Hepatic steatosis: a major trap in liver imaging. *Diagn Interv Imaging* 2013; **94**: 713-727 [PMID: 23751229 DOI: 10.1016/j.diii.2013.03.010]

50 **Hoshiba K**, Demachi H, Miyata S, Matsui O, Takashima T, Tsuji M, Miwa A. Fatty infiltration of the liver distal to a metastatic liver tumor. *Abdom Imaging* 1997; **22**: 496-498 [PMID: 9233885 DOI: 10.1007/s002619900246]

51 **Matsui O**, Kadoya M, Takahashi S, Yoshikawa J, Gabata T, Takashima T, Kitagawa K. Focal sparing of segment IV in fatty livers shown by sonography and CT: correlation with aberrant gastric venous drainage. *AJR Am J Roentgenol* 1995; **164**: 1137-1140 [PMID: 7717220 DOI: 10.2214/ajr.164.5.7717220]

52 **Aubin B**, Denys A, Lafortune M, Déry R, Breton G. Focal sparing of liver parenchyma in steatosis: role of the gallbladder and its vessels. *J Ultrasound Med* 1995; **14**: 77-80 [PMID: 8568966 DOI: 10.7863/jum.1995.14.2.77]

World Journal of Gastroenterology

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World J Gastroenterol 2021 December 14; 27(46): 7909-7924

DOI: 10.3748/wjg.v27.i46.7909

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Enteric nervous system and inflammatory bowel diseases: Correlated impacts and therapeutic approaches through the P2X7 receptor

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Author contributions: Magalhães HIR was responsible for the literature review and analysis and wrote the review; Castelucci P performed the critical interpretation and revised the manuscript for intellectual content.

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

Supported by the São Paulo Research (FAPESP, Brazil), No. 2014/25927-2 and No. 2018/07862-1; and the National Council for Scientific and Technological Development (CNPq, Brazil).

Country/Territory of origin: Brazil

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A

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Abstract

The enteric nervous system (ENS) consists of thousands of small ganglia arranged in the submucosal and myenteric plexuses, which can be negatively affected by Crohn's disease and ulcerative colitis - inflammatory bowel diseases (IBDs). IBDs are complex and multifactorial disorders characterized by chronic and recurrent inflammation of the intestine, and the symptoms of IBDs may include abdominal pain, diarrhea, rectal bleeding, and weight loss. The P2X7 receptor has become a promising therapeutic target for IBDs, especially owing to its wide expression and, in the case of other purinergic receptors, in both human and model animal enteric cells. However, little is known about the actual involvement between the activation of the P2X7 receptor and the cascade of subsequent events and how all these activities associated with chemical signals interfere with the functionality of the affected or treated intestine. In this review, an integrated view is provided, correlating the structural organization of the ENS and the effects of IBDs, focusing on cellular constituents and how therapeutic approaches through the P2X7 receptor can assist in both protection from damage and tissue preservation.

Key Words: Chemical coding; Enteric nervous system; Gastroenterology; Inflammatory bowel diseases; P2X7 receptor; Purinergic signaling

Core Tip: This review summarizes the impacts caused by inflammatory bowel diseases

Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: July 10, 2021
Peer-review started: July 10, 2021
First decision: August 19, 2021
Revised: August 19, 2021
Accepted: November 25, 2021
Article in press: November 25, 2021
Published online: December 14, 2021

P-Reviewer: Xu B
S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

on enteric nervous system cells and brings together the findings of the most recent literature on therapeutic approaches through the P2X7 receptor. Despite the great advancement of knowledge in the field, data on the mechanisms and effects of neuronal loss during colitis are still scarce. Furthermore, clinical trials that would make the use of P2X7 receptor antagonists in human patients feasible are lacking. In the laboratory, the results of animal models reinforce that the P2X7 receptor may be an important future target for the treatment of intestinal disorders.

Citation: Magalhães HIR, Castelucci P. Enteric nervous system and inflammatory bowel diseases: Correlated impacts and therapeutic approaches through the P2X7 receptor. *World J Gastroenterol* 2021; 27(46): 7909-7924
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7909.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7909

INTRODUCTION

The gastrointestinal (GI) tract is a set of organs responsible for performing several complex functions that are essential for an individual's survival, including mainly food transportation, the digestion and absorption of nutrients, and the secretion of water, electrolytes, and mucus[1]. In the GI tract, there is an extensive intrinsic nervous system responsible for the control and coordination of local motility, the movement of fluids through the mucous epithelium, changes in blood flow, and interactions with the immune system[2]. Sometimes, this influence continues even if there is complete separation of the GI tract from the central nervous system (CNS)[2,3].

The enteric nervous system (ENS) is composed of thousands of small ganglia interconnected by their neural fibers and is arranged in two plexuses. The myenteric plexus is located between the fibers of the muscular layer throughout the GI tract, and the submucosal plexus is located in the submucosal layer of the small and large intestines[2,4,5]. Thus, the ENS shares many synaptic and ultrastructural characteristics of the neuronal interrelationship of the GI tract and the CNS[6], with many similarities demonstrated between them, which are reflected in neurological diseases [7]. Enteric innervation has been widely studied, and when preserved and functionally active, enteric innervation is considered equally essential to life as CNS innervation[8].

The study of the ENS has progressed from a healthy context to several pathological models, identifying neuroplastic changes that possibly contribute to modifying intestinal and perception functions in GI disorders[9]. It has been found that purinergic neurotransmission also plays a fundamental role in preserving the internal balance of these organs[10], interacting directly with motor and secretory functions[11] by the expression of several of its receptors on neurons located in the ENS[12]. In addition, the purinergic signaling pathway has also been widely recognized as a fundamental component in the course of inflammation during intestinal diseases[10,13,14].

In this context, the P2X7 receptor appeared to be one of the most correlated representatives in studies of infectious and inflammatory diseases[15]. The most striking differences in the P2X7 receptor in comparison to other purinergic receptors arise not only from its structural conformation but also from a sensitivity that is 10 to 100 times lower for its functional activation, suggesting it as a "danger" detector for tissue damage[16]. Therefore, a better understanding of the behavior of the P2X7 receptor and how it could be affected or modulated in some specific cases is sought, for example, in the treatment of Crohn's disease and ulcerative colitis - inflammatory bowel diseases (IBDs) that cause neuronal death in the ENS and compromise the functionality of the affected organs[17-19].

The great impact of Crohn's disease and ulcerative colitis is that both are capable of influencing all areas of patients' lives, from school and work to social and family life, affecting patients' productivity in each area[20]. In addition, when these conditions are poorly controlled, they can have negative effects on psychosocial well-being[21], increasing even the rates of anxiety and depression according to the severity of the conditions[22]. Worryingly, the occurrence of IBDs cases worldwide increased from 3.7 million to over 6.8 million between 1990 and 2017[23], which makes an individual approach with strong multidisciplinary care increasingly important, as this type of

approach could offer a higher quality of life even for individuals of different ages[20].

Thus, this review aimed to provide an integrated view of the structural organization of the ENS and the deleterious effects arising from IBDs, focusing on the cellular constituents and how therapeutic approaches through the P2X7 receptor can assist in both protection from damage and tissue preservation.

THE ENTERIC NERVOUS SYSTEM

The ENS, also known as the "second brain"[6,24], acts in an essential way in the motility of the esophagus, stomach, and small and large intestines[4,6], modulating the different contraction types of each organ[25]. In addition, the functions of endocrine and exocrine secretion, control of local blood flow, and regulation of inflammatory and immune processes are also related to ENS function[26].

The enteric neural circuit is organized as an interconnected network of enteric neurons and glial cells[4] throughout the entire GI tract and bile and pancreatic ducts [27]. The enteric neural circuit is arranged in two plexuses: the submucosal plexus, which in large mammals is present in two individualized levels (outer/inner) and is located in the outer connective tissue layer and the inner mucosal layer, and the myenteric plexus, located between the longitudinal and circular muscle layers[2,4,5, 28].

Within this complex innervation system, in humans, there are approximately 400 to 600 million neurons[5] grouped into several ganglia that connect[2] through the primary interganglionic tracts, which characterize the primary plexus[4,5]. The secondary and tertiary plexuses are also present in the myenteric plexus, represented by thinner filaments that are arranged parallel to the fibers of the circular musculature [29] and by even thinner filaments that branch among the constituents of the primary plexus[30]. This extensive neuronal network ends up projecting itself toward various effector structures, such as muscular and immune cells and blood vessels[27].

As proposed by Aleksandr S. Dogiel in 1899, the morphological classification of enteric neurons can be based on their conformation and dendritic distribution. Dogiel described type I cells as flattened, slightly elongated, with an angled or star-shaped contour, and, as remarkable characteristics, as having only one axon and four to 20 Lamellar dendrites that frequently extend at a short distance from the cell body[31].

Type II neurons have large round or oval cell bodies and eccentric nuclei[31], and the surface is grooved by bundles of neural fibers[32]. The main characteristic of type II neurons is the presence of several axonal processes that are emitted either directly from the cell body (multipolar neuron) or from a single initial process that branches into short subsidiary axons (pseudounipolar neurons)[4,33]. Such structures run toward the mucosa[34] and sometimes also provide collateral innervation to the submucosal ganglia[35].

Additionally, enteric neurons can also be identified as intrinsic primary afferent neurons (IPANs), interneurons, and motor neurons[4], classified into at least 18 subtypes and using more than 30 neurotransmitters in their synapses[28,30]. Of these neurotransmitters, acetylcholine (ACh) and nitric oxide (NO) stand out as the most abundant[27], as well as adenosine-5′-triphosphate (ATP)[26], vasoactive intestinal polypeptide (VIP), and substance P (SP)[36]. It is not rare that the same chemical compound stimulates neurons that perform distinct functions[26].

IPANs (classified as Dogiel type II) are recognized for responding to chemical stimuli, mucosal deformation and GI muscle tension, translating these signals into a neural impulse that will trigger a local motor reflex[37]. Altogether, IPANs represent approximately 14% and 30% of the neurons of the submucosal and myenteric plexuses, respectively. IPANs often project to form synapses with myenteric interneurons, motor neurons of the longitudinal and circular muscles[38], and with other IPANs[4].

The interneurons of the ENS (classified as Dogiel type I) are interposed with the IPANs and motor neurons[26], acting as mediators that are activated by the first neuron after a stimulus is received in the mucosa[27,39,40]. Thus, four neuronal types have been reported: one ascending (5%)[38], related to the pathways of the propulsive reflexes[41]; and three descending[38], related to local motility reflexes (5%), the conduction of the migratory myoelectric complex in the small intestine (4%), and secretomotor reflexes (2%)[4,30]. The interconnection of motor, secretory, and vasomotor pathways was suggested on the basis of the double projection of some of these neural fibers in both the submucosal and myenteric plexuses[38].

Motor neurons (classified as Dogiel type I) mark direct connections with muscle cells and, according to their neurotransmitter, can be classified as excitatory by

acetylcholine transferase (ChAT) labeling or as inhibitory by neuronal nitric oxide synthase (nNOS) labeling[4,5,36]. In addition, Furness *et al*[30] classified motor neurons as secretomotor/vasodilator neurons (60%), secretomotor neurons that are not vasodilators (29%), and neurons that innervate only enteroendocrine cells. On the basis of distribution analysis, it is already known that this neuronal class is also present in both enteric plexuses[2].

In summary, neurons of the submucosal plexus innervate the mucosal epithelium and submucosal arterioles to control and maintain water and electrolyte balance, luminal secretion and vascular tone, whereas the myenteric plexus promotes motor innervation of both layers of the muscle region[5], controlling the reflex pathways of the motor complex[42]. However, it is worth noting that the former is present only in the small and large intestines, whereas the latter is found continuously from the initial esophageal region to the internal anal sphincter[4].

The great difference in ENS innervation is that because the enteric ganglia possess all the necessary components to generate and complete a complex reflex circuit (IPANs, interneurons, and motor neurons)[28,43], the ENS has the capacity to regulate GI functions even in the absence of extrinsic neural connections[43]. Therefore, several authors have confirmed that ENS action can occur independently of the CNS[4,24,26,36,44,45], even though the latter often initiates or modulates some of the actions of the ENS[18,24,26].

However, according to Furness[5] and Furness *et al*[2], this autonomy does not actually occur. There are dependencies through interactions between local enteric reflexes, reflexes that pass through sympathetic ganglia, and reflexes that pass in return to the CNS[2,5]. Conveniently, these connections can be classified as vagal and thoracolumbar spinal, being represented by pre-enteric neurons that terminate inside the enteric ganglia, controlling and modifying the activities of neurons present there, or even by direct innervation of effector regions, *e.g.*, the striated skeletal muscles of the esophagus and the sphincters of the GI tract[2].

All this structural and functional complexity characterizes the ENS as the largest and most varied division of the peripheral nervous system[46], leading initially John N. Langley[47] to recognize the ENS no longer as a distribution of parasympathetic postganglia but rather as a distinct segment of the autonomic nervous system that, due to its prominence, should stand alongside the sympathetic and parasympathetic divisions.

INFLAMMATORY BOWEL DISEASES AND THEIR IMPACTS ON THE ENTERIC NERVOUS SYSTEM

IBDs, classically subdivided into Crohn's disease and ulcerative colitis[48,49], are complex and multifactorial disorders characterized by chronic and recurrent inflammation of the intestine[50,51]. Usually, debilitating[48], these disorders reach their peak onset in patients between the ages of 15 and 30 years[52], who, on a purely individual basis, may alternate between periods of symptomatic flares and clinical remission[49].

Although the etiology of IBDs is not yet fully understood[53,54], a growing body of evidence has suggested that the occurrence of IBDs is related to genetic predispositions[55,56] and aberrant immune responses in the face of various environmental triggers[56,57], including antigens from the gut microbiota[56,58,59], poor dietary habits, and high antibiotic consumption in childhood and adolescence[57,60]. Worryingly, an increase in both the incidence and prevalence of IBDs has been reported worldwide[23,52,61,62], but this increase is even more pronounced in newly industrialized countries with more westernized societies[63,64].

Commonly, the symptoms of IBDs may include abdominal pain, diarrhea, rectal bleeding, and weight loss. Ulcerative colitis primarily affects the rectum and is limited to the superficial part of the large intestine mucosa[48], and Crohn's disease is manifested by transmural lesions that may extend from the mouth to the anus, promoting possibly irreversible damage[65]. Sometimes the appearance of and gradual increase in intestinal ulcers associated with cumulative destructive effects can cause stenosis, fistulas, and colorectal cancer[66-68]. Therefore, it is clear that IBDs have an expressive influence on the quality of daily life in these patients[20,21].

In this sense, several efforts are being made to more closely mimic these diseases in the laboratory through the use of animal models, either to understand the relationship between their pathophysiological components or to identify the mechanisms and drugs that mitigate the symptomatology[69]. For this, two main substances have been

used quite satisfactorily for colitis induction: dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS). DSS is a soluble polysaccharide supplied in drinking water and chemically interferes with gut mucosal barrier integrity, allowing the dissemination of luminal antigens into underlying tissue. TNBS is a reagent administered rectally in combination with ethanol that disrupts the mucosal barrier, allowing TNBS to induce colitis by haptenating colonic proteins, causing them to become preferential targets for immune cells. In both cases, the onset of acute or chronic lesions is dependent on the concentration and/or the frequency of the administration of each substance[69-73].

Specific to the ENS, reports have pointed out that intestinal inflammation can cause functional and structural changes in neurons[74-76] and necrosis, apoptosis and degeneration in enteric ganglia[17,18,77]. In fact, different authors have already demonstrated important variations in the cell number and neuronal profile of inflamed areas when compared to healthy tissues (Tables 1 and 2). In addition, damage to intestinal innervation during the inflammatory course may cause organ functional losses through modifications in motility patterns, increased excitability with changes in synaptic transmission in neural microcircuits, inadequate secretory responses of the epithelium to incoming stimuli[18,78], and enteric cell death from dependence on multiple caspases[19,79,80]. Despite this, little is still known about the mechanisms behind the loss of enteric innervation linked to IBDs[76].

In view of the therapeutic management of IBDs, the introduction of anti-TNF agents has positively marked this path[81-83], especially as they favor the healing of the mucosal layer with increases in its growth with stimulation[84], and as they demonstrate a greater safety of use when compared to conventional protocols[81,82]. In this same context, the P2X7 receptor is also emerging as a very important medical target for the prevention and treatment of these disorders[10], possibly in a similar way to the above, since its continuous activation may worsen the local inflammatory response[85,86]. However, little is known about the real involvement between the activation of this purinergic receptor and the subsequent cascade of events and how all these activities associated with chemical signaling interfere with the functionality of the affected or treated intestine.

THE PURINERGIC RECEPTORS

ATP is the central nucleotide of body metabolism[87], one of the most abundant molecules in living cells[88], and despite being recognized as an energy substrate[87], ATP also acts systemically in conjunction with adenosine and adenosine diphosphate (ADP). As an example, ATP presents actions in the control of vascular tone and remodeling[89,90] and in growth, differentiation[91], and cell communication[87,88,92,93].

Initially recognized for its fundamental role in several intracellular biochemical processes, the function of ATP as a neurotransmitter was greatly questioned when proposed by Geoffrey Burnstock in 1972[94]. In any case, the discovery of purinergic neurons - as they were named in reference to their relation with purine nucleotides[95] - answered the questions generated about the existence of neurons that are neither cholinergic nor adrenergic[36], and a high level of evidence has been reached on purinergic neurons in the scope of physiological and pathophysiological scientific research[92].

According to Burnstock[96], the presence of purinergic receptors was implicit in the hypothesis of this class of neurotransmission, and these receptors were classified into two types: P1 by the use of adenosine and P2 by the use of ATP and ADP. However, only in 1985 was it proposed on pharmacological grounds that this second type could be further subdivided into two other larger families[97]: P2Y, coupled with G-protein; and P2X, coupled with ion channel-dependent ligands[98]. Four subforms are currently recognized for P1 receptors (A1, A2a, A2b, and A3)[99], eight for P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14)[100], and seven for P2X receptors (P2X1-7)[101,102], making it plausible that purinergic receptors are the most abundant in mammalian tissues[103], found even in cells of neural origin[13,93,103-107].

In the ENS, the presence of purinergic receptors has been widely recognized in enteric neurons and glial cells of humans and other animal species[13,14,108]. In the guinea pig, the P2Y1 receptor has already been identified in the submucosal plexus of the ileum[109], and the P2Y2, P2Y6, P2Y12, P2X2, and P2X3 receptors have been identified in the submucosal and myenteric plexuses of the stomach, jejunum, ileum, and distal colon[110-114].

Table 1 Specific variations in cell number and neuronal profile area according to the respective chemical code observed in the submucosal plexus of the enteric nervous system

Ref.	Species	Colitis	Time	Submucosal plexus	
				Cellular chemical code (change in density compared to healthy tissues, %)	Cellular chemical code (change in profile area compared to healthy tissues, %)
Schneider *et al* [169]	Human	Crohn's disease	6.1±6,3 years	ChAT, nNOS, SP, and NSE (similar to); VIP (> 16%-CT)	N/A
Sigalet *et al*[170]	Rat	TNBS-50% ethanol	5 d	PGP9.5 (<)[1]; VIP (<)[1]; S100 β (<)[1]	N/A
da Silva *et al*[130]	Rat	TNBS-30% ethanol	24 h	P2X7 (< 21%-CT; < 13%-sham) ; Calret (< 11.7%-CT; < 8%-sham); Calb (< 34%-CT; < 30%-sham); HuC/D (< 33.4%-CT; < 28%sham); S100β (< 44.2%-CT; < 33%-sham)	Calbindin (< 25%-CT/sham)

[1]Count change without percentage information.

TNBS: 2,4,6-trinitrobenzenesulfonic acid; N/A: Not applicable; (<): Cell count/area decreased; (>): Cell count/area increased; ir: immunoreactive; CT: Control group; Sham: Sham group; P2X7: P2X7 receptor; ChAT: Acetylcholine transferase enzyme-ir; nNOS: Neuronal nitric oxide synthase enzyme-ir; Calret: Calretinin-ir; Calb: Calbindin-ir; SP: Substance P-ir; VIP: Vasoactive intestinal polypeptide-ir; HuC/D and PGP9. 5: Pan neuronal-ir; NSE: Neuron-specific enolase-ir; S100β: Protein β for calcium S100-ir labeling.

In mouse studies, P2X2, P2X3, and P2X5 receptors were identified in the submucosal and myenteric plexuses of the stomach, jejunum, ileum, and colon[11,115-117]. In rats, P2X2 and P2X3 receptors have been demonstrated in the submucosal and myenteric plexuses from the stomach to the large intestine and rectum[118-123], and P2X6 receptors have been demonstrated in the submucosal plexuses of the jejunum, ileum, and proximal and distal colon and in the myenteric plexuses of the stomach, ileum, and proximal and distal colon[124].

Specifically, the P2X7 receptor has also been visualized in the submucosal and myenteric plexuses of the colon of humans[19] and in the submucosal plexus of the ileum and the myenteric plexus of the stomach and small and large intestines of guinea pigs[125]. In mice, the presence of the P2X7 receptor was identified in the myenteric plexus of the colon[19] and in rats in the submucosal and myenteric plexuses of the esophagus, stomach, jejunum, ileum, large intestine, and distal colon [121,126-133]. Similar to the other purinergic receptors, the P2X7 receptor also presents a wide range of distributions in relation to enteric neurons with different chemical codes that integrate the ENS (Table 3).

The P2X7 receptor

The P2X7 receptor is a trimeric complex that typically contains 595 amino acids (594 in guinea pigs)[134,135]. The P2X7 receptor consists of two transmembrane domains (TM1 and TM2) linked by a large extracellular loop and by two intracellular domains known as the N-terminus and C-terminus[134,136]. The loop acts as a site for transition metal binding and assists in the activation of this receptor *via* ATP[136], allowing the channel formed by TM1 and TM2[86,135,137] to regulate the passage of calcium, sodium, and potassium[13,93,138]. The domains inside the cell modulate the functions and determine the kinetics of the depolarization and expansion of this channel[139]. It is worth noting that in the P2X7 receptor, the intracellular C-terminus is significantly longer than that in the other P2X receptors[134,136].

As another striking feature, the P2X7 receptor also demands higher concentrations of extracellular ATP for its activation than other purinergic receptors do[101], and this is a possible tissue "danger" sensor[101,140]. In response to inflammation[14,128], trauma or injury[91,141], the elevation of ATP causes a prolonged stimulus that induces the transition of the ion channel to a nonselective membrane pore[101,142,143], making the cell permeable to molecules up to 900 daltons[94,101,142,143]. In association, massive calcium influx[144] can contribute to cell death[85,137,145], with subsequent release of greater amounts of ATP[146-148].

Thus, in addition to its already recognized role in neurotransmission[141], the P2X7 receptor is also closely related to most diseases of the body[140], acting in multiple inflammatory processes[85,99149,150], immune responses[10,85,86,99,149,151], metabolism and cell proliferation[149]. The P2X7 receptor may also be responsible for

Table 2 Specific variations in cell number and neuronal profile area according to the respective chemical code observed in the myenteric plexus of the enteric nervous system

Ref.	Species	Colitis	Time	Myenteric plexus	
				Cellular chemical code (change in density compared to healthy tissues, %)	Cellular chemical code (change in profile area compared to healthy tissues, %)
Boyer *et al*[79]	Mice	DNBS-50% ethanol	0.5 - 120 h	HuC/D (< 42%-CT)	N/A
Linden *et al*[17]	Guinea pig	TNBS-30% ethanol	2 - 12 h;1-56 d	HuC/D 12 and 24 h (< 15%-CT); HuC/D 6 and 56 d (< 20%-CT); ChAT, nNOS, calret and NeuN 6 d (=); VIP 6 (>)[1] and 56 d (No differences)	N/A
Sarnelli *et al*[171]	Rat	TNBS-50% ethanol	7 d	HuC/D (< 20%-CT)	N/A
Gulbransen *et al* [19]	Mice	DNBS-50% ethanol	48 h	HuC/D (< 32%-CT)	N/A
Linden[77]	Guinea pig	TNBS-30% ethanol	24 h	HuC/D (< approximately 20%-25%-CT)	N/A
Da Silva *et al*[129]	Rat	TNBS-30% ethanol	24 h	P2X7 (< 11%-CT); ChAT (< 34.9%-CT); nNOS (< 42.3%-CT; < 18%-sham); Calret (< 60.6%-CT; < 15%-sham); Calbindin (< 22.9%-CT); HuC/D (< 33.3%-CT; < 16%-sham); S100β (< 29.2%-CT; < 23%-sham)	nNOS (< 6.6%-CT/sham); ChAT (< 21.2%-CT/sham); Calbindin (>19%-CT); Calretinin (< 2%-sham)
Souza *et al*[133],[2]	Rat	TNBS-30% ethanol	24 h	P2X7 (< 10.6%-sham; < 20.4%-BBG); ChAT (< 34%-sham; < 13.9%-BBG); nNOS (< 22.9%-sham; < 22.2%-BBG); HuC/D (< 15.4%-sham; < 19.5%-BBG); GFAP (< 14.4%-sham; < 17.7%-BBG)	nNOS (< 12%-sham; < 8%-BBG); ChAT and HuC/D (No differences)

[1]Change in count without percentage information.

[2]Data from ileum after colitis. DNBS: Dinitrobenzene sulfonic acid; TNBS: 2,4,6-trinitrobenzenesulfonic acid; N/A: Not applicable; (=): Similarity of cell count; (<): Cell count/area decrease; (>): Cell count/area increase; ir: Immunoreactive; CT: Control group; Sham: Sham group; BBG: Brilliant Blue G-treated animals group; P2X7: P2X7 receptor; ChAT: Acetylcholine transferase enzyme-ir; nNOS: Neuronal nitric oxide synthase enzyme-ir; Calret: Calretinin-ir; VIP: Vasoactive intestinal polypeptide-ir; HuC/D: Neuronal pan-ir; NeuN: Neuronal nuclear antigen-ir; GFAP: Glial fibrillary acid protein-ir; S100β: Protein β for calcium S100-ir labeling.

triggering the stimulation of necrosis and apoptosis after neurological injuries[85,152,153].

Most of the studies involving the ENS have demonstrated a decrease in the number of cells that are immunoreactive to the P2X7 receptor in the submucosal and myenteric plexus following ischemia/reperfusion in the ilea of rats [127,131,132] and intestinal inflammation in rats[128-130,133], mice, and humans[19]. Moreover, the alteration of these same neurons was observed in the ENS of the large intestine of rats subjected to undernourishment protein and renutrition[121].

Antonioli *et al*[128] also observed a higher intensity of immunofluorescence labeling of these cells in the myenteric ganglia of the distal colon of rats with experimentally induced colitis. These findings may reflect higher activation of the P2X7 receptor in the epithelium and lamina propria of the colon in response to inflammation[154] and in human patients with Crohn's disease or ulcerative colitis[155]. Moreover, it has already been shown that the P2X7 receptor also acts in regulating the activation of NF-κB[148,154] and in the release of proinflammatory cytokines (IL-1β, IL-6, IL-18, and TNF)[148,154,156]. In addition, higher colocalization rates between the P2X7 receptor and dendritic cells, T cells, and macrophages in the epithelium and lamina propria of the inflamed colon in humans have also been reported[155].

Thus, it is highlighted that the P2X7 receptor can promote the occurrence and progression of IBDs, altering the local biological behavior[10] and acting as a key factor in the pathogenesis of ulcerative colitis and Crohn's disease[19,154,157], sometimes even being responsible for neuronal loss[19,158]. Soon, effective pharmacological blockade of this receptor will emerge as a new target in the treatment of inflammatory conditions[99].

Table 3 Specific distribution of the P2X7 receptor in relation to cells with different chemical code that integrate the enteric nervous system

Ref.	Species	Tissue	Chemical cellular code (p2x7 receptor expression, %)	
			Submucosal plexus	Myenteric plexus
Hu *et al*[125]	Guinea pig	Ileum	ChAT, calret, NPY and SP	nNos, calret, calb, NPY, SP, and HuC/D
Hu *et al*[125]	Guinea pig	Stomach and intestines	N/A	nNos, calret, calb, NPY, SP, and HuC/D
Vanderwinden *et al*[126]	Rat	Stomach, jejunum, and colon	S100β	S100β
Gulbransen *et al*[19]	Human and mice	Colon	+	+
Girotti *et al*[121]	Rat	Large intestine	P2X7 in 100% of ChAT, calret, and calb; ChAT (22.5%), calret (35%) and calb (12.7%)	P2X7 in 100% of ChAT, nNOS, calret, and calb; ChAT (12.7%), nNOS (35.7%), calret (17.6%) and calb (8.3%)
Palombit *et al*[127]	Rat	Ileum	N/A	P2X7 in 100% of ChAT, nNOS, calret, and calb; ChAT (42.2%), nNOS (24.5%), calret (33.5%), and calb (10.7%)
Antonioli *et al*[128]	Rat	Distal colon	N/A	P2X7 in 100% of HuC/D
Da Silva *et al*[129]	Rat	Distal colon	N/A	P2X7 in 100% of ChAT, nNOS, calret, calb, and S100β
Da Silva *et al*[130]	Rat	Distal colon	P2X7 in 100% of calret, calb, HuC/D, and S100β	N/A

(+): P2X7 receptor positivity without cellular chemical code information; N/A: Not applicable; ir: Immunoreactive; ChAT: Acetylcholine transferase enzyme-ir; nNOS: Neuronal nitric oxide synthase enzyme-ir; Calret: Calretinin-ir; Calb: Calbindin-ir; NPY: Neuropeptide Y-ir; SP: Substance P-ir; HuC/D: Neuronal pan-ir; NF200: Neurofilament 200-ir; GFAP: Glial fibrillary acid protein-ir; S100β: Protein β for calcium S100-ir labeling.

THERAPEUTIC APPROACHES TO THE TREATMENT OF INFLAMMATORY BOWEL DISEASES THROUGH THE P2X7 RECEPTOR

Positive results from the use of P2X7 receptor antagonists have already been demonstrated in the treatment of ischiatic nerve lesions in mice[159], in brain infarction by middle cerebral artery occlusion in rats[160], and in ileal ischemia and reperfusion in rats[131]. During experimentally induced colitis, intraperitoneal application of Brilliant Blue G (BBG) significantly reduced weight loss in rats, the score of mucosal lesions observed through colonoscopy, the macro- and microscopic degrees of inflammation, the number of inflammatory cells, and the deposition of collagen fibers in this organ. Lower levels of P2X7 receptor expression in the epithelium and lamina propria and lower levels of cell apoptosis in the distal colon epithelium were also demonstrated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. In addition, there was a stabilization of low concentrations of TNF-α, IL-1β and NF-κB, elementary members of this inflammatory process[154]. BBG was also effective in protecting intestinal regions distant from the inflammatory focus, as in the case of ileum in relation to distal colitis[56].

Moreover, in the context of colitis, various P2X7 receptor antagonists also slowed disease progression and reduced NF-κB activation, Caspase-1 expression, and concentrations of TNF and IL-1β in the mouse intestine[148]. Microscopic changes[148,154], changes in colonoscopy examination findings[154] and the loss of tight junctions due to inflammatory-cytokine-induced damage were also ameliorated[161]. In knockout (KO) mice, there was also an increase in specimen weight and reductions in histological lesions[155], with a greater preservation of the epithelial barrier, compared to wild-type (WT) animals[162]. Basically, there was no development of this disease in P2X7 receptor KO animals after the induction of inflammation[155,162].

Although all these therapeutic advances are exceptionally remarkable, only Eser *et al*[163] evaluated the use of some P2X7 receptor antagonists in humans with IBDs - a phase IIa study conducted specifically with patients in moderate to severe stages of Crohn's disease. According to the authors, the drug AZD9056 was well tolerated, and although it did not alter the concentrations of C-reactive protein or fecal calprotectin when compared to placebo, it caused a significant improvement in the Crohn's Disease

Activity Index (CDAI) and showing favorable effects on the remission of the disease and marked reduction in abdominal pain during the treatment period[163].

Taken together, this information reinforces the characterization of the P2X7 receptor as a promising target for the treatment of intestinal inflammatory conditions[14,127-133,148,154,155], especially in view of not only its wide expression in macrophages[10,164,165], mast cells[166] and T cells[10,162] but also its their strong involvement in the activation of caspases[167] and the release and regulation of transcription factors and pro-inflammatory cytokines[168,165].

CONCLUSION

It is concluded that IBDs are capable of aggressively and negatively affecting the cellular constituents of the ENS, and further studies are required in this area since knowledge in this area can still be considered, in a certain way, scarce. Studies of structural losses and/or structural deregulations in the enteric plexus may answer numerous questions about intestinal functionality, and therefore, the performance of these studies is of fundamental importance. Thus, it is also clear that the therapeutic approaches carried out through the P2X7 receptor have contributed to the advancement of this knowledge, but unfortunately: (1) We cannot fail to highlight that clinical trials with human patients are still lacking; (2) A better elucidation of the chemical signaling and functional regulation of immune cells upon the activation of this receptor is required; and (3) More quantitative studies on the structural components of the ENS involved in colitis and in its treatment are also required.

REFERENCES

1 **Nagy N**, Goldstein AM. Enteric nervous system development: A crest cell's journey from neural tube to colon. *Semin Cell Dev Biol* 2017; **66**: 94-106 [PMID: 28087321 DOI: 10.1016/j.semcdb.2017.01.006]

2 **Furness JB**, Callaghan BP, Rivera LR, Cho HJ. The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv Exp Med Biol* 2014; **817**: 39-71 [PMID: 24997029 DOI: 10.1007/978-1-4939-0897-4_3]

3 **Bayliss WM**, Starling EH. The movements and innervation of the small intestine. *J Physiol* 1899; **24**: 99-143 [PMID: 16992487 DOI: 10.1113/jphysiol.1899.sp000752]

4 **Furness JB**. The Enteric Nervous System. Oxford: Blackwell Publishing, 2006: 1-102 [DOI: 10.1002/9780470988756]

5 **Furness JB**. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 286-294 [PMID: 22392290 DOI: 10.1038/nrgastro.2012.32]

6 **Gershon MD**. The Second Brain. New York: Harper Collins, 1998 [DOI: 10.1080/15299716.2010.500969]

7 **De Giorgio R**, Guerrini S, Barbara G, Stanghellini V, De Ponti F, Corinaldesi R, Moses PL, Sharkey KA, Mawe GM. Inflammatory neuropathies of the enteric nervous system. *Gastroenterology* 2004; **126**: 1872-1883 [PMID: 15188182 DOI: 10.1053/j.gastro.2004.02.024]

8 **Gershon MD**. Developmental determinants of the independence and complexity of the enteric nervous system. *Trends Neurosci* 2010; **33**: 446-456 [PMID: 20633936 DOI: 10.1016/j.tins.2010.06.002]

9 **Spear ET**, Mawe GM. Enteric neuroplasticity and dysmotility in inflammatory disease: key players and possible therapeutic targets. *Am J Physiol Gastrointest Liver Physiol* 2019; **317**: G853-G861 [PMID: 31604034 DOI: 10.1152/ajpgi.00206.2019]

10 **Liu Y**, Liu X. Research progress of P2X7 receptor in inflammatory bowel disease. *Scand J Gastroenterol* 2019; **54**: 521-527 [PMID: 31056977 DOI: 10.1080/00365521.2019.1609077]

11 **Giaroni C**, Knight GE, Ruan HZ, Glass R, Bardini M, Lecchini S, Frigo G, Burnstock G. P2 receptors in the murine gastrointestinal tract. *Neuropharmacology* 2002; **43**: 1313-1323 [PMID: 12527481 DOI: 10.1016/s0028-3908(02)00294-0]

12 **Ren J**, Bertrand PP. Purinergic receptors and synaptic transmission in enteric neurons. *Purinergic Signal* 2008; **4**: 255-266 [PMID: 18368519 DOI: 10.1007/s11302-007-9088-5]

13 **Kolachala VL**, Bajaj R, Chalasani M, Sitaraman SV. Purinergic receptors in gastrointestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G401-G410 [PMID: 18063703 DOI: 10.1152/ajpgi.00454.2007]

14 **Antonioli L**, Colucci R, Pellegrini C, Giustarini G, Tuccori M, Blandizzi C, Fornai M. The role of purinergic pathways in the pathophysiology of gut diseases: pharmacological modulation and potential therapeutic applications. *Pharmacol Ther* 2013; **139**: 157-188 [PMID: 23588157 DOI: 10.1016/j.pharmthera.2013.04.002]

15 **Savio LEB**, de Andrade Mello P, da Silva CG, Coutinho-Silva R. The P2X7 Receptor in

Inflammatory Diseases: Angel or Demon? *Front Pharmacol* 2018; **9**: 52 [PMID: 29467654 DOI: 10.3389/fphar.2018.00052]

16 **Linden J**, Koch-Nolte F, Dahl G. Purine Release, Metabolism, and Signaling in the Inflammatory Response. *Annu Rev Immunol* 2019; **37**: 325-347 [PMID: 30676821 DOI: 10.1146/annurev-immunol-051116-052406]

17 **Linden DR**, Couvrette JM, Ciolino A, McQuoid C, Blaszyk H, Sharkey KA, Mawe GM. Indiscriminate loss of myenteric neurones in the TNBS-inflamed guinea-pig distal colon. *Neurogastroenterol Motil* 2005; **17**: 751-760 [PMID: 16185315 DOI: 10.1111/j.1365-2982.2005.00703.x]

18 **Lomax AE**, Fernández E, Sharkey KA. Plasticity of the enteric nervous system during intestinal inflammation. *Neurogastroenterol Motil* 2005; **17**: 4-15 [PMID: 15670258 DOI: 10.1111/j.1365-2982.2004.00607.x]

19 **Gulbransen BD**, Bashashati M, Hirota SA, Gui X, Roberts JA, MacDonald JA, Muruve DA, McKay DM, Beck PL, Mawe GM, Thompson RJ, Sharkey KA. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. *Nat Med* 2012; **18**: 600-604 [PMID: 22426419 DOI: 10.1038/nm.2679]

20 **Sairenji T**, Collins KL, Evans DV. An Update on Inflammatory Bowel Disease. *Prim Care* 2017; **44**: 673-692 [PMID: 29132528 DOI: 10.1016/j.pop.2017.07.010]

21 **Bray J**, Fernandes A, Nguyen GC, Otley AR, Heatherington J, Stretton J, Bollegala N, Benchimol EI. The Challenges of Living with Inflammatory Bowel Disease: Summary of a Summit on Patient and Healthcare Provider Perspectives. *Can J Gastroenterol Hepatol* 2016; **2016**: 9430942 [PMID: 27446878 DOI: 10.1155/2016/9430942]

22 **Mikocka-Walus A**, Knowles SR, Keefer L, Graff L. Controversies Revisited: A Systematic Review of the Comorbidity of Depression and Anxiety with Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2016; **22**: 752-762 [PMID: 26841224 DOI: 10.1097/MIB.0000000000000620]

23 **GBD 2017 Inflammatory Bowel Disease Collaborators**. . The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020; **5**: 17-30 [PMID: 31648971 DOI: 10.1016/S2468-1253(19)30333-4]

24 **Gershon MD**. The enteric nervous system: a second brain. *Hosp Pract (1995)* 1999; **34**: 31-32, 35 [PMID: 10418549 DOI: 10.3810/hp.1999.07.153]

25 **Wood JD**. Enteric Nervous System: Neuropathic Gastrointestinal Motility. *Dig Dis Sci* 2016; **61**: 1803-1816 [PMID: 27142673 DOI: 10.1007/s10620-016-4183-5]

26 **Goyal RK**, Hirano I. The enteric nervous system. *N Engl J Med* 1996; **334**: 1106-1115 [PMID: 8598871 DOI: 10.1056/NEJM199604253341707]

27 **Hansen MB**. The enteric nervous system I: organisation and classification. *Pharmacol Toxicol* 2003; **92**: 105-113 [PMID: 12753424 DOI: 10.1034/j.1600-0773.2003.t01-1-920301.x]

28 **Brookes SJ**. Classes of enteric nerve cells in the guinea-pig small intestine. *Anat Rec* 2001; **262**: 58-70 [PMID: 11146429 DOI: 10.1002/1097-0185(20010101)262:1<]

29 **Stöhr P**. Zusammenfassende ergebnisse über die mikroskopische innervation des magen-darmkanals. *Ergeb Anat Entwicklungsgesch* 1952; **34**: 250-401 [DOI: 10.1007/978-3-7091-5436-6_5]

30 **Furness JB**, Clerc N, Lomax AE, Bornstein JC, Kunze WA. Shapes and projections of tertiary plexus neurons of the guinea-pig small intestine. *Cell Tissue Res* 2000; **300**: 383-387 [PMID: 10928268 DOI: 10.1007/s004410000210]

31 **Dogiel AS**. Über den Bau der Ganglien in den Gefl echten des Darmes und der Gallenblase des Menschen und der Säugetiere. *Arch Anat Physiol Leipzig Anat Abt Jg* 1899; 130-158 [DOI: 10.1007/bf02976730]

32 **Pompolo S**, Furness JB. Ultrastructure and synaptic relationships of calbindin-reactive, Dogiel type II neurons, in myenteric ganglia of guinea-pig small intestine. *J Neurocytol* 1988; **17**: 771-782 [PMID: 3230396 DOI: 10.1007/BF01216705]

33 **Hendriks R**, Bornstein JC, Furness JB. An electrophysiological study of the projections of putative sensory neurons within the myenteric plexus of the guinea pig ileum. *Neurosci Lett* 1990; **110**: 286-290 [PMID: 2325901 DOI: 10.1016/0304-3940(90)90861-3]

34 **Song ZM**, Brookes SJ, Costa M. All calbindin-immunoreactive myenteric neurons project to the mucosa of the guinea-pig small intestine. *Neurosci Lett* 1994; **180**: 219-222 [PMID: 7535407 DOI: 10.1016/0304-3940(94)90524-x]

35 **Furness JB**, Trussell DC, Pompolo S, Bornstein JC, Smith TK. Calbindin neurons of the guinea-pig small intestine: quantitative analysis of their numbers and projections. *Cell Tissue Res* 1990; **260**: 261-272 [PMID: 2357722 DOI: 10.1007/BF00318629]

36 **Furness JB**. Types of neurons in the enteric nervous system. *J Auton Nerv Syst* 2000; **81**: 87-96 [PMID: 10869706 DOI: 10.1016/s0165-1838(00)00127-2]

37 **Gallego D**, Mañé N, Gil V, Martínez-Cutillas M, Jiménez M. Mechanisms responsible for neuromuscular relaxation in the gastrointestinal tract. *Rev Esp Enferm Dig* 2016; **108**: 721-731 [PMID: 26938735 DOI: 10.17235/reed.2016.4058/2015]

38 **Costa M**, Brookes SJ, Hennig GW. Anatomy and physiology of the enteric nervous system. *Gut* 2000; **47** Suppl 4: iv15-9; discussion iv26 [PMID: 11076898 DOI: 10.1136/gut.47.suppl_4.iv15]

39 **Kunze WA**, Furness JB. The enteric nervous system and regulation of intestinal motility. *Annu Rev Physiol* 1999; **61**: 117-142 [PMID: 10099684 DOI: 10.1146/annurev.physiol.61.1.117]

40 **Bornstein JC**, Costa M, Grider JR. Enteric motor and interneuronal circuits controlling motility. *Neurogastroenterol Motil* 2004; **16** Suppl 1: 34-38 [PMID: 15066002 DOI: 10.1111/j.1743-3150.2004.00472.x]

41 **Tonini M**, Costa M. A pharmacological analysis of the neuronal circuitry involved in distension-evoked enteric excitatory reflex. *Neuroscience* 1990; **38**: 787-795 [PMID: 1980147 DOI: 10.1016/0306-4522(90)90071-b]

42 **Furness JB**, Jones C, Nurgali K, Clerc N. Intrinsic primary afferent neurons and nerve circuits within the intestine. *Prog Neurobiol* 2004; **72**: 143-164 [PMID: 15063530 DOI: 10.1016/j.pneurobio.2003.12.004]

43 **Furness JB**, Clerc N, Vogalis F, Stebbing MJ. The enteric nervous system and its extrinsic connections. In: Yamada T, Alpers DH, editors. Textbook of Gastroenterology. Philadelphia: Lippincot Williams, 2003: 12-34 [DOI: 10.1002/9781444303254.ch2]

44 **Heanue TA**, Pachnis V. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. *Nat Rev Neurosci* 2007; **8**: 466-479 [PMID: 17514199 DOI: 10.1038/nrn2137]

45 **Chalazonitis A**, Rao M. Enteric nervous system manifestations of neurodegenerative disease. *Brain Res* 2018; **1693**: 207-213 [PMID: 29360466 DOI: 10.1016/j.brainres.2018.01.011]

46 **Sasselli V**, Pachnis V, Burns AJ. The enteric nervous system. *Dev Biol* 2012; **366**: 64-73 [PMID: 22290331 DOI: 10.1016/j.ydbio.2012.01.012]

47 **Langley JN**. The autonomic nervous system: Part 1. Cambridge: Heffer, 1921 [DOI: 10.1093/brain/26.1.1]

48 **Shapiro JM**, Subedi S, LeLeiko NS. Inflammatory Bowel Disease. *Pediatr Rev* 2016; **37**: 337-347 [PMID: 27482063 DOI: 10.1542/pir.2015-0110]

49 **Shivashankar R**, Lichtenstein GR. Mimics of Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2018; **24**: 2315-2321 [PMID: 29947781 DOI: 10.1093/ibd/izy168]

50 **Loddo I**, Romano C. Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis. *Front Immunol* 2015; **6**: 551 [PMID: 26579126 DOI: 10.3389/fimmu.2015.00551]

51 **Liu Y**, Zhao J, Zhao Y, Zong S, Tian Y, Chen S, Li M, Liu H, Zhang Q, Jing X, Sun B, Wang H, Sun T, Yang C. Therapeutic effects of lentinan on inflammatory bowel disease and colitis-associated cancer. *J Cell Mol Med* 2019; **23**: 750-760 [PMID: 30472806 DOI: 10.1111/jcmm.13897]

52 **Kaplan GG**. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 720-727 [PMID: 26323879 DOI: 10.1038/nrgastro.2015.150]

53 **Ke P**, Shao BZ, Xu ZQ, Chen XW, Liu C. Intestinal Autophagy and Its Pharmacological Control in Inflammatory Bowel Disease. *Front Immunol* 2016; **7**: 695 [PMID: 28119697 DOI: 10.3389/fimmu.2016.00695]

54 **Ishisono K**, Mano T, Yabe T, Kitaguchi K. Dietary Fiber Pectin Ameliorates Experimental Colitis in a Neutral Sugar Side Chain-Dependent Manner. *Front Immunol* 2019; **10**: 2979 [PMID: 31921214 DOI: 10.3389/fimmu.2019.02979]

55 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ; NIDDK IBD Genetics Consortium, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E; Belgian-French IBD Consortium; Wellcome Trust Case Control Consortium, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962 [PMID: 18587394 DOI: 10.1038/ng.175]

56 **de Souza HS**, Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 13-27 [PMID: 26627550 DOI: 10.1038/nrgastro.2015.186]

57 **Frolkis A**, Dieleman LA, Barkema HW, Panaccione R, Ghosh S, Fedorak RN, Madsen K, Kaplan GG; Alberta IBD Consortium. Enviroment and the inflammatory bowel diseases. *Can J Gastroenterol* 2013; **27**: e18-e24 [DOI: 10.1155/2013/102859]

58 **Ni J**, Wu GD, Albenberg L, Tomov VT. Gut microbiota and IBD: causation or correlation? *Nat Rev Gastroenterol Hepatol* 2017; **14**: 573-584 [PMID: 28743984 DOI: 10.1038/nrgastro.2017.88]

59 **Baj A**, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic Signaling Along The Microbiota-Gut-Brain Axis. *Int J Mol Sci* 2019; **20** [PMID: 30934533 DOI: 10.3390/ijms20061482]

60 **Vedamurthy A**, Ananthakrishnan AN. Influence of Environmental Factors in the Development and Outcomes of Inflammatory Bowel Disease. *Gastroenterol Hepatol (N Y)* 2019; **15**: 72-82 [PMID: 31011301]

61 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

62 **Kotze PG**, Underwood FE, Damião AOMC, Ferraz JGP, Saad-Hossne R, Toro M, Iade B, Bosques-Padilla F, Teixeira FV, Juliao-Banos F, Simian D, Ghosh S, Panaccione R, Ng SC, Kaplan GG. Progression of Inflammatory Bowel Diseases Throughout Latin America and the Caribbean: A Systematic Review. *Clin Gastroenterol Hepatol* 2020; **18**: 304-312 [PMID: 31252191 DOI:

10.1016/j.cgh.2019.06.030]
63 **Ng SC**, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, Li MF, Ng KK, Kamm MA, Studd C, Bell S, Leong R, de Silva HJ, Kasturiratne A, Mufeena MNF, Ling KL, Ooi CJ, Tan PS, Ong D, Goh KL, Hilmi I, Pisespongsa P, Manatsathit S, Rerknimitr R, Aniwan S, Wang YF, Ouyang Q, Zeng Z, Zhu Z, Chen MH, Hu PJ, Wu K, Wang X, Simadibrata M, Abdullah M, Wu JC, Sung JJY, Chan FKL; Asia–Pacific Crohn's and Colitis Epidemiologic Study (ACCESS) Study Group. Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* 2013; **145**: 158-165.e2 [PMID: 23583432 DOI: 10.1053/j.gastro.2013.04.007]
64 **Ng SC**, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* 2017; **390**: 2769-2778 [PMID: 29050646 DOI: 10.1016/S0140-6736(17)32448-0]
65 **Deepak P**, Fowler KJ, Fletcher JG, Bruining DH. Novel Imaging Approaches in Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2019; **25**: 248-260 [PMID: 30010908 DOI: 10.1093/ibd/izy239]
66 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]
67 **Torres J**, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet* 2017; **389**: 1741-1755 [PMID: 27914655 DOI: 10.1016/S0140-6736(16)31711-1]
68 **Ungaro R**, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet* 2017; **389**: 1756-1770 [PMID: 27914657 DOI: 10.1016/S0140-6736(16)32126-2]
69 **Wirtz S**, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF. Chemically induced mouse models of acute and chronic intestinal inflammation. *Nat Protoc* 2017; **12**: 1295-1309 [PMID: 28569761 DOI: 10.1038/nprot.2017.044]
70 **Morris GP**, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989; **96**: 795-803 [PMID: 2914642]
71 **Kawada M**, Arihiro A, Mizoguchi E. Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 5581-5593 [PMID: 17948932 DOI: 10.3748/wjg.v13.i42.5581]
72 **Chassaing B**, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr Protoc Immunol* 2014; **104**: 15.25.1-15.25.14 [PMID: 24510619 DOI: 10.1002/0471142735.im1525s104]
73 **Bramhall M**, Flórez-Vargas O, Stevens R, Brass A, Cruickshank S. Quality of methods reporting in animal models of colitis. *Inflamm Bowel Dis* 2015; **21**: 1248-1259 [PMID: 25989337 DOI: 10.1097/MIB.0000000000000369]
74 **Sanovic S**, Lamb DP, Blennerhassett MG. Damage to the enteric nervous system in experimental colitis. *Am J Pathol* 1999; **155**: 1051-1057 [PMID: 10514387 DOI: 10.1016/S0002-9440(10)65207-8]
75 **Brierley SM**, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 611-627 [PMID: 25001973 DOI: 10.1038/nrgastro.2014.103]
76 **Stavely R**, Abalo R, Nurgali K. Targeting Enteric Neurons and Plexitis for the Management of Inflammatory Bowel Disease. *Curr Drug Targets* 2020; **21**: 1428-1439 [PMID: 32416686 DOI: 10.2174/1389450121666200516173242]
77 **Linden DR**. Colitis is associated with a loss of intestinofugal neurons. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G1096-G1104 [PMID: 22997196 DOI: 10.1152/ajpgi.00176.2012]
78 **Cirillo C**, Sarnelli G, Cuomo R. Enteric nervous system abnormalities in ulcerative colitis. In: O'Connor MB. Ulcerative Colitis - Epidemiology, Pathogenesis and Complications. IntechOpen, 2011 [DOI: 10.5772/26176]
79 **Boyer L**, Ghoreishi M, Templeman V, Vallance BA, Buchan AM, Jevon G, Jacobson K. Myenteric plexus injury and apoptosis in experimental colitis. *Auton Neurosci* 2005; **117**: 41-53 [PMID: 15620569 DOI: 10.1016/j.autneu.2004.10.006]
80 **Bassotti G**, Villanacci V, Nascimbeni R, Cadei M, Fisogni S, Antonelli E, Corazzi N, Salerni B. Enteric neuroglial apoptosis in inflammatory bowel diseases. *J Crohns Colitis* 2009; **3**: 264-270 [PMID: 21172285 DOI: 10.1016/j.crohns.2009.06.004]
81 **Colombel JF**, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, Schreiber S, Byczkowski D, Li J, Kent JD, Pollack PF. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52-65 [PMID: 17241859 DOI: 10.1053/j.gastro.2006.11.041]
82 **Sandborn WJ**, Colombel JF, D'Haens G, Van Assche G, Wolf D, Kron M, Lazar A, Robinson AM, Yang M, Chao JD, Thakkar R. One-year maintenance outcomes among patients with moderately-to-severely active ulcerative colitis who responded to induction therapy with adalimumab: subgroup analyses from ULTRA 2. *Aliment Pharmacol Ther* 2013; **37**: 204-213 [PMID: 23173821 DOI: 10.1111/apt.12145]
83 **Perin RL**, Damião AOMC, Flores C, Ludvig JC, Magro DO, Miranda EF, Moraes AC, Nones RB, Teixeira FV, Zeroncio M, Kotze PG. VEDOLIZUMAB IN THE MANAGEMENT OF INFLAMMATORY BOWEL DISEASES: A BRAZILIAN OBSERVATIONAL MULTICENTRIC STUDY. *Arq Gastroenterol* 2019; **56**: 312-317 [PMID: 31633731 DOI: 10.1590/S0004-2803.201900000-58]

84 **Rosen MJ**, Dhawan A, Saeed SA. Inflammatory Bowel Disease in Children and Adolescents. *JAMA Pediatr* 2015; **169**: 1053-1060 [PMID: 26414706 DOI: 10.1001/jamapediatrics.2015.1982]

85 **Di Virgilio F**, Dal Ben D, Sarti AC, Giuliani AL, Falzoni S. The P2X7 Receptor in Infection and Inflammation. *Immunity* 2017; **47**: 15-31 [PMID: 28723547 DOI: 10.1016/j.immuni.2017.06.020]

86 **Cao F**, Hu LQ, Yao SR, Hu Y, Wang DG, Fan YG, Pan GX, Tao SS, Zhang Q, Pan HF, Wu GC. P2X7 receptor: A potential therapeutic target for autoimmune diseases. *Autoimmun Rev* 2019; **18**: 767-777 [PMID: 31181327 DOI: 10.1016/j.autrev.2019.06.009]

87 **Rajendran M**, Dane E, Conley J, Tantama M. Imaging Adenosine Triphosphate (ATP). *Biol Bull* 2016; **231**: 73-84 [PMID: 27638696 DOI: 10.1086/689592]

88 **Boué-Grabot E**, Blum D, Ceruti S. Editorial: Purinergic Signaling in Health and Disease. *Front Cell Neurosci* 2020; **14**: 15 [PMID: 32116561 DOI: 10.3389/fncel.2020.00015]

89 **Yamamoto K**, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J. Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. *Nat Med* 2006; **12**: 133-137 [PMID: 16327800 DOI: 10.1038/nm1338]

90 **Burnstock G**. Vessel tone and remodeling. *Nat Med* 2006; **12**: 16-17 [PMID: 16397544 DOI: 10.1038/nm0106-16]

91 **Franke H**, Krügel U, Illes P. P2 receptors and neuronal injury. *Pflugers Arch* 2006; **452**: 622-644 [PMID: 16645849 DOI: 10.1007/s00424-006-0071-8]

92 **Burnstock G**. Physiology and pathophysiology of purinergic neurotransmission. *Physiol Rev* 2007; **87**: 659-797 [PMID: 17429044 DOI: 10.1152/physrev.00043.2006]

93 **Abbracchio MP**, Burnstock G, Verkhratsky A, Zimmermann H. Purinergic signalling in the nervous system: an overview. *Trends Neurosci* 2009; **32**: 19-29 [PMID: 19008000 DOI: 10.1016/j.tins.2008.10.001]

94 **Burnstock G**. Purine and pyrimidine receptors. *Cell Mol Life Sci* 2007; **64**: 1471-1483 [PMID: 17375261 DOI: 10.1007/s00018-007-6497-0]

95 **Burnstock G**. Purinergic nerves. *Pharmacol Rev* 1972; **24**: 509-581 [PMID: 4404211]

96 **Burnstock G**. A basis for distinguishing two types of purinergic receptor. In: Straub RW, Bolis L, editors. Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. New York: Raven, 1978: 107-118 [DOI: 10.1016/0014-5793(79)81367-8]

97 **Burnstock G**, Kennedy C. Is there a basis for distinguishing two types of P2-purinoceptor? *Gen Pharmacol* 1985; **16**: 433-440 [PMID: 2996968 DOI: 10.1016/0306-3623(85)90001-1]

98 **Abbracchio MP**, Burnstock G. Purinoceptors: are there families of P2X and P2Y purinoceptors? *Pharmacol Ther* 1994; **64**: 445-475 [PMID: 7724657 DOI: 10.1016/0163-7258(94)00048-4]

99 **Mehta N**, Kaur M, Singh M, Chand S, Vyas B, Silakari P, Bahia MS, Silakari O. Purinergic receptor P2X7: A novel target for anti-inflammatory therapy. *Bioorg Med Chem* 2014; **22**: 54-88 [DOI: 10.1016/j.bmc.2013.10.054]

100 **Abbracchio MP**, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* 2006; **58**: 281-341 [PMID: 16968944 DOI: 10.1124/pr.58.3.3]

101 **North RA**. Molecular physiology of P2X receptors. *Physiol Rev* 2002; **82**: 1013-1067 [PMID: 12270951 DOI: 10.1152/physrev.00015.2002]

102 **Roberts JA**, Vial C, Digby HR, Agboh KC, Wen H, Atterbury-Thomas A, Evans RJ. Molecular properties of P2X receptors. *Pflugers Arch* 2006; **452**: 486-500 [PMID: 16607539 DOI: 10.1007/s00424-006-0073-6]

103 **Burnstock G**, Knight GE. Cellular distribution and functions of P2 receptor subtypes in different systems. *Int Rev Cytol* 2004; **240**: 31-304 [PMID: 15548415 DOI: 10.1016/s0074-7696(04)40002-3]

104 **Kobayashi K**, Yamanaka H, Noguchi K. Expression of ATP receptors in the rat dorsal root ganglion and spinal cord. *Anat Sci Int* 2013; **88**: 10-16 [PMID: 23179910 DOI: 10.1007/s12565-012-0163-9]

105 **Wang L**, Feng D, Yan H, Wang Z, Pei L. Comparative analysis of P2X1, P2X2, P2X3, and P2X4 receptor subunits in rat nodose ganglion neurons. *PLoS One* 2014; **9**: e96699 [PMID: 24798490 DOI: 10.1371/journal.pone.0096699]

106 **Puchałowicz K**, Baranowska-Bosiacka I, Dziedziejko V, Chlubek D. Purinergic signaling and the functioning of the nervous system cells. *Cell Mol Biol Lett* 2015; **20**: 867-918 [PMID: 26618572 DOI: 10.1515/cmble-2015-0050]

107 **Burnstock G**. Introduction to purinergic signalling in the brain. In: Barańska J, editor. Glioma Signaling: Advances in Experimental Medicine and Biology. Cham: Springer, 2020: 1-12

108 **Christofi FL**, Zhang H, Yu JG, Guzman J, Xue J, Kim M, Wang YZ, Cooke HJ. Differential gene expression of adenosine A1, A2a, A2b, and A3 receptors in the human enteric nervous system. *J Comp Neurol* 2001; **439**: 46-64 [PMID: 11579381 DOI: 10.1002/cne.1334]

109 **Gao N**, Hu HZ, Zhu MX, Fang X, Liu S, Gao C, Wood JD. The P2Y purinergic receptor expressed by enteric neurones in guinea-pig intestine. *Neurogastroenterol Motil* 2006; **18**: 316-323 [PMID: 16553587 DOI: 10.1111/j.1365-2982.2005.00754.x]

110 **Castelucci P**, Robbins HL, Poole DP, Furness JB. The distribution of purine P2X(2) receptors in the guinea-pig enteric nervous system. *Histochem Cell Biol* 2002; **117**: 415-422 [PMID: 12029488 DOI: 10.1007/s00418-002-0404-4]

111 **Van Nassauw L**, Brouns I, Adriaensen D, Burnstock G, Timmermans JP. Neurochemical

identification of enteric neurons expressing P2X(3) receptors in the guinea-pig ileum. *Histochem Cell Biol* 2002; **118**: 193-203 [PMID: 12271355 DOI: 10.1007/s00418-002-0447-6]

112 **Poole DP**, Castelucci P, Robbins HL, Chiocchetti R, Furness JB. The distribution of P2X3 purine receptor subunits in the guinea pig enteric nervous system. *Auton Neurosci* 2002; **101**: 39-47 [PMID: 12462358 DOI: 10.1016/s1566-0702(02)00179-0]

113 **Xiang Z**, Burnstock G. Distribution of P2Y2 receptors in the guinea pig enteric nervous system and its coexistence with P2X2 and P2X3 receptors, neuropeptide Y, nitric oxide synthase and calretinin. *Histochem Cell Biol* 2005; **124**: 379-390 [PMID: 16136347 DOI: 10.1007/s00418-005-0043-7]

114 **Xiang Z**, Burnstock G. Distribution of P2Y6 and P2Y12 receptor: their colocalization with calbindin, calretinin and nitric oxide synthase in the guinea pig enteric nervous system. *Histochem Cell Biol* 2006; **125**: 327-336 [PMID: 16195889 DOI: 10.1007/s00418-005-0071-3]

115 **Ruan HZ**, Burnstock G. The distribution of P2X5 purinergic receptors in the enteric nervous system of mouse. *Cell Tissue Res* 2005; **319**: 191-200 [PMID: 15551155 DOI: 10.1007/s00441-004-1002-7]

116 **Mizuno MS**, Crisma AR, Borelli P, Castelucci P. Expression of the P2X$_2$ receptor in different classes of ileum myenteric neurons in the female obese ob/ob mouse. *World J Gastroenterol* 2012; **18**: 4693-4703 [PMID: 23002338 DOI: 10.3748/wjg.v18.i34.4693]

117 **Mizuno MS**, Crisma AR, Borelli P, Schäfer BT, Silveira MP, Castelucci P. Distribution of the P2X2 receptor and chemical coding in ileal enteric neurons of obese male mice (ob/ob). *World J Gastroenterol* 2014; **20**: 13911-13919 [PMID: 25320527 DOI: 10.3748/wjg.v20.i38.13911]

118 **Xiang Z**, Burnstock G. P2X2 and P2X3 purinoceptors in the rat enteric nervous system. *Histochem Cell Biol* 2004; **121**: 169-179 [PMID: 14767775 DOI: 10.1007/s00418-004-0620-1]

119 **Misawa R**, Girotti PA, Mizuno MS, Liberti EA, Furness JB, Castelucci P. Effects of protein deprivation and re-feeding on P2X2 receptors in enteric neurons. *World J Gastroenterol* 2010; **16**: 3651-3663 [PMID: 20677337 DOI: 10.3748/wjg.v16.i29.3651]

120 **Paulino AS**, Palombit K, Cavriani G, Tavares-de-Lima W, Mizuno MS, Marosti AR, da Silva MV, Girotti PA, Liberti EA, Castelucci P. Effects of ischemia and reperfusion on P2X2 receptor expressing neurons of the rat ileum enteric nervous system. *Dig Dis Sci* 2011; **56**: 2262-2275 [PMID: 21409380 DOI: 10.1007/s10620-011-1588-z]

121 **Girotti PA**, Misawa R, Palombit K, Mendes CE, Bittencourt JC, Castelucci P. Differential effects of undernourishment on the differentiation and maturation of rat enteric neurons. *Cell Tissue Res* 2013; **353**: 367-380 [PMID: 23644765 DOI: 10.1007/s00441-013-1620-z]

122 **Marosti AR**, da Silva MV, Palombit K, Mendes CE, Tavares-de-Lima W, Castelucci P. Differential effects of intestinal ischemia and reperfusion in rat enteric neurons and glial cells expressing P2X2 receptors. *Histol Histopathol* 2015; **30**: 489-501 [PMID: 25400134 DOI: 10.14670/hh-30.489]

123 **Mendes CE**, Palombit K, Vieira C, Silva I, Correia-de-Sá P, Castelucci P. The Effect of Ischemia and Reperfusion on Enteric Glial Cells and Contractile Activity in the Ileum. *Dig Dis Sci* 2015; **60**: 2677-2689 [PMID: 25917048 DOI: 10.1007/s10620-015-3663-3]

124 **Yu Q**, Zhao Z, Sun J, Guo W, Fu J, Burnstock G, He C, Xiang Z. Expression of P2X6 receptors in the enteric nervous system of the rat gastrointestinal tract. *Histochem Cell Biol* 2010; **133**: 177-188 [PMID: 19946698 DOI: 10.1007/s00418-009-0659-0]

125 **Hu HZ**, Gao N, Lin Z, Gao C, Liu S, Ren J, Xia Y, Wood JD. P2X(7) receptors in the enteric nervous system of guinea-pig small intestine. *J Comp Neurol* 2001; **440**: 299-310 [PMID: 11745625 DOI: 10.1002/cne.1387]

126 **Vanderwinden JM**, Timmermans JP, Schiffmann SN. Glial cells, but not interstitial cells, express P2X7, an ionotropic purinergic receptor, in rat gastrointestinal musculature. *Cell Tissue Res* 2003; **312**: 149-154 [PMID: 12684872 DOI: 10.1007/s00441-003-0716-2]

127 **Palombit K**, Mendes CE, Tavares-de-Lima W, Silveira MP, Castelucci P. Effects of ischemia and reperfusion on subpopulations of rat enteric neurons expressing the P2X7 receptor. *Dig Dis Sci* 2013; **58**: 3429-3439 [PMID: 23990036 DOI: 10.1007/s10620-013-2847-y]

128 **Antonioli L**, Giron MC, Colucci R, Pellegrini C, Sacco D, Caputi V, Orso G, Tuccori M, Scarpignato C, Blandizzi C, Fornai M. Involvement of the P2X7 purinergic receptor in colonic motor dysfunction associated with bowel inflammation in rats. *PLoS One* 2014; **9**: e116253 [PMID: 25549098 DOI: 10.1371/journal.pone.0116253]

129 **Da Silva MV**, Marosti AR, Mendes CE, Palombit K, Castelucci P. Differential effects of experimental ulcerative colitis on P2X7 receptor expression in enteric neurons. *Histochem Cell Biol* 2015; **143**: 171-184 [PMID: 25201348 DOI: 10.1007/s00418-014-1270-6]

130 **Da Silva MV**, Marosti AR, Mendes CE, Palombit K, Castelucci P. Submucosal neurons and enteric glial cells expressing the P2X7 receptor in rat experimental colitis. *Acta Histochem* 2017; **119**: 481-494 [PMID: 28501138 DOI: 10.1016/j.acthis.2017.05.001]

131 **Palombit K**, Mendes CE, Tavares-de-Lima W, Barreto-Chaves ML, Castelucci P. Blockage of the P2X7 Receptor Attenuates Harmful Changes Produced by Ischemia and Reperfusion in the Myenteric Plexus. *Dig Dis Sci* 2019; **64**: 1815-1829 [PMID: 30734238 DOI: 10.1007/s10620-019-05496-8]

132 **Mendes CE**, Palombit K, Tavares-de-Lima W, Castelucci P. Enteric glial cells immunoreactive for P2X7 receptor are affected in the ileum following ischemia and reperfusion. *Acta Histochem* 2019; **121**: 665-679 [PMID: 31202513 DOI: 10.1016/j.acthis.2019.06.001]

133 **Souza RF**, Evangelinellis MM, Mendes CE, Righetti M, Lourenço MCS, Castelucci P. P2X7 receptor antagonist recovers ileum myenteric neurons after experimental ulcerative colitis. *World J*

Gastrointest Pathophysiol 2020; **11**: 84-103 [PMID: 32587788 DOI: 10.4291/wjgp.v11.i4.84]

134 **Surprenant A**, Rassendren F, Kawashima E, North RA, Buell G. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* 1996; **272**: 735-738 [PMID: 8614837 DOI: 10.1126/science.272.5262.735]

135 **Sluyter R**. The P2X7 receptor. In: Atassi M, editor. Protein Reviews. Advances in Experimental Medicine and Biology. Singapura: Springer, 2017

136 **Liu X**, Ma W, Surprenant A, Jiang LH. Identification of the amino acid residues in the extracellular domain of rat P2X(7) receptor involved in functional inhibition by acidic pH. *Br J Pharmacol* 2009; **156**: 135-142 [PMID: 19068080 DOI: 10.1111/j.1476-5381.2008.00002.x]

137 **Zhang WJ**, Zhu ZM, Liu ZX. The role and pharmacological properties of the P2X7 receptor in neuropathic pain. *Brain Res Bull* 2020; **155**: 19-28 [PMID: 31778766 DOI: 10.1016/j.brainresbull.2019.11.006]

138 **North RA**, Jarvis MF. P2X receptors as drug targets. *Mol Pharmacol* 2013; **83**: 759-769 [PMID: 23253448 DOI: 10.1124/mol.112.083758]

139 **Coddou C**, Yan Z, Obsil T, Huidobro-Toro JP, Stojilkovic SS. Activation and regulation of purinergic P2X receptor channels. *Pharmacol Rev* 2011; **63**: 641-683 [PMID: 21737531 DOI: 10.1124/pr.110.003129]

140 **Burnstock G**. P2X ion channel receptors and inflammation. *Purinergic Signal* 2016; **12**: 59-67 [PMID: 26739702 DOI: 10.1007/s11302-015-9493-0]

141 **Sperlágh B**, Illes P. P2X7 receptor: an emerging target in central nervous system diseases. *Trends Pharmacol Sci* 2014; **35**: 537-547 [PMID: 25223574 DOI: 10.1016/j.tips.2014.08.002]

142 **North RA**, Verkhratsky A. Purinergic transmission in the central nervous system. *Pflugers Arch* 2006; **452**: 479-485 [PMID: 16688467 DOI: 10.1007/s00424-006-0060-y]

143 **Dubyak GR**. P2X7 receptor regulation of non-classical secretion from immune effector cells. *Cell Microbiol* 2012; **14**: 1697-1706 [PMID: 22882764 DOI: 10.1111/cmi.12001]

144 **Matyśniak D**, Nowak N, Chumak V, Pomorski P. P2X7 receptor activity landscape in rat and human glioma cell lines. *Acta Biochim Pol* 2020; **67**: 7-14 [PMID: 32187491 DOI: 10.18388/abp.2020_2848]

145 **Ohishi A**, Keno Y, Marumiya A, Sudo Y, Uda Y, Matsuda K, Morita Y, Furuta T, Nishida K, Nagasawa K. Expression level of P2X7 receptor is a determinant of ATP-induced death of mouse cultured neurons. *Neuroscience* 2016; **319**: 35-45 [PMID: 26812038 DOI: 10.1016/j.neuroscience.2016.01.048]

146 **Burnstock G**. Pathophysiology and therapeutic potential of purinergic signaling. *Pharmacol Rev* 2006; **58**: 58-86 [PMID: 16507883 DOI: 10.1124/pr.58.1.5]

147 **Deaglio S**, Robson SC. Ectonucleotidases as regulators of purinergic signaling in thrombosis, inflammation, and immunity. *Adv Pharmacol* 2011; **61**: 301-332 [PMID: 21586363 DOI: 10.1016/b978-0-12-385526-8.00010-2]

148 **Wan P**, Liu X, Xiong Y, Ren Y, Chen J, Lu N, Guo Y, Bai A. Extracellular ATP mediates inflammatory responses in colitis *via* P2 × 7 receptor signaling. *Sci Rep* 2016; **6**: 19108 [PMID: 26739809 DOI: 10.1038/srep19108]

149 **Young CNJ**, Górecki DC. P2RX7 Purinoceptor as a Therapeutic Target-The Second Coming? *Front Chem* 2018; **6**: 248 [PMID: 30003075 DOI: 10.3389/fchem.2018.00248]

150 **Zhang Q**, Hu F, Guo F, Zhou Q, Xiang H, Shang D. Emodin attenuates adenosine triphosphateinduced pancreatic ductal cell injury in vitro *via* the inhibition of the P2X7/NLRP3 signaling pathway. *Oncol Rep* 2019; **42**: 1589-1597 [PMID: 31524270 DOI: 10.3892/or.2019.7270]

151 **Di Virgilio F**, Giuliani AL. Purinergic signalling in autoimmunity: A role for the P2X7R in systemic lupus erythematosus? *Biomed J* 2016; **39**: 326-338 [PMID: 27884379 DOI: 10.1016/j.bj.2016.08.006]

152 **Volonté C**, Amadio S, Cavaliere F, D'Ambrosi N, Vacca F, Bernardi G. Extracellular ATP and neurodegeneration. *Curr Drug Targets CNS Neurol Disord* 2003; **2**: 403-412 [PMID: 14683468 DOI: 10.2174/1568007033482643]

153 **Franke H**, Illes P. Involvement of P2 receptors in the growth and survival of neurons in the CNS. *Pharmacol Ther* 2006; **109**: 297-324 [PMID: 16102837 DOI: 10.1016/j.pharmthera.2005.06.002]

154 **Marques CC**, Castelo-Branco MT, Pacheco RG, Buongusto F, do Rosário A Jr, Schanaider A, Coutinho-Silva R, de Souza HS. Prophylactic systemic P2X7 receptor blockade prevents experimental colitis. *Biochim Biophys Acta* 2014; **1842**: 65-78 [PMID: 24184714 DOI: 10.1016/j.bbadis.2013.10.012]

155 **Neves AR**, Castelo-Branco MT, Figliuolo VR, Bernardazzi C, Buongusto F, Yoshimoto A, Nanini HF, Coutinho CM, Carneiro AJ, Coutinho-Silva R, de Souza HS. Overexpression of ATP-activated P2X7 receptors in the intestinal mucosa is implicated in the pathogenesis of Crohn's disease. *Inflamm Bowel Dis* 2014; **20**: 444-457 [PMID: 24412990 DOI: 10.1097/01.mib.0000441201.10454.06]

156 **Luna-Gomes T**, Santana PT, Coutinho-Silva R. Silica-induced inflammasome activation in macrophages: role of ATP and P2X7 receptor. *Immunobiology* 2015; **220**: 1101-1106 [PMID: 26024943 DOI: 10.1016/j.imbio.2015.05.004]

157 **Diezmos EF**, Bertrand PP, Liu L. Purinergic Signaling in Gut Inflammation: The Role of Connexins and Pannexins. *Front Neurosci* 2016; **10**: 311 [PMID: 27445679 DOI: 10.3389/fnins.2016.00311]

158 **Roberts JA**, Lukewich MK, Sharkey KA, Furness JB, Mawe GM, Lomax AE. The roles of purinergic signaling during gastrointestinal inflammation. *Curr Opin Pharmacol* 2012; **12**: 659-666

[PMID: 23063457 DOI: 10.1016/j.coph.2012.09.011]

159 **Ribeiro T**, Oliveira JT, Almeida FM, Tomaz MA, Melo PA, Marques SA, de Andrade GM, Martinez AMB. Blockade of ATP P2X7 receptor enhances ischiatic nerve regeneration in mice following a crush injury. *Brain Res* 2017; **1669**: 69-78 [PMID: 28554806 DOI: 10.1016/j.brainres.2017.05.025]

160 **Arbeloa J**, Pérez-Samartín A, Gottlieb M, Matute C. P2X7 receptor blockade prevents ATP excitotoxicity in neurons and reduces brain damage after ischemia. *Neurobiol Dis* 2012; **45**: 954-961 [PMID: 22186422 DOI: 10.1016/j.nbd.2011.12.014]

161 **Diezmos EF**, Markus I, Perera DS, Gan S, Zhang L, Sandow SL, Bertrand PP, Liu L. Blockade of Pannexin-1 Channels and Purinergic P2X7 Receptors Shows Protective Effects Against Cytokines-Induced Colitis of Human Colonic Mucosa. *Front Pharmacol* 2018; **9**: 865 [PMID: 30127744 DOI: 10.3389/fphar.2018.00865]

162 **Figliuolo VR**, Savio LEB, Safya H, Nanini H, Bernardazzi C, Abalo A, de Souza HSP, Kanellopoulos J, Bobé P, Coutinho CMLM, Coutinho-Silva R. P2X7 receptor promotes intestinal inflammation in chemically induced colitis and triggers death of mucosal regulatory T cells. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 1183-1194 [PMID: 28286160 DOI: 10.1016/j.bbadis.2017.03.004]

163 **Eser A**, Colombel JF, Rutgeerts P, Vermeire S, Vogelsang H, Braddock M, Persson T, Reinisch W. Safety and Efficacy of an Oral Inhibitor of the Purinergic Receptor P2X7 in Adult Patients with Moderately to Severely Active Crohn's Disease: A Randomized Placebo-controlled, Double-blind, Phase IIa Study. *Inflamm Bowel Dis* 2015; **21**: 2247-2253 [PMID: 26197451 DOI: 10.1097/mib.0000000000000514]

164 **Wiley JS**, Sluyter R, Gu BJ, Stokes L, Fuller SJ. The human P2X7 receptor and its role in innate immunity. *Tissue Antigens* 2011; **78**: 321-332 [PMID: 21988719 DOI: 10.1111/j.1399-0039.2011.01780.x]

165 **Burnstock G**, Jacobson KA, Christofi FL. Purinergic drug targets for gastrointestinal disorders. *Curr Opin Pharmacol* 2017; **37**: 131-141 [PMID: 29149731 DOI: 10.1016/j.coph.2017.10.011]

166 **Kurashima Y**, Amiya T, Nochi T, Fujisawa K, Haraguchi T, Iba H, Tsutsui H, Sato S, Nakajima S, Iijima H, Kubo M, Kunisawa J, Kiyono H. Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors. *Nat Commun* 2012; **3**: 1034 [PMID: 22948816 DOI: 10.1038/ncomms2023]

167 **Kopp R**, Krautloher A, Ramírez-Fernández A, Nicke A. P2X7 Interactions and Signaling - Making Head or Tail of It. *Front Mol Neurosci* 2019; **12**: 183 [PMID: 31440138 DOI: 10.3389/fnmol.2019.00183]

168 **Franke H**, Verkhratsky A, Burnstock G, Illes P. Pathophysiology of astroglial purinergic signalling. *Purinergic Signal* 2012; **8**: 629-657 [PMID: 22544529 DOI: 10.1007/s11302-012-9300-0]

169 **Schneider J**, Jehle EC, Starlinger MJ, Neunlist M, Michel K, Hoppe S, Schemann M. Neurotransmitter coding of enteric neurones in the submucous plexus is changed in non-inflamed rectum of patients with Crohn's disease. *Neurogastroenterol Motil* 2001; **13**: 255-264 [PMID: 11437988 DOI: 10.1046/j.1365-2982.2001.00265.x]

170 **Sigalet DL**, Wallace L, De Heuval E, Sharkey KA. The effects of glucagon-like peptide 2 on enteric neurons in intestinal inflammation. *Neurogastroenterol Motil* 2010; **22**: 1318-e350 [PMID: 20718942 DOI: 10.1111/j.1365-2982.2010.01585.x]

171 **Sarnelli G**, De Giorgio R, Gentile F, Calì G, Grandone I, Rocco A, Cosenza V, Cuomo R, D'Argenio G. Myenteric neuronal loss in rats with experimental colitis: role of tissue transglutaminase-induced apoptosis. *Dig Liver Dis* 2009; **41**: 185-193 [PMID: 18635410 DOI: 10.1016/j.dld.2008.06.004]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2021 December 14; 27(46): 7925-7942

DOI: 10.3748/wjg.v27.i46.7925

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

COVID-19 and gut immunomodulation

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Author contributions: Roy K and Agarwal S contributed equally; Roy K, Agarwal S, Banerjee R, Paul MK, and Purbey PK contributed to the draft preparation of the article; Paul MK and Purbey PK conceptualized and edited the article.

Conflict-of-interest statement: No Conflict-of-interest.

Country/Territory of origin: United States

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

The disease coronavirus disease 2019 (COVID-19) is a severe respiratory illness that has emerged as a devastating health problem worldwide. The disease outcome is heterogeneous, and severity is likely dependent on the immunity of infected individuals and comorbidities. Although symptoms of the disease are primarily associated with respiratory problems, additional infection or failure of other vital organs are being reported. Emerging reports suggest a quite common co-existence of gastrointestinal (GI) tract symptoms in addition to respiratory symptoms in many COVID-19 patients, and some patients show just the GI symptoms. The possible cause of the GI symptoms could be due to direct infection of the epithelial cells of the gut, which is supported by the fact that (1) The intestinal epithelium expresses a high level of angiotensin-converting enzyme-2 and transmembrane protease serine 2 protein that are required for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry into the cells; (2) About half of the severe COVID-19 patients show viral RNA in their feces and various parts of the GI tract; and (3) SARS-CoV-2 can directly infect gut epithelial cells *in vitro* (gut epithelial cells and organoids) and *in vivo* (rhesus monkey). The GI tract seems to be a site of active innate and adaptive immune responses to SARS-CoV-2 as clinically, stool samples of COVID-19 patients possess proinflammatory cytokines (interleukin 8), calprotectin (neutrophils activity), and immunoglobulin A antibodies. In addition to direct immune activation by the virus,

Received: February 18, 2021
Peer-review started: February 18, 2021
First decision: May 13, 2021
Revised: June 9, 2021
Accepted: November 29, 2021
Article in press: November 29, 2021
Published online: December 14, 2021

P-Reviewer: Atoum M, Fan Y, Fei C
S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

impairment of GI epithelium integrity can evoke immune response under the influence of systemic cytokines, hypoxia, and changes in gut microbiota (dysbiosis) due to infection of the respiratory system, which is confirmed by the observation that not all of the GI symptomatic patients are viral RNA positive. This review comprehensively summarizes the possible GI immunomodulation by SARS-CoV-2 that could lead to GI symptoms, their association with disease severity, and potential therapeutic interventions.

Key Words: COVID-19; Gastrointestinal symptoms; Pathogenesis; Innate immune response; Adaptive immune response; Gut microbiota; Dysbiosis; Therapeutics; Probiotic; Pre-existing diseases

Core Tip: Coronavirus disease 2019 (COVID-19) is a global pandemic. Many COVID-19 patients either present gastrointestinal (GI) symptoms in addition to respiratory symptoms or just GI symptoms. Syndrome coronavirus 2 (SARS-CoV-2) directly infects GI epithelial cells as they express significant levels of angiotensin-converting enzyme-2 and transmembrane protease serine 2 protein, required for SARS-CoV-2 entry. This article reviews gut infection and GI immunomodulation by SARS-CoV-2, leading to spectrum of GI symptoms and pathogenesis in COVID-19-patients. Special emphases are given on the innate and acquired immune responses in the GI tract due to intestinal and non-intestinal SARS-CoV-2 infection, COVID-19 severity in people with pre-existing intestinal diseases, role of gut microbiota, and possible therapeutic interventions are discussed.

Citation: Roy K, Agarwal S, Banerjee R, Paul MK, Purbey PK. COVID-19 and gut immunomodulation. *World J Gastroenterol* 2021; 27(46): 7925-7942
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7925.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7925

INTRODUCTION

In December 2019, pneumonia cases with unrecognized etiology were reported in the Wuhan city of China, causing fever and acute respiratory distress. The causative agent is a novel coronavirus named syndrome coronavirus 2 (SARS-CoV-2), and the disease is referred to as coronavirus disease 2019 (COVID-19)[1,2]. SARS-CoV-2 belongs to the order "Nidovirales"; family of "Coronaviridae", and subfamily "Orthocoronavirinae" [3]. Coronaviruses are single-stranded RNA viruses, usually zoonotic but have regularly affected humans to cause a major health crisis[4,5]. COVID-19 rapidly spread like an epidemic in China, followed by worldwide transmission of the infection and, therefore, was declared a pandemic and global crisis by the World Health Organization (WHO) in March 2020. As of May 23, 2021, the WHO COVID-19 dashboard reported 166352007 confirmed cases of COVID-19, and 3449189 deaths worldwide, making this one of the worst pandemics in the 21st century. The high rate of human-to-human transmission, asymptomatic carriers, and the absence of therapeutic intervention led to the global pandemic.

The evolution of new variants of the virus has made the situation even worse. New SARS-CoV-2 strains are emerging like B.1.351 was detected in South Africa, B.1.207 in Nigeria, while strain B.1.1.7 was identified in the United Kingdom in December 2020 and is highly infectious. The new strains like B.1.1.7 strain harbor several mutations, especially in the S protein, including the N501Y (asparagine to tyrosine substitution), 69/70 deletion. P681H and enhances the virus-angiotensin-converting enzyme-2 (ACE-2) binding efficacy, thereby making the variants highly contagious. B.1.617.1, B.1.617.2, and B.1.617.3 are the three subtypes of the Indian variant reported in October 2020 is highly infectious and causing fresh waves of infection in many countries around the world. Three important mutations in the sequence coding for the viral spike protein co-occur in variant B.1.617.1: L452R, E484Q, and P681R. B.1.617.2 is also linked to the L452R, T478K, and P681R mutations[6]. Indications suggest that

these variants can trigger severe disease conditions or higher fatality rates. The complete impact of these mutations is not yet understood and is still being researched, but comprehensive genomic strain surveillance is needed to better understand the strain-specific infection, pathogenesis, epidemiological and therapeutic aspects. Several potential therapeutic and prophylactic interventions are under investigation or have undergone randomized controlled trials. Great strides have been made in vaccine development, and COVID-19 vaccines are now approved for mass use in several countries. Raising hopes for curbing the COVID-19 crisis and WHO's guidelines on wearing a mask, social distancing, and sanitization needs to be strictly followed to bend down the infection curve.

Though COVID-19 mainly causes respiratory illness, many patients experience gastrointestinal (GI) symptoms, including nausea, vomiting, belly pain, appetite loss, and diarrhea. GI symptoms are often associated with the presence of CoV2 RNA in many patients' stool (feces) samples[7]. Though the mechanism of lung infection is widely studied, there is a dearth of information regarding the enteric phase of SARS-CoV-2, especially the immune contexture and response. The gut microbiome is considered to play a key role in regulating the impact of SARS-CoV-2, and significant alterations in the microbiota profiles are reported in COVID-19 patients. The role of the gut-lung axis and the severe respiratory distress associated with gut imbalance is also very relevant[8]. COVID researchers have reported a disturbance of the gut microbiota and its association with lung and gut infections, which can cause hindrance in the gut-lung axis. Recent data suggest that GI symptoms might be a warning sign of a more serious condition with poor prognosis. Because of the GI infection and COVID severity, the present paper deals with a complete review of the COVID-19-associated gut-infection, pathogenesis, innate and acquired immune responses, gut microbiota, and possible therapeutic intervention. Figure 1, is a schematic showing SARS-CoV-2 infection and activation of cell death-associated release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), associated inflammation and host intracellular immune response.

COVID-19 PATHOGENESIS AND INFECTION PROCESS

Human-to-human transmission of COVID-19 can occur when the infectious respiratory droplets of patients are transmitted as droplets or aerosol that finally gets deposited into the nasal, oral, and conjunctival mucosa of an uninfected human being. SARS-CoV-2 prominently attacks the lungs and infects other organs such as the gut, heart, blood vessels, kidney, cortex, and central nervous system[9]. SARS-CoV-2 infects host cells when the viral spike (S) protein binds to the cell surface receptor ACE2. Thereby, ACE2 is the crucial cellular receptor for the entry of SARS-CoV-2[10]. Two functional domains are found in the S protein: A receptor-binding domain and a second domain with S1/S2 cleavage site containing multiple arginine residues that must be cleaved by cell proteases for cellular entry. The furin-mediated pre-cleavage of the S1/S2 site leads to further activation of viral fusion to the cells by transmembrane protease serine 2 protein (TMPRSS2)[11,12]. ACE2 receptors and TMPRSS2 are expressed in various human cells susceptible to viral infection, including epithelial cells in the lungs, small intestine, and colon, tubular cells of the kidney, neuronal and glial cells in the brain, enterocytes, vascular endothelial cells, smooth muscle cells and cardiomyocytes[13]. Viruses are shed in the feces long after the resolution of the pulmonary symptoms, making the fecal-oral route of SARS-CoV-2 transmission a possibility. Single-cell transcriptomics data suggest that the GI epithelium, especially the enterocytes lining of the ileum and colon, shows a higher frequency of coexpression of both the ACE2 and TMPRSS2 and therefore, are conducive for SARS-CoV-2 interaction and infection, which may explain the GI pathogenesis[14,15]. The viral entry is associated with the release of proinflammatory cytokines, immune cell infiltration, and overall immune activation leading to inflammation. The infection-associated GI-specific symptoms include anorexia, watery diarrhea, nausea and vomiting, and associated abdominal pain[14] (Figures 1 and 2).

COVID-19 AND GI SYMPTOMS

Similar to lung infection, the GI-infection by SARS-CoV-2 triggers an antiviral immune response characterized by the release of interferon (IFN), cytokines, and chemokines in the infected cells. Figure 2 presents a brief overview of the GI infection routes and

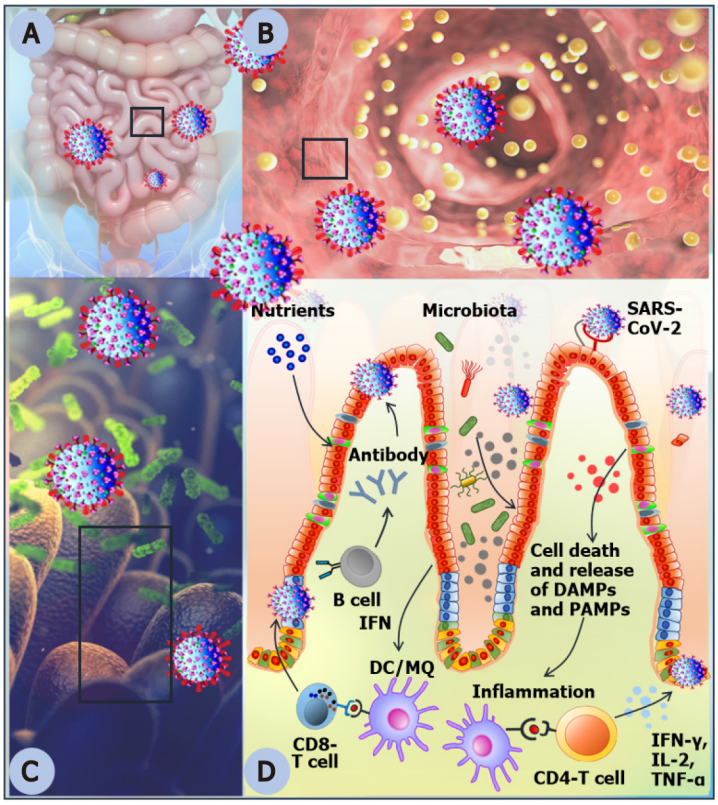

Figure 1 COVID-19 and gut Immunomodulation. A: Model is showing gut infection; B: Zoomed in area of the gut; C: Zoomed in representation of an area showing the intestinal crypts; D: Zoomed in C, showing a histological representation of intestinal crypts. The intestinal epithelium is folded and organized into crypts and villus. Villus is the finger-like projections gutting out towards the lumen of the intestine (red cells). The crypts base (shown in yellow and green cells) houses the intestinal stem cells, while the blue cells comprise the transit-amplifying cells. SARS-CoV-2 activates angiotensin-converting enzyme 2 receptors, and epithelial cell death-associated release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). DAMPs and PAMPs are considered a danger signal by immune cells, especially the dendritic cells, macrophages, and innate immune cells. This damage recognition is associated with proinflammatory cytokine production (like Interferon, tumor necrosis factor-α), followed by immune infiltration and virus-specific B and T cell response. CD8+ T cells undergo clonal expansion and kill the infected cells and launch an antiviral attack. B cells differentiation to plasma cells can lead to antiviral antibody production and subsequent neutralization of SARS-CoV-2[10,14]. Some images (Free Stock Media) are downloaded from Canva.com using subscription. IFN: Interferon; TNF-α: Tumor necrosis factor-α; DAMP: Damage-associated molecular patterns; PAMP: Pathogen-associated molecular patterns; DC: Dendritic cells; MQ: Macrophages.

symptoms. These inflammatory mediators promote infiltration of neutrophils, macrophages, and T cells to the site of infection, resulting in enteric inflammation that may lead to diarrhea and other GI symptoms[10]. Studies have shown that the elevated fecal levels of calprotectin (a marker protein expressed mainly by neutrophils) in patients with COVID-19 adds to the growing evidence that SARS-CoV-2 infection triggers an inflammatory response in the intestine. Calprotectin concentrations were found to be significantly higher in COVID-19 patients who had suffered from diarrhea along with elevated serum interleukin (IL)-6 levels[16]. An alternate mechanism implicated in GI symptoms in COVID-19 patients is oxygen deprivation [17]. Hypoxia is one of the major clinical symptoms in COVID-19 patients known to influence intestinal homeostasis, including microbiota composition and immune function. It is shown that oxygen deprivation (exacerbated hypoxia) can contribute to GI disorders and inflammatory disease severity[18].

The tissues that are targeted by SARS-CoV-2 go through an early phase of infection where a high viral load induces intestinal symptoms such as vomiting and diarrhea associated with COVID-19 during the initial phase in some patients. Thus, diarrhea should also generate awareness of a possible SARS-CoV-2 infection and should be investigated to reach an early diagnosis of COVID-19 to slow down its transmission instead of waiting for the respiratory symptoms to develop.

The first results linking GI symptoms with COVID-19 were obtained from a study conducted in COVID-19 confirmed patients in Wuhan, China[19]. In this study, 204 patients with COVID-19 who presented at three hospitals were analyzed. Although most patients presented with respiratory symptoms, many patients also presented with GI -specific symptoms. It is possible that GI symptoms associated with COVID-19 could be underreported due to the focus on fatal respiratory symptoms. However, a

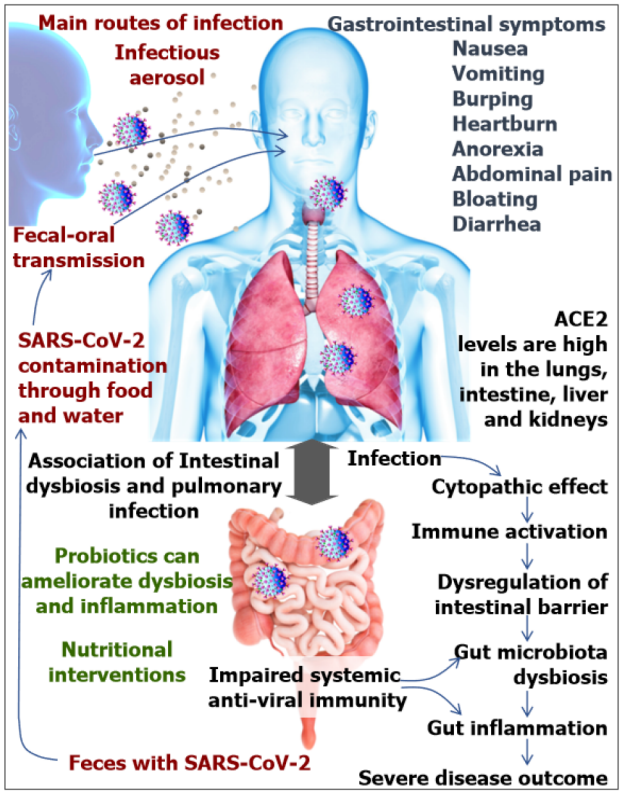

Figure 2 Illustrative model showing gastrointestinal infection routes and symptoms. The figure shows the main routes of infection. Infectious respiratory droplets or aerosols deposited on the nasal, oral, or conjunctiva may lead to person-to-person spread. SARS-CoV-2 is detected in feces of infected patients may infect by fecal-oral transmission. The right upper section of the figure also discusses the significant gastrointestinal symptoms associated with COVID-19 infection. The receptors (angiotensin-converting enzyme 2 and TMPRSS2) of SARS-CoV-2 are detected on various organs, especially the lungs, intestine, liver and kidneys. The right lower section describes the infection process leading to intestinal symptoms. After SARS-CoV-2 infection, cytopathic effect occurs due to infection and associated immune activation leading to compromised intestinal barrier function, microbial dysbiosis, and severe symptoms. Many studies have established the link between healthy intestinal flora and the gut-lung axis. COVID-19 severely induces the intestinal microbiota dysbiosis and affects the gut-lung axis, especially the immune response. Probiotics and appropriate nutritional supplements can help protect from SARS-CoV-2 associated symptoms[10,14]. Some images (Free Stock Media) are downloaded from Canva.com using subscription. ACE2: Angiotensin-converting enzyme 2.

study by Pan *et al*[20] reported that patients without GI symptoms were more likely to recover and be discharged than those with GI symptoms (60% *vs* 34%). This data indicates that GI symptoms like diarrhea may be associated with a worse outcome requiring respiratory assistance and intensive care admission. It was also found that patients with COVID-19, especially those with digestive symptoms, remained for a long time from the onset of symptoms to hospital admission with an average time of 9 d compared to patients with only respiratory symptoms who had an average admission time of 7.3 d[19]. This may indicate that those with digestive symptoms waited longer to be diagnosed in the hospital, as they were unsuspected of being SARS-CoV-2 positive in the absence of respiratory symptoms[21]. Besides, prolonged hospital stay could also be due to treatment time needed to resolve multiple symptoms in patients with GI and respiratory infections.

Wang *et al*[22] analyzed the biodistribution of SARS-CoV-2 in different tissues of patients with confirmed COVID-19[22]. In this study, SARS-CoV-2 was detected in multiple tissue specimens collected from 205 COVID-19 patients. Bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%). However, the virus was also detected in feces suggesting that the infectious virions are secreted from the virus-infected GI cells. The virus has also been detected in GI histological samples and by endoscopy[23]. Therefore, the fecal-oral transmission could be a possible route for the viral spread. To further investigate the presence of SARS-CoV-2 in feces, Xiao *et al*[24] examined the viral RNA in feces from 73 patients with SARS-CoV-2 during their hospitalizations. Out of the 73 hospitalized patients infected with SARS-CoV-2, 39 (53.42%) tested positive for SARS-CoV-2 RNA in their stool. The study also found that 17 (23.29%) patients continued to have positive stool results after showing negative outcomes in the respiratory samples. Overall, these data suggest viral GI infection and a potential fecal-oral transmission that can last even after viral clearance in the respiratory tract, and also advocates implementing testing of the virus in feces by real-

time reverse transcription polymerase chain reaction for disease monitoring and surveillance.

IMMUNOMODULATION IN GI TRACT DUE TO INTESTINAL AND NON-INTESTINAL INFECTIONS

Clinical data suggest that co-infection of GI tract along with respiratory tract are quite prevalent[25]. Xiao *et al*[24] has reported the presence of replicating viruses in the epithelium of the GI tract[24], and the *in vitro* models of cell and organoid culture of human intestinal epithelial cells (hIECs) support efficient SARS-CoV-2 infection, replication and production of infectious de novo virus particles[25]. Intestinal viral load seems to show a stronger association with the severity of respiratory and GI symptoms in COVID-19 patients[26]. Recently, in a non-human primate (rhesus monkey) model of SARS-CoV-2 infection, *in vivo* infection of GI tract triggered reduced proliferation and increased apoptosis of intestinal epithelial and goblet cells along with intestinal inflammation by macrophages has been reported by performing immunohistochemistry for proliferation (Ki67), apoptosis (cleaved caspase 3), and recruited macrophages (CD68+), and multiplex cytokine assay of GI tract tissues[27]. These reports support immune modulation in the GI tract due to direct infection of GI tract cells by the virus or due to changes in the GI tract integrity and microbiota under the influence of systemic cytokines and hypoxic conditions or a combination of all. GI tract is a site of active immune reaction to generate tolerant immunity against various commensal pathogens and an effective immunity to fight the pathogenic infectious agents, such as bacteria, viruses, parasites, *etc.* Direct or indirect modulation in the GI tract's immune activation during SARS-CoV-2 infection seems a reason for observed GI symptoms in COVID-19 patients.

Innate immune response to SARS-CoV-2

The initial protection against pathogens is established by innate immunity. Although more studies are needed, it is reasonably convincing that intestinal epithelium gets infected and is associated with some sort of GI symptoms. Virally infected cells can recognize the virus and virus-associated molecular patterns to elicit initial innate immune pathways to release cytokines and chemokines to recruit body's innate-immune cells such as neutrophils, macrophages, *etc.* to the infected area of the gut, which further augments the inflammation in order to restrict the viral replication. This inflammatory response also promotes antigen processing and presentation to establish the adaptive immune response. However, some of the inflammatory cytokines are known to increase permeability of the intestinal lumen to the commensal microbes and may contribute to the onset of the GI-symptoms. The possible host immune responses during COVID-19 infection is discussed in this review.

Innate immune response mechanism to SARS-CoV-2: As explained in previous sections, it is evident that SARS-CoV-2 can infect various tissues of GI-tract followed by intestinal cell death, macrophage recruitment and release of various pro inflammatory cytokines to compromise the intestinal barrier[27]. Therefore, it is likely that SARS-CoV-2 infection of intestinal epithelial cells (IEcs) would trigger a coordinated innate immune response due to the recognition of SARS-CoV-2 associated molecular patterns (PAMPs), similar to that reported in the lung's epithelial cells[25,28]. The initial cytokine released by the infected cells can further recruit immune cells (neutrophils, macrophages, lymphocytes *etc.*), in the gut microenvironment to amplify the inflammatory response by recognition of PAMS and cytokines by their specialized receptors to restrict the virus propagation[29,30]. Notably, this early innate immune response is essential to facilitate the emergence of a more specific adaptive immune response by lymphocytes. The nature, timing and strength of innate and adaptive immune responses have been reported to be determining factors for the COVID-19 patient's symptoms[31]. Several components of inflammation exist but we have limited knowledge on the nature of inflammatory pathways triggered in the GI-tract by SARS-CoV-2.

One of the important components of inflammation is IFN response that includes large number of genes exerting antiviral effect. Recent report in a monkey model has shown many proinflammatory cytokines in the GI tract but they show no clear evidence on the IFN-I response genes and thus further omics studies may shed some lights in this regard[27]. It is apparent that asymptomatic and mild/moderate symptomatic patients develop a compelling early innate immune response to

successful viral clearance. While, patients with severe symptoms (especially the elderly and those with pre-existing health conditions) exhibit a dysfunctional early innate immune response against SARS-CoV-2 to allow the dissemination of infection leading to life-threatening complications[32,33]. In general, inadequate early innate immune response and failure to generate enough antiviral IFNs allow immune evasion, viral propagation, the spread of infection and subsequently cell death, and the release of PAMPs and DAMPs to cause cytokine storm. However, there is no strong correlation between viral load and severity of the disease highlighting the role of genetic or physiological state of the individual in developing the severe symptoms. Currently, we have little knowledge about the contribution of GI tract infection and inflammation towards cytokine storm and organ damage, which needs further exploration in the clinical and experimental setup. However, in the rhesus monkey model, it is evident that infection of GI-tract can contribute to systemic inflammation and inflammation to lungs[27].

IEcs and goblet cells undergo apoptosis[27]; however, other form of inflammatory cell death could be operational, which needs to be investigated in a preclinical and clinical setup as various types of cell death can occur due to the activation of innate immune recognition of PAMPs and DAMPs. The inflammatory cell death includes Pyroptosis, Apoptosis and Necroptosis, also termed as Panoptosis[30]. Pyroptosis is an inflammasome or Gasdermin mediated phenomena that involve caspase1, 4, and 5 activations (in humans) and gasdermin mediated pore formation and release of Il1b and IL-18. Recent data suggested a role of SARS-CoV-2 infection induced pyroptosis in peripheral blood mononuclear cells through NLRP3 (NLR family pyrin domain-containing 3) inflammasome activation, cleavage of caspase-1, and secretion of IL-1β and IL-18[34]. Necroptosis is a mixed-lineage kinase domain-like pseudokinase (MLKL)-mediated inflammatory cell death, during which oligomerized MLKL is translocated to form channels in the plasma membrane, which has been documented in SARS-CoV-2 infection[35]. Karki *et al*[35] have shown that a combination of just tumor necrosis factor (TNF)-α and IFN-γ can exert significant cell death in bone marrow-derived macrophages and their blockage can abrogate the cell death and severe symptoms in COVID-19 situation. We guess, possibly similar kind of cell death also operates in GI-tract as TNF-α and IFN-γ are induced in SARS-CoV-2 infected GI-tract[35]. Here, as part of an innate immune response, we elaborate on the evidence of IFN (IFN-I and IFN-III) and proinflammatory cytokines production in the context of human GI tract cells that may have consequences towards GI symptoms.

Induction of IFN and cytokines in the cells of the GI tract upon SARS-CoV-2 infection: To successfully combat and generate immune memory against SARS-CoV-2, the host' cells must generate an early innate immune response that includes the production of antiviral IFN and proinflammatory cytokines soon upon viral detection [36]. The severity of COVID-19 disease has been correlated with a defective or lower level of systemic IFN production but an elevated level of proinflammatory cytokines [37-39]. Since, infection of GI-tract contribute to the systemic cytokine pool[27], a detailed transcriptomic profile of GI-tract in non-human primate model can reveal some clues in future. In the *in vitro* models, similar to lung epithelial cells, the IEcs and intestinal organoids induce both type-I (IFN-I) and type-III IFN (IFN-III)[25,26,40]. Interestingly, SARS-CoV-2 induces stronger IFN-stimulated genes than SARS-CoV in the intestinal organoids, which seems similar to that observed in lung epithelial cells [26]. Analysis of feces of COVID-19 patients has revealed a significant association of elevated proinflammatory cytokine (IL-8) and lower level of anti-inflammatory cytokine (IL-10) in the COVID-19 patients as compared to healthy people, which indicate an inflammation/immune response in the intestine[26]. Post infection, expression of cytokines is evident in the time course analysis of 23 cytokines in the GI-tract of SARS-CoV-2 infected rhesus monkey[27]. Current studies analyzing the IEcs response upon SARS-CoV-2 infection have little data on IFN and ISG at later time points (24 h or longer). Also, analysis of cytokines in gut biopsy samples from various disease category may be useful. Future studies can be carried out to investigate other proinflammatory cytokines profiles that are usually observed in other viruses or bacterial infections. A comparative study would be necessary to dissect the molecular differences in response between IEcs and lung's epithelial cells. Whether intestinal inflammation contributes to the systemic cytokine pool (which seems convincing in rhesus monkey model), caused various types of cell death in intestinal epithelium and resident immune cells would be important aspects to explore.

Adaptive immune response to SARS-CoV-2 in the GI tract

The adaptive immune response mediated by B and T lymphocytes is usually

pathogen-specific and develops slowly relative to the innate immune response. B cells and T cells in the intestine are continually interacting with a vast amount of antigen-derived from diet and commensal microbes and maintain immune homeostasis. The interaction of gut-associated antigen and lymphocytes primarily happens in the gut-associated lymphoid tissues, including the Peyer's patches, isolated lymphoid follicles, and gut-draining mesenteric lymph nodes, leading to maturation and differentiation of lymphocytes[41]. We will discuss the potential adaptive immune response to SARS-CoV-2 infection.

Lymphopenia in COVID-19

Non-severe COVID patients show a near-normal number of circulating lymphocytes, while severe patients show a reduced number of circulating lymphocytes, a condition known as lymphopenia[42,43]. Whereas, a detailed analysis of lymphocyte subsets shows a significant reduction in T cells and NK cells, but without any alteration in B cell number in severe patients[22,42,43]. COVID-19 patients with pre-existing metabolic disease, like diabetes, show a higher proportion of severe infection[44], and lymphopenia has been linked to the severity of Crohn's disease[45]. Lymphopenia in severe COVID-19 patients may result from the synergistic effect of inflammation and metabolic disorder. The cellular mechanism of lymphopenia could be due to the following reasons and beyond. First, inhibition of lymphocyte proliferation in severe disease. Pre-existing metabolic disease, such as diabetes, enhances the propensity of the severity of COVID-19[46], and metabolic molecules, such as elevated blood lactic acid levels, may inhibit lymphocyte proliferation[47]. Second, higher lymphocyte death in severe disease. The potential mechanism of lymphocyte death could be due to metabolic disorder, inflammation, damage of lymphatic organs, and direct infection of lymphocytes[47]. Third, reduced lymphocyte production by skewed hematopoietic lineage cell fate decision. Metabolic disease (ulcerative colitis) and inflammation can skew hematopoietic fate decision towards the myelopoiesis with a concomitant decrease in lymphopoiesis[22,47,48]. Forth, infiltration of lymphocytes at the site of infection.

B cell and antibody-mediated immunity in COVID-19

In general, the intestine offers a model example of the diversity of antibody-secreting cells (ASCs) and comprises at least three subpopulations in humans[49]. Binding of antigen to antigen-specific B cells generates activated B cells that differentiates into ASCs with the help of T helper cells. It has been shown that 70% of non-severe COVID-19 patients have high and persistent SARS-CoV-2 neutralizing immunoglobulin (Ig)G in the sera after their recovery[50]. Antibody isotype analysis shows SARS-CoV-2 specific serum IgA and IgM in non-severe patients[51,52]. A longitudinal study in recovered patients showed that IgG antibodies are relatively stable up to 105 d post symptom onset while IgA and IgM antibodies rapidly decay[52]. Interestingly, an anti-SARS-CoV-2 antibody found in the mucosal fluid (saliva, nasal fluid, and tear fluid) and COVID-19 recovered patients have anti-SARS-CoV-2 specific IgG and IgA in saliva, indicating that antibody in the GI tract could be as crucial as antibodies in the serum for protective immunity[52-54]. ASCs in the intestine is a significant source of IgA producing cells in human. This indicates that the GI tract plays an essential role in generating anti-SARS-CoV-2 antibodies and protective immunity against SARS-CoV-2. The antibody can protect SARS-CoV-2 infection possibly by the following; antibody-mediated neutralization of the pathogen, phagocytosis of infected cells, and antibody-dependent cellular cytotoxicity. Patient-generated SARS-CoV-2-specific antibody can neutralize the virus SARS-CoV-2 in *ex-vivo* condition[53,55]. Furthermore, a mounting adaptive response in GI is supported by the prevalent presence of IgA in the stool of severe SARS-CoV-2 patients[26]. However, the importance and potency of antibody-mediated neutralization of SARS-CoV-2 *in vivo* require future studies in the model systems.

Longitudinal studies have shown that IgG and IgA levels to SARS-CoV-2 were significantly elevated as the disease progresses[52,56,57]. Anti-SARS-CoV-2 IgG was detected within the first week after onset of the symptoms in approximately 40% of patients. Within 15 d (late stage of infection), antibody levels increased by almost 100% of patients[57]. In general, severe patients showed a significantly higher IgG and IgA level compared to non-severe patients at late stages of infection. Surprisingly, the antibody level increases as the disease worsen in the severe group; on the contrary, the disease cured (patients recovered) in the non-severe group[51,56,57]. A few studies have shown that the severity of the disease positively correlated with an increased amount of IgG against S-protein and N-protein, especially in elderly patients[58].

Similarly, a very high level of anti-SARS-CoV-2 IgA correlated with severe acute respiratory distress syndrome[51]. Recent studies have shown that antibody levels (especially those of IgG and IgA) and B cell repertoire are highly dependent on the nature of microbiota in the gut[59,60]. Similarly, B cells and antibodies in the gut could affect the composition of the microbiota. B cell knock-out mice (a proxy for antibody deficiency) and AID deficiency mice (don't have secretory IgA in the gut) have reduced microbial diversity and alter the composition of gut microbiota[61]. Therefore, antibodies and microbiota have a feedback loop to maintain a healthy immune response. The current understanding is that people with dysbiosis (imbalance microbiota) have a prevalence of COVID-19[62]. Alternatively, it could be possible that high IgA levels in the severe patients who recovered have altered the composition of the microbiota and may have a long-term health effect. Figure 3 schematically represents the development of COVID-19 progress and its relationship to changes in the gut flora and disease progression.

T cell and cellular immunity in COVID-19

Cellular immunity is mediated by T cells, and microbiota profoundly affects T cell activation and differentiation, as observed in B cells. Dysbiosis can prompt multiple immune disorders mediated by T cells[63]. T cells have numerous subsets ($CD4^+$ T cells, $CD8^+$ T cells, and Treg) and distinct biological functions. $CD4^+$ T cells primarily regulate the function of other immune cells, $CD8^+$ T (cytotoxic T cells) cells can produce granzyme, and perforin results in the elimination of virus-infected cell, and regulatory T cell (Treg), which can restrain other activated T cells' function[64,65]. COVID-19 recovered patients have SARS-CoV-2 reactive IFN $^+$ T cells and granzyme B producing $CD8^+$ T cells[66,67]. The correlation of IFN $^+$ T cells and granzyme B producing $CD8^+$ T cells in recovered patients may indicate activated T cells mediated elimination of the virus-infected cells[54]. Interestingly, it has been observed that asymptomatic or mild SARS-CoV-2 infected patients have SARS-CoV-2 specific $CD4^+$ T cells, which could be due to cross-reactive $CD4^+$ T cell recognition between the common cold and SARS-CoV-2 coronaviruses[66,67].

The T cell functions are dysregulated in many severe patients[67,68]. The source of dysregulated T cell functions in severe disease could be due to the following reasons. First, it has been shown that both severe and non-severe patients have a comparable proportion of activated T cells suggesting functionality of activated T cells may be restrained by other immune cells, such as Treg, in non-severe patients[32,42]. In line with this, Qin *et al*[42] showed that severe patients have fewer Treg (specifically, induced Treg). Several studies have shown that GI tract dysbiosis can alter Treg/$CD4^+$ T cell axis and may have a pathogenic outcome[69]. The generation of fewer Treg in severe patients can be a synergistic effect of inflammation and mucosal microbiota imbalance. Second, T cells are exhausted in severe patients than non-severe COVID-19 patients[67,68]. Dysbiosis can promote T cells exhaustion[70]. So, it could be possible that T cell exhaustion in severe patients is a combined effect of hyper inflammation and imbalanced GI microbiota. However, we can't exclude other possibilities (such as bystander T cells) of dysregulated T cells in severe disease.

COVID-19 IN PEOPLE WITH PRE-EXISTING INTESTINAL DISEASES

Patients with chronic GI conditions may be at an increased risk of severe COVID-related illness, therefore management of these patients becomes important. Although the primary source of transmission for SARS-CoV-2 is respiratory droplets, there is increasing evidence supporting the possibility of a fecal-oral route of transmission. Patients with active ulcerative colitis and Crohn's disease have a greater tissue concentration of ACE2, increasing the possibility of an infection[71]. Additionally, the level of serine protease TMPRSS2, is about ten times higher in patients with inflammatory bowel disease (IBD) than in healthy subjects, suggesting an increased risk of infection in these patients[72]. Brenner *et al*[73] created the Surveillance Epidemiology of Coronavirus Under Research Exclusion for IBD (SECURE-IBD) database to identify potential IBD-associated COVID-19 risk factors. Out of 525 patients with IBD and COVID-19, severe infection (defined as intensive care unit admission, ventilator use, or death) was reported in seven percent of patients. Potential risk factors in these patients include increasing age, ≥ 2 comorbidities like diabetes mellitus, chronic inflammatory disease, and systemic glucocorticoids use, but not with anti-TNF therapy[73]. Using anti-TNF antibodies has been shown to reduce inflammatory cell death during experimental COVID-19 situation[35].

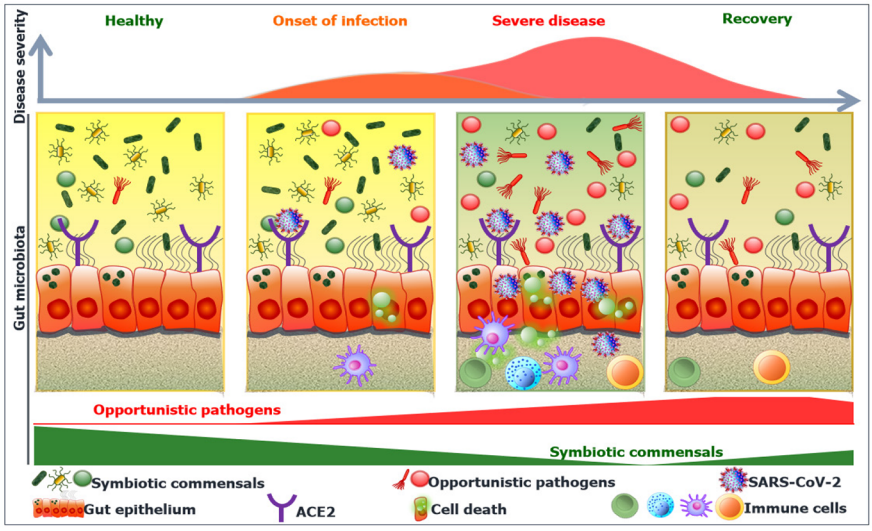

Figure 3 Schematic diagram showing COVID-19 disease progression and correlation with alterations with gut microbiota. The progression of gut microbiome alteration and its association with clinical symptoms and gut dysbiosis is evident. Cartoon inspired by[10,62,85]. ACE2: Angiotensin-converting enzyme 2.

The clinical presentation of several GI diseases (*e.g.*, Crohn's disease, ulcerative colitis) can mimic COVID-19 infection. Examples include diseases that manifest with diarrhea, nausea, vomiting, and/or anorexia. In a study by Mao *et al*[74], preliminary data have suggested that the prevalence of COVID-19 is not higher in IBD patients as compared to the general population[74]. Other studies have suggested that patients with IBD in remission are not at higher risk for SARS-CoV-2 virus infection and that such patients should continue maintenance therapy to sustain remission[74-76]. Digestive complications related to IBD relapse could be confused with symptoms of COVID infection and may skew the data for COVID symptoms in IBD patients. Discontinuing maintenance therapy for IBD has been associated with disease relapse and may lead to an increase of adverse outcomes such as hospitalizations, surgeries, and/or glucocorticoid therapy like prednisone that may increase the risk for severe COVID-19[76].

Patients with a flare of Crohn's disease/ulcerative colitis or active IBD, in the absence of COVID-19, may benefit from anti-inflammatory or biologic therapy to induce remission. Mild IBD therapeutic options include oral budesonide, aminosalicylates, and topical (rectal) therapy. While, the usual options for treating moderately to severely active IBD include biologic therapies (*e.g.*, anti-TNF agents, anti-integrin agents, and anti-interleukin agents)[76] are still viable. However, if systemic glucocorticoids are deemed necessary, the lowest dose of glucocorticoid with an appropriate clinical response is used for a short duration before transitioning to another therapy that is glucocorticoid-sparing[76]. Management of a patient hospitalized with severe ulcerative colitis in the absence of COVID-19 may include treatment with a glucocorticoid (like methylprednisolone) and in unresponsive cases medical therapy may be escalated to infliximab[76]. Surgery is an alternative option for patients who do not improve with medical therapy. Additionally, in the COVID-19 era, the initial use of infliximab at a dose of 5 mg/kg rather than glucocorticoid therapy is a reasonable approach.

IBD patients with known or suspected COVID-19 should have individualized medication regimen adjustments in order to balance the risk of disease flare[77]. The goal is to reduce immunosuppression during active viral infection to lower the risk of COVID-19-related complications (*e.g.*, pneumonia). Patients with suspected or confirmed COVID-19 infection can be treated with Budesonide, Aminosalicylates, including sulfasalazine, topical rectal therapy (*e.g.*, topical glucocorticoid), and antibiotics. However, Glucocorticoids require dose adjustment based on the severity of COVID-19 infection and Immunomodulators like thiotropines, methotrexate; Tofacitinib (Janus kinase inhibitor); biologic agents like anti-TNF agents, ustekinumab, or vedolizumab are held or delayed in patients with active symptoms of COVID-19 until symptoms resolve[78,79].

However, the association of comorbidities, and their effect on the prognosis of COVID-19 needs to be further evaluated. In a recent study, 18 (1%) of 1590 COVID-19

cases had a history of cancer of which three had a history of colorectal cancer, one each of colonic tubular adenocarcinoma, rectal carcinoma, and colorectal carcinoma. It was also noted that patients with a history of cancer and positive SARS-CoV-2 virus were observed to have a higher risk of severe events[80]. Several strategies have been proposed, such as delaying of adjuvant chemotherapy or elective surgery on a patient-by-patient basis, stronger personal protection provisions, and more intensive surveillance or treatment[80].

In a cross-sectional survey of 86602 individuals, 53130 reported prior abdominal pain, acid reflux, heartburn, and regurgitation with 6.4 percent COVID positivity. Proton pump inhibitors (PPI) users were shown to be considerably more likely than non-users to report a positive COVID-19 test result, with a dose-dependent increase in the likelihood of a positive test result, and further studies are required to ascertain the link. PPI increase the risk of enteric infections due to PPI-induced hypochlorhydria. The usage of Histamine-2 receptor antagonist was not associated with an increase in risk[81].

GUT MICROBE AND COVID-19

The gut microbiota, which includes approximately 10^{14} resident bacteria, archaea, virus, and fungi, regulates not only the metabolism and host immunity but also the overall health. The gut and the lung microbiota seem to bi-directionally modulate each other and maintain a healthy gut-lung axis and is reported to be altered in COVID-19 patients and other diseases[82]. Lung infections can also significantly change the composition of gut microbiota, a process collectively termed as "gut microbial dysbiosis." Viral respiratory infections are also known to induce inappetence and significantly impact the gut microbiota[7]. Severe pulmonary SARS-CoV-2 infection is associated with a hyperactive immune reaction and "cytokine storm". Inflammatory mediators cause significant lung cytopathy and hyper-permeability, leading to a viral transfer to the gut *via* circulation or some other unknown mechanisms. The inflammatory mediators also damage the intestinal barrier leading to the leakage of intestinal microorganisms and associated metabolites into the main bloodstream that may further the inflammation and GI symptoms.

Moreover, microorganism-associated molecular patterns and PAMPs are recognized by host immune mediators and evoke a strong detrimental immunological reaction in organs, including the lungs and intestine. This vicious cycle of chronic immune activation leads to tissue inflammation and damage. Giron *et al*[83], 2020 study the role of COVID-19-associated lung injury, systemic inflammation, and disruption of the gut's barrier functions, resulting in the enhanced vulnerability of microbial products [83]. Thus, COVID-19 affects the gut lung axis and induces microbial dysbiosis.

There is a dearth of information regarding the direct *vs* the indirect effect of SARS-CoV-2 on gut microbiota. Zuo *et al*[84], 2020 analyzed fecal microbiome from COVID-19 patients using shotgun metagenomic sequencing technology and detected higher opportunistic pathogens (including, *Clostridium hathewayi*, *Actinomyces viscosus*, and *Bacteriodes nordii*) and a concomitant decrease in beneficial commensals (including, *Faecalibacterium prausnitzii*, *Lachnospiraceae bacterium 5_1_63FAA*, *Eubacterium rectale*, *Ruminococcus obeum*, and *Dorea formicigenerans*[84]. Interestingly all patients in this study cohort did not present GI symptoms. Data regarding the use of probiotics and nutritional intervention can further confirm the hypothesis that SARS-CoV-2 associated disease severity may be dictated by the patient's microbiota status. Probiotics are live microbes, when consumed, can provide gut health. Several studies have shown that the administration of probiotics in COVID patients can ameliorate gut dysbiosis and improve host immune response[85,86]. In the absence of specific data, further investigation regarding the particular role of probiotics and supplements, microbial type or nutritional component needs investigation in larger SARS-CoV-2 infected patient cohorts[87].

Elderly people (> 60 years) are associated with severe symptoms and higher mortality rates. The link between aging and progressive alteration of detrimental gut microbiota is well worked out[88]. An increasing number of reports suggest that a strong relationship exists between the gut microbiome and SARS-CoV-2 infection severity. Therefore, the heightened risk of aged patients may be associated with microbial dysbiosis, leaky gut, inflammation, and a dysfunctional gut-lung axis in addition to pre-existing conditions. A major question that has not been addressed is why certain developed countries have significantly higher mortality rates as compared to some underdeveloped or developing nations. Amongst many possibilities, the role

of lung and gut microbiome and resulting interference with systemic immunity may also help explain the global disparities in COVID-19 associated disease severity and death[89]. To have a comprehensive idea investigation with a larger data set is warranted.

THERAPEUTICS OPTIONS FOR COVID-19

Though infection prevention, control strategies, and preventive treatment are the mainstay of the current management of COVID-19. Some glimmer of hope has arrived in the form of COVID-19 vaccines' approval for emergency use by many nations, but currently, no safe and effective treatment exists. The possible list of emerging therapeutics for the treatment of COVID-19 is steadily expanding and evolving, and that too in a short period of time. The United States Food and Drug Administration (FDA) has approved emergency use authorizations (EUAs) for a few medications and therapies and several others are under clinical trials[90]. This review will concentrate on the strategies especially aimed at prophylactic and therapeutic modulating the host immune system. Pre-infection immunoprophylaxis depends on the immune activation of the host immune system before infection and disease initiation. At the same time, therapeutic intervention strategies are focused to repair the immune systems during the duration of the illness, post-infection. Several therapeutic targets including IFN-I, TNF, JAK/STAT, IL-1, IL-6, GMCSF, convalescent plasma, and complements, are under investigation. A comprehensive list of all prophylactic and therapeutic molecules undergoing clinical trials is available online (https://www.who.int/ictrp/en/; clinicaltrials.gov).

Approved therapeutics for COVID-19

Several antiviral molecules are undergoing clinical trials, and Remdesevir has been approved by United States FDA for therapeutic management of COVID-19 patients [91]. Remdesevir (Veklury), being a nucleoside analog, prevents viral replication by inhibiting the viral RNA-dependent RNA polymerase (RdRp) activity, shortened recovery time, and reduced mortality rates. Eli Lilly and Company has also received EUA for a combinatorial use of Baricitinib (Olumiant; an inhibitor of JAK kinase) with Remdesevir in patients requiring supplemental oxygen.

The antibody cocktail of Casirivimab and Imdevimab by Regeneron Pharmaceuticals, Inc. has also obtained the EUA by the United States FDA for the treatment of mild to moderate COVID-19. Casirivimab and Imdevimab are monoclonal antibodies against spike protein of SARS-CoV-2, are supposed to neutralize the viral entry. Eli Lilly COVID-19 neutralizing antibody bamlanivimab (LY-CoV555) is also directed against the spike protein and has received EUA for non-hospitalized adults. Convalescent plasma (CP) is a passive immune therapy approach, where COVID-19 recovered patient can donate plasma rich in SARS-CoV-2-specific neutralizing antibodies to persons at high risk of contracting COVID-19[92]. United States FDA has provided a EUA for the use of CP as a treatment option for COVID-19 patients.

The potential vaccine can change the course of the COVID-19 pandemic. Researchers have used several technological vaccine development platforms, including nucleic acid-based (DNA and RNA), virus mimicking particle subunit vaccine, peptide vaccines, attenuated virus-based vaccines. mRNA vaccines developed by Pfizer-BioNTech and Moderna have received EUA from the United States FDA, bringing a big sigh of relief. mRNA vaccines prompt the cell to express the viral spike protein, which elicits a strong immune reaction against the infecting SARS-CoV-2 virus. The Oxford-AstraZeneca's COVID-19 vaccine has also received authorization in many countries. The Oxford-AstraZeneca vaccine uses the gene for the coronavirus S protein (double-stranded DNA) packed in an adenovirus. Other vaccine producers like Johnson & Johnson/Janssen Pharmaceuticals and Gam-COVID-Vac (Sputnik V) developed by the Gamaleya Research Institute of Epidemiology and Microbiology are also effective and are being used for mass vaccination in many countries. Bharat Biotech and the Indian Council of Medical Research collaborated to create Covaxin (codenamed BBV152), an inactivated virus-based COVID-19 vaccine. CoronaVac (inactivated vaccine) is produced by Sinovac is also used to vaccinate to fight against COVID-19. The new evolving strains with mutations in the S protein create a possibility of decreased susceptibility to monoclonal antibodies, vaccines and therapeutic agents. Scientists are investigating these mutations to help explain how quickly they can be spread and if vaccines will be effective.

Under trial therapeutics for COVID-19

Several strategies to target the uncontrolled host immune system have been attempted and are currently in clinical trials (clinicaltrials.gov). TNF-α, a proinflammatory cytokine, exhibits a positive correlation with advances in disease stages. Preliminary clinical data suggest the effectiveness of anti-TNF-α in reducing the cytokine storm as well as tissue inflammation. TNF-α-blockers, both small molecule and antibodies (Adalimumab and Otilimab), are currently under trial[93]. Patients with IBD with COVID respond better to anti-TNF-α blockers than alternative agents[94]. Dysregulated early IFN-I response may eventually lead to COVID complications, and an early IFN-Iα/β treatment with broad antiviral response can ameliorate disease progression [95]. IL-6 is associated with 'cytokine storm', and inhibition of IL-6 using a monoclonal antibody (Tocilizumab, Sarilumab) is under clinical trial and can be a potential treatment option. Initial clinical studies in China and a case study in France suggested a rapid favorable outcome on the therapeutic value of the anti-IL-6 receptor antibody [96]. More investigation is warranted as IL-6 is also reported to prevent enterocyte cell death after injury and help proliferation and repair[97]. Sanofi's KEVZARA Phase III trial investigating the efficacy of an anti-IL-6 receptor antibody in severe and critically ill patients did not yield promising results. Hence, further investigation is warranted to understand better the therapeutic advantage of inhibiting IL-6 signaling in COVID patients. Other than current therapeutics, patients suffering from systemic inflammation and IBD and associated diarrhea may benefit from the potential use of pro and pre-biotics[91,98].

CONCLUSION

SARS-CoV-2 has spread exponentially as a pandemic throughout the world. Scientists and researchers all over the world are working tirelessly to develop potential coronavirus treatment options. The United States FDA has recently granted EAU for several therapeutic modalities for targeting COVID-19. Pfizer and Moderna are producing United States FDA approved vaccines in millions of doses for the prophylactic use in COVID-19 patients. In this review, we have attempted to describe the link between COVID-19 associated GI infection, immune responses, and disease outcomes. SARS-CoV-2 primarily causes severe respiratory symptoms but also affects the GI system in many patients. The role of SARS-CoV-2 in gut infection, route of infection, relation with disease severity, localized *vs* systemic immune reaction, altered microbiota, dysbiosis, and the mechanism underlying pre-existing conditions and therapy. Many queries remain unexplored, especially in the context of GI infection, and need further investigation. The bidirectional gut-lung axis has been implicated in the homeostasis of the immune system. GI inflammation and dysbiosis may contribute to systemic inflammation and affect lung and other organs' health, and may be associated with severe COVID consequences. The vice versa may also be confirmed and the underlying mechanism that pathologically upsets the gut-lung communications during COVID-19 infection is not clearly understood. The role of probiotics in enhancing the immune system and the attenuation of dysbiosis may be a promising approach for reducing the GI-symptoms and preventing the COVID-19 severity. The emergence of new strains like B.1.207, B.1.351, B.1.1.7, B.1.617.1, B.1.617.2, and B.1.617.3 can impact GI significantly and therefore strain surveillance is important and its role in gut infection also needs to be studied. Hence the use of bioinformatics, mutational analysis, structural modeling to better understand the spike-ACE2 interaction, and the use of organoid and non-human primate models to study the viral infection process and therapeutic screening are key in the fight against COVID-19.

ACKNOWLEDGEMENTS

Manash K Paul acknowledges Steven Dubinett, Brigitte Gomperts, and Volker Hartenstein from UCLA for providing constant support and mentoring.

REFERENCES

1 **Lai CC**, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J*

Antimicrob Agents 2020; **55**: 105924 [PMID: 32081636 DOI: 10.1016/j.ijantimicag.2020.105924]
2 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]
3 **Banerjee A**, Kulcsar K, Misra V, Frieman M, Mossman K. Bats and Coronaviruses. *Viruses* 2019; **11** [PMID: 30634396 DOI: 10.3390/v11010041]
4 **Schoeman D**, Fielding BC. Coronavirus envelope protein: current knowledge. *Virol J* 2019; **16**: 69 [PMID: 31133031 DOI: 10.1186/s12985-019-1182-0]
5 **Viana SD**, Nunes S, Reis F. ACE2 imbalance as a key player for the poor outcomes in COVID-19 patients with age-related comorbidities - Role of gut microbiota dysbiosis. *Ageing Res Rev* 2020; **62**: 101123 [PMID: 32683039 DOI: 10.1016/j.arr.2020.101123]
6 **Singh J**, Rahman SA, Ehtesham NZ, Hira S, Hasnain SE. SARS-CoV-2 variants of concern are emerging in India. *Nat Med* 2021; **27**: 1131-1133 [PMID: 34045737 DOI: 10.1038/s41591-021-01397-4]
7 **Groves HT**, Higham SL, Moffatt MF, Cox MJ, Tregoning JS. Respiratory Viral Infection Alters the Gut Microbiota by Inducing Inappetence. *mBio* 2020; **11** [PMID: 32071269 DOI: 10.1128/mBio.03236-19]
8 **Lake MA**. What we know so far: COVID-19 current clinical knowledge and research. *Clin Med (Lond)* 2020; **20**: 124-127 [PMID: 32139372 DOI: 10.7861/clinmed.2019-coron]
9 **Banerjee R**, Roy P, Das S, Paul MK. A hybrid model integrating warm heat and ultraviolet germicidal irradiation might efficiently disinfect respirators and personal protective equipment. *Am J Infect Control* 2021; **49**: 309-318 [PMID: 32735810 DOI: 10.1016/j.ajic.2020.07.022]
10 **Villapol S**. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res* 2020; **226**: 57-69 [PMID: 32827705 DOI: 10.1016/j.trsl.2020.08.004]
11 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280.e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]
12 **Coutard B**, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res* 2020; **176**: 104742 [PMID: 32057769 DOI: 10.1016/j.antiviral.2020.104742]
13 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]
14 **Kumar A**, Faiq MA, Pareek V, Raza K, Narayan RK, Prasoon P, Kumar P, Kulandhasamy M, Kumari C, Kant K, Singh HN, Qadri R, Pandey SN, Kumar S. Relevance of SARS-CoV-2 related factors ACE2 and TMPRSS2 expressions in gastrointestinal tissue with pathogenesis of digestive symptoms, diabetes-associated mortality, and disease recurrence in COVID-19 patients. *Med Hypotheses* 2020; **144**: 110271 [PMID: 33254575 DOI: 10.1016/j.mehy.2020.110271]
15 **Zhang H**, Kang Z, Gong H, Xu D, Wang J, Li Z, Cui X, Xiao J, Zhan J, Meng T, Zhou W, Liu J, Xu H. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut* 2020; **69**: 1010-1018 [DOI: 10.1136/gutjnl-2020-320953]
16 **Effenberger M**, Grabherr F, Mayr L, Schwaerzler J, Nairz M, Seifert M, Hilbe R, Seiwald S, Scholl-Buergi S, Fritsche G, Bellmann-Weiler R, Weiss G, Müller T, Adolph TE, Tilg H. Faecal calprotectin indicates intestinal inflammation in COVID-19. *Gut* 2020; **69**: 1543-1544 [PMID: 32312790 DOI: 10.1136/gutjnl-2020-321388]
17 **Cavezzi A**, Troiani E, Corrao S. COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. *Clin Pract* 2020; **10**: 1271 [PMID: 32509258 DOI: 10.4081/cp.2020.1271]
18 **Singhal R**, Shah YM. Oxygen battle in the gut: Hypoxia and hypoxia-inducible factors in metabolic and inflammatory responses in the intestine. *J Biol Chem* 2020; **295**: 10493-10505 [PMID: 32503843 DOI: 10.1074/jbc.REV120.011188]
19 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]
20 **Pan Y**, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 2020; **20**: 411-412 [PMID: 32105638 DOI: 10.1016/S1473-3099(20)30113-4]
21 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]
22 **Wang W**, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA* 2020; **323**: 1843-1844 [PMID: 32159775 DOI: 10.1001/jama.2020.3786]
23 **Lin L**, Jiang X, Zhang Z, Huang S, Fang Z, Gu Z, Gao L, Shi H, Mai L, Liu Y, Lin X, Lai R, Yan Z, Li X, Shan H. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020; **69**: 997-1001 [PMID: 32241899 DOI: 10.1136/gutjnl-2020-321013]

24 **Xiao F**, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 2020; **158**: 1831-1833.e3 [PMID: 32142773 DOI: 10.1053/j.gastro.2020.02.055]

25 **Stanifer ML**, Kee C, Cortese M, Zumaran CM, Triana S, Mukenhirn M, Kraeusslich HG, Alexandrov T, Bartenschlager R, Boulant S. Critical Role of Type III Interferon in Controlling SARS-CoV-2 Infection in Human Intestinal Epithelial Cells. *Cell Rep* 2020; **32**: 107863 [PMID: 32610043 DOI: 10.1016/j.celrep.2020.107863]

26 **Britton GJ**, Chen-Liaw A, Cossarini F, Livanos AE, Spindler MP, Plitt T, Eggers J, Mogno I, Gonzalez-Reiche AS, Siu S, Tankelevich M, Grinspan LT, Dixon RE, Jha D, van de Guchte A, Khan Z, Martinez-Delgado G, Amanat F, Hoagland DA, tenOever BR, Dubinsky MC, Merad M, van Bakel H, Krammer F, Bongers G, Mehandru S, Faith JJ. Limited intestinal inflammation despite diarrhea, fecal viral RNA and SARS-CoV-2-specific IgA in patients with acute COVID-19. *medRxiv* 2020 [PMID: 32909002 DOI: 10.1101/2020.09.03.20183947]

27 **Jiao L**, Li H, Xu J, Yang M, Ma C, Li J, Zhao S, Wang H, Yang Y, Yu W, Wang J, Yang J, Long H, Gao J, Ding K, Wu D, Kuang D, Zhao Y, Liu J, Lu S, Liu H, Peng X. The Gastrointestinal Tract Is an Alternative Route for SARS-CoV-2 Infection in a Nonhuman Primate Model. *Gastroenterology* 2021; **160**: 1647-1661 [PMID: 33307034 DOI: 10.1053/j.gastro.2020.12.001]

28 **Scaldaferri F**, Ianiro G, Privitera G, Lopetuso LR, Vetrone LM, Petito V, Pugliese D, Neri M, Cammarota G, Ringel Y, Costamagna G, Gasbarrini A, Boskoski I, Armuzzi A. The Thrilling Journey of SARS-CoV-2 into the Intestine: From Pathogenesis to Future Clinical Implications. *Inflamm Bowel Dis* 2020; **26**: 1306-1314 [PMID: 32720978 DOI: 10.1093/ibd/izaa181]

29 **Reikine S**, Nguyen JB, Modis Y. Pattern Recognition and Signaling Mechanisms of RIG-I and MDA5. *Front Immunol* 2014; **5**: 342 [PMID: 25101084 DOI: 10.3389/fimmu.2014.00342]

30 **Lee S**, Channappanavar R, Kanneganti TD. Coronaviruses: Innate Immunity, Inflammasome Activation, Inflammatory Cell Death, and Cytokines. *Trends Immunol* 2020; **41**: 1083-1099 [PMID: 33153908 DOI: 10.1016/j.it.2020.10.005]

31 **Zohar T**, Loos C, Fischinger S, Atyeo C, Wang C, Slein MD, Burke J, Yu J, Feldman J, Hauser BM, Caradonna T, Schmidt AG, Cai Y, Streeck H, Ryan ET, Barouch DH, Charles RC, Lauffenburger DA, Alter G. Compromised Humoral Functional Evolution Tracks with SARS-CoV-2 Mortality. *Cell* 2020; **183**: 1508-1519.e12 [PMID: 33207184 DOI: 10.1016/j.cell.2020.10.052]

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

33 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

34 **Rodrigues TS**, de Sá KSG, Ishimoto AY, Becerra A, Oliveira S, Almeida L, Gonçalves AV, Perucello DB, Andrade WA, Castro R, Veras FP, Toller-Kawahisa JE, Nascimento DC, de Lima MHF, Silva CMS, Caetite DB, Martins RB, Castro IA, Pontelli MC, de Barros FC, do Amaral NB, Giannini MC, Bonjorno LP, Lopes MIF, Santana RC, Vilar FC, Auxiliadora-Martins M, Luppino-Assad R, de Almeida SCL, de Oliveira FR, Batah SS, Siyuan L, Benatti MN, Cunha TM, Alves-Filho JC, Cunha FQ, Cunha LD, Frantz FG, Kohlsdorf T, Fabro AT, Arruda E, de Oliveira RDR, Louzada-Junior P, Zamboni DS. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. *J Exp Med* 2021; **218**: e20201707 [PMID: 33231615 DOI: 10.1084/jem.20201707]

35 **Karki R**, Sharma BR, Tuladhar S, Williams EP, Zalduondo L, Samir P, Zheng M, Sundaram B, Banoth B, Malireddi RKS, Schreiner P, Neale G, Vogel P, Webby R, Jonsson CB, Kanneganti TD. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. *Cell* 2021; **184**: 149-168.e17 [PMID: 33278357 DOI: 10.1016/j.cell.2020.11.025]

36 **Kikkert M**. Innate Immune Evasion by Human Respiratory RNA Viruses. *J Innate Immun* 2020; **12**: 4-20 [PMID: 31610541 DOI: 10.1159/000503030]

37 **Cao X**. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol* 2020; **20**: 269-270 [PMID: 32273594 DOI: 10.1038/s41577-020-0308-3]

38 **Prompetchara E**, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. *Asian Pac J Allergy Immunol* 2020; **38**: 1-9 [PMID: 32105090 DOI: 10.12932/AP-200220-0772]

39 **Magro C**, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, Baxter-Stoltzfus A, Laurence J. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl Res* 2020; **220**: 1-13 [PMID: 32299776 DOI: 10.1016/j.trsl.2020.04.007]

40 **Blanco-Melo D**, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* 2020; **181**: 1036-1045.e9 [PMID: 32416070 DOI: 10.1016/j.cell.2020.04.026]

41 **Silva-Sanchez A**, Randall TD. Anatomical Uniqueness of the Mucosal Immune System (GALT, NALT, iBALT) for the Induction and Regulation of Mucosal Immunity and Tolerance. *Mucosal*

Vacci 2020; 21-54 [DOI: 10.1016/b978-0-12-811924-2.00002-x]
42 **Qin C**, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian DS. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020; **71**: 762-768 [PMID: 32161940 DOI: 10.1093/cid/ciaa248]
43 **Vabret N**, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, Levantovsky R, Malle L, Moreira A, Park MD, Pia L, Risson E, Saffern M, Salomé B, Esai Selvan M, Spindler MP, Tan J, van der Heide V, Gregory JK, Alexandropoulos K, Bhardwaj N, Brown BD, Greenbaum B, Gümüş ZH, Homann D, Horowitz A, Kamphorst AO, Curotto de Lafaille MA, Mehandru S, Merad M, Samstein RM; Sinai Immunology Review Project. Immunology of COVID-19: Current State of the Science. *Immunity* 2020; **52**: 910-941 [PMID: 32505227 DOI: 10.1016/j.immuni.2020.05.002]
44 **The Lancet Diabetes Endocrinology**. COVID-19 and diabetes: a co-conspiracy? *Lancet Diabetes Endocrinol* 2020; **8**: 801 [PMID: 32946812 DOI: 10.1016/S2213-8587(20)30315-6]
45 **Taxonera C**, Mendoza JL, Ortega L, Pérez MI, Díaz-Rubio M. Adalimumab reversed a severe lymphopenia in a patient with Crohn's disease. *J Crohns Colitis* 2012; **6**: 488-491 [PMID: 22398051 DOI: 10.1016/j.crohns.2011.10.016]
46 **Yang J**, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis* 2020; **94**: 91-95 [PMID: 32173574 DOI: 10.1016/j.ijid.2020.03.017]
47 **Tan L**, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, Miao H. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther* 2020; **5**: 33 [PMID: 32296069 DOI: 10.1038/s41392-020-0148-4]
48 **Trottier MD**, Irwin R, Li Y, McCabe LR, Fraker PJ. Enhanced production of early lineages of monocytic and granulocytic cells in mice with colitis. *Proc Natl Acad Sci U S A* 2012; **109**: 16594-16599 [PMID: 23012474 DOI: 10.1073/pnas.1213854109]
49 **Landsverk OJ**, Snir O, Casado RB, Richter L, Mold JE, Réu P, Horneland R, Paulsen V, Yaqub S, Aandahl EM, Øyen OM, Thorarensen HS, Salehpour M, Possnert G, Frisén J, Sollid LM, Baekkevold ES, Jahnsen FL. Antibody-secreting plasma cells persist for decades in human intestine. *J Exp Med* 2017; **214**: 309-317 [PMID: 28104812 DOI: 10.1084/jem.20161590]
50 **Wu F**, Wang A, Liu M, Wang Q, Chen J, Xia S, Ling Y, Zhang Y, Xun J, Lu L, Jiang S, Lu H, Wen Y, Huang J. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *BMJ* 2020 [DOI: 10.1101/2020.03.30.20047365]
51 **Cervia C**, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A, Raeber ME, Adamo S, Weigang S, Emmenegger M, Hasler S, Bosshard PP, De Cecco E, Bächli E, Rudiger A, Stüssi-Helbling M, Huber LC, Zinkernagel AS, Schaer DJ, Aguzzi A, Kochs G, Held U, Probst-Müller E, Rampini SK, Boyman O. Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. *J Allergy Clin Immunol* 2021; **147**: 545-557.e9 [PMID: 33221383 DOI: 10.1016/j.jaci.2020.10.040]
52 **Isho B**, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH, Li Z, Chao G, Rojas OL, Bang YM, Pu A, Christie-Holmes N, Gervais C, Ceccarelli D, Samavarchi-Tehrani P, Guvenc F, Budylowski P, Li A, Paterson A, Yue FY, Marin LM, Caldwell L, Wrana JL, Colwill K, Sicheri F, Mubareka S, Gray-Owen SD, Drews SJ, Siqueira WL, Barrios-Rodiles M, Ostrowski M, Rini JM, Durocher Y, McGeer AJ, Gommerman JL, Gingras AC. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol* 2020; **5** [PMID: 33033173 DOI: 10.1126/sciimmunol.abe5511]
53 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270-273 [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7]
54 **Thevarajan I**, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, Jia X, Nicholson S, Catton M, Cowie B, Tong SYC, Lewin SR, Kedzierska K. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nat Med* 2020; **26**: 453-455 [PMID: 32284614 DOI: 10.1038/s41591-020-0819-2]
55 **Wölfel R**, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020; **581**: 465-469 [PMID: 32235945 DOI: 10.1038/s41586-020-2196-x]
56 **Long QX**, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, Liao P, Qiu JF, Lin Y, Cai XF, Wang DQ, Hu Y, Ren JH, Tang N, Xu YY, Yu LH, Mo Z, Gong F, Zhang XL, Tian WG, Hu L, Zhang XX, Xiang JL, Du HX, Liu HW, Lang CH, Luo XH, Wu SB, Cui XP, Zhou Z, Zhu MM, Wang J, Xue CJ, Li XF, Wang L, Li ZJ, Wang K, Niu CC, Yang QJ, Tang XJ, Zhang Y, Liu XM, Li JJ, Zhang DC, Zhang F, Liu P, Yuan J, Li Q, Hu JL, Chen J, Huang AL. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020; **26**: 845-848 [PMID: 32350462 DOI: 10.1038/s41591-020-0897-1]
57 **Zhao J**, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Qian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B, Zhang T, Fu Y, Ge S, Liu L, Zhang J, Xia N, Zhang Z. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. *Clin Infect Dis* 2020; **71**: 2027-2034 [PMID: 32221519 DOI: 10.1093/cid/ciaa344]
58 **Jiang HW**, Li Y, Zhang HN, Wang W, Men D, Yang X, Qi H, Zhou J, Tao SC. Global profiling of

SARS-CoV-2 specific IgG/ IgM responses of convalescents using a proteome microarray. *BMJ* 2020 [DOI: 10.1101/2020.03.20.20039495]

59 **Li H**, Limenitakis JP, Greiff V, Yilmaz B, Schären O, Urbaniak C, Zünd M, Lawson MAE, Young ID, Rupp S, Heikenwälder M, McCoy KD, Hapfelmeier S, Ganal-Vonarburg SC, Macpherson AJ. Mucosal or systemic microbiota exposures shape the B cell repertoire. *Nature* 2020; **584**: 274-278 [PMID: 32760003 DOI: 10.1038/s41586-020-2564-6]

60 **Nowosad CR**, Mesin L, Castro TBR, Wichmann C, Donaldson GP, Araki T, Schiepers A, Lockhart AAK, Bilate AM, Mucida D, Victora GD. Tunable dynamics of B cell selection in gut germinal centres. *Nature* 2020; **588**: 321-326 [PMID: 33116306 DOI: 10.1038/s41586-020-2865-9]

61 **Kubinak JL**, Round JL. Do antibodies select a healthy microbiota? *Nat Rev Immunol* 2016; **16**: 767-774 [PMID: 27818504 DOI: 10.1038/nri.2016.114]

62 **Dhar D**, Mohanty A. Gut microbiota and Covid-19- possible link and implications. *Virus Res* 2020; **285**: 198018 [PMID: 32430279 DOI: 10.1016/j.virusres.2020.198018]

63 **Honda K**, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature* 2016; **535**: 75-84 [PMID: 27383982 DOI: 10.1038/nature18848]

64 **Sakaguchi S**, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol* 2009; **21**: 1105-1111 [PMID: 19737784 DOI: 10.1093/intimm/dxp095]

65 **Schmidt ME**, Varga SM. The CD8 T Cell Response to Respiratory Virus Infections. *Front Immunol* 2018; **9**: 678 [PMID: 29686673 DOI: 10.3389/fimmu.2018.00678]

66 **Grifoni A**, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, Rawlings SA, Sutherland A, Premkumar L, Jadi RS, Marrama D, de Silva AM, Frazier A, Carlin AF, Greenbaum JA, Peters B, Krammer F, Smith DM, Crotty S, Sette A. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* 2020; **181**: 1489-1501.e15 [PMID: 32473127 DOI: 10.1016/j.cell.2020.05.015]

67 **Braun J**, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, Hippenstiel S, Dingeldey M, Kruse B, Fauchere F, Baysal E, Mangold M, Henze L, Lauster R, Mall MA, Beyer K, Röhmel J, Voigt S, Schmitz J, Miltenyi S, Demuth I, Müller MA, Hocke A, Witzenrath M, Suttorp N, Kern F, Reimer U, Wenschuh H, Drosten C, Corman VM, Giesecke-Thiel C, Sander LE, Thiel A. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020; **587**: 270-274 [PMID: 32726801 DOI: 10.1038/s41586-020-2598-9]

68 **Diao B**, Wang C, Tan Y, Chen X, Liu Y, Ning L, Chen L, Li M, Wang G, Yuan Z, Feng Z, Zhang Y, Wu Y, Chen Y. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). *Front Immunol* 2020; **11**: 827 [PMID: 32425950 DOI: 10.3389/fimmu.2020.00827]

69 **Pandiyan P**, Bhaskaran N, Zou M, Schneider E, Jayaraman S, Huehn J. Microbiome Dependent Regulation of T_{regs} and Th17 Cells in Mucosa. *Front Immunol* 2019; **10**: 426 [PMID: 30906299 DOI: 10.3389/fimmu.2019.00426]

70 **Yu AI**, Zhao L, Eaton KA, Ho S, Chen J, Poe S, Becker J, Gonzalez A, McKinstry D, Hasso M, Mendoza-Castrejon J, Whitfield J, Koumpouras C, Schloss PD, Martens EC, Chen GY. Gut Microbiota Modulate CD8 T Cell Responses to Influence Colitis-Associated Tumorigenesis. *Cell Rep* 2020; **31**: 107471 [PMID: 32268087 DOI: 10.1016/j.celrep.2020.03.035]

71 **Garg M**, Royce SG, Tikellis C, Shallue C, Batu D, Velkoska E, Burrell LM, Patel SK, Beswick L, Jackson A, Britto K, Lukies M, Sluka P, Wardan H, Hirokawa Y, Tan CW, Faux M, Burgess AW, Hosking P, Monagle S, Thomas M, Gibson PR, Lubel J. Imbalance of the renin-angiotensin system may contribute to inflammation and fibrosis in IBD: a novel therapeutic target? *Gut* 2020; **69**: 841-851 [PMID: 31409604 DOI: 10.1136/gutjnl-2019-318512]

72 **Jablaoui A**, Kriaa A, Mkaouar H, Akermi N, Soussou S, Wysocka M, Wołoszyn D, Amouri A, Gargouri A, Maguin E, Lesner A, Rhimi M. Fecal Serine Protease Profiling in Inflammatory Bowel Diseases. *Front Cell Infect Microbiol* 2020; **10**: 21 [PMID: 32117798 DOI: 10.3389/fcimb.2020.00021]

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

74 **Mao R**, Liang J, Shen J, Ghosh S, Zhu LR, Yang H, Wu KC, Chen MH; Chinese Society of IBD, Chinese Elite IBD Union; Chinese IBD Quality Care Evaluation Center Committee. Implications of COVID-19 for patients with pre-existing digestive diseases. *Lancet Gastroenterol Hepatol* 2020; **5**: 425-427 [PMID: 32171057 DOI: 10.1016/S2468-1253(20)30076-5]

75 **Norsa L**, Indriolo A, Sansotta N, Cosimo P, Greco S, D'Antiga L. Uneventful Course in Patients With Inflammatory Bowel Disease During the Severe Acute Respiratory Syndrome Coronavirus 2 Outbreak in Northern Italy. *Gastroenterology* 2020; **159**: 371-372 [PMID: 32247695 DOI: 10.1053/j.gastro.2020.03.062]

76 **Rubin DT**, Feuerstein JD, Wang AY, Cohen RD. AGA Clinical Practice Update on Management of Inflammatory Bowel Disease During the COVID-19 Pandemic: Expert Commentary. *Gastroenterology* 2020; **159**: 350-357 [PMID: 32283100 DOI: 10.1053/j.gastro.2020.04.012]

77 **D'Amico F**, Peyrin-Biroulet L, Danese S. Inflammatory Bowel Diseases and COVID-19: The Invisible Enemy. *Gastroenterology* 2020; **158**: 2302-2304 [PMID: 32305331 DOI:

10.1053/j.gastro.2020.04.032]
78 **Kennedy NA**, Jones GR, Lamb CA, Appleby R, Arnott I, Beattie RM, Bloom S, Brooks AJ, Cooney R, Dart RJ, Edwards C, Fraser A, Gaya DR, Ghosh S, Greveson K, Hansen R, Hart A, Hawthorne AB, Hayee B, Limdi JK, Murray CD, Parkes GC, Parkes M, Patel K, Pollok RC, Powell N, Probert CS, Raine T, Sebastian S, Selinger C, Smith PJ, Stansfield C, Younge L, Lindsay JO, Irving PM, Lees CW. British Society of Gastroenterology guidance for management of inflammatory bowel disease during the COVID-19 pandemic. *Gut* 2020; **69**: 984-990 [PMID: 32303607 DOI: 10.1136/gutjnl-2020-321244]
79 **Rubin DT**, Abreu MT, Rai V, Siegel CA; International Organization for the Study of Inflammatory Bowel Disease. Management of Patients With Crohn's Disease and Ulcerative Colitis During the Coronavirus Disease-2019 Pandemic: Results of an International Meeting. *Gastroenterology* 2020; **159**: 6-13.e6 [PMID: 32272113 DOI: 10.1053/j.gastro.2020.04.002]
80 **Liang W**, Guan W, Chen R, Wang W, Li J, Xu K, Li C, Ai Q, Lu W, Liang H, Li S, He J. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *Lancet Oncol* 2020; **21**: 335-337 [PMID: 32066541 DOI: 10.1016/S1470-2045(20)30096-6]
81 **Almario CV**, Chey WD, Spiegel BMR. Increased Risk of COVID-19 Among Users of Proton Pump Inhibitors. *Am J Gastroenterol* 2020; **115**: 1707-1715 [PMID: 32852340 DOI: 10.14309/ajg.0000000000000798]
82 **Ahlawat S**, Asha, Sharma KK. Immunological co-ordination between gut and lungs in SARS-CoV-2 infection. *Virus Res* 2020; **286**: 198103 [PMID: 32717345 DOI: 10.1016/j.virusres.2020.198103]
83 **Giron LB**, Dweep H, Yin X, Wang H, Damra M, Goldman AR, Gorman N, Palmer CS, Tang HY, Shaikh MW, Forsyth CB, Balk RA, Zilberstein NF, Liu Q, Kossenkov A, Keshavarzian A, Landay A, Abdel-Mohsen M. Plasma Markers of Disrupted Gut Permeability in Severe COVID-19 Patients. *Front Immunol* 2021; **12**: 686240 [PMID: 34177935 DOI: 10.3389/fimmu.2021.686240]
84 **Zuo T**, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology* 2020; **159**: 944-955.e8 [PMID: 32442562 DOI: 10.1053/j.gastro.2020.05.048]
85 **Baud D**, Dimopoulou Agri V, Gibson GR, Reid G, Giannoni E. Using Probiotics to Flatten the Curve of Coronavirus Disease COVID-2019 Pandemic. *Front Public Health* 2020; **8**: 186 [PMID: 32574290 DOI: 10.3389/fpubh.2020.00186]
86 **Sundararaman A**, Ray M, Ravindra PV, Halami PM. Role of probiotics to combat viral infections with emphasis on COVID-19. *Appl Microbiol Biotechnol* 2020; **104**: 8089-8104 [PMID: 32813065 DOI: 10.1007/s00253-020-10832-4]
87 **Infusino F**, Marazzato M, Mancone M, Fedele F, Mastroianni CM, Severino P, Ceccarelli G, Santinelli L, Cavarretta E, Marullo AGM, Miraldi F, Carnevale R, Nocella C, Biondi-Zoccai G, Pagnini C, Schiavon S, Pugliese F, Frati G, d'Ettorre G. Diet Supplementation, Probiotics, and Nutraceuticals in SARS-CoV-2 Infection: A Scoping Review. *Nutrients* 2020; **12** [PMID: 32521760 DOI: 10.3390/nu12061718]
88 **Xu C**, Zhu H, Qiu P. Aging progression of human gut microbiota. *BMC Microbiol* 2019; **19**: 236 [PMID: 31660868 DOI: 10.1186/s12866-019-1616-2]
89 **Kumar P**, Chander B. COVID 19 mortality: Probable role of microbiome to explain disparity. *Med Hypotheses* 2020; **144**: 110209 [PMID: 33254516 DOI: 10.1016/j.mehy.2020.110209]
90 Meeting the challenge of long COVID. *Nat Med* 2020; **26**: 1803 [PMID: 33288947 DOI: 10.1038/s41591-020-01177-6]
91 **van der Lelie D**, Taghavi S. COVID-19 and the Gut Microbiome: More than a Gut Feeling. *mSystems* 2020; **5** [PMID: 32694127 DOI: 10.1128/mSystems.00453-20]
92 **Casadevall A**, Pirofski LA. The convalescent sera option for containing COVID-19. *J Clin Invest* 2020; **130**: 1545-1548 [PMID: 32167489 DOI: 10.1172/JCI138003]
93 **Mahase E**. Covid-19: Anti-TNF drug adalimumab to be trialled for patients in the community. *BMJ* 2020; **371**: m3847 [PMID: 33004419 DOI: 10.1136/bmj.m3847]
94 **Robinson PC**, Richards D, Tanner HL, Feldmann M. Accumulating evidence suggests anti-TNF therapy needs to be given trial priority in COVID-19 treatment. *Lancet Rheumatol* 2020; **2**: e653-e655 [PMID: 33521660 DOI: 10.1016/S2665-9913(20)30309-X]
95 **Sallard E**, Lescure FX, Yazdanpanah Y, Mentre F, Peiffer-Smadja N. Type 1 interferons as a potential treatment against COVID-19. *Antiviral Res* 2020; **178**: 104791 [PMID: 32275914 DOI: 10.1016/j.antiviral.2020.104791]
96 **Feldmann M**, Maini RN, Woody JN, Holgate ST, Winter G, Rowland M, Richards D, Hussell T. Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. *Lancet* 2020; **395**: 1407-1409 [PMID: 32278362 DOI: 10.1016/S0140-6736(20)30858-8]
97 **Kuhn KA**, Manieri NA, Liu TC, Stappenbeck TS. IL-6 stimulates intestinal epithelial proliferation and repair after injury. *PLoS One* 2014; **9**: e114195 [PMID: 25478789 DOI: 10.1371/journal.pone.0114195]
98 **Olaimat AN**, Aolymat I, Al-Holy M, Ayyash M, Abu Ghoush M, Al-Nabulsi AA, Osaili T, Apostolopoulos V, Liu SQ, Shah NP. The potential application of probiotics and prebiotics for the prevention and treatment of COVID-19. *NPJ Sci Food* 2020; **4**: 17 [PMID: 33083549 DOI: 10.1038/s41538-020-00078-9]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2021 December 14; 27(46): 7943-7955

DOI: 10.3748/wjg.v27.i46.7943

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Transmembrane serine protease 2 and angiotensin-converting enzyme 2 anti-inflammatory receptors for COVID-19/inflammatory bowel diseases treatment

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Author contributions: Lashgari NA, Roudsari NM, and Momtaz S collected and/or interpreted the data and wrote the manuscript; Abdolghaffari AH and Momtaz S provided the study material, conceived, designed, and finally approved the manuscript; all authors have read and approved the manuscript.

Conflict-of-interest statement: The authors have no conflicts of interest to disclose.

Country/Territory of origin: Iran

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0

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Abstract

Inflammatory bowel diseases (IBD) refer to a subgroup of chronic, progressive, long-term, and relapsing inflammatory disorders. IBD may spontaneously grow in the colon, and in severe cases may result in tumor lesions such as invasive carcinoma in inflamed regions of the intestine. Recent epidemiological reports indicate that old age and underlying diseases such as IBD contribute to severity and mortality in patients with coronavirus disease 2019 (COVID-19). Currently, the ongoing COVID-19 pandemic caused serious morbidity and mortality worldwide. It has also been shown that the transmembrane serine protease 2 is an essential factor for viral activation and viral engulfment. Generally, viral entry causes a 'cytokine storm' that induces excessive generation of proinflammatory cytokines/chemokines including interleukin (IL)-6, IL-2, IL-7, tumor necrosis factor-α, and interferon-γ. Future research could concentrate on developing inflammatory immunological responses that are efficient to encounter COVID-19.

Grade D (Fair): 0
Grade E (Poor): 0

Received: March 16, 2021
Peer-review started: March 16, 2021
First decision: May 1, 2021
Revised: May 12, 2021
Accepted: November 28, 2021
Article in press: November 28, 2021
Published online: December 14, 2021

P-Reviewer: Moreira TMM, Soares RLS
S-Editor: Fan JR
L-Editor: Filipodia
P-Editor: Fan JR

Current analysis elucidates the role of inflammation and immune responses during IBD infection with COVID-19 and provides a list of possible targets for IBD-regulated therapies in particular. Data from clinical, *in vitro*, and *in vivo* studies were collected in English from PubMed, Google Scholar, Scopus, and the Cochrane library until May 2021.

Key Words: Inflammatory bowel diseases; COVID-19; Transmembrane serine protease 2; Inflammation; Pro-inflammatory; Immunological responses

Core Tip: This article provides clinical evidence on synthetic or natural-based transmembrane serine protease 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) inhibitors, which are able to reduce coronavirus disease 2019-induced inflammation and cytokine storms in inflammatory bowel disease patients. Hence, targeting TMPRSS2 and ACE2 could be noticed as a novel approach for inflammatory bowel diseases treatment.

Citation: Lashgari NA, Momeni Roudsari N, Momtaz S, Abdolghaffari AH. Transmembrane serine protease 2 and angiotensin-converting enzyme 2 anti-inflammatory receptors for COVID-19/inflammatory bowel diseases treatment. *World J Gastroenterol* 2021; 27(46): 7943-7955
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7943.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7943

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a primarily respiratory ailment that is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), additionally named as 2019 novel COVID. It is profoundly overwhelming with case casualty rates of 2%-3%. Since its appearance in December 2019 in China, COVID-19 has quickly spread and influenced populaces in virtually all areas of the world[1,2]. Old-age patients and those with chronic conditions are more prone to dreariness and mortality in COVID-19. This high mortality is implicated in misrepresented and misled invulnerable reactions that cause cytokine storms. In brief, SARS-CoV-2 infects the angiotensin-converting enzyme 2 (ACE2) expressing epithelial cells in the lung and/or the intestine, leading to a massive production of mediators that induce the immune cell activation. Overactivation of immune cells leads to severe complications including acute respiratory distress syndrome, shock, and multiorgan failure[3,4].

Inflammatory bowel diseases (IBD) include two major types: Ulcerative colitis and Crohn's disease. IBD is characterized with persistent resistant interceded sicknesses that regularly require immunomodulatory and immunosuppressive treatments[5,6]. Therefore, patients with IBD are at high risk to different shrewd viral and bacterial contaminations. There is no solid evidence that patients with IBD are at higher risk for COVID-19 infection, although it has been indicated that patients with IBD who are pregnant are more vulnerable[7]. The current study discusses the impact of COVID-19 on IBD[8,9]. We provide evidence on mediatory effects of the transmembrane serine protease 2 (TMPRSS2) and ACE2 signaling pathways against inflammation and introduces the synthetic or natural TMPRSS2 and ACE2 inhibitors as probable approaches for IBD treatment in the COVID-19 situation[9,10].

LITERATURE SEARCH

PubMed, Google Scholar, Scopus, and Cochrane Library were searched and relevant clinical, *in vivo*, and *in vitro* articles (in English) were collected until May 2021. Search terms included "corona virus" OR "COVID-19" AND "inflammatory bowel disease" OR "IBD" OR "inflammation" AND "TMPRSS2" OR "ACE2" AND "TMPRSS2 inhibitors"

OR "ACE2 inhibitors".

COVID-19 PATHOGENESIS

Variations in potency of the SARS-CoV-2 cell entry may account for discovering new solutions to deal with the virus. It has been reported that the entrance of SARS-CoV-2 to the human cells victimizes the SARS-CoV receptor ACE2 and TMPRSS2 for the spike (S) supermolecule priming. It is debatable whether the metallopeptidase domain seventeen [a disintegrin and metalloprotease domain 17 (ADAM17), also referred to as the tumor necrosis factor (TNF)-α-converting accelerator] located in the ACE2 ectodomain shedding may or may not counteract the virus entry by increasing the number of soluble ACE2, or it solely contributes to the ACE1/ACE2 unbalancing, inflammation, and occlusion[11]. The ACE2-receptor/S-protein interaction could be a key factor for success of virus infection and willingness. Similarly, single ester polymorphisms located inside the TMPRSS2 factor (21q22.3) can play a more important role in respiratory disorder[12]. ACE1 and ACE2 collaborate with the renin-angiotensin system to balance the native vasoconstrictor/proliferative ACE1/angiotensin II/angiotensin II type 1/angiotensin (Ang) II/Ang type 1 receptor (ACE1/Ang-II type 1/AT1-axis), and vasodilator/antiproliferative (ACE2/Ang1-7/mitochondrial assembly-axis) actions. This ends up in the protection of organs and blood vessels by the decoagulants, medicinal drugs, anti-proliferation, anti-fibrosis, anti-alveolar vegetative cell caspase-mediated cell death, and anti-oxidative stress activities that are able to antagonize the Ang-II effects[11,13].

TMPRSS2 AND ACE2 STRUCTURE AND RELATED SIGNALING PATHWAYS

In a complex pathophysiological condition like COVID-19, the ACE2 cytoplasmic tail cleavage intervened by TMPRSS2 is a significant event to be considered (Figure 1). Cleavage of the ACE2 tail by TMPRSS2 increases viral load in objective cells, and TMPRSS2 could facilitate the SARS-CoV-2 passage *via* the SARS-S cleavage, which induces the S protein for film combination. The ACE2 cleavage may enhance viral uptake through the cathepsin L-subordinate pathway, resulting in viral integration with the endosomal layer and eventually cell contamination[11,14]. In spite of similar explicitness of TMPRSS2 and ADAM17 for ACE2, they act opposite for cleavage of ACE2. To start with, the divisions produced by the cleavage of these proteases have distinctive subatomic sizes, mainly due to various cleavage locales. Second, cleavage of ACE2 by ADAM17 forms the ACE2 ectodomain, which is shed into the extracellular medium, as the soluble ACE biologically dynamic structure[15,16]. *In vitro* studies have shown that the ACE2 ectodomain does not separate from the TMPRRS2-induced ACE2 cleavage. This was evidenced by a C-terminal intracellular cleavage. In this manner, the distinctions in the cleavage destinations and its organic outcomes might be basic. For sure, just the soluble ACE2 structure would have a defensive impact on prevention of viral particle aggregations[17]. Therefore, overexpression of ADAM17 and TMPRSS2 could be a primary factor in inflammation storm that is characterized by negative features such as renin-angiotensin system lopsidedness, intense irritation, and intravascular coagulation in older populations with COVID-19 comorbidities. Initiation of inflammation cycles is a key element for SARS-CoV-2 contamination[18,19].

TMRPSS2 AND ACE2 INFLAMMATORY PATHWAY

ACE2 is the main receptor for SARS-CoV-2, providing additional insurance against the destructive impacts of viral diseases. Moreover, as referenced above, solid confirmations indicate that the outflow of ACE2 is dependent on the companion of hormonal, hereditary, and age-related systems[20,21]. Overaction of ADAM17 in both COVID-19 and the plasma level of ACE2 has been confirmed by several reports. Overexpression of the *ADAM17* gene and its protein level have been implicated in several inflammatory conditions including IBD[22,23]. High levels of inflammatory cytokines and chemokines in COVID-19 patients are accounted for by more elevated levels of interleukin (IL)-2, IL-7, IL-10, granulocyte colony-stimulating factor, interferon

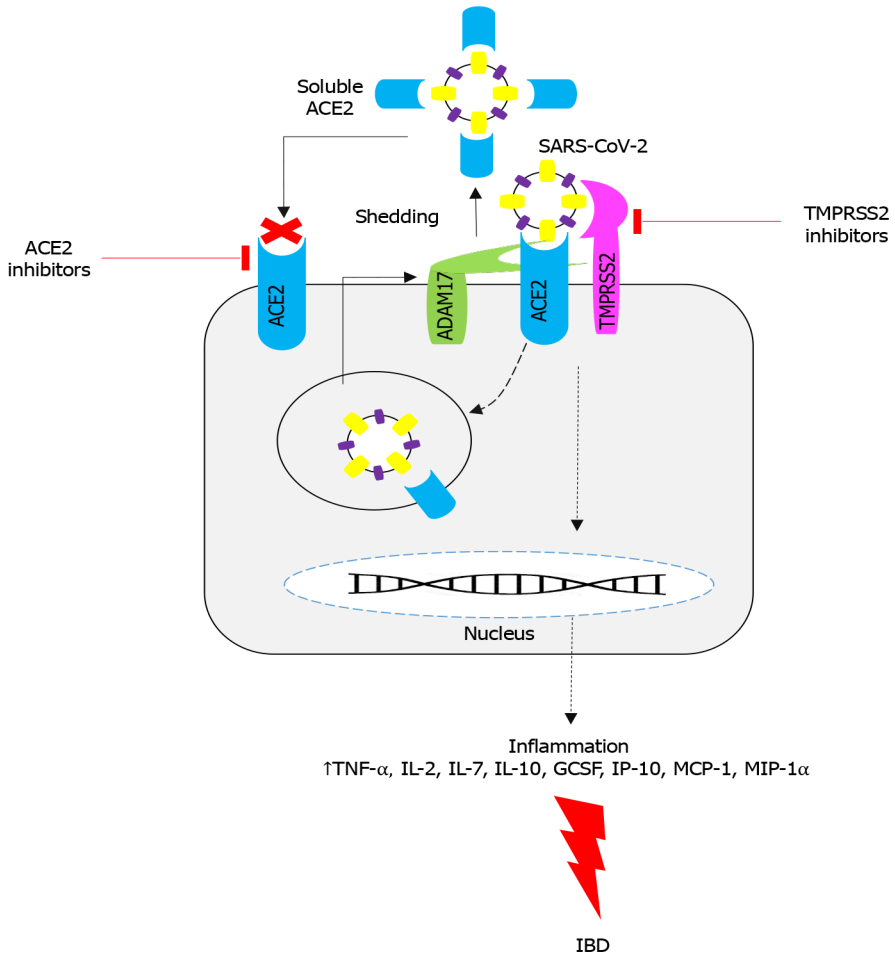

Figure 1 Coronavirus disease 2019 induced inflammatory bowel diseases mechanism. ADAM17: A disintegrin and metalloprotease domain 17; ACE2: Angiotensin-converting enzyme 2; TMPRSS2: Transmembrane serine protease 2; SARS-Cov-2: Severe acute respiratory syndrome coronavirus 2; GCSF: Granulocyte colony-stimulating factor; IP-10: Interferon gamma-induced protein 10; MCP1: Monocyte chemoattractant protein 1; MIP1-α: Macrophage inflammatory proteins-α; IBD: Inflammatory bowel diseases.

gamma-induced protein 10, monocyte chemoattractant protein 1, macrophage inflammatory proteins-1A, and TNF-α. A significant effect of the "fiery wave" in COVID-19 indicates the cytokine storm may be firmly connected with the seriousness of the infection[24,25]. The 'cytokine storm' is a significant target for research about the pathogenic cycles in SARS-CoV–2 contaminations and is a way to recognize new restorative targets. On the other hand, blockade of SARS-CoV–ACE2 in the ACE2 cytoplasmic domain pathway results in upregulation of ADAM17 activity. Upregulated ADAM17 increases the ACE2 ectodomain proteolytic cleavage[26,27]. Similar to the ACE2 tail cleavage, ADAM17 upregulation is essential for SARS-CoV infection. Finally, excessive activity of ADAM17 induces proinflammatory mediators, thus upregulating the inflammatory pathway during SARS-CoV-2 infection. ACE2 can be cleaved by the activity of TMPRSS2 protease[28,29]. TMPRSS2-induced ACE2 cytoplasmic tail cleavage may incite the viral uptake through a cathepsin L-subordinate pathway. Of note, acute respiratory distress syndrome is a delayed consequence of an aberrant generation of proinflammatory cytokines/chemokines or the 'cytokine storm' by effector cells[30-32].

COVID-19 INDUCED IBD: CORRELATIONS AND OVERLAPPING OF INFLAMMATORY PATHOGENESIS

The SARS-CoV-2 receptor ACE2 and TMPRSS2 receptor are central factors in COVID-19-induced IBD pathogenesis (Table 1 and Figure 1). These receptors are often found within the lower respiratory lot of pneumocytes and the gastrointestinal tract[7]. The ACE2 receptors are frequently located within the terminal ileum and colon. It was

Table 1 Clinical evidences of coronavirus disease 2019-induced inflammatory bowel diseases treatment

Ref.	Clinical studies	Model of IBD	Intervention	Duration of treatment	Numbers of animals in intervention group and control group	Outcomes	Adverse effects
Nowak *et al*[25]	Clinical trial	IBD in COVID-19	-	-	138 treatment naïve IBD patients (cases) and 154 controls	↑ACE2/TMPRSS2 expression; ↑ Inflammation	-
Brenner *et al*[67]	18 yr (with IBD), the Pediatric IBD Porto Group	-	TNF antagonist monotherapy (48%), followed by sulfasalazine/mesalamine (23%)	March 2020-October 2020	Hospitalized cases (n = 14); Outpatient cases (n = 195)	Sulfasalazine/Mesalamine and steroid therapy were associated with increased hospitalization risk and TNF antagonist monotherapy was associated with decreased risk parallel those reported in adult IBD patients. PIBD patients have a relatively low risk of severe COVID-19, even when receiving biologic and/or other immune-suppressive therapies for their IBD	-
Norsa *et al*[85]	Clinical trial	Crohn disease and Ulcer colitis	Anti-inflammatory (Salicylates); thiopurines or methotrexate; biologics (Infliximab, Adalimumab, Ustekinumab, Vedolizumab, Golimumab); steroids; Other immunosuppressants (Tacrolimus, Cyclosporin, Mofetil Micofenolate)	February 2020-March 2020	Crohn disease = 186; Ulcer colitis = 336	IBD improvement: ↓TNF-α; ↓ Inflammation; ↓ACE2/TMPRSS2 expression	-
Mazza *et al*[86]	Clinical trial	Ulcerative colitis	Methylprednisolone (40 mg/d); prednisone dosage at the time of patient's death was 25 mg daily	December 2019-February 2020	-	IBD improvement; Improvement in COVID-19 symptoms; ↓ Inflammation	-
Tursi *et al* [87]	Clinical trial	Crohn's disease	Adalimumab	-	-	Maintain of IBD remission during COVID-19; Managing/preventing COVID-driven pneumonia: ↓TNF-α ; ↓Inflammation; ↓ACE2/TMPRSS2 expression	-
Bodini *et al*[88]	Clinical trial	IBD	Immunosuppressants/biological treatment	3 wk	48 patients	IBD improvement; Improvement in; COVID-19 symptoms	Increase the risk of infection
Tursi *et al* [89]	Clinical trial	Crohn's disease	Mesalazine (3 g/d) and Adalimumab 40 mg subcutaneously	-	74 cases	IBD improvement; Improvement in COVID-19 symptoms; ↓TNF-α; ↓ Inflammation; ↓ACE2/TMPRSS2 expression	-
Allocca *et al*[90]	Clinical trial	IBD	Biological treatment	-	162 IBD patients	IBD improvement; Improvement in COVID-19 symptoms	-
Jacobs *et al*[91]	Clinical trial	Ulcerative colitis	Tofacitinib (10 mg twice daily)	5 mo	-	IBD improvement; Improvement in COVID-19 symptoms	Increase the risk of infection
Gutin *et al*[69]	Clinical trial	Ulcerative colitis	Biological treatment	February 2020-March 2020	522 patients	IBD improvement; Improvement in COVID-19 symptoms: ↓TNF-α; ↓ Inflammation; ↓ACE2/TMPRSS2 expression	
Taxonera *et al*[92]	Clinical trial	Crohn's disease	Immunomodulatory/biologics	-	n = 12	IBD improvement; Improvement in COVID-19 symptoms; ↓TNF-α; ↓ Inflammation; ↓ACE2/TMPRSS2 expression	
Allocca *et al*[93]	Clinical trial	-	Immunosuppressant or biologics	-	n = 15	IBD improvement; Improvement in COVID-19 symptoms; ↓TNF-α; ↓ Inflammation	-
Mak *et al* [94]	Clinical trial	IBD in COVID-19	Thirty (75%) were on 5-Aminosalicylates acid, 15 (37.5%) on	-	n = 63	IBD improvement; Improvement in COVID-19 symptoms; ↓	-

			immunosuppressants (14 Thiopurine, one Tacrolimus), 11 (27.5%) on corticosteroids and 7 (17.5%) on biologics (3 Infliximab, 1 Adalimumab, 2 Vedolizumab and 1 Ustekinumab)			Inflammation	
Bardasi and Alvisi [95]	Clinical trial	Crohn's disease in COVID-19	Subcutaneous administration of 40 mg Adalimumab	6 mo	-	IBD improvement; Improvement in COVID-19 symptoms ↓ Inflammation	-
Ashton *et al*[96]	Clinical trial	IBD in COVID-19	Anti-TNF therapy (Infliximab or Adalimumab)	-	*n* = 122	IBD improvement; Improvement in COVID-19 symptoms: ↓TNF-α; ↓ Inflammation	-

ACE2: Angiotensin-converting enzyme 2; TMPRSS2: Transmembrane serine protease 2; IBD: Inflammatory bowel diseases; TNF: Tumor necrosis factor.

shown that the convergence of these receptors was higher in IBD patients, in both the energetic and calm stages of the disease[33]. The ACE2 receptors are a part of the renin angiotensin-aldosterone system that is assumed to play critical roles in controlling the provocative handle. Terminal ileum and the colon are the most affected areas in IBD [34]. IBD is also correlated with upregulation of inflammatory cytokines and the ACE2 receptors. As we discuss in this article, patients with IBD do not seem powerless against COVID-19[35]. In this context, a few theories have been proposed. For instance, the renin angiotensin-aldosterone system has two specific pathways involved in irritation course. Multiple studies confirmed that ACE2 is upregulated in IBD, and in the SARS-CoV-2 condition ACE2 exacerbates the disease symptoms. Accordingly, prevention of the ACE2 protein expression has been suggested for controlling both COVID-19 and IBD[36,37]. While the ACE-angiotensin receptor 1 pathway is favorable for inflammation, the ACE2 pathway helps in tissue security. Given the enteric inflammation in IBD, it has been suggested that the ACE2 receptors and the host cell surface proteases like TMPRSS2 may suppress SARS-CoV-2[38,39]. The ACE2 level was shown to be downregulated in colonic aggravation in animal models; thereby, some IBD drugs such as steroids and biologics were found useful for cutting down the ACE2 in infected cells. Another report declared no change in ACE2 receptors or TMPRSS2 in IBD patients when diverged from controls[40,41].

IBD IN COVID-19: TREATMENT APPROACH

As mentioned, there are limited data on the possible impact of SARS-CoV-2 contamination on patients with IBD. Various methodologies can be utilized alone or concurrently to conquer the infection. Blockage of the ACE2 receptors and the viral S protein are the main focus of current investigations on SARS-CoV-2 regulation. So far, we discussed that blockage of the TMPRSS2 receptor and/or the ACE2/TMPRSS2 complex is likewise a plausible approach to modulate this infection. In this context, a number of synthetic or natural TMPRSS2 and ACE2 inhibitors that are able to mediate the TMPRSS2 and ACE2 signaling have been explored.

Natural agents targeting TMRPSS2 and ACE2 to manage the COVID-19 and IBD overlap

Medicinal plants are the greatest age-old wellspring of remedially valuable phytochemicals that are utilized to keep up human's wellbeing and to forestall and treat numerous infections. Medicinal plants and spices are used in Ayurveda, a conventional and optional restorative treatment in light of comprehensive body recuperating, which began in the Indian subcontinent[42,43]. Enormous investigations are right now centered on understanding the remedial viability and the activity of these phytochemicals. An improved dietary regimen along with natural medicinal formulations may provide preventive strategies for intense respiratory diseases, aspiratory fibrosis, pneumonia, sepsis, and numerous organ failure, which are hallmarks of serious COVID-19 contamination[44]. Also, a significant number of these phytochemicals help the insusceptible framework and instills insurance against infective diseases. It was shown that oxidative stress and many other reasons, notwithstanding existing comorbidities, add to a large number of difficulties related to coronavirus disease. Herein, we introduce plant species that contain various phyt-

ochemicals with antiviral, antifibrotic, cell reinforcement, mitigating, and immunomodulatory properties[45]. These phytochemicals, when used in blend, could have synergistic impacts, either as prophylactic or as steady specialists to limit certain clinical manifestations observed in COVID-19-contaminated patients. Moreover, certain types of microscopic organisms, green growth, and parasites may have remedial impacts against pneumonic fibrosis and intense lung injury[46].

ACE2 is found in the outer layer of the human cell that is accounted as a likely coupling site for the S protein. A couple of experiments have shown that there is a strong link between ACE2 and the S protein. Thus, blockade of ACE2 by phytochemicals is a strategy to fight SARS-CoV-2[47]. Several studies reported that SARS-CoV-2 is able to infect the central nervous system through TMRPSS2 and ACE2 receptors. It was also shown that ACE2 participates in neuroprotective responses, hence playing a critical role in treatment of COVID-19. Phytochemicals such as baicalin, scutellarin, and hesperetin can bind to ACE2 and prevent neurological impairments caused by COVID-19.

It was shown that hesperidin, chrysin, and emodin are also effective for COVID-19 treatment by attenuating the harmful effect of viral infection within cells[48]. Kaempferol, quercetin, and fisetin can bind with human angiotensin-converting enzyme-S-protein. In silico studies demonstrated that quercetin, quercetin 3 glucuronide-7-glucoside, quercetin 3-vicianoside, absinthin, glabridin, and gallic acid have strong affinity toward ACE2 to suppress COVID-19. Nuclear docking examination elucidated that dithymoquinone (aquinone) encounters the COVID-19 neurological side effects through blockade of ACE2. An in silico study reported that two chalcones namely azobechalcone and isolophirachalcone and some alkaloids (*i.e.* fangchinoline and tetrandrine) had high limiting proclivity to the S protein of SARS-CoV-2[49]. Flavonoids reduce the ACE2 expression through inducing the nuclear factor erythroid 2-related factor 2, thus fighting SARS-CoV-2 by means of their antioxidant properties. Kaempferol, quercetin, and fisetin are promising flavonoids against COVID-19-induced adverse neurological effects. Stilbenes, in particular resveratrol, are promising candidates for COVID-19 treatments, mainly by disturbing the formation of the S protein and the ACE2 receptor complex[50]. A variety of phenolic compounds including naringenin, hesperetin, hesperidin, and baicalin (alone or in combination) showed inhibitory effects on ACE2 activity and can be considered as potential treatments for COVID-19[51].

Different studies exhibited that some other phenolic compounds such as cinnamaldehyde as well as terpenoids such as carvacrol, geraniol, anethole, L-4-terpineol, cinnamyl acidic, thymol, and pulegone possess antiviral activities through blockade of the viral S protein[52,53]. It was reported that the binding affinity of ACE2 linkage with scutellarin (a flavonoid glycoside) and glycyrrhizin (a triterpenoid) was stronger than baicalin, hesperetin, and nicotianamine[54].

Limonoids and triterpenoids also displayed similar inhibitory effects on ACE2. Another in silico study similarly demonstrated that limonin, obacunone, ursolic destructive, glycyrrhizin destructive, 7-deacetyl-7-benzoylgedunin, maslinic acid, and corosolic acid effectively target SARS-CoV-2 proteins[55]. In this line, nimbin (a triterpenoid) and curcumin exhibited high limiting proclivity on ACE2 and the S protein [56]. Epigallocatechin-3-gallate and theaflavin gallate were shown to have inhibitory effects on the S-protein central channel of SARS-CoV-2. Moreover, three alkaloids, including cepharanthine, fangchinoline, and tetrandrine, inhibited the S protein of Human coronavirus Subtype OC43 (Human-CoV-OC43) expression, while tetrandrine exhibited moderating effects on viral sicknesses. An indazole alkaloid isolated from the seeds of *Nigella sativa*, called nigellidine, was shown to bind the dynamic areas of SARS-CoV-2, thereby paralyzing the virus. In another study, anthraquinone emodin blocked the ACE2 and S protein conjunction[57,58].

Chemical agents targeting TMRPSS2 and ACE2 to manage the treatment of COVID-19 and IBD overlap

Various classes of medications, with different powers and immunosuppressive potentials, are used for IBD treatment (Table 1 and Figure 1). At present, limited data are available for the utilization of different medications in IBD under the COVID-19 condition, henceforth the level of proof is not yet certain[59]. Current suggestions, proposed by specialists and different social orders, are overwhelmingly based on the recounted proof from the utilization of these medications during other viral pandemics like SARS and Middle East respiratory syndrome coronavirus or a few distributed case reports[60]. By and large, usage of intense immunosuppressants in IBD patients should be limited, except if totally essential. Notwithstanding, patients

who are on stable upkeep portions may keep on doing as such with close contact with their physicians[61,62].

Salicylates: Salicylates are usually utilized in either oral form or as a bowel purge. They have a neighborhood activity and are improbable to influence the course of COVID-19 when are used in IBD patients, thereby they may be securely proceeded in dosages[63].

Corticosteroids: Corticosteroids are the most common drugs that are used in IBD, mainly due to their intense calming effects. Therefore, steroids may be valuable in suppression of COVID-19, particularly in conditions like intense lung injury, intense respiratory trouble disorder, and septic shock. During the SARS and Middle East respiratory syndrome pandemic, corticosteroids treatment helped to postpone viremia [64,65], while there were no general improvement in terms of septic shock or psychosis, *etc.*[66]. Given the absence of adequacy, the World Health Organization suggested that routine corticosteroids ought to be avoided except in explicit circumstances. Steroids are possibly kept away from the first line therapies in recently analyzed IBD patients. Notwithstanding, considering their tremendous advantages in IBD, it was suggested that the main steroids might be beneficial at low doses in patients with COVID-19 and IBD, specifically in patients that are already on treatment [67,68]. Steroids with limited site of action, for example budesonide, seem harmless to be used. Infliximab might be a therapeutic option for COVID-19 positive patients with mild respiratory symptoms[61,69].

Cyclosporin: Cyclosporin is used for serious ulcerative colitis as an option in contrast to steroids. Although, some data pointed out that cyclosporine can inhibit the coronavirus replication proteins *in vitro*, its prescription is controversial in patients with COVID-19 due to its strong immunosuppressive properties[70-72].

Azathioprine and methotrexate: Azathioprine is a thiopurine that is often used for IBD treatment, particularly for upkeep treatment. Curiously, past investigations have demonstrated that thiopurine analogs have both immediate and roundabout activities on smothering antiviral movement. They also hinder viral proteases once the host proteins were engaged with viral replication[73,74]. Depending on the perception of genuine viral contaminations in IBD patients who are using thiopurine, the treatment time can be estimated. Interruption in treatment up to 14 d after recuperation from COVID-19 has been suggested. Methotrexate can perhaps continue without issues[75, 76].

Biologics: Current data show that infliximab and adalimumab (TNF-α inhibitors) have no unfavorable effects on the clinical course of COVID-19[62,77]. One reason speculated is the strong mitigating impact of TNF blockage, which may indeed constrict the cytokine storm in serious types of COVID-19[78,79]. Co-administration of medicines (*i.e.* thiopurine and infliximab) might be an option. Also, monotherapy with natural products may be considered[60,80,81]. Vedolizumab (an adversary of α4β7 integrin) is significantly explicit for movement on the gut, hence it is favorable for fundamental or pneumonic responses in COVID-19[62,82]. Ustekinumab is an approved clinical therapy for patients with IBD. Ustekinumab is a cytokine antibody and an inhibitor of IL-12 and IL-23. Currently, there are no major concerns about usage of ustekinumab in patients with IBD and COVID-19. Vedolizumab or ustekinumab might be the primary therapeutic options for individuals at higher risk of COVID-19 if biological treatments are thought of[79,83,84].

CONCLUSION

Information on the physiologic and pathophysiologic functions of ACE2/TMPRSS2 is still scant. ACE2/TMPRSS2 is very much described in the cardiovascular and renal frameworks. Yet little data exist regarding other organ frameworks, for example the gastrointestinal system. Moreover, specific function of the ACE2/TMPRSS2 axis in pathologic conditions was traditionally restricted to cardiovascular illnesses. Although, considering the ACE2/TMPRSS2 as a multifunctional protein has accomplished significance as of late.

The current COVID-19 pandemic has featured the importance of ACE2/TMPRSS2 as a receptor for SARS-CoV-2, yet research is expected to determine whether the ACE2/TMPRSS2 levels enhance the pathogenesis of COVID-19 or could benefit the course of illness by diminishing the malicious impacts of Ang II. Moreover, the

relationship between ACE2/TMPRSS2, the intestinal amino corrosive vehicle, and IBD merits further consideration in patients with IBD. At last, association of ACE2/TMPRSS2 to integrins raises concerns and expectations, particularly because there were just two articles regarding the matter. Taking everything into account, investigating the multifunctional nature of ACE2/TMPRSS2 in IBD (by describing its appearance/movement in the blood, gut, as well as excrement of patients with IBD and solid control patients) will develop the knowledge on the pathophysiology of this illness. In accordance with this objective, recognizable proof of other biomarkers of infection movement, treatment reaction, and new medication target, as well as setting of the novel helpful alternatives is required to affect tolerant consideration.

REFERENCES

1 **Stopsack KH**, Mucci LA, Antonarakis ES, Nelson PS, Kantoff PW. *TMPRSS2* and COVID-19: Serendipity or Opportunity for Intervention? *Cancer Discov* 2020; **10**: 779-782 [PMID: 32276929 DOI: 10.1158/2159-8290.CD-20-0451]

2 **Scaldaferri F**, Pugliese D, Privitera G, Onali S, Lopetuso LR, Rizzatti G, Settanni CR, Pizzoferrato M, Schiavoni E, Turchini L, Amatucci V, Napolitano D, Bernabei T, Mora V, Laterza L, Papa A, Guidi L, Rapaccini GL, Gasbarrini A, Armuzzi A. Impact of COVID-19 pandemic on the daily management of biotechnological therapy in inflammatory bowel disease patients: Reorganisational response in a high-volume Italian inflammatory bowel disease centre. *United European Gastroenterol J* 2020; **8**: 775-781 [PMID: 32438878 DOI: 10.1177/2050640620929133]

3 **Marini JJ**, Gattinoni L. Management of COVID-19 Respiratory Distress. *JAMA* 2020; **323**: 2329-2330 [PMID: 32329799 DOI: 10.1001/jama.2020.6825]

4 **Li X**, Ma X. Acute respiratory failure in COVID-19: is it "typical" ARDS? *Crit Care* 2020; **24**: 198 [PMID: 32375845 DOI: 10.1186/s13054-020-02911-9]

5 **Lashgari NA**, Roudsari NM, Momtaz S, Ghanaatian N, Kohansal P, Farzaei MH, Afshari K, Sahebkar A, Abdolghaffari AH. Targeting Mammalian Target of Rapamycin: Prospects for the Treatment of Inflammatory Bowel Diseases. *Curr Med Chem* 2021; **28**: 1605-1624 [PMID: 32364064 DOI: 10.2174/0929867327666200504081503]

6 **Lashgari NA**, Roudsari NM, Zandi N, Pazoki B, Rezaei A, Hashemi M, Momtaz S, Rahimi R, Shayan M, Dehpour AR, Abdolghaffari AH. Current overview of opioids in progression of inflammatory bowel disease; pharmacological and clinical considerations. *Mol Biol Rep* 2021; **48**: 855-874 [PMID: 33394234 DOI: 10.1007/s11033-020-06095-x]

7 **Neurath MF**. COVID-19 and immunomodulation in IBD. *Gut* 2020; **69**: 1335-1342 [PMID: 32303609 DOI: 10.1136/gutjnl-2020-321269]

8 **Reinsch S**, Stallmach A, Grunert PC. The COVID-19 Pandemic: Fears and Overprotection in Pediatric Patients with Inflammatory Bowel Disease and Their Families. *Pediatr Gastroenterol Hepatol Nutr* 2021; **24**: 65-74 [PMID: 33505895 DOI: 10.5223/pghn.2021.24.1.65]

9 **Yang C**, Xiao SY. COVID-19 and inflammatory bowel disease: A pathophysiological assessment. *Biomed Pharmacother* 2021; **135**: 111233 [PMID: 33433350 DOI: 10.1016/j.biopha.2021.111233]

10 **Bourgonje AR**, Abdulle AE, Timens W, Hillebrands JL, Navis GJ, Gordijn SJ, Bolling MC, Dijkstra G, Voors AA, Osterhaus AD, van der Voort PH, Mulder DJ, van Goor H. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol* 2020; **251**: 228-248 [PMID: 32418199 DOI: 10.1002/path.5471]

11 **Suárez-Fariñas M**, Tokuyama M, Wei G, Huang R, Livanos A, Jha D, Levescot A, Irizar H, Kosoy R, Cording S, Wang W, Losic B, Ungaro RC, Di'Narzo A, Martinez-Delgado G, Suprun M, Corley MJ, Stojmirovic A, Houten SM, Peters L, Curran M, Brodmerkel C, Perrigoue J, Friedman JR, Hao K, Schadt EE, Zhu J, Ko HM, Cho J, Dubinsky MC, Sands BE, Ndhlovu L, Cerf-Bensusan N, Kasarskis A, Colombel JF, Harpaz N, Argmann C, Mehandru S. Intestinal Inflammation Modulates the Expression of ACE2 and TMPRSS2 and Potentially Overlaps With the Pathogenesis of SARS-CoV-2-related Disease. *Gastroenterology* 2021; **160**: 287-301.e20 [PMID: 32980345 DOI: 10.1053/j.gastro.2020.09.029]

12 **Batchu SN**, Kaur H, Yerra VG, Advani SL, Kabir MG, Liu Y, Klein T, Advani A. Lung and Kidney ACE2 and TMPRSS2 in Renin-Angiotensin System Blocker-Treated Comorbid Diabetic Mice Mimicking Host Factors That Have Been Linked to Severe COVID-19. *Diabetes* 2021; **70**: 759-771 [PMID: 33310740 DOI: 10.2337/db20-0765]

13 **Sakamoto A**, Kawakami R, Kawai K, Gianatti A, Pellegrini D, Kutys R, Guo L, Mori M, Cornelissen A, Sato Y, Bellasi A, Faggi L, Hong C, Romero M, Guagliumi G, Virmani R, Finn AV. ACE2 (Angiotensin-Converting Enzyme 2) and TMPRSS2 (Transmembrane Serine Protease 2) Expression and Localization of SARS-CoV-2 Infection in the Human Heart. *Arterioscler Thromb Vasc Biol* 2021; **41**: 542-544 [PMID: 33086866 DOI: 10.1161/ATVBAHA.120.315229]

14 **Kermani NZ**, Song WJ, Badi Y, Versi A, Guo Y, Sun K, Bhavsar P, Howarth P, Dahlen SE, Sterk PJ, Djukanovic R, Adcock IM, Chung KF; U-BIOPRED Consortium. Sputum ACE2, TMPRSS2 and FURIN gene expression in severe neutrophilic asthma. *Respir Res* 2021; **22**: 10 [PMID: 33413387 DOI: 10.1186/s12931-020-01605-8]

15 **Wang H**, Sun X, VonCannon JL, Kon ND, Ferrario CM, Groban L. Estrogen receptors are linked to angiotensin-converting enzyme 2 (ACE2), ADAM metallopeptidase domain 17 (ADAM-17), and transmembrane protease serine 2 (TMPRSS2) expression in the human atrium: insights into COVID-19. Hypertens Res 2021; 1-3 [DOI: 10.1038/s41440-021-00626-0]

16 **Calcagnile M**, Forgez P, Iannelli A, Bucci C, Alifano M, Alifano P. Molecular docking simulation reveals ACE2 polymorphisms that may increase the affinity of ACE2 with the SARS-CoV-2 Spike protein. *Biochimie* 2021; **180**: 143-148 [PMID: 33181224 DOI: 10.1016/j.biochi.2020.11.004]

17 **Zarubin A**, Stepanov V, Markov A, Kolesnikov N, Marusin A, Khitrinskaya I, Swarovskaya M, Litvinov S, Ekomasova N, Dzhaubermezov M, Maksimova N, Sukhomyasova A, Shtygasheva O, Khusnutdinova E, Radzhabov M, Kharkov V. Structural Variability, Expression Profile, and Pharmacogenetic Properties of TMPRSS2 Gene as a Potential Target for COVID-19 Therapy. *Genes (Basel)* 2020; **12** [PMID: 33375616 DOI: 10.3390/genes12010019]

18 **Ozono S**, Zhang Y, Ode H, Sano K, Tan TS, Imai K, Miyoshi K, Kishigami S, Ueno T, Iwatani Y, Suzuki T, Tokunaga K. SARS-CoV-2 D614G spike mutation increases entry efficiency with enhanced ACE2-binding affinity. *Nat Commun* 2021; **12**: 848 [PMID: 33558493 DOI: 10.1038/s41467-021-21118-2]

19 **Beyerstedt S**, Casaro EB, Rangel ÉB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis 2021; 1-15 [DOI: 10.1007/s10096-020-04138-6]

20 **Toyonaga T**, Araba KC, Kennedy MM, Keith BP, Wolber EA, Beasley C, Steinbach EC, Schaner MR, Jain A, Long MD, Barnes EL, Herfarth HH, Isaacs KL, Hansen JJ, Kapadia MR, Guillem JG, Gulati AS, Sethupathy P, Furey TS, Ehre C, Sheikh SZ. Increased colonic expression of ACE2 associates with poor prognosis in Crohn's disease. *Sci Rep* 2021; **11**: 13533 [PMID: 34188154 DOI: 10.1038/s41598-021-92979-2]

21 **Kyrou I**, Randeva HS, Spandidos DA, Karteris E. Not only ACE2-the quest for additional host cell mediators of SARS-CoV-2 infection: Neuropilin-1 (NRP1) as a novel SARS-CoV-2 host cell entry mediator implicated in COVID-19. *Signal Transduct Target Ther* 2021; **6**: 21 [PMID: 33462185 DOI: 10.1038/s41392-020-00460-9]

22 **Asselta R**, Paraboschi EM, Mantovani A, Duga S. *ACE2* and *TMPRSS2* variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. *Aging (Albany NY)* 2020; **12**: 10087-10098 [PMID: 32501810 DOI: 10.18632/aging.103415]

23 **Dong M**, Zhang J, Ma X, Tan J, Chen L, Liu S, Xin Y, Zhuang L. ACE2, TMPRSS2 distribution and extrapulmonary organ injury in patients with COVID-19. *Biomed Pharmacother* 2020; **131**: 110678 [PMID: 32861070 DOI: 10.1016/j.biopha.2020.110678]

24 **Xiao L**, Sakagami H, Miwa N. ACE2: The key Molecule for Understanding the Pathophysiology of Severe and Critical Conditions of COVID-19: Demon or Angel? *Viruses* 2020; **12** [PMID: 32354022 DOI: 10.3390/v12050491]

25 **Nowak JK**, Lindstrøm JC, Kalla R, Ricanek P, Halfvarson J, Satsangi J. Age, Inflammation, and Disease Location Are Critical Determinants of Intestinal Expression of SARS-CoV-2 Receptor *ACE2* and *TMPRSS2* in Inflammatory Bowel Disease. *Gastroenterology* 2020; **159**: 1151-1154.e2 [PMID: 32413354 DOI: 10.1053/j.gastro.2020.05.030]

26 **de Lucena TMC**, da Silva Santos AF, de Lima BR, de Albuquerque Borborema ME, de Azevêdo Silva J. Mechanism of inflammatory response in associated comorbidities in COVID-19. *Diabetes Metab Syndr* 2020; **14**: 597-600 [PMID: 32417709 DOI: 10.1016/j.dsx.2020.05.025]

27 **Rothan HA**, Byrareddy SN. The potential threat of multisystem inflammatory syndrome in children during the COVID-19 pandemic. *Pediatr Allergy Immunol* 2021; **32**: 17-22 [PMID: 32897642 DOI: 10.1111/pai.13361]

28 **Zipeto D**, Palmeira JDF, Argañaraz GA, Argañaraz ER. ACE2/ADAM17/TMPRSS2 Interplay May Be the Main Risk Factor for COVID-19. *Front Immunol* 2020; **11**: 576745 [PMID: 33117379 DOI: 10.3389/fimmu.2020.576745]

29 **Abassi Z**, Higazi AAR, Kinaneh S, Armaly Z, Skorecki K, Heyman SN. ACE2, COVID-19 Infection, Inflammation, and Coagulopathy: Missing Pieces in the Puzzle. *Front Physiol* 2020; **11**: 574753 [PMID: 33123031 DOI: 10.3389/fphys.2020.574753]

30 **Burgueño JF**, Reich A, Hazime H, Quintero MA, Fernandez I, Fritsch J, Santander AM, Brito N, Damas OM, Deshpande A, Kerman DH, Zhang L, Gao Z, Ban Y, Wang L, Pignac-Kobinger J, Abreu MT. Expression of SARS-CoV-2 Entry Molecules ACE2 and TMPRSS2 in the Gut of Patients With IBD. *Inflamm Bowel Dis* 2020; **26**: 797-808 [PMID: 32333601 DOI: 10.1093/ibd/izaa085]

31 **Potdar AA**, Dube S, Naito T, Botwin G, Haritunians T, Li D, Yang S, Bilsborough J, Denson LA, Daly M, Targan SR, Fleshner P, Braun J, Kugathasan S, Stappenbeck TS, McGovern DPB. Reduced expression of COVID-19 host receptor, *ACE2* is associated with small bowel inflammation, more severe disease, and response to anti-TNF therapy in Crohn's disease. *medRxiv* 2020 [PMID: 32511625 DOI: 10.1101/2020.04.19.20070995]

32 **Azimi A**. TMPRSS2 inhibitors, Bromhexine, Aprotinin, Camostat and Nafamostat as potential treatments for COVID-19. 2020 [DOI: 10.31226/osf.io/a3rvm]

33 **Queiroz NSF**, Barros LL, Azevedo MFC, Oba J, Sobrado CW, Carlos AS, Milani LR, Sipahi AM, Damião AOMC. Management of inflammatory bowel disease patients in the COVID-19 pandemic era: a Brazilian tertiary referral center guidance. *Clinics (Sao Paulo)* 2020; **75**: e1909 [PMID: 32321117 DOI: 10.6061/clinics/2020/e1909]

34 **Fragoso RP**, Rodrigues M. COVID-19 and pediatric inflammatory bowel disease: How to manage it?

Clinics (Sao Paulo) 2020; **75**: e1962 [PMID: 32520223 DOI: 10.6061/clinics/2020/e1962]

35 **Al-Ani A**, Prentice R, Rentsch C, Johnson D, Ardalan Z, Heerasing N, Garg M, Campbell S, Sasadeusz J, Macrae F. Prevention, diagnosis and management of COVID-19 in the inflammatory bowel disease patient. Aliment Pharmacol Ther 2020 [DOI: 10.1111/apt.15779]

36 **An P**, Ji M, Ren H, Su J, Ding NS, Kang J, Yin A, Zhou Q, Shen L, Zhao L, Jiang X, Xiao Y, Tan W, Lv X, Li J, Liu S, Zhou J, Chen H, Xu Y, Liu J, Chen M, Cao J, Zhou Z, Tan S, Yu H, Dong W, Ding Y. Prevention of COVID-19 in patients with inflammatory bowel disease in Wuhan, China. *Lancet Gastroenterol Hepatol* 2020; **5**: 525-527 [PMID: 32311321 DOI: 10.1016/S2468-1253(20)30121-7]

37 **Prentice RE**, Tjandra D, Garg M, Lubel JS, Fourlanos S, Johnson D, Al-Ani A, Christensen B. Letter: ACE2, IBD and COVID-19-why IBD patients may be at reduced risk of COVID-19. *Aliment Pharmacol Ther* 2020; **52**: 1422-1423 [PMID: 33105980 DOI: 10.1111/apt.16063]

38 **Reuken PA**, Wüst M, Löffler B, Bauer M, Stallmach A. Letter: SARS-CoV-2-induced gastrointestinal inflammation. *Aliment Pharmacol Ther* 2020; **52**: 1748-1749 [PMID: 33205881 DOI: 10.1111/apt.16087]

39 **Sultan K**, Mone A, Durbin L, Khuwaja S, Swaminath A. Review of inflammatory bowel disease and COVID-19. *World J Gastroenterol* 2020; **26**: 5534-5542 [PMID: 33088153 DOI: 10.3748/wjg.v26.i37.5534]

40 **Viana SD**, Nunes S, Reis F. ACE2 imbalance as a key player for the poor outcomes in COVID-19 patients with age-related comorbidities - Role of gut microbiota dysbiosis. *Ageing Res Rev* 2020; **62**: 101123 [PMID: 32683039 DOI: 10.1016/j.arr.2020.101123]

41 **Kavaliers M**, Innes DG. Novelty-induced opioid analgesia in deer mice (Peromyscus maniculatus): sex and population differences. *Behav Neural Biol* 1988; **49**: 54-60 [PMID: 3345191 DOI: 10.1016/j.dld.2020.12.012]

42 **Bhuiyan FR**, Howlader S, Raihan T, Hasan M. Plants Metabolites: Possibility of Natural Therapeutics Against the COVID-19 Pandemic. *Front Med (Lausanne)* 2020; **7**: 444 [PMID: 32850918 DOI: 10.3389/fmed.2020.00444]

43 **Panyod S**, Ho CT, Sheen LY. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. *J Tradit Complement Med* 2020; **10**: 420-427 [PMID: 32691006 DOI: 10.1016/j.jtcme.2020.05.004]

44 **Huang J**, Tao G, Liu J, Cai J, Huang Z, Chen JX. Current Prevention of COVID-19: Natural Products and Herbal Medicine. *Front Pharmacol* 2020; **11**: 588508 [PMID: 33178026 DOI: 10.3389/fphar.2020.588508]

45 **Boozari M**, Hosseinzadeh H. Natural products for COVID-19 prevention and treatment regarding to previous coronavirus infections and novel studies. Phytother Res 2020 [DOI: 10.1002/ptr.6873]

46 **Naik B**, Gupta N, Ojha R, Singh S, Prajapati VK, Prusty D. High throughput virtual screening reveals SARS-CoV-2 multi-target binding natural compounds to lead instant therapy for COVID-19 treatment. *Int J Biol Macromol* 2020; **160**: 1-17 [PMID: 32470577 DOI: 10.1016/j.ijbiomac.2020.05.184]

47 **da Silva Antonio A**, Wiedemann LSM, Veiga-Junior VF. Natural products' role against COVID-19. RSC Adv 2020; 10: 23379-23393 [DOI: 10.1039/d0ra03774e]

48 **Chen H**, Du Q. Potential natural compounds for preventing SARS-CoV-2 (2019-nCoV) infection. 2020 Preprint. Available from: 202001.0358.v3

49 **Seadawy MG**, Gad AF, Shamel M, Elharty B, Mohamed MF, Elfiky AA, Ahmed A, Zekri ARN. in vitro: Natural Compounds (Thymol, Carvacrol, Hesperidine, And Thymoquinone) Against SARS-CoV2 Strain Isolated From Egyptian Patients. 2020 Preprint. Available from: 2020.11.07.367649

50 **Muchtaridi M**, Fauzi M, Khairul Ikram NK, Mohd Gazzali A, Wahab HA. Natural Flavonoids as Potential Angiotensin-Converting Enzyme 2 Inhibitors for Anti-SARS-CoV-2. *Molecules* 2020; **25** [PMID: 32882868 DOI: 10.3390/molecules25173980]

51 **Dabaghian F**, Khanavi M, Zarshenas MM. Bioactive compounds with possible inhibitory activity of Angiotensin-Converting Enzyme-II; a gate to manage and prevent COVID-19. *Med Hypotheses* 2020; **143**: 109841 [PMID: 32425303 DOI: 10.1016/j.mehy.2020.109841]

52 **Kulkarni SA**, Nagarajan SK, Ramesh V, Palaniyandi V, Selvam SP, Madhavan T. Computational evaluation of major components from plant essential oils as potent inhibitors of SARS-CoV-2 spike protein. *J Mol Struct* 2020; **1221**: 128823 [PMID: 32834111 DOI: 10.1016/j.molstruc.2020.128823]

53 **Rathinavel T**, Meganathan B, Kumarasamy S, Ammashi S, Thangaswamy S, Ragunathan Y, Palanisamy S. Potential COVID-19 Drug from Natural Phenolic Compounds through In Silico Virtual Screening Approach. Biointerface Res Appl Chem 2020; 10161-10173 [DOI: 10.33263/briac113.1016110173]

54 **Solnier J**, Fladerer JP. Flavonoids: A complementary approach to conventional therapy of COVID-19? Phytochem Rev 2020; 1-23 [DOI: 10.1007/s11101-020-09720-6]

55 **Luo P**, Liu D, Li J. Pharmacological perspective: glycyrrhizin may be an efficacious therapeutic agent for COVID-19. *Int J Antimicrob Agents* 2020; **55**: 105995 [PMID: 32335281 DOI: 10.1016/j.ijantimicag.2020.105995]

56 **Dowluru KS**, Rao KRS. Phylogenetic analysis and In silico Screening of drug targets for ACE2 in Human and Spike Glycoprotein in Sars-CoV-2 for control of COVID19. 2020 Preprint. Available from: rs.3.rs-23862/v1

57 **Mhatre S**, Naik S, Patravale V. A molecular docking study of EGCG and theaflavin digallate with the druggable targets of SARS-CoV-2. *Comput Biol Med* 2021; **129**: 104137 [PMID: 33302163 DOI: 10.1016/j.compbiomed.2020.104137]

58 **Jang M**, Park R, Park YI, Cha YE, Yamamoto A, Lee JI, Park J. EGCG, a green tea polyphenol, inhibits human coronavirus replication in vitro. *Biochem Biophys Res Commun* 2021; **547**: 23-28 [PMID: 33588235 DOI: 10.1016/j.bbrc.2021.02.016]

59 **Lichtenstein GR**, Rubin DT. Coronavirus and Patients With Inflammatory Bowel Disease: Management Strategies for the Practicing Clinician. *Am J Gastroenterol* 2020; **115**: 1566-1569 [PMID: 32833732 DOI: 10.14309/ajg.0000000000000817]

60 **Burke KE**, Kochar B, Allegretti JR, Winter RW, Lochhead P, Khalili H, Colizzo FP, Hamilton MJ, Chan WW, Ananthakrishnan AN. Immunosuppressive Therapy and Risk of COVID-19 Infection in Patients With Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2021; **27**: 155-161 [PMID: 33089863 DOI: 10.1093/ibd/izaa278]

61 **Sebastian S**, Walker GJ, Kennedy NA, Conley TE, Patel KV, Subramanian S, Kent AJ, Segal JP, Brookes MJ, Bhala N, Gonzalez HA, Hicks LC, Mehta SJ, Lamb CA; PROTECT-ASUC Study Group. Assessment, endoscopy, and treatment in patients with acute severe ulcerative colitis during the COVID-19 pandemic (PROTECT-ASUC): a multicentre, observational, case-control study. *Lancet Gastroenterol Hepatol* 2021; **6**: 271-281 [PMID: 33545083 DOI: 10.1016/S2468-1253(21)00016-9]

62 **Zingone F**, Buda A, Savarino EV. Starting a biologic therapy in IBD patients amidst COVID-19: hold, careful monitoring or testing? *J Crohns Colitis* 2020 [PMID: 32426800 DOI: 10.1093/ecco-jcc/jjaa102]

63 **Attauabi M**, Seidelin J, Burisch J; Danish COVID-IBD Study Group. Association between 5-aminosalicylates in patients with IBD and risk of severe COVID-19: an artefactual result of research methodology? *Gut* 2021; **70**: 2020-2022 [PMID: 33658323 DOI: 10.1136/gutjnl-2021-324397]

64 **Halpin DMG**, Singh D, Hadfield RM. Inhaled corticosteroids and COVID-19: a systematic review and clinical perspective. *Eur Respir J* 2020; **55** [PMID: 32341100 DOI: 10.1183/13993003.01009-2020]

65 **Saad AF**, Chappell L, Saade GR, Pacheco LD. Corticosteroids in the Management of Pregnant Patients With Coronavirus Disease (COVID-19). *Obstet Gynecol* 2020; **136**: 823-826 [PMID: 32769659 DOI: 10.1097/AOG.0000000000004103]

66 **Tlayjeh H**, Mhish OH, Enani MA, Alruwaili A, Tleyjeh R, Thalib L, Hassett L, Arabi YM, Kashour T, Tleyjeh IM. Association of corticosteroids use and outcomes in COVID-19 patients: A systematic review and meta-analysis. J Infect Public Health 2020 [DOI: 10.1016/j.jiph.2020.09.008]

67 **Brenner EJ**, Pigneur B, Focht G, Zhang X, Ungaro RC, Colombel JF, Turner D, Kappelman MD, Ruemmele FM. Benign Evolution of SARS-Cov2 Infections in Children With Inflammatory Bowel Disease: Results From Two International Databases. *Clin Gastroenterol Hepatol* 2021; **19**: 394-396.e5 [PMID: 33059040 DOI: 10.1016/j.cgh.2020.10.010]

68 **El Ouali S**, Rubin DT, Cohen BL, Regueiro MD, Rieder F. Optimal inflammatory bowel disease management during the global coronavirus disease 2019 pandemic. *Curr Opin Gastroenterol* 2021; **37**: 313-319 [PMID: 33859104 DOI: 10.1097/MOG.0000000000000741]

69 **Gutin LS**, Lam AY, Velayos FS, Santos SA. Going Viral: Management of IBD in the Era of the COVID-19 Pandemic. *Dig Dis Sci* 2020; **65**: 1571-1575 [PMID: 32363528 DOI: 10.1007/s10620-020-06299-y]

70 **Schuurmans MM**, Hage R. Cyclosporine A and COVID-19 - The COQUIMA cohort. *EClinicalMedicine* 2021; **31**: 100680 [PMID: 33385129 DOI: 10.1016/j.eclinm.2020.100680]

71 Pathania YS. Cyclosporine: hope for severe COVID-19? BMJ Support Palliat Care 2021 [DOI: 10.1136/bmjspcare-2020-002681]

72 **Al-Ani AH**, Prentice RE, Christensen B. Letter to the Editor: Care of the Patient with IBD Requiring Hospitalisation During the COVID-19 Pandemic. J Crohns Colitis 2020 [DOI: 10.1093/ecco-jcc/jjaa183]

73 **Ghoshal UC**, Sahu S, Biswas SN, Singh P, Chaudhary M, Ghoshal U, Tiwari P, Rai S, Mishra SK. Care of inflammatory bowel disease patients during coronavirus disease-19 pandemic using digital health-care technology. JGH Open 2021 [DOI: 10.1002/jgh3.12498]

74 **Mansouri P**, Farshi S, Nickhah N, Nobari NN, Chalangari R, Nilforoushzadeh MA. Immunosuppressive/Immunomodulatory Therapies in Dermatology and COVID-19. Clin Dermatol 2021 [DOI: 10.1016/j.clindermatol.2021.01.003]

75 **D'Silva KM**, Serling-Boyd N, Hsu TY, Sparks JA, Wallace ZS. SARS-CoV-2 antibody response after COVID-19 in patients with rheumatic disease. Ann Rheum Dis 2021 [DOI: 10.1136/annrheumdis-2021-220166]

76 **Kasperkiewicz M**. COVID-19 outbreak and autoimmune bullous diseases: A systematic review of published cases. *J Am Acad Dermatol* 2021; **84**: 563-568 [PMID: 32781181 DOI: 10.1016/j.jaad.2020.08.012]

77 **Festa S**, Aratari A, De Biasio F, Fascì-Spurio F, Papi C. Screening for active COVID-19 infection prior to biologic therapy in IBD patients: Let's not increase our uncertainty without reducing our concerns. Dig Liver Dis 2020; 52: 1246-1247 [DOI: 10.1016/j.dld.2020.05.037]

78 **Danese S**, Cecconi M, Spinelli A. Management of IBD during the COVID-19 outbreak: resetting clinical priorities. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 253-255 [PMID: 32214232 DOI: 10.1038/s41575-020-0294-8]

79 **Weissman S**, Aziz M, Smith WL, Elias S, Swaminath A, Feuerstein JD. Safety of biologics in inflammatory bowel disease patients with COVID-19. *Int J Colorectal Dis* 2021; **36**: 2051-2055 [PMID: 34131784 DOI: 10.1007/s00384-021-03977-9]

80 **Gargallo-Puyuelo CJ**, Laredo V, Gomollón F. Thiopurines in Inflammatory Bowel Disease. How to Optimize Thiopurines in the Biologic Era? *Front Med (Lausanne)* 2021; **8**: 681907 [PMID: 34336887 DOI: 10.3389/fmed.2021.681907]

81 **Aysha AA**, Rentsch C, Prentice R, Johnson D, Bryant RV, Ward MG, Costello SP, Lewindon P, Ghaly S, Connor SJ, Begun J, Christensen B. Practical management of inflammatory bowel disease patients during the COVID-19 pandemic: expert commentary from the Gastroenterological Society of Australia Inflammatory Bowel Disease faculty. *Intern Med J* 2020; **50**: 798-804 [PMID: 32656985 DOI: 10.1111/imj.14889]

82 **Weidinger C**, Hegazy AN, Glauben R, Siegmund B. COVID-19—from mucosal immunology to IBD patients. Mucosal Immunol 2021; 1-8 [DOI: 10.1038/s41385-021-00384-9]

83 **Shields S**, Dunlop A, Seenan JP, Macdonald J. Disease monitoring of biologic treatment in IBD: early impact and future implications of COVID-19 pandemic. BMJ 2020 [DOI: 10.1136/flgastro-2020-101563]

84 **Fung M**, Babik JM. COVID-19 in Immunocompromised Hosts: What We Know So Far. *Clin Infect Dis* 2021; **72**: 340-350 [PMID: 33501974 DOI: 10.1093/cid/ciaa863]

85 **Norsa L**, Indriolo A, Sansotta N, Cosimo P, Greco S, D'Antiga L. Uneventful course in IBD patients during SARS-CoV-2 outbreak in northern Italy. Gastroenterology 2020; 159: 371-372 [DOI: 10.1053/j.gastro.2020.03.062]

86 **Mazza S**, Sorce A, Peyvandi F, Vecchi M, Caprioli F. A fatal case of COVID-19 pneumonia occurring in a patient with severe acute ulcerative colitis. *Gut* 2020; **69**: 1148-1149 [PMID: 32245909 DOI: 10.1136/gutjnl-2020-321183]

87 **Tursi A**, Papa A. Impact of Anti-TNFα Antibodies on the Risk of Covid-19 and its Severity in Patients with Inflammatory Bowel Diseases. *J Crohns Colitis* 2020; **14**: 1646-1647 [PMID: 32303756 DOI: 10.1093/ecco-jcc/jjaa076]

88 **Bodini G**, Demarzo MG, Casagrande E, De Maria C, Kayali S, Ziola S, Giannini EG. Concerns related to COVID-19 pandemic among patients with inflammatory bowel disease and its influence on patient management. *Eur J Clin Invest* 2020; **50**: e13233 [PMID: 32294238 DOI: 10.1111/eci.13233]

89 **Tursi A**, Angarano G, Monno L, Saracino A, Signorile F, Ricciardi A, Papa A. COVID-19 infection in Crohn's disease under treatment with adalimumab. Gut 2020; 69: 1364-1365 [DOI: 10.1136/gutjnl-2020-321240]

90 **Allocca M**, Fiorino G, Furfaro F, Gilardi D, Radice S, D'Amico F, Zilli A, Danese S. Maintaining the Quality Standards of Care for Inflammatory Bowel Disease Patients During the COVID-19 Pandemic. *Clin Gastroenterol Hepatol* 2020; **18**: 1882-1883 [PMID: 32304737 DOI: 10.1016/j.cgh.2020.04.028]

91 **Jacobs J**, Clark-Snustad K, Lee S. Case Report of a SARS-CoV-2 Infection in a Patient With Ulcerative Colitis on Tofacitinib. *Inflamm Bowel Dis* 2020; **26**: e64 [PMID: 32342098 DOI: 10.1093/ibd/izaa093]

92 **Taxonera C**, Sagastagoitia I, Alba C, Mañas N, Olivares D, Rey E. 2019 novel coronavirus disease (COVID-19) in patients with inflammatory bowel diseases. *Aliment Pharmacol Ther* 2020; **52**: 276-283 [PMID: 32359205 DOI: 10.1111/apt.15804]

93 **Allocca M**, Fiorino G, Zallot C, Furfaro F, Gilardi D, Radice S, Danese S, Peyrin-Biroulet L. Incidence and Patterns of COVID-19 Among Inflammatory Bowel Disease Patients From the Nancy and Milan Cohorts. *Clin Gastroenterol Hepatol* 2020; **18**: 2134-2135 [PMID: 32360811 DOI: 10.1016/j.cgh.2020.04.071]

94 **Mak JWY**, Weng MT, Wei SC, Ng SC. Zero COVID-19 infection in inflammatory bowel disease patients: Findings from population-based inflammatory bowel disease registries in Hong Kong and Taiwan. *J Gastroenterol Hepatol* 2021; **36**: 171-173 [PMID: 32589798 DOI: 10.1111/jgh.15164]

95 **Bardasi G**, Alvisi P. SARS-CoV-2 infection in severe pediatric Crohn's disease. What about anti-tumor necrosis factor α therapy? *Dig Liver Dis* 2020; **52**: 1244-1245 [PMID: 32703727 DOI: 10.1016/j.dld.2020.06.047]

96 **Ashton JJ**, Kammermeier J, Spray C, Russell RK, Hansen R, Howarth LJ, Torrente F, Deb P, Renji E, Muhammed R, Paul T, Kiparissi F, Epstein J, Lawson M, Hope B, Zamvar V, Narula P, Kadir A, Devadason D, Bhavsar H, Beattie RM. Impact of COVID-19 on diagnosis and management of paediatric inflammatory bowel disease during lockdown: a UK nationwide study. *Arch Dis Child* 2020; **105**: 1186-1191 [PMID: 32732316 DOI: 10.1136/archdischild-2020-319751]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2021 December 14; 27(46): 7956-7968

DOI: 10.3748/wjg.v27.i46.7956

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

T cells in pancreatic cancer stroma

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Author contributions: Goulart MR and drafted the original manuscript and composed the original figure; Goulart MR, Stasinos K, Fincham REA, Delvecchio FR and Kocher HM contributed to the review and editing of the manuscript and figure.

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

Supported by PCRF Post-doctoral Fellowship (to Goulart MR); Lewin International Surgical Oncology Fellowship funded by Barts Health NHS Trust and Barts Charity(to Stasinos K); PhD Studentship Awarded by Barts Charity (London, UK) and A*STAR (Singapore) (to Fincham REA); and Cancer Research UK Post-doctoral Fellowship (to Delvecchio FR).

Country/Territory of origin: United Kingdom

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly devastating disease with a dismal 5-year survival rate. PDAC has a complex tumour microenvironment; characterised by a robust desmoplastic stroma, extensive infiltration of immune-suppressive cells such as immature myeloid cells, tumour-associated macrophages, neutrophils and regulatory T cells, and the presence of exhausted and senescent T cells. The cross-talk between cells in this fibrotic tumour establishes an immune-privileged microenvironment that supports tumour cell escape from immune-surveillance, disease progression and spread to distant organs. PDAC tumours, considered to be non-immunogenic or cold, express low mutation burden, low infiltration of $CD8^+$ cytotoxic lymphocytes that are localised along the invasive margin of the tumour border in the surrounding fibrotic tissue, and often display an exhausted phenotype. Here, we review the role of T cells in pancreatic cancer, examine the complex interactions of these crucial effector units within pancreatic cancer stroma and shed light on the increasingly attractive use of T cells as therapy.

Key Words: Immunosuppression; T cell exhaustion; Tumour microenvironment; Pancreatic ductal adenocarcinoma; Pancreatic cancer stroma

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

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: April 9, 2021
Peer-review started: April 9, 2021
First decision: May 17, 2021
Revised: May 18, 2021
Accepted: November 28, 2021
Article in press: November 28, 2021
Published online: December 14, 2021

P-Reviewer: Falasca M
S-Editor: Gao CC
L-Editor: A
P-Editor: Gao CC

Core Tip: Pancreatic ductal adenocarcinoma (PDAC) is a highly devastating disease with a dismal 5-year survival of less than 5% in patients with metastatic disease, and is predicted to become the second cause of cancer-related death by 2030. Here, we discuss the complexity of the PDAC immunosuppressive tumour microenvironment, the mechanisms involved in T cell dysfunction, and potential immunotherapeutic strategies for treating PDAC.

Citation: Goulart MR, Stasinos K, Fincham REA, Delvecchio FR, Kocher HM. T cells in pancreatic cancer stroma. *World J Gastroenterol* 2021; 27(46): 7956-7968
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7956.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7956

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a highly devastating disease with a dismal 5-year survival of less than 5% in patients with metastatic disease[1], and is predicted to become the second cause of cancer-related death by 2030[2]. Late detection and incredibly aggressive biology are significant challenges determining therapeutic failure[3,4]. PDAC has a complex tumour microenvironment (TME) characterised by a robust desmoplastic stroma[5], and an expanded pool of immunosuppressive immune cells shielding the malignant cells harbouring aberrant expression of oncogenic pathways. The interplay between various cell types in this fibrotic TME supports tumour cell escape from immunosurveillance, disease progression and spread to distant organs [6,7], highlighting this cancer's ability to evade immune recognition and its extra-ordinary metastatic potential. In this review, we discuss the interactions between T cells and the other components of the PDAC TME and highlight the impact of these interactions on the phenotype and function of T cells. Emerging immune-therapeutic strategies employed in overcoming T cell dysfunction and improve patient survival are also discussed.

PDAC IMMUNE LANDSCAPE

PDAC carcinogenesis is characterised by an abundant fibro-inflammatory reaction and subsequent oncogene activation on epithelial cells, resulting in a pro-tumorigenic microenvironment[8]. At early stages of cancer development, oncogenic *KRAS* expression in pancreatic cells results in the formation of pancreatic intraepithelial neoplasia (PanIN), and drives an inflammatory reaction that modulates the recruitment and infiltration of immunosuppressive myeloid and lymphoid cell subsets. *KRAS*-mutated pancreatic cells regulate the maintenance of immunoregulatory microenvironment by inducing the release of interleukin (IL)-6, IL-10 and transforming growth factor (TGF-β) cytokines. In the setting of sustained chronic inflammation, PanIN progression to malignant lesion is accompanied by mutations in genes such as TP53, CDKN2A and SMAD4 frequently, which further contribute to shape the immune microenvironment. For example, the mutant tumour suppressor gene TP53 are implicated in sustaining the tissue damage and chronic inflammation by enhancing the expression of NF-kB, secretion of vascular endothelial growth factor (VEGF) and activation of fibroblasts. Decreased infiltration of T and B cells and elevated numbers of Tregs were significantly correlated with CDKN2A mutations while SMAD4 mutations are involved with enhanced invasion, metastasis and immunosuppressive effects of TGF-β on immune response[9].

Chemotactic factors associated with the recruitment of dysfunctional bone marrow-derived myeloid cells include granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony-stimulating factor (G-CSF), IL-3, VEGF, and the interaction of the C-X-C chemokine ligand 12 (CXCL12)/C-X-C chemokine receptor 4 (CXCR4) or C-C chemokine ligand 2 (CCL2)/C-C chemokine receptor 2 (CCR2), amongst others [10]. Stromal-associated fibroblasts produce C-X-C chemokine ligand 13 (CXCL13), which recruits IL-35-producing regulatory B cells (Breg) into the TME, further contributing to PDAC progression through IL35-mediated stimulation of tumour cell proliferation[10]. Copious infiltration of immature myeloid cells, tumour-associated

macrophages (TAMs), neutrophils and regulatory immune cells ultimately establishes an immune-privileged microenvironment that protects the malignant cells from T cell immunosurveillance and sustains tumour growth[11].

Therefore, pancreatic cancer evolves to establish a complex and heterogeneous immune microenvironment, characterised by high numbers of strongly suppressive immune cells, and a modest infiltration of lymphocytes with anti-tumour properties [12-14]. As such, PDAC tumours are considered to be non-immunogenic or cold, displaying low infiltration of $CD8^+$ cytotoxic lymphocytes (CTLs) that are localised along the invasive margin of the tumour border or trapped in the surrounding fibrotic tissue but are not present within the tumour core. Moreover, infiltrated $CD8^+$ T cells in PDAC tumours often display minimal signs of activation[11,15,16]. T cell exclusion from TME has been demonstrated both in genetically engineered KPC ($KRas^{LSL\_G12D/+}$, $Trp53^{LSL\_R172H/+}$, Pdx1-Cre) mouse models[16] and PDAC patients[17].

Macrophages compose the most abundant immune cells in PDAC[11]. They play a critical role in the exclusion of T cells from tumours, maintenance of fibrosis through the secretion of pro-fibrotic cytokines[18] and induction of angiogenesis by secreting VEGF[19]. Increases in TAMs correlate with poor prognosis[20,21]. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immunosuppressive cells, including immature monocytes, granulocytes and dendritic cells (DCs). These cells show potent ability to inhibit proliferation and induce apoptosis of both $CD4^+$ and $CD8^+$ T cells, secrete elevated amounts of immunosuppressive cytokines IL-10 and TGF-β, which collaborate to the recruitment of regulatory $CD4^+$ T cells (Tregs), and decrease the infiltration of natural killer (NK) and NKT cells into the tumour[22]. MDSCs accumulation has been described in the spleen, tumours and metastatic lesions in KPC models of PDAC, and its accumulation negatively correlated with $CD8^+$ T cells infiltration[19].

Likewise, Tregs upregulate the expression of CTL-associated antigen 4 (CTLA-4) [23], interact with DCs suppressing the expression of the co-stimulatory ligands, such as CD80 and CD86, necessary for T cell activation, secrete immunosuppressive cytokines, and directly suppress $CD8^+$ T cells anti-tumour immunity[24]. Infiltration of Tregs occurs at early stages of PDAC formation, and increased numbers of both circulating and intra-tumoural. Tregs have been observed in pancreatic cancer patients [19]. Additionally, the presence of tumour-infiltrating IL-17-producing CD4+ T cells and γδT cells also contribute to tumour immune evasion and progression[11,25].

Similar to PDAC, in inflammatory conditions of the pancreas, such as pancreatitis, the inflammatory reaction leads to the infiltration of myeloid cells, such as monocytes and neutrophils. Although macrophages comprise a significant population within the inflamed pancreas, T cells are also present, and infiltration of $CD4^+$ T cells has been implicated in the progression of acute pancreatitis in mice. As pancreatitis progresses, the ratio of $CD4^+$ and $CD8^+$ T cell increases, with increased numbers of immunosuppressive Tregs observed in patients with chronic pancreatitis.

T CELL INTERACTIONS AND IMMUNE DYSFUNCTION IN PANCREATIC CANCER

T cell infiltration is observed in patients with surgically-resected PDAC and correlates with improved outcomes suggesting the anti-tumour potential of tumour-infiltrating $CD4^+$ and $CD8^+$ T cells[26]. However, as PDAC progresses, tumour-infiltrating T cell composition shifts to a decrease in $CD8^+$ T cells and elevated percentage of Tregs within the $CD4^+$ T cell subset[27]. While $CD4^+$ Tregs are a prominent feature of the immune infiltrate, $CD8^+$ T cells are rare in the PDAC microenvironment[24]. Consequently, PDAC is considered to be a poorly immune responsive cancer, with T cells present within the tumour microenvironment often showing lack of activation, or an exhausted phenotype[28-30]. This observation demonstrates that infiltrated $CD8^+$ T cell may recognise and mount a response against these tumours, but the unfavourable TME halts optimal cytotoxic function.

Spatial localisation of the immune cells in these tumours reflect the challenging biology of PDAC TME. Tumour-infiltrating $CD8^+$ T cells are localised at the periphery, within the surrounding fibrotic stroma in PDAC tissues[6,21,31,32]. $CD8^+$ T cells migrate away from the juxta-tumoural compartment by favouring their movement towards CXCL12-rich stroma laid by activated pancreatic stellate cells (PSCs)[6]. The proximity of intra-tumoral $CD8^+$ T cells to tumour cells correlates with patient survival [32].

PSCs play a central role in shaping the architecture of PDAC by modulating the ECM components and producing a physical barrier that limits T cell infiltration, migration and direct interaction with neoplastic cells[33]. These cells can also act as non-professional antigen-presenting cells (APCs) and secrete cytokines and growth factors that boost the recruitment of immunosuppressive cells and inhibit T cell responses, resulting in increased disease aggressiveness and decreased overall survival[34]. Therefore, in conjunction with the immunosuppressive cells, PSCs are crucial players in the orchestration of an immuno-privileged PDAC microenvironment by combination of secreted cytokines, chemokines and extra-cellular matrix proteins as well as direct cell-cell contact.

Cancer cell-intrinsic factors also impact T cell function. Overexpression of immune checkpoint mediators like programmed death-1 receptor (PD-1)-ligand (PD-L1) is one mechanism by which cancers suppress T cell immunity. PD-L1 is overexpressed in PDAC cells, and this overexpression correlates with worse prognosis[20]. Pancreatic cancer cells can also downregulate Fas, a cell surface receptor associated with the induction of Fas-mediated apoptosis in tumour cells. $CD8^+$ T cells use the Fas-FasL and perforin–granzyme pathways as major effector mechanisms of cytotoxicity, and loss of Fas expression in PDAC tumours result in cancer immune evasion[7,35]. Spatial localisation and T cell interactions within the PDAC tumour microenvironment are shown in Figure 1.

PDAC has a low mutation burden, resulting in low neoantigen burden and the scarcity of tumour-infiltrating effector T cells. Only a few PDAC tumour antigens capable of inducing an anti-tumour immune response have been identified. Low mutation burden with minimal expression of neoantigens, and consequently marginal T cell infiltration is a classical feature in KPC models[36], and in PDAC patients[29,37]. In a recent study aimed to identify T cell neoantigens in long-term survival patients, it appears that the total neoantigen burden does not correlate with increased survival, but the presence of high-quality neoantigens played an essential role in the immunosurveillance of long-term survival patients. This study also highlighted the correlation of prolonged survival with granzyme B^+ $CD8^+$ T cells[26]. In keeping with this hypothesis, the total number of infiltrated $CD8^+$ T cells after vaccine immunotherapy did not show correlation with survival, but the subset of granzyme B^+ $CD8^+$ T cells was associated with long-term survivors[38]. These findings suggested that T cell quality may be more important than the total number of T cells for adequate anti-tumour immunity[39].

Identification of multiple dense lymphocyte aggregates, known as tertiary lymphoid structures (TLS) has also been observed in PDAC[38]. Importantly, detection of TLS in tumour tissue of PDAC patients was an independent prognostic factor for prolonged survival[40-42]. Although TLS can occur intra-tumoral or at the tumour periphery, only the presence of intra-tumoral TLS correlates with survival[41]. TLS aggregates contain T- and B-cell areas co-localised with myeloid and follicular DCs, and high-endothelial venules, displaying similar organisation to secondary lymphoid organs. They comprise ectopic lymphoid sites where T-cell activation and proliferation takes place[41]. Nevertheless, PDAC immune microenvironment is enriched with both exhausted and senescent T cells, and a diverse pool of highly immunosuppressive cells [43].

T CELL PHENOTYPE AND FUNCTIONS

Mature T cells can be classified as $CD8^+$ T cells (CTLs) and $CD4^+$ helper T cells (Th), which further differentiate into Th1, Th2, Th17 and Tregs[17]. $CD4^+$ Th1 cells secrete the pro-inflammatory cytokine interferon-γ (IFN-γ) which activates and supports CTLs cytotoxicity, while $CD4^+$ Th2 cells exhibit tumour-promoting functions by producing a plethora of cytokines, sustaining fibrosis through ECM and collagen deposition, and contributing to the differentiation of macrophages into a M2-immunosuppressive phenotype[44]. Polarisation towards Th2 cell subset is a common trait in pancreatic cancer, and this shift from Th1 to Th2 cells is correlated with decreased patient survival[45]. In PDAC patients, $CD4^+$ Th17 cells functions are mediated by the secretion of IL-17 cytokine. Although not very well understood, infiltration of this population has been associated with immune tolerance and reduced survival in murine models[46]. Tregs are an essential component of the T cell population. PDAC patients have increased numbers of Tregs that are inversely associated with $CD8^+$ T cells, therefore, they are often used as a negative prognostic biomarker in PDAC[45]. These cells can be identified by the expression of $CD4^+CD25^+FOXP3^+$ phenotype[24]

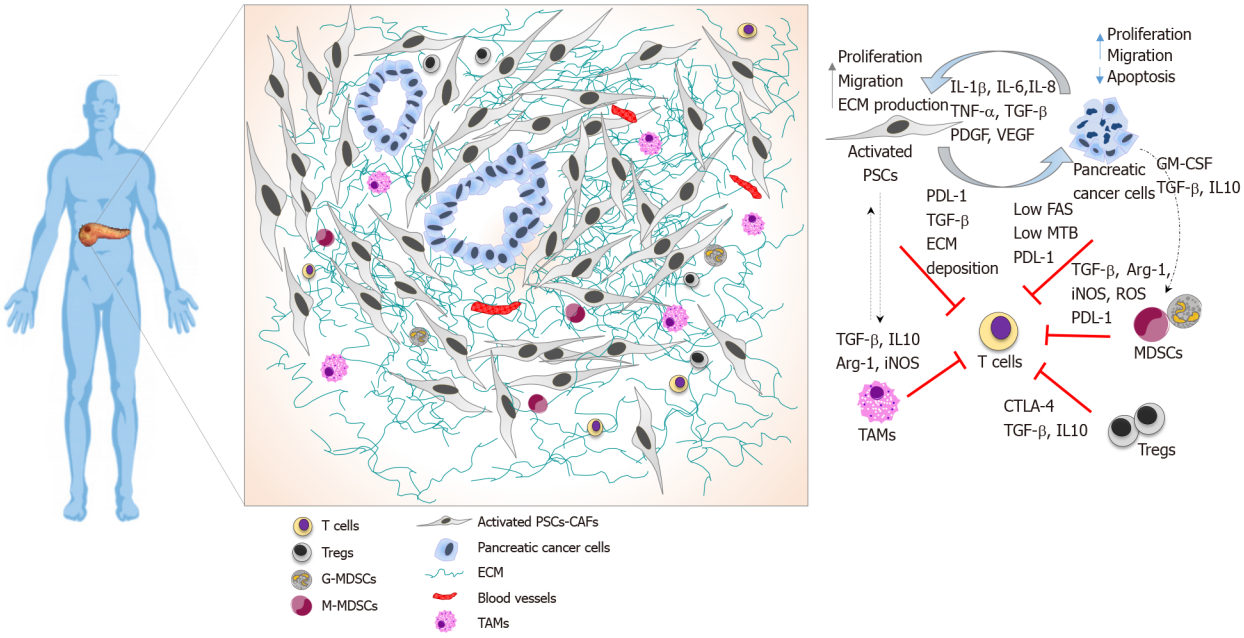

Figure 1 Pancreatic ductal adenocarcinoma immune landscape and T cell immunosuppression. Illustrative image showing spatial localisation of T cells in the pancreatic ductal adenocarcinoma tumour microenvironment and cellular interactions that collectively prevent T cell infiltration and function. T cells are localised at the periphery of tumours preventing direct contact with cancer cells. Pancreatic stellate cells produce elevated amounts of extracellular matrix driving a fibrotic tissue that entraps infiltrated T cells, alongside with immunosuppressive cytokine to and expression of programmed death-ligand 1 (PDL-1). Pancreatic cancer cells avoid T cell killing by downregulating Fas, exhibiting low tumour mutational burden, expressing PDL-1 and secreting growth factors and cytokines that recruits immunosuppressive cells. Myeloid-derived-suppressor cells express PDL-1 and suppress T cells functions by several mechanisms, including depleting of arginase 1, the release of reactive oxygen species, and secretion of cytokines. Tregs directly suppress T cells, express cytotoxic T-lymphocyte-associated protein 4 and secrete cytokines. TAMs play a role in sequestering T cells at the periphery and secrete immunosuppressive cytokines. PSC: Pancreatic stellate cells; TAMs: Tumour-associated macrophages; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; GM-CSF: Granulocyte-macrophage colony-stimulating factor; Arg-1: Arginase 1; PDL-1: Programmed death-ligand 1; iNOS: Inducible nitric oxide; MDSC: Myeloid-derived-suppressor cells; ROS: Reactive oxygen species; ECM: Extracellular matrix; TBM: Tumour mutational burden.

(Table 1).

CTLs are the preferred immune cells for targeting tumours. For durable and efficient immune responses, naïve T cells are primed in the lymph nodes with tumour antigens through interactions with APCs. Upon activation, they rapidly proliferate, differentiate into antigen-specific CTLs and migrate to tumour sites to perform their cytotoxic functions[47]. Elimination of tumour cells by CTLs occurs *via* the release of cytotoxic granzymes, IFN-γ and tumour necrosis factor α (TNF-α), or by induction of FasL-mediated apoptosis[48]. Following a cytotoxic immune response, the majority of CTLs will undergo apoptosis while a small fraction of them will further differentiate into diverse subsets of multipotent, long-lived memory $CD8^+$ T cells endowed with self-renewal ability[47]. The integration of three coordinated signals regulates T cells activation, expansion, survival, and memory formation: T cell receptor (TCR) stimulation by antigens, engagement of co-stimulatory molecules (CD28, CD27, 4-1BB, and OX40) expressed by $CD8^+$ T cells, and the release of inflammatory cytokines. In the absence of co-stimulatory signals, antigenic stimulation induces tolerance or clonal deletion in peripheral lymphoid organs[49]. The pro-inflammatory cytokines IL-12, IL-2 and IFN-γ, are crucial for satisfactory naïve $CD8^+$ T cell activation, expansion and differentiation whereas IL-7 and IL-15 are predominantly required for formation maintenance of memory $CD8^+$ T cells. In pancreatic cancer patients, both number and functions are altered within the $CD8^+$ T cell population. These patients show a decrease in circulating $CD8^+$ T cells and a decrease in perforin expression within these cells compared to healthy subjects. Moreover, intra-tumoural $CD8^+$ T infiltrates often display abnormal exhausted phenotype[44].

Memory $CD8^+$ T cells immediately proliferate upon antigen stimulation, execute cytotoxic functions, secrete effector cytokines, persist in greater numbers and exist in different metabolic, transcriptional, and epigenetic states[50]. Importantly, while the correlation between the numbers of memory $CD8^+$ T cells and the efficacy of T cell immunity is firmly established, the quality (or functional ability) of memory $CD8^+$ T cells also determines the degree of protection[47,48,50]. While memory T cell population are heterogeneous and consist of multiple subsets, the central memory T cells (T_{CM}) and effector memory T cell (T_{EM}) subsets have been best characterised. T_{CM}

Table 1 T cell phenotype and functions

T cell phenotype	Surface markers	Immune response	Effector functions
Cytotoxic T cell			
CTLs	CD8	Tumour killing	IFN-γ, TNF-α cytokines, granzymes, FasL
Helper T cell			
Th1	CD4 STAT4 T-bet	Tumour killing	IFN-γ, IL-2 cytokines, increase CTL activity
Th2	CD4 STAT6 GATA3	Tumour tolerance	IL-4, IL-5, IL-13 cytokines, decrease CTL activity
Th17	STAT3 RORγt	Tumour tolerance	IL-17 cytokine
γδ T cells	TCRγ/δ	Tumour tolerance	IL-4, IL-10, TGF-β cytokines and CTL activity
Regulatory T cell			
Tregs	CD4 CD25 FOXP3	Tumour tolerance	IL-10, TGF-β cytokines, CTLA-4

CTL: Cytotoxic lymphocyte; IFN-γ: Interferon-γ; TNF-α: Tumour necrosis factor α; IL: Interleukin; TGF-β: Transforming growth factor β.

cells express high levels of CD62L and CCR7 and efficiently home to lymph nodes, whereas T_{EM} cells lack these molecules and reside mainly in non-lymphoid peripheral tissues but are able to migrate rapidy in response to cytokine gradient. T_{CM} and T_{EM} subsets can also be identified along with a terminally differentiated $CD8^+$ T subset that expresses CD45RA (T_{EMRA}). This way, the T_{CM} subset is classified as $CD45RA^-$ $CD27^{high}$ $CCR7^+$ cells and T_{EM} subset as $CD45RA^-$ $CD27^{low}$ $CCR7^-$ cells. In contrast, T_{EMRA} subset can be identified as $CD45RA^+CD27^{lo}$ $CCR7^-$ cells, and naïve T cells as $CD45RA^+CD27^{high}$ $CCR7^+$ cells, but there are other methods of differentiating these sub-types[47,50].

A handful of other markers have been described to differentiate T cell populations during the effector-to-memory transition states. Increased expression of IL-7Rα (CD127) is functionally required for long-term survival and can be used to identify memory precursor $CD8^+$ T cells. Other proteins co-expressing with $CD127^+$ $CD8^+$ T cells include Bcl-2, CD27, CXCR3, and CD28. Cells expressing these set of markers have the most remarkable capacity to develop into central memory $CD8^+$ T cells (T_{CM}), showing elevated ability to proliferate upon antigen stimulation, increased IL-2 secretion, and self-renewal. Conversely, $CD8^+$ T cells with increased expression of KLRG1, CD57 and decreased expression CD127, CD27, CXCR3, and CD28 are associated with effector or memory $CD8^+$ T cells that display cytotoxicity, elevated IFN-γ production and short-life span. Therefore, $KLRG1^+$ $CD127^-$ $CD8^+$ T cells can be considered effector memory $CD8^+$ T cells (T_{EM}), at least in murine models, though human equivalent data is awaited[47,50].

Transcriptional factors promote the development and function of T_{EM} and T_{CM} cells. Expression of T-bet, Blimp1, ID2, and STAT4 is associated with T_{EM} cells, while high expression of TCF1, BCL-6, ID3, and STAT3 is linked to the formation of T_{CM} cells[49,50]. Interesting, in B cells, Blimp-1 and BCL-6 are essential for the development of germinal centre B cells and long-lived plasma cell through reciprocally antagonising each other[51], suggesting that this set of transcription factors acts in a similar fashion, in the regulation of effector- memory T-cell transition. Moreover, Tcf7 and Lef1 transcription factors are found in self-renewing multipotent $CD8^+$ T cells known as memory stem cells[52].

T CELL EXHAUSTION

Exhausted T cells differ from other dysfunctional T cells, including anergic T cells and senescent T cells. Anergic T cells are induced by suboptimal stimulation showing cells with low proliferative capacity and minimal effector function. Senescent T cells initiate from repeated stimulation, resulting in cells with low proliferative capacity, low expression of inhibitory receptors but show high effector functions despite shortened telomeres. Differently, exhausted T cells result from persistent antigenic stimulation causing Tcells with low proliferative capacity, low to moderate effector functions and elevated expression of multiple inhibitory receptors[53].

In cancers such as PDAC, T cells that go through the activation process will later differentiate into memory-like cells and will ultimately become terminally differen-

tiated exhausted T cells. Exhausted T cells result from persistent antigen exposure featuring cells with low proliferative capacity, increased apoptosis, loss of their cytotoxic function, and elevated expression of multiple inhibitory receptors also known as immune checkpoints such as PD-1, CTLA-4, T cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte activation gene 3 (LAG-3), or T cell immunoreceptor with Ig and ITIM domains (TIGIT)[43,53]. Each inhibitory receptor binds to its ligand, typically expressed by APCs and tumour cells in the TME.

The surface receptor PD-1 (CD279) is the primary receptor involved in T cell inhibitory signalling. PD-1 has two ligands: PD-L1 (CD274) and PD-L2 (CD273) can be found on the surface of antigen-presenting, MDSCs, TAMs and cancer cells. IFN-γ is the main trigger for PD-L1 and PD-L2 upregulation, while induction of PD-1 expression on T cells results from cell receptor (TCR) stimulation or secretion of the cytokines IL-2, IL-7, IL-15, IL-21, and TGF-β. Engagement of PD-L1 or PD-L2 with PD-1 receptor on T cells, inhibits dephosphorylation of TCR signalling components, specifically CD28, resulting in decreased IL-2, IFN-γ, and TNF-α cytokine production, survival, proliferation and effector functions[54].

CTLA-4 (CD152) is a B7/CD28 family member that is constitutively expressed by Tregs. CTLA-4-mediated immunosuppression occurs by limiting signalling through the co-stimulatory receptor CD28 during antigen-presentation, by either binding or deleting CD80 and CD86 from APCs[55]. Thus, indirectly reducing T cell activation and immune responses to tumour antigens. Other T cell subsets such as $CD4^+$ T cells can also upregulate this receptor upon activation[56]. LAG-3 exerts inhibitory effects on T cells through MHCII binding, which results in decreased T cell activation and cytotoxicity, and increased suppressive function in Tregs. In PDAC, upregulation of this receptor is observed in association with upregulation of both PD-1 and CTL-4[57].

The inhibitory receptor TIGIT compete with CD226 to bind the ligands CD112 and CD155 while Tim-3 binds to Galectin-9 and CEACAM1 proteins to inhibit T cell function[58]. Of note, upregulation of Tim-3 in patients with PDAC is correlated with decreased patient survival[57]. Transcription factors involved in the formation of dysfunctional T cells include T-bet, Eomes, Foxo1, Blimp-1, NFAT and IRF-4[53].

A recent study using multiplex immunohistochemistry imaging combined with single-RNA sequencing to evaluate T cell landscape and function in patients with pancreatic cancer, demonstrated that infiltrated $CD8^+$ T cells displayed a senescent phenotype, identified by the expression of $CD57^+CD27^-CD28^-$ or $CD45RA^+$ $CD27^{-/Low}$ $CD28^{-/Low}$ or an exhausted phenotype with elevated expression of $TIGIT^+$ and $CD39^+$ markers alongside PD-$1^{low/intermediate}$ expression[30]. Senescent and exhausted T cells as well as Tregs were also identified within the $CD4^+$ population. Additionally, intra-tumoural Tregs exhibit highly suppressive phenotypes, highlighted by the expression of multiple (TIGIT, ICOS, CD39) inhibitory markers[30].

IMMUNOTHERAPY AND PANCREATIC CANCER

Strategies aiming to leverage the activity of CTLs or the reversal of T cell dysfunction are widespread and have shown clinical success across a variety of cancer[27,29,59]. However, efforts to translate immunotherapy to PDAC, have been met with substantial challenges. The presence of tumour-infiltrating lymphocytes (TILs) with effector and memory functions within the tumour microenvironment and the positive correlation between $CD8^+$ T effector memory cells and patient survival highlight the significance of the T cell immune infiltrate in limiting cancer progression[48]. Hence, the lack of efficacy in existing immunotherapies reflects the challenging non-immunogenic PDAC TME[11,38,48,60].

Chimeric antigen receptor (CAR) T cells or tumour vaccines alone have not demonstrated a survival benefit in PDAC tumours[11,35,48,61]. However, work is ongoing on demonstrating novel targetable antigens or switchable CAR T cells which get activated on reaching the tumour[62,63]. Although most infiltrated $CD8^+$ T cells in the PDAC stroma display features of an exhausted phenotype, demonstrated by cell surface expression of multiple inhibitory receptors, immunotherapy with single-agent immune checkpoint blockade (ICB) has been disappointing. KPC mouse models did not show anti-tumour responses to either CTLA4, PD-1, PDL-1 monotherapy or CTL-4 combined with PD-1/PDL-1 blockade[19,64]. Similarly, human clinical trials using ICB demonstrated insufficient clinical activity and minimal improvement on prognosis, with clinical benefit observed in only highly selected patients[27,39]. Equally, monotherapy with CTLA-4 antibodies and in combination with chemotherapy has not shown ideal clinical activity[59]. Furthermore, exciting avenues for targeting novel

antigens such as CEACAM7 offers hope for CAR-T cell therapy[62,65,66].

The vast majority of trials targeted towards T cells in pancreatic cancer are centred around the use of immune inhibitory receptors against PD-1 and CTLA-4[67]. Most of these trials have enrolled patients with metastatic or borderline resectable pancreatic cancer and assessed the response to either single or double agent immunotherapy or combination therapy with chemotherapy/radiotherapy. The results regarding progression free survival or overall survival have been so far underwhelming[68]. In a meta-analysis on checkpoint inhibitors overall survival and progression-free survival showed no improvement in single agent therapy but a small number of studies on combination therapy have been more promising[69]. It is feasible that the limited tumor mutational burden of pancreatic cancer compared to immunotherapy responsive tumours, such as melanoma or non-small cell lung cancer, may be the key differentiating factor. The phase II KEYNOTE-185 study trying to assess the efficacy of pembrolizumab on patients with non-colorectal microsatellite unstable/mismatch repair deficient cancers enrolled 22 patients with pancreatic cancer, of which four patients showed response to treatment with increase in progression-free survival and median survival[70]. These results, although encouraging, demonstrate that there key barriers around identifying correct groups of patients that would benefit from T cell targeted therapies.

There are various explanations for ICB failure in PDAC tumours including low mutational burden and expression of neoantigens, minimal intra-tumoural infiltration of $CD8^+$ T cells, expression of multiple inhibitory receptors in $CD8^+$ T cells that infiltrate tumours, as well as decreased tumour and myeloid expression cell expression of PDL-1[31,57]. To improve PADC response to ICB, combined approaches have been investigated. Multi-agent immunotherapeutic protocols targeting multiple inhibitory receptors is a promising approach, and has proved more effective than single inhibitory receptor blockade in reversing dysfunctional $CD8^+$ T cells PDAC[27,71]. In the same way, strategies with the goal to prime effector $CD8^+$ T cells to increase their immunogenicity and responsiveness before the use of checkpoint inhibitor treatment represents an exciting opportunity in cancer immunotherapy[12,27,59,72,73]. Combinatory approaches utilising GM-CSF-secreting tumour cells vaccine (GVAX), to induce upregulation of PD-L1 expression into the PDAC TME, prior CTLA-4 and anti-PD-1/PDL-1 blockade has shown promising results in PDAC patients[57,72], and a dual blockade targeting CXCR4 and PD-1 demonstrated improvement in T cell infiltration with a decline in MDSCs[57].

Strategies with the co-stimulatory molecule agonist CD40 used to enhance APC capabilities of macrophages[74] combined with gemcitabine, PD-1 and CTL-4 ICB resulted in increased T-cell priming and infiltration in PDAC tumours[64,72]. Extraction and *in vitro* expansion of TILs from PDAC tumours also have been explored [40] and the results demonstrated autologous T cell killing activity[75,76].

CONCLUSION

The PDAC tumour microenvironment is characterised by complex fibrotic stroma with substantial infiltration of tumour-promoting immunosuppressive cells and pronounced T cell exhaustion, favouring immune evasion that results in immunotherapeutic failures and poor clinical outcome. Therefore, understanding the complexity of PDAC immune landscape and the mechanisms involved in T cell dysfunction may contribute to identifying new immunotherapeutic strategies for treating PDAC and monitoring such response with novel technologies such as ctDNA to assess tumour lysis[77]. As such, unsuccessful immunotherapies could be reversed using combined approaches targeting multiple pathways that obstruct T cell anti-tumour immunity along with other strategies to target stroma[78,79].

A variety of preclinical studies highlighting the influence of PDAC stromal components on T cell anti-tumour responses provided rationale for the development of clinical trials incorporating combined approaches to enhance T cell responses[80]. CXCL12 from cancer-associated fibroblasts synergizes with anti-PD-L1 blockade resulting in activation of T cells and tumour regression in mice[6,81]. Similarly, dual blockade of TGF-β and anti-PD1 resulted in increased T cell responses and tumour regression[82]. Moreover, targeting of myeloid cells with CSF1R in combination with PD-1 or CTLA-4 blockade[83] or focal adhesion kinases inhibitors has been shown to decrease infiltration of suppressive myeloid populations with concomitant activation of T cells, and improved survival in mice models[84].

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]

2 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]

3 **Ryan DP**, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med* 2014; **371**: 2140-2141 [PMID: 25427123 DOI: 10.1056/NEJMc1412266]

4 **Neuzillet C**, Rousseau B, Kocher H, Bourget P, Tournigand C. Unravelling the pharmacologic opportunities and future directions for targeted therapies in gastro-intestinal cancers Part 1: GI carcinomas. *Pharmacol Ther* 2017; **174**: 145-172 [PMID: 28223233 DOI: 10.1016/j.pharmthera.2017.02.028]

5 **Froeling FE**, Kocher HM. Homeostatic restoration of desmoplastic stroma rather than its ablation slows pancreatic cancer progression. *Gastroenterology* 2015; **148**: 849-850 [PMID: 25724458 DOI: 10.1053/j.gastro.2015.02.043]

6 **Ene-Obong A**, Clear AJ, Watt J, Wang J, Fatah R, Riches JC, Marshall JF, Chin-Aleong J, Chelala C, Gribben JG, Ramsay AG, Kocher HM. Activated pancreatic stellate cells sequester CD8+ T cells to reduce their infiltration of the juxtatumoral compartment of pancreatic ductal adenocarcinoma. *Gastroenterology* 2013; **145**: 1121-1132 [PMID: 23891972 DOI: 10.1053/j.gastro.2013.07.025]

7 **Ren B**, Cui M, Yang G, Wang H, Feng M, You L, Zhao Y. Tumor microenvironment participates in metastasis of pancreatic cancer. *Mol Cancer* 2018; **17**: 108 [PMID: 30060755 DOI: 10.1186/s12943-018-0858-1]

8 **Dibra D**, Mishra L, Li S. Molecular mechanisms of oncogene-induced inflammation and inflammation-sustained oncogene activation in gastrointestinal tumors: an under-appreciated symbiotic relationship. *Biochim Biophys Acta* 2014; **1846**: 152-160 [PMID: 24821201 DOI: 10.1016/j.bbcan.2014.05.001]

9 **Sun H**, Zhang B, Li H. The Roles of Frequently Mutated Genes of Pancreatic Cancer in Regulation of Tumor Microenvironment. *Technol Cancer Res Treat* 2020; **19**: 1533033820920969 [PMID: 32372692 DOI: 10.1177/1533033820920969]

10 **Pylayeva-Gupta Y**, Das S, Handler JS, Hajdu CH, Coffre M, Koralov SB, Bar-Sagi D. IL35-Producing B Cells Promote the Development of Pancreatic Neoplasia. *Cancer Discov* 2016; **6**: 247-255 [PMID: 26715643 DOI: 10.1158/2159-8290.CD-15-0843]

11 **Stone ML**, Beatty GL. Cellular determinants and therapeutic implications of inflammation in pancreatic cancer. *Pharmacol Ther* 2019; **201**: 202-213 [PMID: 31158393 DOI: 10.1016/j.pharmthera.2019.05.012]

12 **Orth M**, Metzger P, Gerum S, Mayerle J, Schneider G, Belka C, Schnurr M, Lauber K. Pancreatic ductal adenocarcinoma: biological hallmarks, current status, and future perspectives of combined modality treatment approaches. *Radiat Oncol* 2019; **14**: 141 [PMID: 31395068 DOI: 10.1186/s13014-019-1345-6]

13 **Lee B**, Gibbs P. Inflammation, Biomarkers and Immuno-Oncology Pathways in Pancreatic Cancer. *J Pers Med* 2019; **9** [PMID: 31035449 DOI: 10.3390/jpm9020020]

14 **de Santiago I**, Yau C, Heij L, Middleton MR, Markowetz F, Grabsch HI, Dustin ML, Sivakumar S. Immunophenotypes of pancreatic ductal adenocarcinoma: Meta-analysis of transcriptional subtypes. *Int J Cancer* 2019; **145**: 1125-1137 [PMID: 30720864 DOI: 10.1002/ijc.32186]

15 **Uzunparmak B**, Sahin IH. Pancreatic cancer microenvironment: a current dilemma. *Clin Transl Med* 2019; **8**: 2 [PMID: 30645701 DOI: 10.1186/s40169-019-0221-1]

16 **Clark CE**, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res* 2007; **67**: 9518-9527 [PMID: 17909062 DOI: 10.1158/0008-5472.CAN-07-0175]

17 **Ino Y**, Yamazaki-Itoh R, Shimada K, Iwasaki M, Kosuge T, Kanai Y, Hiraoka N. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br J Cancer* 2013; **108**: 914-923 [PMID: 23385730 DOI: 10.1038/bjc.2013.32]

18 **Nielsen SR**, Quaranta V, Linford A, Emeagi P, Rainer C, Santos A, Ireland L, Sakai T, Sakai K, Kim YS, Engle D, Campbell F, Palmer D, Ko JH, Tuveson DA, Hirsch E, Mielgo A, Schmid MC. Macrophage-secreted granulin supports pancreatic cancer metastasis by inducing liver fibrosis. *Nat Cell Biol* 2016; **18**: 549-560 [PMID: 27088855 DOI: 10.1038/ncb3340]

19 **Clark CE**, Beatty GL, Vonderheide RH. Immunosurveillance of pancreatic adenocarcinoma: insights from genetically engineered mouse models of cancer. *Cancer Lett* 2009; **279**: 1-7 [PMID: 19013709 DOI: 10.1016/j.canlet.2008.09.037]

20 **Knudsen ES**, Vail P, Balaji U, Ngo H, Botros IW, Makarov V, Riaz N, Balachandran V, Leach S, Thompson DM, Chan TA, Witkiewicz AK. Stratification of Pancreatic Ductal Adenocarcinoma: Combinatorial Genetic, Stromal, and Immunologic Markers. *Clin Cancer Res* 2017; **23**: 4429-4440 [PMID: 28348045 DOI: 10.1158/1078-0432.CCR-17-0162]

21 **Mahajan UM**, Langhoff E, Goni E, Costello E, Greenhalf W, Halloran C, Ormanns S, Kruger S, Boeck S, Ribback S, Beyer G, Dombroswki F, Weiss FU, Neoptolemos JP, Werner J, D'Haese JG, Bazhin A, Peterhansl J, Pichlmeier S, Büchler MW, Kleeff J, Ganeh P, Sendler M, Palmer DH, Kohlmann T, Rad R, Regel I, Lerch MM, Mayerle J. Immune Cell and Stromal Signature Associated

With Progression-Free Survival of Patients With Resected Pancreatic Ductal Adenocarcinoma. *Gastroenterology* 2018; **155**: 1625-1639.e2 [PMID: 30092175 DOI: 10.1053/j.gastro.2018.08.009]

22 **Kumar V**, Patel S, Tcyganov E, Gabrilovich DI. The Nature of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *Trends Immunol* 2016; **37**: 208-220 [PMID: 26858199 DOI: 10.1016/j.it.2016.01.004]

23 **Wing K**, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 2008; **322**: 271-275 [PMID: 18845758 DOI: 10.1126/science.1160062]

24 **Jang JE**, Hajdu CH, Liot C, Miller G, Dustin ML, Bar-Sagi D. Crosstalk between Regulatory T Cells and Tumor-Associated Dendritic Cells Negates Anti-tumor Immunity in Pancreatic Cancer. *Cell Rep* 2017; **20**: 558-571 [PMID: 28723561 DOI: 10.1016/j.celrep.2017.06.062]

25 **Daley D**, Zambirinis CP, Seifert L, Akkad N, Mohan N, Werba G, Barilla R, Torres-Hernandez A, Hundeyin M, Mani VRK, Avanzi A, Tippens D, Narayanan R, Jang JE, Newman E, Pillarisetty VG, Dustin ML, Bar-Sagi D, Hajdu C, Miller G. γδ T Cells Support Pancreatic Oncogenesis by Restraining αβ T Cell Activation. *Cell* 2016; **166**: 1485-1499.e15 [PMID: 27569912 DOI: 10.1016/j.cell.2016.07.046]

26 **Balachandran VP**, Łuksza M, Zhao JN, Makarov V, Moral JA, Remark R, Herbst B, Askan G, Bhanot U, Senbabaoglu Y, Wells DK, Cary CIO, Grbovic-Huezo O, Attiyeh M, Medina B, Zhang J, Loo J, Saglimbeni J, Abu-Akeel M, Zappasodi R, Riaz N, Smoragiewicz M, Kelley ZL, Basturk O; Australian Pancreatic Cancer Genome Initiative; Garvan Institute of Medical Research; Prince of Wales Hospital; Royal North Shore Hospital; University of Glasgow; St Vincent's Hospital; QIMR Berghofer Medical Research Institute; University of Melbourne, Centre for Cancer Research; University of Queensland, Institute for Molecular Bioscience; Bankstown Hospital; Liverpool Hospital; Royal Prince Alfred Hospital, Chris O'Brien Lifehouse; Westmead Hospital; Fremantle Hospital; St John of God Healthcare; Royal Adelaide Hospital; Flinders Medical Centre; Envoi Pathology; Princess Alexandria Hospital; Austin Hospital; Johns Hopkins Medical Institutes; ARC-Net Centre for Applied Research on Cancer, Gönen M, Levine AJ, Allen PJ, Fearon DT, Merad M, Gnjatic S, Iacobuzio-Donahue CA, Wolchok JD, DeMatteo RP, Chan TA, Greenbaum BD, Merghoub T, Leach SD. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. *Nature* 2017; **551**: 512-516 [PMID: 29132146 DOI: 10.1038/nature24462]

27 **Johnson BA 3rd**, Yarchoan M, Lee V, Laheru DA, Jaffee EM. Strategies for Increasing Pancreatic Tumor Immunogenicity. *Clin Cancer Res* 2017; **23**: 1656-1669 [PMID: 28373364 DOI: 10.1158/1078-0432.CCR-16-2318]

28 **Tumeh PC**, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014; **515**: 568-571 [PMID: 25428505 DOI: 10.1038/nature13954]

29 **Bailey P**, Chang DK, Forget MA, Lucas FA, Alvarez HA, Haymaker C, Chattopadhyay C, Kim SH, Ekmekcioglu S, Grimm EA, Biankin AV, Hwu P, Maitra A, Roszik J. Exploiting the neoantigen landscape for immunotherapy of pancreatic ductal adenocarcinoma. *Sci Rep* 2016; **6**: 35848 [PMID: 27762323 DOI: 10.1038/srep35848]

30 **Sivakumar S**, Abu-Shah E, Ahern DJ, Arbe-Barnes EH, Mangal N, Reddy S, Rendek A, Easton A, Kurz E, Silva M, Soonawalla Z, Heij LR, Bashford-Rogers R, Middleton MR, Dustin ML. Activated regulatory T-cells, dysfunctional and senescent T-cells dominate the microenvironment of pancreatic cancer. 2020 Preprint. Available from: bioRxiv:2020.06.20.163071 [DOI: 10.1101/2020.06.20.163071]

31 **Stromnes IM**, Hulbert A, Pierce RH, Greenberg PD, Hingorani SR. T-cell Localization, Activation, and Clonal Expansion in Human Pancreatic Ductal Adenocarcinoma. *Cancer Immunol Res* 2017; **5**: 978-991 [PMID: 29066497 DOI: 10.1158/2326-6066.CIR-16-0322]

32 **Carstens JL**, Correa de Sampaio P, Yang D, Barua S, Wang H, Rao A, Allison JP, LeBleu VS, Kalluri R. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. *Nat Commun* 2017; **8**: 15095 [PMID: 28447602 DOI: 10.1038/ncomms15095]

33 **Li C**, Cui L, Yang L, Wang B, Zhuo Y, Zhang L, Wang X, Zhang Q, Zhang S. Pancreatic Stellate Cells Promote Tumor Progression by Promoting an Immunosuppressive Microenvironment in Murine Models of Pancreatic Cancer. *Pancreas* 2020; **49**: 120-127 [PMID: 31856087 DOI: 10.1097/MPA.0000000000001464]

34 **Elyada E**, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffi G, Lucito MS, Sivajothi S, Armstrong TD, Engle DD, Yu KH, Hao Y, Wolfgang CL, Park Y, Preall J, Jaffee EM, Califano A, Robson P, Tuveson DA. Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. *Cancer Discov* 2019; **9**: 1102-1123 [PMID: 31197017 DOI: 10.1158/2159-8290.CD-19-0094]

35 **Tesfaye AA**, Kamgar M, Azmi A, Philip PA. The evolution into personalized therapies in pancreatic ductal adenocarcinoma: challenges and opportunities. *Expert Rev Anticancer Ther* 2018; **18**: 131-148 [PMID: 29254387 DOI: 10.1080/14737140.2018.1417844]

36 **Evans RA**, Diamond MS, Rech AJ, Chao T, Richardson MW, Lin JH, Bajor DL, Byrne KT, Stanger BZ, Riley JL, Markosyan N, Winograd R, Vonderheide RH. Lack of immunoediting in murine pancreatic cancer reversed with neoantigen. *JCI Insight* 2016; **1** [PMID: 27642636 DOI: 10.1172/jci.insight.88328]

37 **Yarchoan M**, Johnson BA 3rd, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. *Nat Rev Cancer* 2017; **17**: 569 [PMID: 28835723 DOI: 10.1038/nrc.2017.74]

38 **Lutz ER**, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, Solt S, Dorman A, Wamwea A, Yager A, Laheru D, Wolfgang CL, Wang J, Hruban RH, Anders RA, Jaffee EM, Zheng L. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. *Cancer Immunol Res* 2014; **2**: 616-631 [PMID: 24942756 DOI: 10.1158/2326-6066.CIR-14-0027]

39 **Upadhrasta S**, Zheng L. Strategies in Developing Immunotherapy for Pancreatic Cancer: Recognizing and Correcting Multiple Immune "Defects" in the Tumor Microenvironment. *J Clin Med* 2019; **8** [PMID: 31527414 DOI: 10.3390/jcm8091472]

40 **Poschke I**, Faryna M, Bergmann F, Flossdorf M, Lauenstein C, Hermes J, Hinz U, Hank T, Ehrenberg R, Volkmar M, Loewer M, Glimm H, Hackert T, Sprick MR, Höfer T, Trumpp A, Halama N, Hassel JC, Strobel O, Büchler M, Sahin U, Offringa R. Identification of a tumor-reactive T-cell repertoire in the immune infiltrate of patients with resectable pancreatic ductal adenocarcinoma. *Oncoimmunology* 2016; **5**: e1240859 [PMID: 28123878 DOI: 10.1080/2162402X.2016.1240859]

41 **Hiraoka N**, Ino Y, Yamazaki-Itoh R, Kanai Y, Kosuge T, Shimada K. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. *Br J Cancer* 2015; **112**: 1782-1790 [PMID: 25942397 DOI: 10.1038/bjc.2015.145]

42 **Castino GF**, Cortese N, Capretti G, Serio S, Di Caro G, Mineri R, Magrini E, Grizzi F, Cappello P, Novelli F, Spaggiari P, Roncalli M, Ridolfi C, Gavazzi F, Zerbi A, Allavena P, Marchesi F. Spatial distribution of B cells predicts prognosis in human pancreatic adenocarcinoma. *Oncoimmunology* 2016; **5**: e1085147 [PMID: 27141376 DOI: 10.1080/2162402X.2015.1085147]

43 **Papalampros A**, Vailas M, Ntostoglou K, Chiloeches ML, Sakellariou S, Chouliari NV, Samaras MG, Veltsista PD, Theodorou SDP, Margetis AT, Bergonzini A, Karydakis L, Hasemaki N, Havaki S, Moustakas II, Chatzigeorgiou A, Karamitros T, Patsea E, Kittas C, Lazaris AC, Felekouras E, Gorgoulis VG, Frisan T, Pateras IS. Unique Spatial Immune Profiling in Pancreatic Ductal Adenocarcinoma with Enrichment of Exhausted and Senescent T Cells and Diffused CD47-SIRPα Expression. *Cancers (Basel)* 2020; **12** [PMID: 32645996 DOI: 10.3390/cancers12071825]

44 **Foucher ED**, Ghigo C, Chouaib S, Galon J, Iovanna J, Olive D. Pancreatic Ductal Adenocarcinoma: A Strong Imbalance of Good and Bad Immunological Cops in the Tumor Microenvironment. *Front Immunol* 2018; **9**: 1044 [PMID: 29868007 DOI: 10.3389/fimmu.2018.01044]

45 **Protti MP**, De Monte L. Immune infiltrates as predictive markers of survival in pancreatic cancer patients. *Front Physiol* 2013; **4**: 210 [PMID: 23950747 DOI: 10.3389/fphys.2013.00210]

46 **Barilla RM**, Diskin B, Caso RC, Lee KB, Mohan N, Buttar C, Adam S, Sekendiz Z, Wang J, Salas RD, Cassini MF, Karlen J, Sundberg B, Akbar H, Levchenko D, Gakhal I, Gutierrez J, Wang W, Hundeyin M, Torres-Hernandez A, Leinwand J, Kurz E, Rossi JAK, Mishra A, Liria M, Sanchez G, Panta J, Loke P, Aykut B, Miller G. Specialized dendritic cells induce tumor-promoting IL-10^{+}IL-17^{+} FoxP3^{neg} regulatory CD4^{+} T cells in pancreatic carcinoma. *Nat Commun* 2019; **10**: 1424 [PMID: 30926808 DOI: 10.1038/s41467-019-09416-2]

47 **Cui W**, Kaech SM. Generation of effector CD8+ T cells and their conversion to memory T cells. *Immunol Rev* 2010; **236**: 151-166 [PMID: 20636815 DOI: 10.1111/j.1600-065X.2010.00926.x]

48 **Fridman WH**, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* 2017; **14**: 717-734 [PMID: 28741618 DOI: 10.1038/nrclinonc.2017.101]

49 **Mescher MF**, Curtsinger JM, Agarwal P, Casey KA, Gerner M, Hammerbeck CD, Popescu F, Xiao Z. Signals required for programming effector and memory development by CD8+ T cells. *Immunol Rev* 2006; **211**: 81-92 [PMID: 16824119 DOI: 10.1111/j.0105-2896.2006.00382.x]

50 **Martin MD**, Badovinac VP. Defining Memory CD8 T Cell. *Front Immunol* 2018; **9**: 2692 [PMID: 30515169 DOI: 10.3389/fimmu.2018.02692]

51 **Crotty S**, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. *Nat Immunol* 2010; **11**: 114-120 [PMID: 20084069 DOI: 10.1038/ni.1837]

52 **Gattinoni L**, Zhong XS, Palmer DC, Ji Y, Hinrichs CS, Yu Z, Wrzesinski C, Boni A, Cassard L, Garvin LM, Paulos CM, Muranski P, Restifo NP. Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. *Nat Med* 2009; **15**: 808-813 [PMID: 19525962 DOI: 10.1038/nm.1982]

53 **Schietinger A**, Greenberg PD. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. *Trends Immunol* 2014; **35**: 51-60 [PMID: 24210163 DOI: 10.1016/j.it.2013.10.001]

54 **Seidel JA**, Otsuka A, Kabashima K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. *Front Oncol* 2018; **8**: 86 [PMID: 29644214 DOI: 10.3389/fonc.2018.00086]

55 **Curran MA**, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci U S A* 2010; **107**: 4275-4280 [PMID: 20160101 DOI: 10.1073/pnas.0915174107]

56 **Cristescu R**, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, Sher X, Liu XQ, Lu H, Nebozhyn M, Zhang C, Lunceford JK, Joe A, Cheng J, Webber AL, Ibrahim N, Plimack ER, Ott PA, Seiwert TY, Ribas A, McClanahan TK, Tomassini JE, Loboda A, Kaufman D. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018; **362** [PMID: 30309915 DOI: 10.1126/science.aar3593]

57 **Saka D**, Gökalp M, Piyade B, Cevik NC, Arik Sever E, Unutmaz D, Ceyhan GO, Demir IE, Asimgil

H. Mechanisms of T-Cell Exhaustion in Pancreatic Cancer. *Cancers (Basel)* 2020; **12** [PMID: 32823814 DOI: 10.3390/cancers12082274]

58 **Xia A**, Zhang Y, Xu J, Yin T, Lu XJ. T Cell Dysfunction in Cancer Immunity and Immunotherapy. *Front Immunol* 2019; **10**: 1719 [PMID: 31379886 DOI: 10.3389/fimmu.2019.01719]

59 **Young K**, Hughes DJ, Cunningham D, Starling N. Immunotherapy and pancreatic cancer: unique challenges and potential opportunities. *Ther Adv Med Oncol* 2018; **10**: 1758835918816281 [PMID: 30574212 DOI: 10.1177/1758835918816281]

60 **Strauss J**, Alewine C, Figg WD, Duffy A. Targeting the microenvironment of pancreatic cancer: overcoming treatment barriers and improving local immune responses. *Clin Transl Oncol* 2016; **18**: 653-659 [PMID: 26661112 DOI: 10.1007/s12094-015-1459-8]

61 **Ott PA**, Wu CJ. Cancer Vaccines: Steering T Cells Down the Right Path to Eradicate Tumors. *Cancer Discov* 2019; **9**: 476-481 [PMID: 30862723 DOI: 10.1158/2159-8290.CD-18-1357]

62 **Raj D**, Nikolaidi M, Garces I, Lorizio D, Castro NM, Caiafa SG, Moore K, Brown NF, Kocher HM, Duan X, Nelson BH, Lemoine NR, Marshall JF. CEACAM7 Is an Effective Target for CAR T-cell Therapy of Pancreatic Ductal Adenocarcinoma. *Clin Cancer Res* 2021; **27**: 1538-1552 [PMID: 33479048 DOI: 10.1158/1078-0432.CCR-19-2163]

63 **Raj D**, Yang MH, Rodgers D, Hampton EN, Begum J, Mustafa A, Lorizio D, Garces I, Propper D, Kench JG, Kocher HM, Young TS, Aicher A, Heeschen C. Switchable CAR-T cells mediate remission in metastatic pancreatic ductal adenocarcinoma. *Gut* 2019; **68**: 1052-1064 [PMID: 30121627 DOI: 10.1136/gutjnl-2018-316595]

64 **Winograd R**, Byrne KT, Evans RA, Odorizzi PM, Meyer AR, Bajor DL, Clendenin C, Stanger BZ, Furth EE, Wherry EJ, Vonderheide RH. Induction of T-cell Immunity Overcomes Complete Resistance to PD-1 and CTLA-4 Blockade and Improves Survival in Pancreatic Carcinoma. *Cancer Immunol Res* 2015; **3**: 399-411 [PMID: 25678581 DOI: 10.1158/2326-6066.CIR-14-0215]

65 **Reader CS**, Vallath S, Steele CW, Haider S, Brentnall A, Desai A, Moore KM, Jamieson NB, Chang D, Bailey P, Scarpa A, Lawlor R, Chelala C, Keyse SM, Biankin A, Morton JP, Evans TJ, Barry ST, Sansom OJ, Kocher HM, Marshall JF. The integrin αvβ6 drives pancreatic cancer through diverse mechanisms and represents an effective target for therapy. *J Pathol* 2019; **249**: 332-342 [PMID: 31259422 DOI: 10.1002/path.5320]

66 **Haider S**, Wang J, Nagano A, Desai A, Arumugam P, Dumartin L, Fitzgibbon J, Hagemann T, Marshall JF, Kocher HM, Crnogorac-Jurcevic T, Scarpa A, Lemoine NR, Chelala C. A multi-gene signature predicts outcome in patients with pancreatic ductal adenocarcinoma. *Genome Med* 2014; **6**: 105 [PMID: 25587357 DOI: 10.1186/s13073-014-0105-3]

67 **Katayama ES**, Hue JJ, Bajor DL, Ocuin LM, Ammori JB, Hardacre JM, Winter JM. A comprehensive analysis of clinical trials in pancreatic cancer: what is coming down the pike? *Oncotarget* 2020; **11**: 3489-3501 [PMID: 33014285 DOI: 10.18632/oncotarget.27727]

68 **Roth MT**, Cardin DB, Berlin JD. Recent advances in the treatment of pancreatic cancer. *F1000Res* 2020; **9** [PMID: 32148767 DOI: 10.12688/f1000research.21981.1]

69 **Henriksen A**, Dyhl-Polk A, Chen I, Nielsen D. Checkpoint inhibitors in pancreatic cancer. *Cancer Treat Rev* 2019; **78**: 17-30 [PMID: 31325788 DOI: 10.1016/j.ctrv.2019.06.005]

70 **Marabelle A**, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, Geva R, Gottfried M, Penel N, Hansen AR, Piha-Paul SA, Doi T, Gao B, Chung HC, Lopez-Martin J, Bang YJ, Frommer RS, Shah M, Ghori R, Joe AK, Pruitt SK, Diaz LA Jr. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* 2020; **38**: 1-10 [PMID: 31682550 DOI: 10.1200/JCO.19.02105]

71 **Torphy RJ**, Schulick RD, Zhu Y. Understanding the immune landscape and tumor microenvironment of pancreatic cancer to improve immunotherapy. *Mol Carcinog* 2020; **59**: 775-782 [PMID: 32166821 DOI: 10.1002/mc.23179]

72 **Vonderheide RH**. The Immune Revolution: A Case for Priming, Not Checkpoint. *Cancer Cell* 2018; **33**: 563-569 [PMID: 29634944 DOI: 10.1016/j.ccell.2018.03.008]

73 **Karamitopoulou E**. Tumour microenvironment of pancreatic cancer: immune landscape is dictated by molecular and histopathological features. *Br J Cancer* 2019; **121**: 5-14 [PMID: 31110329 DOI: 10.1038/s41416-019-0479-5]

74 **Beatty GL**, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, Sharp LL, Torigian DA, O'Dwyer PJ, Vonderheide RH. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 2011; **331**: 1612-1616 [PMID: 21436454 DOI: 10.1126/science.1198443]

75 **Meng Q**, Liu Z, Rangelova E, Poiret T, Ambati A, Rane L, Xie S, Verbeke C, Dodoo E, Del Chiaro M, Löhr M, Segersvärd R, Maeurer MJ. Expansion of Tumor-reactive T Cells From Patients With Pancreatic Cancer. *J Immunother* 2016; **39**: 81-89 [PMID: 26849077 DOI: 10.1097/CJI.0000000000000111]

76 **Hall M**, Liu H, Malafa M, Centeno B, Hodul PJ, Pimiento J, Pilon-Thomas S, Sarnaik AA. Expansion of tumor-infiltrating lymphocytes (TIL) from human pancreatic tumors. *J Immunother Cancer* 2016; **4**: 61 [PMID: 27777771 DOI: 10.1186/s40425-016-0164-7]

77 **Sivapalan L**, Kocher HM, Ross-Adams H, Chelala C. Molecular profiling of ctDNA in pancreatic cancer: Opportunities and challenges for clinical application. *Pancreatology* 2021; **21**: 363-378 [PMID: 33451936 DOI: 10.1016/j.pan.2020.12.017]

78 **Kocher HM**, Basu B, Froeling FEM, Sarker D, Slater S, Carlin D, deSouza NM, De Paepe KN,

Goulart MR, Hughes C, Imrali A, Roberts R, Pawula M, Houghton R, Lawrence C, Yogeswaran Y, Mousa K, Coetzee C, Sasieni P, Prendergast A, Propper DJ. Phase I clinical trial repurposing all-trans retinoic acid as a stromal targeting agent for pancreatic cancer. *Nat Commun* 2020; **11**: 4841 [PMID: 32973176 DOI: 10.1038/s41467-020-18636-w]

79 **Neuzillet C**, Tijeras-Raballand A, Ragulan C, Cros J, Patil Y, Martinet M, Erkan M, Kleeff J, Wilson J, Apte M, Tosolini M, Wilson AS, Delvecchio FR, Bousquet C, Paradis V, Hammel P, Sadanandam A, Kocher HM. Inter- and intra-tumoural heterogeneity in cancer-associated fibroblasts of human pancreatic ductal adenocarcinoma. *J Pathol* 2019; **248**: 51-65 [PMID: 30575030 DOI: 10.1002/path.5224]

80 **Ware MB**, El-Rayes BF, Lesinski GB. Mirage or long-awaited oasis: reinvigorating T-cell responses in pancreatic cancer. *J Immunother Cancer* 2020; **8** [PMID: 32843336 DOI: 10.1136/jitc-2020-001100]

81 **Feig C**, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, Teichmann SA, Janowitz T, Jodrell DI, Tuveson DA, Fearon DT. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci U S A* 2013; **110**: 20212-20217 [PMID: 24277834 DOI: 10.1073/pnas.1320318110]

82 **Principe DR**, Park A, Dorman MJ, Kumar S, Viswakarma N, Rubin J, Torres C, McKinney R, Munshi HG, Grippo PJ, Rana A. TGFβ Blockade Augments PD-1 Inhibition to Promote T-Cell-Mediated Regression of Pancreatic Cancer. *Mol Cancer Ther* 2019; **18**: 613-620 [PMID: 30587556 DOI: 10.1158/1535-7163.MCT-18-0850]

83 **Zhu Y**, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, Wang-Gillam A, Goedegebuure SP, Linehan DC, DeNardo DG. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res* 2014; **74**: 5057-5069 [PMID: 25082815 DOI: 10.1158/0008-5472.CAN-13-3723]

84 **Jiang H**, Liu X, Knolhoff BL, Hegde S, Lee KB, Jiang H, Fields RC, Pachter JA, Lim KH, DeNardo DG. Development of resistance to FAK inhibition in pancreatic cancer is linked to stromal depletion. *Gut* 2020; **69**: 122-132 [PMID: 31076405 DOI: 10.1136/gutjnl-2018-317424]

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World J Gastroenterol 2021 December 14; 27(46): 7969-7981

DOI: 10.3748/wjg.v27.i46.7969

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

COVID-19 status quo: Emphasis on gastrointestinal and liver manifestations

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Conflict-of-interest statement: There is no conflict of interest.

Country/Territory of origin: United States

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0

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Abstract

The coronavirus disease 2019 (COVID-19) has caused one of the worst public health crises in modern history. Even though severe acute respiratory syndrome coronavirus 2 primarily affects the respiratory tract, gastrointestinal manifestations are well described in literature. This review will discuss the epidemiology, virology, manifestations, immunosuppressant states, and lessons learned from COVID-19. Observations: At the time of writing, COVID-19 had infected more than 111 million people and caused over 2.5 million deaths worldwide. Multiple medical comorbidities including obesity, pre-existing liver condition and the use of proton pump inhibitor have been described as risk factor for severe COVID-19. COVID-19 most frequently causes diarrhea (12.4%), nausea/vomiting (9%) and elevation in liver enzymes (15%-20%). The current data does not suggest that patients on immunomodulators have a significantly increased risk of mortality from COVID-19. The current guidelines from American Gastroenterological Association and American Association for the Study of Liver Diseases do not recommend pre-emptive changes in patients on immunosuppression if the patients have not been infected with COVID-19. Conclusions and relevance: The COVID-19 pandemic has prompted a change in structure and shape of gastroenterology departmental activities. Endoscopy should be performed only when necessary and with strict protective measures. Online consultations in the form of telehealth services and home drug deliveries have revolutionized the field.

Grade D (Fair): 0
Grade E (Poor): 0

Received: April 28, 2021
Peer-review started: April 28, 2021
First decision: June 13, 2021
Revised: July 23, 2021
Accepted: November 25, 2021
Article in press: November 25, 2021
Published online: December 14, 2021

P-Reviewer: Amir M, Goenka MK, Xie M
S-Editor: Liu M
L-Editor: A
P-Editor: Liu M

Key Words: COVID-19; Status quo; Epidemiology; Virology; Gastrointestinal manifestations; Liver manifestations; Immunosuppressed states

Core Tip: The coronavirus disease 2019 (COVID-19) has caused one of the worst public health crises in modern history. Even though severe acute respiratory syndrome coronavirus 2 primarily affects the respiratory tract, gastrointestinal manifestations are well described in literature. This review will discuss the epidemiology, virology, manifestations, immunosuppressant states, and lessons learned from COVID-19.

Citation: Bhurwal A, Minacapelli CD, Orosz E, Gupta K, Tait C, Dalal I, Zhang C, Zhao E, Rustgi VK. COVID-19 status quo: Emphasis on gastrointestinal and liver manifestations. *World J Gastroenterol* 2021; 27(46): 7969-7981
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7969.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7969

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is considered one of the fastest expanding pandemics since the Spanish flu of 1918, and one of the most impactful public health crises in modern history. As of February 2021, the coronavirus causing COVID-19, also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had infected more than 111 million people and caused over 2.5 million deaths worldwide, including 500000 in the United States[1].

It is hypothesized that SARS-CoV-2 originated from animal reservoirs and adapted to human-to-human transmission[2,3]. The first cases of severe pneumonia-like conditions were diagnosed in Wuhan, China at the end of 2019[4]. In February 2020, the international virus classification commission termed the novel coronavirus SARS-CoV-2, and its clinical disease was termed COVID-19 by the World Health Organization (WHO) [5,6]. The case fatality ratio (CFR), defined as the proportion of individuals dying of a disease, was estimated to be up to 3%[7]. Till date, CFR remains the best tool to express the severity of COVID-19 infection among confirmed cases (Table 1). In addition to CFR, many other COVID-19 risk assessment tools have been developed that try to gauge the severity of the novel disease[8] within ethnically diverse populations[9].

SARS-CoV-2 pathogenesis manifests primarily as a respiratory viral syndrome causing symptoms such as cough, fever, general malaise, dyspnea, and respiratory distress, and in a proportion of cases causes severe pulmonary manifestations with respiratory failure and death[10]. SARS-CoV-2 propagates from the respiratory tract to other organs such as the gastrointestinal (GI) tract and liver[10-12].

Due to the availability of the genomic sequence of the viral RNA, scientists and researchers have been able to understand the SARS-CoV-2 virus and develop treatment strategies. This review will discuss the epidemiology, virology, manifestations, immunosuppressant states, and lessons learned from COVID-19.

EPIDEMIOLOGY

The high degree of infectivity of the COVID-19 virus is attributed to its novelty in the human host. It can be can be measured by the basic reproduction number or R0, which is a statistical tool used to describe the contagiousness of a virus. It is estimated that the SARS-CoV-2 R0 is between 2 and 3, signifying that each infected person is likely to spread the infection to 2 to 3 additional people[13,14]. The secondary attack rate characterizes the contagiousness of a virus in the close contact setting, which considers how social behaviors may influence transmissibility[15,16]. Jing *et al*[17] estimated the secondary attack rate of COVID-19 to be 12.4% amongst close relatives and 17.1% amongst those who share the same residential address.

Table 1 Calculated case fatality rate globally and regional according to World Health Organization reports

Region	Cumulative confirmed cases	Cumulative death cases	CFR
Global	111762965	2479678	2.22%
Americas	49700102	1182591	2.38%
Europa	37974729	848644	2.23%
South East Asia	13415064	205814	1.53%
Eastern Mediterranean	6266689	142986	2.28%
Africa	2811106	71159	2.53%
Western Pacific	1594530	28471	1.79%

Estimated calculation with January 24, 2021 Data - World Health Organization Coronavirus (COVID-19) Dashboard, Available from: https://covid19.who.int/. CFR: Case fatality ratio.

There are many medical comorbidities identified as risk factors for increased COVID-19 severity and mortality. In a summary report from China, pre-existing comorbid conditions increase fatality rate by 10.5% for cardiovascular disease, 7.3% for diabetes, 6.3% for chronic respiratory disease, 6.0% for hypertension, and 5.6% for cancer[18]. In a retrospective cohort study of 403 COVID-19 patients from a racially diverse, urban hospital, Rustgi *et al*[9] identified chronic kidney disease, hypertension, congestive heart failure, coronary artery disease, malignancy, dementia, cerebrovascular disease, seizures, and COPD to be associated with increased mortality. The centers for disease control (CDC) lists diabetes and BMI as conditions associated with increased risk of severe illness[19]. In an analysis of nearly 300000 COVID-19 cases in the United States, the mortality rate was 12 times as high among patients with reported comorbidities compared to those with none[20]. Understanding the significance of these risk factors can be vital when triaging and treating patients with COVID-19.

Few GI and liver-specific risk factors have also been identified. In a retrospective study of 2780 COVID-19 patients, Galiero *et al*[21] examined the effect of pre-existing liver disease (including NAFLD, NASH, and cirrhosis) on mortality. Patients with liver disease had a significantly higher risk of mortality. Another gastroenterology-specific risk factor identified is the use of proton pump inhibitors (PPIs). Luxenburger *et al*[22] reported that in hospitalized patients with COVID-19, the use of a PPI significantly increased the risk of developing secondary infection (48.4% *vs* 20.0%, $P \leq 0.001$), ARDS (27.4% *vs* 12.2%, $P = 0.02$), and mortality (19.4% *vs* 5.6%, $P = 0.01$)[22]. One proposed mechanism is that PPI use suppresses gastric acid production leading to increased gastric microbiota which, in turn, can lead to micro-aspiration and subsequent bacterial colonization of the lung[23]. In addition, there is growing evidence that PPIs can also modulate immune responses by inhibiting neutrophil function with anti-inflammatory activity[24].

Age is another notable variable that affects mortality rates with older patients at much higher risk of death. One study in China showed that the mortality rate could be up to 3 times higher in patients who are 80 years or older[18]. Race and ethnicity have also been shown to affect mortality rates, though there is limited literature. Rustgi *et al*[9] showed that White, Blacks, Asians, and Hispanics all have significantly different mortality rates[9]. In a meta-analysis of more than 3 million reported global cases, male patients compared to females had increased odds of ICU admission (OR, 2.84; 95%CI: 2.06-3.92) and mortality (OR,1.39; 95%CI: 1.31-1.47)[25]. COVID-19 mortality rates also vary significantly by country. For example, France had a CFR of 15.2% whereas Korea had a CFR of 2.1%[26]. Unique delivery systems and healthcare infrastructure in these countries play a major role in influencing COVID-19 mortality.

VIROLOGY

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus of the Coronaviridae family[2]. The coronaviruses can infect a wide range of vertebrates, including snakes, bats, pangolins, and humans. Sequence similarities with the bat and pangolin coronavirus virus RaTG13 strains suggest that the SARS-CoV-2 has a

zoonotic origin[2,3,27].

Human infection happens by aerosol droplets or carried on fomites. Upon inhalation, the SARS-CoV-2 enters host respiratory cells *via* the angiotensin-converting enzyme 2 (ACE2) receptor and activating receptors such as the transmembrane protease serine 2 or cathepsin (Figure 1)[27,28]. Viral replication in the infected cells causes immune cells to proliferate and produce large amounts of cytokines and chemokines such as TNF-alpha, interferon-gamma, interleukin 6 (IL-6), IL-8, and IL-10 (Figure 1)[28,29]. This process causes a cascade of inflammatory reactions with toxic damage to the lungs (Figure 2). These mechanisms have also been utilized as targets for therapy. After the initial focus on hydroxychloroquine, emphasis has more recently been on polymerase inhibitors (Remdesivir), binding agents such as convalescent plasma therapy and IL-6 inhibitors such as Tocilizumab[30,31]. Vaccines, such as mRNA-based (Pfizer-BioNTech, Moderna), adenovirus-based (AstraZeneca, Sputnik V, Convidicea, ZF2001), inactivated viral particles (CoronaVac, BBIBP-CorV, Covaxin, CoviVac), non-replicating viral vector (Janssen), and peptide (EpiVacCorona) (Figure 3) are areas of active evolution[30-33]. Adenovirus based intra-nasal COVID vaccines are currently undergoing evaluation *via* clinical trials. These vaccines with different mechanisms of action trigger immune responses and are of great benefit to systematically stop the COVID-19 pandemic[34].

GI symptoms such as diarrhea, nausea, vomiting, or abdominal pain have been reported in approximately 10%-15% of COVID-19 patients before, during or after clinical disease[28,35]. Stool samples from infected patients may test positive for the presence of SARS-CoV-2[36]. In vitro studies have demonstrated that enterocyte organoids may harbor and be capable of supporting SARS-CoV-2 replication[37]. In addition, in-vivo reports indicate that viral RNA is detectable by RT-PCR in biopsies from the esophagus, stomach, duodenum, and rectum[35]. These limited studies suggest that the SARS-CoV-2 virus can actively infect and replicate in the GI tract causing direct organ dysfunction.

The liver may suffer injury in 35%-56% of COVID-19 patients as shown by elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase and/or bilirubin levels[12,38,39]. Interestingly, SARS-CoV-2 RNA sequences have been detected by RT-PCR in liver tissues of infected individuals, suggesting that hepatic injury may be related to direct viral infection[40]. Biopsy findings have demonstrated non-specific inflammatory changes such as hepatocyte swelling and steatosis, mild proliferation of hepatic sinusoid cells, hyperplasia of Kupffer cells and infiltration of lymphocytes[41-43]. SARS-CoV-2 injury in the liver may be mediated by high ACE-2 expression in liver cholangiocytes as well as TROP-2 Liver progenitor cells[41].

Ischemia-perfusion injury has been reported as a complication of COVID-19 in both the GI and liver and has been more frequently observed in those COVID-19 patients admitted to intensive care units[44-46]. This injury may be due to coagulopathy, vasculopathy, hypoxia and shock caused by COVID-19 and thromboembolic events [47-49]. Under these conditions, there is an increase in reactive oxygen species which, in turn, activate transcription factors and initiate the release of various pro-inflammatory factors that lead to tissue damage (Figure 2)[49,50].

GI MANIFESTATIONS

GI symptoms are common in COVID-19[51]. The most commonly reported GI manifestations are diarrhea, nausea, vomiting, and abdominal pain[52]. Loss of appetite and dysgeusia have also been described[53]. Viral particles have been isolated in fecal samples suggesting the possibility of fecal transmission of the virus[54]. A minority of patients with positive stool testing lacked GI symptoms suggesting asymptomatic carriage of disease[55]. These findings highlight the importance of fecal-aerosol-mucosal transmission among individuals exposed to contaminated feces, including public toilets or areas with poor sanitation. This provides a concerning avenue for infectious spread in under-developed regions of the globe, including many regions in Africa and South Asia which lack comprehensive wastewater treatment facilities. Disease control guidelines have emphasized effective management and disinfection of potentially contaminated feces in COVID19 patients, and aggressive vaccination programs in areas at higher risk for fecal-oral spread[43].

Initial case series from China revealed diarrhea as the most prevalent GI symptom, occurring in 2%-36% of cases, followed by nausea (1%-17%), vomiting (1%-6%), and abdominal pain (2%-6%)[56]. As the pandemic has spread, additional reviews and

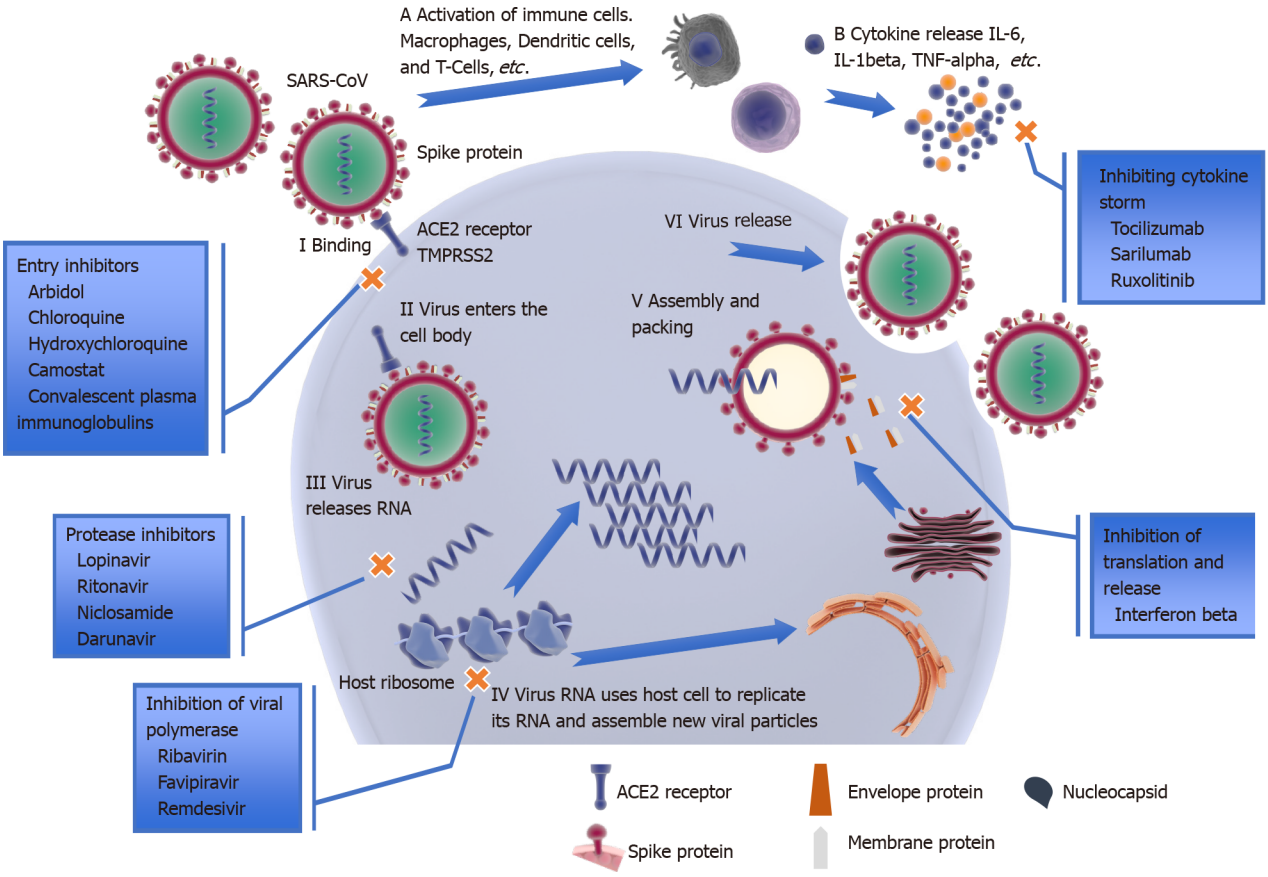

Figure 1 Schematic representation of severe acute respiratory syndrome coronavirus 2 life cycle causing coronavirus disease 2019. Angiotensin-converting enzyme 2 receptors located on the cell surface of ciliated epithelial cells in the respiratory airways, and in type II pneumocytes in the alveoli, bind to virus spike proteins (I). The virus enters the cell body (II) and releases its RNA (III) using host cells to create new virus particles by replication of RNA and translation of polyproteins (IV). New viral particles are assembled (V) and released by exocytosis (VI). Library of Science & Medical Illustrations were utilized in part to create this figure. https://creativecommons.org/licenses/by-nc-sa/4.0/.

meta-analyses have confirmed the prevalence of GI symptoms in other population groups, although much of the reported data remains from cohorts of Chinese patients. A large meta-analysis of 59254 patients predominantly from the Hubei province (75.8), showed that 9% of all patients experience GI symptoms (Table 2)[57]. A more recent meta-analysis by Tariq *et al*[52] focused specifically on GI manifestations of disease, including 12797 patients from 11 countries. Of the patients included, 12.4% reported diarrhea and 9% nausea and/or vomiting. Abdominal pain was also reported in 6.2% of patients. Comparative analysis by patient location revealed a significantly higher proportion of symptoms of diarrhea and nausea/vomiting in the non-China subgroup while loss of appetite was similar between groups.

Anosmia and ageusia are frequently reported with important implications. Ageusia was reported in 20% of patients in one recent review while the rates of anosmia varied greatly across studies from 22%-68%[53]. Anosmia and dysgeusia were more likely to be associated with concurrent nausea or loss of appetite (16.9 *vs* 6.5%, $P = 0.006$).

GI symptoms generally occur with modest frequency compared with respiratory symptoms and fever; however, there are a small number of patients who present with GI symptoms as the only manifestation of disease[58]. Among these patients with isolated symptoms, there is frequently a more delayed hospital presentation compared to respiratory symptoms (9.0 d *vs* 7.3 d)[59]. The American Gastroenterological Association (AGA) has recommended COVID-19 testing in patients with new onset GI symptoms as these may precede pulmonary symptoms[60]. Several studies have attempted to correlate GI symptoms with severity of disease and mortality with mixed results. A recent United States-based case-control series of 150 patients with GI symptoms did not demonstrate increased mortality, intubation, or hospital length of stay compared with controls who lacked GI symptoms[61]. This is in contrast to some initial series from China which linked digestive symptoms to longer length of stay [62]. Further investigation into this area is needed.

Table 2 Gastrointestinal manifestations in coronavirus disease 2019

Ref.	Number of subjects	Diarrhea	Nausea	Vomiting[1]	Abdominal pain
Lin *et al*[35], 2020	95	24%	18%	4%	2%
Wong *et al*[56], 2020	2230	2%-36%	1%-17%	1%-6%	2%-6%
Tariq *et al*[52], 2020	12797	12%	9%[1]	9%[1]	6%

[1]Study presented "nausea and/or vomiting" as one statistic.

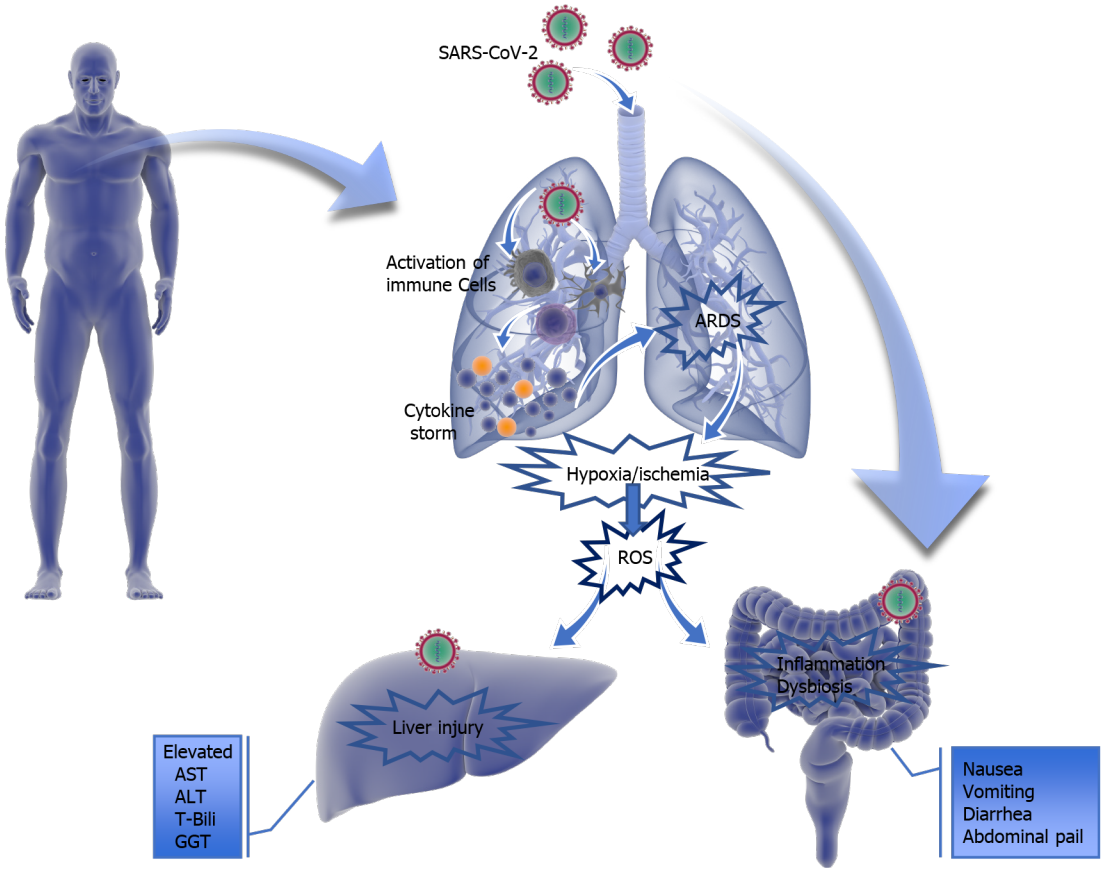

Figure 2 Overview of severe acute respiratory syndrome coronavirus 2 affecting multiple body systems directly or indirectly. Library of Science & Medical Illustrations were utilized in part to create BioNTech this figure. https://creativecommons.org/licenses/by-nc-sa/4.0/.

While critically ill patients still experience many of the same GI symptoms including diarrhea and vomiting, more severe complications noted have included bowel ischemia (3.8%), ileus (55.8%), and Ogilvie-like syndrome (1.9%), as demonstrated in one series of 141 COVID ICU patients(44). A recent review compared GI complications between critically ill patients with COVID and those with non-COVID ARDS and found that the COVID cohort developed more GI complications (74% *vs* 37%, $P < 0.001$)[63]. While many symptoms have been identified, data describing the significance of the GI symptoms in predicting disease course and outcomes has been limited, variable, and sometimes contradictory.

COVID-19 AND LIVER MANIFESTATIONS

COVID-19 infection has been shown to directly affect the liver and cause laboratory abnormalities *via* previously described mechanisms based on abundant ACE2 receptors found on hepatocytes and cholangiocytes to directly enter cells and cause significant liver dysfunction and injury[55,63]. Hospitals in China reported abnormal liver enzymes in approximately 14%-76% of cases hospitalized for COVID-19[64,65].

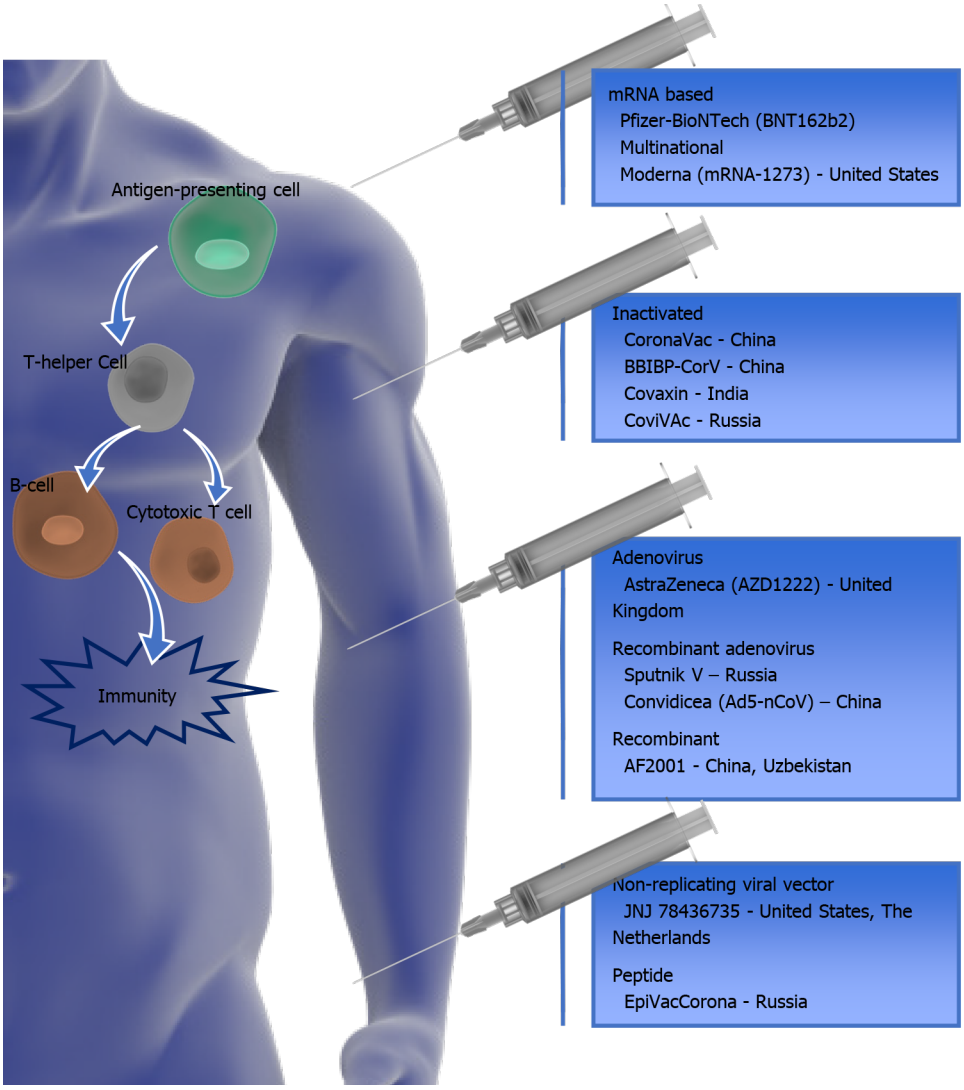

Figure 3 Summary of authorized/approved coronavirus disease 2019 vaccines around the world and their mechanism. The immune response starts by antigen-presenting cells engulfing the virus and activating T-helper cells. These T-helper cells enable an immune response *via* B cells (antibodies) and Cytotoxic T cells to destroy virus-infected cells. Library of Science & Medical Illustrations were utilized in part to create this figure. https://creativecommons.org/licenses/by-nc-sa/4.0/.

According to a systematic review and meta-analysis of 47 studies with 10890 total patients with COVID-19, approximately 15% to 20% were identified to have abnormal liver enzymes with a higher prevalence identified in studies performed outside of China (Table 3)[60].

Hospitalized patients found to have abnormal liver enzymes, may have also shown a higher likelihood of developing severe disease, as well as increased risk of intensive care admission and death[57]. This lack of data has recently prompted the AGA to recommend obtaining baseline liver enzymes and consider monitoring liver enzymes in patients throughout the course of their infection[60].

Treatments for COVID-19 have been associated with elevated liver enzymes and subsequent injury, most notably with remdesivir use. Remdesivir use in early trials and series was associated with 10%-50% of patients developing transient, mild to moderate (< 5 times upper limit or normal) elevations in AST and ALT within 5 da of therapy. Nine percent of patients in reported trials showed at least moderate elevations, but resolved with discontinuation and were not associated with clinically significant injury. Pharmacology guidelines recommend close monitoring of liver enzymes and early discontinuation of infusions if elevations rise > 10 times the upper limit of normal[66,67]. Dexamethasone remains a treatment for severe COVID-19 infection. It should be acknowledged that prolonged use of corticosteroid therapy can cause hepatic steatosis as well as increase the risk of developing reactivation of latent infections, such as viral hepatitis B.

Table 3 Liver manifestations in coronavirus disease 2019

Ref.	Number of subjects	Abnormal LFTs (any)	ALT	AST	Tbili
Lin *et al*[35], 2020	95	-	5%	4%	23%
Wang *et al*[38], 2020	105	56%	16%	9%	2%
Fan *et al*[39], 2020	148	37%	18%	22%	6%
Zhang *et al*[63], 2020	1628	14%-53%	-	-	-
Cai *et al*[65], 2020	417	76%	-	-	-
Sultan *et al*[60], 2020	10890	-	15%	15%	17%

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

COVID-19 AND IMMUNOSUPPRESSED STATES

Corticosteroids, immunomodulators (thiopurines, methotrexate) and biologic therapies (such as anti-TNF agents) are frequently used to manage inflammatory bowel disease (IBD), liver transplant recipients and patients with autoimmune hepatitis[68]. These medications block the intracellular signals necessary for host immunity and are associated with high rates of viral and bacterial infections including pneumonia[69,70]. Therefore, it is plausible that immunosuppressed patients would increase the risk of infection with SARS-CoV-2. However, it is also plausible that these medications reduce mortality in COVID-19 by blocking the cytokine storm of SARS-CoV-2[71]. Hence, how a gastroenterologist should handle immunosuppressive therapies in patients with suspected or confirmed COVID-19 is a challenging clinical question.

The SECURE-IBD (Surveillance Epidemiology of Coronavirus Under Research Exclusion – IBD) database consists of reported cases of COVID-19 in IBD patients[72]. The data suggests that the prevalence of severe COVID-19 is low in patients on immunomodulator therapy and biologic therapy; 25% needed hospitalization on immunomodulator monotherapy whereas 19% needed hospitalization while on anti-TNF agents[73]. Fortunately, mortality rates were low with 2% in the immunomodulator monotherapy group and 1% in the anti-TNF cohort[72]. Thus, the AGA recommends that IBD therapies be continued with a goal of maintaining remission and adjustment being made as necessary[74].

Similar to SECURE-IBD, SECURE-Cirrhosis (Surveillance Epidemiology of Coronavirus Under Research Exclusion - Cirrhosis) is a registry for all COVID-19 cases in patients with chronic liver disease as well as liver transplant recipients[75]. The data from this registry suggests that patients with chronic liver disease but without cirrhosis have a similar risk of mortality from COVID-19 as patients without liver disease. However, patients with cirrhosis have an increased risk with mortality of 32%[75,76]. The data from the registry also suggests that liver transplantation was not associated with an increase in mortality with SARS-CoV-2 infection[77]. The American Association For The Study Of Liver Diseases (AASLD) recommends that anticipatory changes in immunosuppressive regimen should not be made for post-transplant patients and autoimmune patients without COVID-19[78]. In patients on immunosuppression, AASLD recommends lowering the dosages based on the general principles to manage infections in these patients[78].

In summary, the current data does not suggest that patients on immunomodulators have an increased risk of mortality from COVID-19. The current guidelines from AGA and AASLD do not recommend pre-emptive changes in patients on immunosuppression if the patients have not been infected with COVID-19. However, the dosages may be adjusted in patients with COVID-19 on the basis of general principles[74,78].

CONCLUSION

The WHO and CDC have developed ongoing recommendations to be followed during the COVID-19 pandemic. The mechanism of injury and cascade of events due to COVID-19 (Figure 2) have been studied in great detail. These have helped develop targets for therapy and vaccines. The current literature does not reveal that immunosuppressed patients are at higher risk of COVID-19 infection[74,78]. However,

the impact of the COVID-19 vaccine on specific organs such as liver and GI tract are still uncertain. Further research is necessary to evaluate the long-term effects of the vaccine in relation to GI tract. Despite lack of long-term data, patients and physicians are encouraged to get vaccinated as universal vaccination is of great societal and global benefit.

The pandemic has prompted a change in structure and shape of gastroenterology departmental activities. 27% of the centers in the United States and Canada had implemented routine endotracheal intubation for upper endoscopic procedures[79]. The reshaping has been aimed to address urgent and emergent needs of the community and decreasing patient exposures in the hospital. Most of the practices had altered coverage schedule for the physicians. Strict protective measures during endoscopic procedures such as gowns, gloves, face shields, N95 masks, hairnets, double gloves, shoe covers, have also been implemented[79]. The patients are screened at arrival for symptoms and exposures. During the pandemic, only highly urgent endoscopic procedures are being performed on COVID-19 patients[80]. Endoscopy should be performed only when necessary and in a negative pressure flow room for COVID-19 patients if such a room is available. Where a negative pressure flow room for COVID-19 is not possible, strict sanitation measures are recommended[80].

Online consultations in the form of telehealth services and home drug deliveries have been important. Virtual clinics have been started by majority of the institutions as chronic digestive diseases can be managed *via* online consultations. Approximately 96% (70/73) of the practices had adopted telehealth as revealed in the survey of 62 U.S and 11 Canadian Centers in May 2020[80].

The evaluation of inpatients should be judicious to prevent unnecessary exposure of patients, hospital personnel to COVID-19. Clearly, society as well as healthcare delivery will continue to evolve and adapt for this and future crisis.

REFERENCES

1 **World Health Organization**. Dashboard WCD. [cited 10 March 2014]. Available from: https://covid19.who.int

2 **Andersen KG**, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. *Nat Med* 2020; **26**: 450-452 [PMID: 32284615 DOI: 10.1038/s41591-020-0820-9]

3 **Rasmussen AL**. On the origins of SARS-CoV-2. *Nat Med* 2021; **27**: 9 [PMID: 33442004 DOI: 10.1038/s41591-020-01205-5]

4 **Zhou P**, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579**: 270-273 [PMID: 32015507 DOI: 10.1038/s41586-020-2012-7]

5 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

6 **Coronaviridae Study Group of the International Committee on Taxonomy of Viruses**. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020; **5**: 536-544 [PMID: 32123347 DOI: 10.1038/s41564-020-0695-z]

7 **Spychalski P**, Błażyńska-Spychalska A, Kobiela J. Estimating case fatality rates of COVID-19. *Lancet Infect Dis* 2020; **20**: 774-775 [PMID: 32243815 DOI: 10.1016/S1473-3099(20)30246-2]

8 **Liang W**, Liang H, Ou L, Chen B, Chen A, Li C, Li Y, Guan W, Sang L, Lu J, Xu Y, Chen G, Guo H, Guo J, Chen Z, Zhao Y, Li S, Zhang N, Zhong N, He J; China Medical Treatment Expert Group for COVID-19. Development and Validation of a Clinical Risk Score to Predict the Occurrence of Critical Illness in Hospitalized Patients With COVID-19. *JAMA Intern Med* 2020; **180**: 1081-1089 [PMID: 32396163 DOI: 10.1001/jamainternmed.2020.2033]

9 **Rustgi V**, Makar M, Minacapelli CD, Gupta K, Bhurwal A, Li Y, Catalano C, Panettieri R. In-Hospital Mortality and Prediction in an Urban U.S. Population With COVID-19. *Cureus* 2020; **12**: e11786 [PMID: 33409033 DOI: 10.7759/cureus.11786]

10 **Lai CC**, Ko WC, Lee PI, Jean SS, Hsueh PR. Extra-respiratory manifestations of COVID-19. *Int J Antimicrob Agents* 2020; **56**: 106024 [PMID: 32450197 DOI: 10.1016/j.ijantimicag.2020.106024]

11 **Gulati A**, Pomeranz C, Qamar Z, Thomas S, Frisch D, George G, Summer R, DeSimone J, Sundaram B. A Comprehensive Review of Manifestations of Novel Coronaviruses in the Context of Deadly COVID-19 Global Pandemic. *Am J Med Sci* 2020; **360**: 5-34 [PMID: 32620220 DOI: 10.1016/j.amjms.2020.05.006]

12 **Makar M**, John Pisano T, Minacapelli CD, Rustgi V. Gastrointestinal Findings in a Patient With COVID-19. *ACG Case Rep J* 2020; **7**: e00392 [PMID: 32637437 DOI: 10.14309/crj.0000000000000392]

13 **Delamater PL**, Street EJ, Leslie TF, Yang YT, Jacobsen KH. Complexity of the Basic Reproduction Number (R_0). *Emerg Infect Dis* 2019; **25**: 1-4 [PMID: 30560777 DOI: 10.3201/eid2501.171901]

14 **Sharma V DU**. Epidemiology of COVID-19: Implications for a Gastroenterologist. *J Digest Endosc* 2020; **11**: 8-12 [DOI: 10.1055/s-0040-1712549]

15 **Dhar Chowdhury S**, Oommen AM. Epidemiology of COVID-19. *J Digest Endosc* 2020; **11**: 3-7 [DOI: 10.1055/s-0040-1712187]

16 **Center of Diease Control and Prevention**. An Introduction to Applied Epidemiology and Biostatistics. Lesson 3: Measures of Risk. In: Principles of Epidemiology in Public Health Practice. 3th ed. U.S. Department of Health & Human Services, 2012

17 **Jing QL**, Liu MJ, Zhang ZB, Fang LQ, Yuan J, Zhang AR, Dean NE, Luo L, Ma MM, Longini I, Kenah E, Lu Y, Ma Y, Jalali N, Yang ZC, Yang Y. Household secondary attack rate of COVID-19 and associated determinants in Guangzhou, China: a retrospective cohort study. *Lancet Infect Dis* 2020; **20**: 1141-1150 [PMID: 32562601 DOI: 10.1016/S1473-3099(20)30471-0]

18 **Wu Z**, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020; **323**: 1239-1242 [PMID: 32091533 DOI: 10.1001/jama.2020.2648]

19 **Centers for Disease Control and Prevention**. COVID-19. Your Health. People with Certain Medical Conditions. [cited 10 March 2014]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html

20 **Stokes EK**, Zambrano LD, Anderson KN, Marder EP, Raz KM, El Burai Felix S, Tie Y, Fullerton KE. Coronavirus Disease 2019 Case Surveillance - United States, January 22-May 30, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 759-765 [PMID: 32555134 DOI: 10.15585/mmwr.mm6924e2]

21 **Galiero R**, Pafundi PC, Simeon V, Rinaldi L, Perrella A, Vetrano E, Caturano A, Alfano M, Beccia D, Nevola R, Marfella R, Sardu C, Coppola C, Scarano F, Maggi P, De Lucia Sposito P, Vocciante L, Rescigno C, Sbreglia C, Fraganza F, Parrella R, Romano A, Calabria G, Polverino B, Pagano A, Bologna C, Amitrano M, Esposito V, Coppola N, Maturo N, Adinolfi LE, Chiodini P, Sasso FC; COVOCA Study Group. Impact of chronic liver disease upon admission on COVID-19 in-hospital mortality: Findings from COVOCA study. *PLoS One* 2020; **15**: e0243700 [PMID: 33301529 DOI: 10.1371/journal.pone.0243700]

22 **Luxenburger H**, Sturm L, Biever P, Rieg S, Duerschmied D, Schultheiss M, Neumann-Haefelin C, Thimme R, Bettinger D. Treatment with proton pump inhibitors increases the risk of secondary infections and ARDS in hospitalized patients with COVID-19: coincidence or underestimated risk factor? *J Intern Med* 2021; **289**: 121-124 [PMID: 32608546 DOI: 10.1111/joim.13121]

23 **Corsonello A**, Lattanzio F, Bustacchini S, Garasto S, Cozza A, Schepisi R, Lenci F, Luciani F, Maggio MG, Ticinesi A, Butto V, Tagliaferri S, Corica F. Adverse Events of Proton Pump Inhibitors: Potential Mechanisms. *Curr Drug Metab* 2018; **19**: 142-154 [PMID: 29219052 DOI: 10.2174/1389200219666171207125351]

24 **Namazi MR**, Jowkar F. A succinct review of the general and immunological pharmacologic effects of proton pump inhibitors. *J Clin Pharm Ther* 2008; **33**: 215-217 [PMID: 18452407 DOI: 10.1111/j.1365-2710.2008.00907.x]

25 **Peckham H**, de Gruijter NM, Raine C, Radziszewska A, Ciurtin C, Wedderburn LR, Rosser EC, Webb K, Deakin CT. Male sex identified by global COVID-19 meta-analysis as a risk factor for death and ITU admission. *Nat Commun* 2020; **11**: 6317 [PMID: 33298944 DOI: 10.1038/s41467-020-19741-6]

26 **Bulut C**, Kato Y. Epidemiology of COVID-19. *Turk J Med Sci* 2020; **50**: 563-570 [PMID: 32299206 DOI: 10.3906/sag-2004-172]

27 **Wrobel AG**, Benton DJ, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ. SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects. *Nat Struct Mol Biol* 2020; **27**: 763-767 [PMID: 32647346 DOI: 10.1038/s41594-020-0468-7]

28 **Trypsteen W**, Van Cleemput J, Snippenberg WV, Gerlo S, Vandekerckhove L. On the whereabouts of SARS-CoV-2 in the human body: A systematic review. *PLoS Pathog* 2020; **16**: e1009037 [PMID: 33125439 DOI: 10.1371/journal.ppat.1009037]

29 **Xu ZS**, Shu T, Kang L, Wu D, Zhou X, Liao BW, Sun XL, Wang YY. Temporal profiling of plasma cytokines, chemokines and growth factors from mild, severe and fatal COVID-19 patients. *Signal Transduct Target Ther* 2020; **5**: 100 [PMID: 32561706 DOI: 10.1038/s41392-020-0211-1]

30 **Bansal V**, Mahapure KS, Bhurwal A, Gupta I, Hassanain S, Makadia J, Madas N, Armaly P, Singh R, Mehra I, O'Horo JC, Kashyap R. Mortality Benefit of Remdesivir in COVID-19: A Systematic Review and Meta-Analysis. *Front Med (Lausanne)* 2020; **7**: 606429 [PMID: 33585508 DOI: 10.3389/fmed.2020.606429]

31 **Janiaud P**, Axfors C, Schmitt AM, Gloy V, Ebrahimi F, Hepprich M, Smith ER, Haber NA, Khanna N, Moher D, Goodman SN, Ioannidis JPA, Hemkens LG. Association of Convalescent Plasma Treatment With Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis. *JAMA* 2021; **325**: 1185-1195 [PMID: 33635310 DOI: 10.1001/jama.2021.2747]

32 **Ghazy RM**, Almaghraby A, Shaaban R, Kamal A, Beshir H, Moursi A, Ramadan A, Taha SHN. A systematic review and meta-analysis on chloroquine and hydroxychloroquine as monotherapy or combined with azithromycin in COVID-19 treatment. *Sci Rep* 2020; **10**: 22139 [PMID: 33335141 DOI: 10.1038/s41598-020-77748-x]

33 **Forni G**, Mantovani A; COVID-19 Commission of Accademia Nazionale dei Lincei, Rome. COVID-

19 vaccines: where we stand and challenges ahead. *Cell Death Differ* 2021; **28**: 626-639 [PMID: 33479399 DOI: 10.1038/s41418-020-00720-9]

34 **Deplanque D**, Launay O. Efficacy of COVID-19 vaccines: From clinical trials to real life. *Therapie* 2021; **76**: 277-283 [PMID: 34049688 DOI: 10.1016/j.therap.2021.05.004]

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

36 **Han C**, Duan C, Zhang S, Spiegel B, Shi H, Wang W, Zhang L, Lin R, Liu J, Ding Z, Hou X. Digestive Symptoms in COVID-19 Patients With Mild Disease Severity: Clinical Presentation, Stool Viral RNA Testing, and Outcomes. *Am J Gastroenterol* 2020; **115**: 916-923 [PMID: 32301761 DOI: 10.14309/ajg.0000000000000664]

37 **Lamers MM**, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, Paul van Schayck J, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schipper D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. SARS-CoV-2 productively infects human gut enterocytes. *Science* 2020; **369**: 50-54 [PMID: 32358202 DOI: 10.1126/science.abc1669]

38 **Wang Q**, Zhao H, Liu LG, Wang YB, Zhang T, Li MH, Xu YL, Gao GJ, Xiong HF, Fan Y, Cao Y, Ding R, Wang JJ, Cheng C, Xie W. Pattern of liver injury in adult patients with COVID-19: a retrospective analysis of 105 patients. *Mil Med Res* 2020; **7**: 28 [PMID: 32507110 DOI: 10.1186/s40779-020-00256-6]

39 **Fan Z**, Chen L, Li J, Cheng X, Yang J, Tian C, Zhang Y, Huang S, Liu Z, Cheng J. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; **18**: 1561-1566 [PMID: 32283325 DOI: 10.1016/j.cgh.2020.04.002]

40 **Cheung CCL**, Goh D, Lim X, Tien TZ, Lim JCT, Lee JN, Tan B, Tay ZEA, Wan WY, Chen EX, Nerurkar SN, Loong S, Cheow PC, Chan CY, Koh YX, Tan TT, Kalimuddin S, Tai WMD, Ng JL, Low JG, Yeong J, Lim KH. Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from five recovered patients with COVID-19. *Gut* 2021 [PMID: 34083386 DOI: 10.1136/gutjnl-2021-324280]

41 **Tian D**, Ye Q. Hepatic complications of COVID-19 and its treatment. *J Med Virol* 2020; **92**: 1818-1824 [PMID: 32437004 DOI: 10.1002/jmv.26036]

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

43 **Song M**, Li ZL, Zhou YJ, Tian G, Ye T, Zeng ZR, Deng J, Wan H, Li Q, Liu JB. Gastrointestinal involvement of COVID-19 and potential faecal transmission of SARS-CoV-2. *J Zhejiang Univ Sci B* 2020; **21**: 749-751 [PMID: 32893532 DOI: 10.1631/jzus.B2000253]

44 **Kaafarani HMA**, El Moheb M, Hwabejire JO, Naar L, Christensen MA, Breen K, Gaitanidis A, Alser O, Mashbari H, Bankhead-Kendall B, Mokhtari A, Maurer L, Kapoen C, Langeveld K, El Hechi MW, Lee J, Mendoza AE, Saillant NN, Parks J, Fawley J, King DR, Fagenholz PJ, Velmahos GC. Gastrointestinal Complications in Critically Ill Patients With COVID-19. *Ann Surg* 2020; **272**: e61-e62 [PMID: 32675498 DOI: 10.1097/SLA.0000000000004004]

45 **Hu LL**, Wang WJ, Zhu QJ, Yang L. [Novel coronavirus pneumonia-related liver injury: etiological analysis and treatment strategy]. *Zhonghua Gan Zang Bing Za Zhi* 2020; **28**: 97-99 [PMID: 32075364 DOI: 10.3760/cma.j.issn.1007-3418.2020.02.001]

46 **Yang L**, Wang W, Wang X, Zhao J, Xiao L, Gui W, Fan H, Xia J, Li Z, Yan J, Alasbahi A, Zhu Q, Hou X. Creg in Hepatocytes Ameliorates Liver Ischemia/Reperfusion Injury in a TAK1-Dependent Manner in Mice. *Hepatology* 2019; **69**: 294-313 [PMID: 30076625 DOI: 10.1002/hep.30203]

47 **Keshavarz P**, Rafiee F, Kavandi H, Goudarzi S, Heidari F, Gholamrezanezhad A. Ischemic gastrointestinal complications of COVID-19: a systematic review on imaging presentation. *Clin Imaging* 2021; **73**: 86-95 [PMID: 33341452 DOI: 10.1016/j.clinimag.2020.11.054]

48 **Connors JM**, Levy JH. COVID-19 and its implications for thrombosis and anticoagulation. *Blood* 2020; **135**: 2033-2040 [PMID: 32339221 DOI: 10.1182/blood.2020006000]

49 **Zhao JN**, Fan Y, Wu SD. Liver injury in COVID-19: A minireview. *World J Clin Cases* 2020; **8**: 4303-4310 [PMID: 33083389 DOI: 10.12998/wjcc.v8.i19.4303]

50 **Zhang XJ**, Cheng X, Yan ZZ, Fang J, Wang X, Wang W, Liu ZY, Shen LJ, Zhang P, Wang PX, Liao R, Ji YX, Wang JY, Tian S, Zhu XY, Zhang Y, Tian RF, Wang L, Ma XL, Huang Z, She ZG, Li H. An ALOX12-12-HETE-GPR31 signaling axis is a key mediator of hepatic ischemia-reperfusion injury. *Nat Med* 2018; **24**: 73-83 [PMID: 29227475 DOI: 10.1038/nm.4451]

51 **Cha MH**, Regueiro M, Sandhu DS. Gastrointestinal and hepatic manifestations of COVID-19: A comprehensive review. *World J Gastroenterol* 2020; **26**: 2323-2332 [PMID: 32476796 DOI: 10.3748/wjg.v26.i19.2323]

52 **Tariq R**, Saha S, Furqan F, Hassett L, Pardi D, Khanna S. Prevalence and Mortality of COVID-19 Patients With Gastrointestinal Symptoms: A Systematic Review and Meta-analysis. *Mayo Clin Proc* 2020; **95**: 1632-1648 [PMID: 32753138 DOI: 10.1016/j.mayocp.2020.06.003]

53 **Carrillo-Larco RM**, Altez-Fernandez C. Anosmia and dysgeusia in COVID-19: A systematic review. *Wellcome Open Res* 2020; **5**: 94 [PMID: 32587902 DOI: 10.12688/wellcomeopenres.15917.1]

54 **Tian Y**, Rong L, Nian W, He Y. Review article: gastrointestinal features in COVID-19 and the

possibility of faecal transmission. *Aliment Pharmacol Ther* 2020; **51**: 843-851 [PMID: 32222988 DOI: 10.1111/apt.15731]

55 **Zhang J**, Wang S, Xue Y. Fecal specimen diagnosis 2019 novel coronavirus-infected pneumonia. *J Med Virol* 2020; **92**: 680-682 [PMID: 32124995 DOI: 10.1002/jmv.25742]

56 **Wong SH**, Lui RN, Sung JJ. Covid-19 and the digestive system. *J Gastroenterol Hepatol* 2020; **35**: 744-748 [PMID: 32215956 DOI: 10.1111/jgh.15047]

57 **Borges do Nascimento IJ**, Cacic N, Abdulazeem HM, von Groote TC, Jayarajah U, Weerasekara I, Esfahani MA, Civile VT, Marusic A, Jeroncic A, Carvas Junior N, Pericic TP, Zakarija-Grkovic I, Meirelles Guimarães SM, Luigi Bragazzi N, Bjorklund M, Sofi-Mahmudi A, Altujjar M, Tian M, Arcani DMC, O'Mathúna DP, Marcolino MS. Novel Coronavirus Infection (COVID-19) in Humans: A Scoping Review and Meta-Analysis. *J Clin Med* 2020; **9**: 941 [PMID: 32235486 DOI: 10.3390/jcm9040941]

58 **Liang W**, Feng Z, Rao S, Xiao C, Xue X, Lin Z, Zhang Q, Qi W. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. *Gut* 2020; **69**: 1141-1143 [PMID: 32102928 DOI: 10.1136/gutjnl-2020-320832]

59 **Patel KP**, Patel PA, Vunnam RR, Hewlett AT, Jain R, Jing R, Vunnam SR. Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. *J Clin Virol* 2020; **128**: 104386 [PMID: 32388469 DOI: 10.1016/j.jcv.2020.104386]

60 **Sultan S**, Altayar O, Siddique SM, Davitkov P, Feuerstein JD, Lim JK, Falck-Ytter Y, El-Serag HB; AGA Institute. AGA Institute Rapid Review of the Gastrointestinal and Liver Manifestations of COVID-19, Meta-Analysis of International Data, and Recommendations for the Consultative Management of Patients with COVID-19. *Gastroenterology* 2020; **159**: 320-334.e27 [PMID: 32407808 DOI: 10.1053/j.gastro.2020.05.001]

61 **Ramachandran P**, Onukogu I, Ghanta S, Gajendran M, Perisetti A, Goyal H, Aggarwal A. Gastrointestinal Symptoms and Outcomes in Hospitalized Coronavirus Disease 2019 Patients. *Dig Dis* 2020; **38**: 373-379 [PMID: 32599601 DOI: 10.1159/000509774]

62 **El Moheb M**, Naar L, Christensen MA, Kapoen C, Maurer LR, Farhat M, Kaafarani HMA. Gastrointestinal Complications in Critically Ill Patients With and Without COVID-19. *JAMA* 2020; **324**: 1899-1901 [PMID: 32970139 DOI: 10.1001/jama.2020.19400]

63 **Zhang C**, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020; **5**: 428-430 [PMID: 32145190 DOI: 10.1016/S2468-1253(20)30057-1]

64 **Xu L**, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. *Liver Int* 2020; **40**: 998-1004 [PMID: 32170806 DOI: 10.1111/liv.14435]

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

66 **Agarwal A**, Chen A, Ravindran N, To C, Thuluvath PJ. Gastrointestinal and Liver Manifestations of COVID-19. *J Clin Exp Hepatol* 2020; **10**: 263-265 [PMID: 32405183 DOI: 10.1016/j.jceh.2020.03.001]

67 LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases, 2012 [PMID: 31643176]

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

69 **Lichtenstein GR**, Loftus EV, Isaacs KL, Regueiro MD, Gerson LB, Sands BE. ACG Clinical Guideline: Management of Crohn's Disease in Adults. *Am J Gastroenterol* 2018; **113**: 481-517 [PMID: 29610508 DOI: 10.1038/ajg.2018.27]

70 **Kirchgesner J**, Lemaitre M, Carrat F, Zureik M, Carbonnel F, Dray-Spira R. Risk of Serious and Opportunistic Infections Associated With Treatment of Inflammatory Bowel Diseases. *Gastroenterology* 2018; **155**: 337-346.e10 [PMID: 29655835 DOI: 10.1053/j.gastro.2018.04.012]

71 **Long MD**, Martin C, Sandler RS, Kappelman MD. Increased risk of pneumonia among patients with inflammatory bowel disease. *Am J Gastroenterol* 2013; **108**: 240-248 [PMID: 23295276 DOI: 10.1038/ajg.2012.406]

72 **Current Data**. Secure-IBD database. [cited 10 January 2021]. Available from: https://covidibd.org/current-data/

73 **Maude SL**, Barrett D, Teachey DT, Grupp SA. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *Cancer J* 2014; **20**: 119-122 [PMID: 24667956 DOI: 10.1097/PPO.0000000000000035]

74 **Rubin DT**, Feuerstein JD, Wang AY, Cohen RD. AGA Clinical Practice Update on Management of Inflammatory Bowel Disease During the COVID-19 Pandemic: Expert Commentary. *Gastroenterology* 2020; **159**: 350-357 [PMID: 32283100 DOI: 10.1053/j.gastro.2020.04.012]

75 **SECURE-Liver Registry**. SECURE-Cirrhosis. [cited 10 January 2021]. Available from: https://covidcirrhosis.web.unc.edu/

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

77 **Webb GJ**, Marjot T, Cook JA, Aloman C, Armstrong MJ, Brenner EJ, Catana MA, Cargill T,

Dhanasekaran R, García-Juárez I, Hagström H, Kennedy JM, Marshall A, Masson S, Mercer CJ, Perumalswami PV, Ruiz I, Thaker S, Ufere NN, Barnes E, Barritt AS 4th, Moon AM. Outcomes following SARS-CoV-2 infection in liver transplant recipients: an international registry study. *Lancet Gastroenterol Hepatol* 2020; **5**: 1008-1016 [PMID: 32866433 DOI: 10.1016/S2468-1253(20)30271-5]

78 **Fix OK**, Hameed B, Fontana RJ, Kwok RM, McGuire BM, Mulligan DC, Pratt DS, Russo MW, Schilsky ML, Verna EC, Loomba R, Cohen DE, Bezerra JA, Reddy KR, Chung RT. Clinical Best Practice Advice for Hepatology and Liver Transplant Providers During the COVID-19 Pandemic: AASLD Expert Panel Consensus Statement. *Hepatology* 2020; **72**: 287-304 [PMID: 32298473 DOI: 10.1002/hep.31281]

79 **Forbes N**, Smith ZL, Spitzer RL, Keswani RN, Wani SB, Elmunzer BJ; North American Alliance for the Study of Digestive Manifestations of COVID-19. Changes in Gastroenterology and Endoscopy Practices in Response to the Coronavirus Disease 2019 Pandemic: Results From a North American Survey. *Gastroenterology* 2020; **159**: 772-774.e13 [PMID: 32376410 DOI: 10.1053/j.gastro.2020.04.071]

80 **Paramasivan P**. Infection Control Practices in Endoscopy Room in COVID-19 Era. *J Digest Endosc* 2020; 11: 67-68 [DOI: 10.1055/s-0040-1712599]

World Journal of Gastroenterology

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World J Gastroenterol 2021 December 14; 27(46): 7982-7994

DOI: 10.3748/wjg.v27.i46.7982

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Recombinant protein *Schistosoma japonicum*-derived molecule attenuates dextran sulfate sodium-induced colitis by inhibiting miRNA-217-5p to alleviate apoptosis

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Author contributions: Sun X contributed to the experiment design; Wu ZD, Yang RB, and Chen F contributed to the experiment implementation; Liu JH and Hu YY analyzed the data; Zhang LC and Wang LF conceived the experiments, performed the experiments, and wrote the manuscript; all authors participated in critical revision of the manuscript and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Sun Yat-sen University Institutional Review Board.(Approval No. SYSU-IACUC-2019-B517).

Conflict-of-interest statement: The authors declare no competing interests.

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Abstract

BACKGROUND

Inflammatory bowel disease (IBD) affects millions of people worldwide and has emerged as a growing problem in industrialized nations. The lack of therapeutic targets has limited the treatment of IBD. Studies found that parasitic nematode infections can ameliorate clinical and experimental colitis. Our previous study found that rSj16, a 16-kDa secreted protein of *Schistosoma japonicum* produced by *Escherichia coli*, has protective effects on dextran sulfate sodium (DSS)-induced colitis in mice. Apoptosis is an important factor in the pathogenesis of colitis. However, it is not clear whether the effect of rSj16 on colitis is related to apoptosis.

AIM

To investigate whether the protective effects of rSj16 on colitis is related to apoptosis and its mechanism.

METHODS

In-vivo, colitis was induced by DSS. The severity of colitis was assessed. WB was used to detect the changes of apoptosis-related genes in colon tissues. Q-PCR was

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: I have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Supported by the National Natural Science Foundation of China, No. 81902081 and No. 81871682; the Natural Science Foundation of Guangdong Province, No. 2020A1515011573 and No. 2019A1515012068; China Postdoctoral Science Foundation, No. 2018M640858 and No. 2019T120771; Fundamental Research Funds for the Central Universities, No. 19ykpy170, No. 17ykpy09 and No. 19ykpy29; National Science and Technology Major Project, No. 2018ZX10101002-001; the 111 Project, No. B12003; and the Natural Science Foundation of Guangdong Province, No. 2021A1515010976.

Country/Territory of origin: China

Specialty type: Medicine, research and experimental

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

used to detect the changes of miRNA-217-5p and HNF1B. *In-vitro*, WB was used to detect the changes of apoptosis-related genes in intestinal epithelial cells. TUNNEL staining and flow cytometry were used to detect cell apoptosis.

RESULTS

rSj16 attenuates clinical activity in DSS-induced colitis mice. TUNNEL staining and WB results showed that apoptosis was increased in colon tissue after treatment with DSS, and the apoptosis of colon tissue was significantly reduced after treatment with rSj16. Compared with normal mice, the expression of miR-217-5p was increased in colon tissue of DSS-induced colitis mice. In addition, the miR-217-5p target gene *hnf1b* was decreased after administration of DSS. After treatment with rSj16, the expression of miR-217-5p was decreased and the expression of HNF1B was increased compared with the DSS-treated group. When Etoposide was used in combination with miR-217-5p mimic on MODE-K cells, the expression of cleaved-Caspase-3 and Bax was increased, and Bcl-2 was decreased compared with only Etoposide treatment, the expression of HNF1B was significantly reduced, suggesting that miR-217-5p acts as a pro-apoptotic in colon epithelial cells and down-regulates the target gene *hnf1b*. After rSj16 administration in MODE-K cells, miR-217-5p expression was significantly decreased, HNF1B expression was increased, and apoptosis was reduced.

CONCLUSION

The protective effects of rSj16 on colitis is related to apoptosis and miRNA-217-5p may be a further target for therapeutic intervention against IBD.

Key Words: *Schistosoma japonicum*; rSj16; Inflammatory bowel disease; Apoptosis; miRNA-217-5p

Core Tip: The lack of therapeutic targets has limited the treatment of inflammatory bowel disease (IBD). Parasitic nematode infections can ameliorate clinical and experimental colitis. Our previous study found that rSj16, a 16-kDa secreted protein of *Schistosoma japonicum* produced by Escherichia coli, has protective effects on dextran sulfate sodium (DSS)-induced colitis in mice. We found that rSj16 can inhibit DSS-induced apoptosis in the colons of mice with colitis. In addition, we found that the inhibitory effect of rSj16 on apoptosis was associated with decreased miR-217-5p, and that hepatocyte nuclear factor 1 beta was increased after treatment with rSj16. These results highlight a novel therapeutic target that may be used to treat IBD.

Citation: Zhang LC, Wu XY, Yang RB, Chen F, Liu JH, Hu YY, Wu ZD, Wang LF, Sun X. Recombinant protein *Schistosoma japonicum*-derived molecule attenuates dextran sulfate sodium-induced colitis by inhibiting miRNA-217-5p to alleviate apoptosis. *World J Gastroenterol* 2021; 27(46): 7982-7994
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7982.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7982

INTRODUCTION

Inflammatory bowel disease (IBD) affects millions of people worldwide and has emerged as a growing problem in industrialized nations[1]. The two distinct forms of IBD, ulcerative colitis and Crohn's disease, are characterized by intermittent, chronic or progressive inflammation[2]. The etiologies of both forms are multifactorial, including immunoregulatory factors, genetic susceptibility, environmental changes, and abnormalities of gut microbiota. Traditional treatments for IBD include 5-aminosalicylic acid agents, steroids, and antimicrobials. However, as these drugs have limitations and many patients cannot achieve remission, a research focus in this field is to devise biological therapies for the treatment of IBD.

Received: June 16, 2021
Peer-review started: June 16, 2021
First decision: June 30, 2021
Revised: July 9, 2021
Accepted: November 29, 2021
Article in press: November 29, 2021
Published online: December 14, 2021

P-Reviewer: Prasetyo EP, Saber A, Yang X
S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

Recent studies have demonstrated that helminth *Schistosoma* can protect against IBD. In a DSS-induced mouse colitis model, an attenuated inflammatory response was found in those infected with *Schistosoma japonicum* (*S. japonicum*)[3]. *Schistosoma mansoni* (*S. mansoni*) egg antigen has a beneficial modulatory effect in a DSS-induced mice colitis model[4]. In adult male mice with colitis, *S. mansoni* infection modulates the colitis mice immune system, suppressing colitis and limiting dysbiosis of intestinal microbiome[5]. Infection with *S. mansoni* also attenuates disease in rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis[6]. Our previous study confirmed that exosomes derived from dendritic cells treated with *S. japonicum* soluble egg antigen attenuate DSS-induced colitis in mice[7]. Furthermore, we have also shown that rSj16 has protective effects on DSS-induced mouse colitis[8].

Apoptosis is an important factor in the pathogenesis of colitis. Abnormal apoptosis of intestinal epithelial cells (IECs) is frequently found in IBD[9,10]. IEC apoptosis results in disruption of intestinal barrier integrity, and may allow the infiltration of bacteria, triggering an inflammatory cascade[11]. Aberrant IEC apoptosis stimulates the production of tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ), both of which further induce IEC apoptosis[12]. MicroRNAs are critical post-transcriptional regulators of gene expression and key mediators of pathophysiology of inflammatory bowel disease (IBD)[13]. However, the molecular basis of IEC apoptosis in the pathogenesis of IBD remains unclear.

MicroRNAs (miRNAs) are small non-coding RNAs that are about 22 nucleotides long. MiRNAs negatively regulate gene expression at the post-transcriptional or translational level by complementary binding to the 3′-untranslated region(UTR). MiRNAs control genes involved in cellular processes such as inflammation, cell-cycle regulation, stress response, differentiation, apoptosis, and migration[14,15]. Studies have shown that miRNAs play an important role in IBD. For example, miR-301a promotes intestinal mucosal inflammation by inducing IL-17A and TNF-α in IBD[16]. MiR-31 is increased in colon tissues of patients with IBD, reduces inflammatory signaling and promotes colon regeneration[17]. Myeloid-derived miR-223 Limits intestinal inflammation by constraining the nlrp3 inflammasome[18]. Upregulation of miR-665 promotes apoptosis and colitis in inflammatory bowel disease by repressing the endoplasmic reticulum stress components XBP1 and ORMDL3[19]. In Shamran's study, miR-217 may induce Sirt-1 and provide protection against intestinal inflammation[20]. The hepatocyte nuclear factor (HNF) superfamily of transcription factors is essential for the development and maintenance of a variety of humans and mice tissues, and is further classified into four families, HNF1, FOXA, HNF4, and ONECUT, based on their functional domains. In gut, HNFs are expressed in IECs, which regulate a variety of physiological functions, including differentiation, barrier function, and metabolism[21]. Hepatic nuclear factor-4α (HNF4α) mRNA level was also downregulated in mouse model of ileitis (SAMP) compared with control mice[22]. Hepatocyte nuclear factor-1beta (HNF1B) is the most important liver-specific transcription factor, with responsibility for sequence-specific DNA binding. HNF1B is reportedly a target of miR-217, with a role in circ-TTBK2- and miR-217-mediated modulation of malignant glioma progression[23].

In this study, we investigate whether the protective effects of rSj16 on colitis is related to apoptosis and its mechanism. miRNA may function through regulating the expression of encoding genes in IBD[16]. We explore the relationship between rSj16, miR-217-5p and IBD, providing theoretical support for the clinical application of rSj16 in the treatment of IBD.

MATERIALS AND METHODS

Animals and ethics

Male BALB/c mice (aged 6 wk, 18-20 g) were purchased from the Experimental Animal Center of Guangdong. All animal experimental procedures were approved by the Medical Research Ethics Committee of Sun Yat-sen University (SYSU-IACUC-2019-B517) and conformed to the Chinese National Institute of Health Guide for the Care and Use of Laboratory Animals.

Induction and treatment of colitis

Recombinant protein (rSj16) was expressed and purified as described previously[8]. A total of 15 mice were randomly assigned to three groups. Acute colitis was induced by administering water with 3% (wt/vol) DSS (36–50 kDa; MP Biomedicals, Illkirch, France) to mice over a period of 7 d. The control mice (n = 5) received drinking water.

Over the same period, rSj16 was administered to the colitis mice (n = 5) *via* intraperitoneal (i.p.) injection (100 μg per mouse) on each day from 1 to 7. Control groups (n = 5) received the same volume of vehicle (phosphate buffered saline; PBS) over the same time frame. The mice were fed standard mouse chow.

Clinical scoring

During treatment, mice were observed daily. Changes in body weight, occurrence of diarrhea and bleeding were recorded. Blood in the feces was determined using a Hemoccult assay kit (Nanjing Jiancheng Bio-engineering Institute, China). A clinical disease score (disease activity index, DAI) was evaluated based on weight loss, diarrhea, and bleeding as described previously[8].

Macroscopic assessment and histologic analysis

Mice were sacrificed on day 7. Colon length was measured, and the macroscopic scores of colons were assessed by an independent observer who was blinded to treatment status[7]. The colons were fixed in 4% formaldehyde and then embedded in paraffin. Colon sections were prepared and stained with hematoxylin and eosin (H & E). Histopathological scores were determined in a blinded fashion, according to the criteria described in our previous study.

Cell culture and treatment

Mouse intestinal epithelial cell line, MODE-K cells were purchased from the BeNa Culture Collection (BNCC, China). The cells were cultured with Dulbecco's modified eagle medium (DMEM) (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.), 100 IU/mL penicillin and 100 mg/mL streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.), incubated in a 5% CO_2 environment at 37 °C. The cells were seeded one day prior to transfection in 12-well cell culture plates. Cells were incubated in a serum-free medium for starvation overnight, then stimulated with miRNA mimic (Assay ID: MIMAT0000679) or mimic control (50 nM, Ruibo, Guangzhou, China) using RNAi MAX (Invitrogen, United States). MiRNA mimics are miRNAs that mimic endogenous miRNAs and can be synthesized by chemical synthesis to enhance the function of endogenous miRNAs.

Flow cytometry

MODE-K cells were seeded in 6-well plates and treated with mimic control, miRNA mimic, miRNA mimic + Etoposide (MedChemExpress, United States, 25 μM)[24], and miRNA mimic + Etoposide + rSj16 (4 μg/mL) for 48 h. Adherent and floating cells were collected and resuspended in 100 μl binding buffer. Each group of cells was stained with 2 μl Annexin-V FITC and propidium iodide (PI, BD Biosciences) at room temperature for 15 min. Samples were analyzed using a CytoFLEX S flow cytometer (Beckman Coulter, United States).

Western blot

MODE-K cells were homogenized with a protein extraction reagent buffer (RIPA; Beyotime Institute of Biotechnology) containing protease and phosphatase inhibitors. Protein concentration was measured using the bicinchoninic acid assay (Beyotime Institute of Biotechnology, China). Equal amounts of proteins were separated by 10% SDS-PAGE and transferred to a polyvinylidene fluoride blotting membrane (GE Healthcare Life Sciences, United Kingdom). The membrane was blocked by 10% milk. The membrane was incubated with a primary antibody (proteintech cleaved-Caspase3, Cat No. 19677-1-AP; Bax, Cat No. 50599-2-Ig; Bcl-2, Cat No. 12789-1-AP; HNF1B, Cat No. 12533-1-AP; GAPDH Sigma-Aldrich G9295) diluted 1:1,000 overnight at 4 °C, followed by incubation with the secondary antibody (ProteinTech Group, Inc.; anti-mouse, cat. no. SA00001-1; anti-rabbit, cat. no. SA00001-2) diluted 1:2000 at room temperature for 2 h. Immunodetection was performed using enhanced chemiluminescence reagent (Thermo Fisher Scientific, Inc.) and visualized using chemiluminescence gel imaging system (Tanon-5200 Multi, Shanghai China). ImageJ (×64) software (National Institutes of Health) was used to quantify the results[25].

TUNEL staining method

MODE-K cells treated with mimic control, miRNA mimic, miRNA mimic + Etoposide, and miRNA mimic + Etoposide + rSj16 were inoculated into 24-well plates for 48 h then fixed with 4% paraformaldehyde for 15 min at room temperature. Diluted TUNEL staining fluid (Beyotime Institute of Biotechnology) was added to cells and colon histological sections according to the manufacturer's instructions. PBS was used

to wash cells, followed by DNA staining with DAPI at room temperature for 10 min, and the staining was observed using a Leica DMI4000B fluorescence microscope (magnification, ×10), positive cells were quantified by Image J software.

RNA extraction and quantitative reverse-transcription PCR

Total RNA was harvested using TRIzol according to manufacturer's instructions, including MODE-K cells and 50 mg mouse colon tissue samples. Complementary DNA (cDNA) was synthesized from 1.0 μg of total RNA with oligo (dT) primers using a cDNA Synthesis Kit (Takara, Japan). The expression of mRNA and miRNA was determined using a SYBR Green Master Mix kit (Takara, Japan), primer sequences are shown in Table 1. GAPDH or U6 were used as an internal control, and the fold change was calculated by the $2^{-\Delta\Delta CT}$ method.

Dual-luciferase reporter assay

HEK 293T cells were transfected using the HNF1B UTR reporter plasmid together with miR-217-5p mimic or control mimic for 48 h using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc). Following this period, cells were lysed using the Dual-Glo® Reagent (Dual-Glo® Luciferase Assay System; Promega Corporation). Renilla luciferase assay substrate and firefly luciferase detection reagent were added and luciferase activities were detected using the Infinite F500 Multimarker Analyser (TECAN, Austria) according to the manufacturer's instructions. Renilla luciferase was used as an internal reference, with luciferase activity normalized to Renilla luciferase activity[26].

Statistical analysis

All data are expressed as means ± SD. Results were compared between the two groups using an unpaired two-sample *t*-test. Multiple comparisons between more than two groups were analysed by one-way ANOVA test or Kruskal–Wallis test (non-parametric). The value of $P < 0.05$ was considered statistically significant.

RESULTS

rSj16 protects against acute DSS-induced colitis

As found in our previous study[8], after DSS administration, the mice lost weight over time. At the same time, the DAI of mice with colitis also increased with time. After treatment with rSj16, body weight loss and DAI were both significantly alleviated in mice with colitis (Figure 1A and B). Colon length was significantly reduced by application of DSS, and was restored after rSj16 treatment (Figure 1C and D). Mean colon macroscopic scores were significantly suppressed in DSS + rSj16 group compared with DSS + PBS group (Figure 1E). Additionally, H&E histopathology results showed that treatment with rSj16 significantly reduced inflammation (Figure 1F). Consistent with this, histopathological scores after treatment with rSj16 + DSS were significantly lower than after treatment with DSS + PBS (Figure 1G and Table 2).

rSj16 inhibit DSS induced apoptosis of colon epithelial cells

IEC apoptosis is increased in affected areas of IBD[27], leading to the disruption of intestinal barrier integrity that may allow bacteria to penetrate into the intestinal wall from the intestinal cavity and trigger an inflammatory cascade, including the production of pro-inflammatory cytokines, to remove the invading bacteria[28,29]. In the present study, to investigate the mechanism by which rSj16 alleviates DSS-induced acute colitis, 3% DSS was administered to mice daily for 7 d, and western blot was performed to detect the apoptosis of colon epithelial cells in mice. As shown in Figure 2A, the pro-apoptotic protein cleaved-Caspase-3 and Bax were increased, while the anti-apoptotic protein Bcl-2 was decreased after treatment with DSS + PBS compared with the Water + PBS, indicating that DSS can induce apoptosis of colon epithelial cells. In addition, pro-apoptotic Bax was decreased and anti-apoptotic protein Bcl-2 was increased after treatment with rSj16 + DSS compared with DSS + PBS. These results demonstrate that rSj16 may significantly inhibit DSS-induced colon epithelial cells apoptosis (Figure 2A). TUNEL staining, indicating apoptosis, was increased in colon tissue and the number of TUNEL positive cells decreased significantly after administration of rSj16 (Figure 2B).

Table 1 Quantitative real time PCR primer sequences

Gene	Forward (5'-3')	Reverse (5'-3')
hnf1b	CCCATCCTCAAAGAGCTCCA	AGAGGTGGGATTGGTTCAGG
GAPDH(Mouse)	ACTCCACTCACGGCAAATTC	TCTCCATGGTGGTGAAGACA
miR-217-5p	UACUGCAUCAGGAACUGACUGGA	mRQ3' Primer (Takara, Kyoto, Japan)
U6	Takara, Kyoto, Japan	Takara, Kyoto, Japan

Table 2 Values of the evaluation indexes

	Body weight loss on day 7 (%) (mean ± SD)	DAI on day 7 (mean ± SD)	Colon length (mean ± SD)	Macroscopic scores (mean ± SD)	Histopathological scores (mean ± SD)	*n*
Water + PBS	125.30 ± 6.30	0.00 ± 0.00	9.70 ± 0.56	0.00 ± 0.00	0.00 ± 0.00	5
DSS + PBS	89.11 ± 8.02	5.20 ± 0.45	6.58 ± 0.48	7.8 ± 1.30	13.00 ± 2.55	5
DSS + rSj16	106.00 ± 5.97	1.40 ± 0.89	8.12 ± 0.35	3.20 ± 0.84	4.4 ± 1.14	5

DSS: Dextran sulfate sodium.

rSj16 inhibits the expression of miR-217-5p in the colon of mice with DSS-induced colitis

Research has shown that down-regulation of miR-217-5p may reduce the apoptosis of cardiomyocyte derived cell lines[30]. We found that, compared with Water-treated mice, the expression of miR-217-5p was increased in colon tissue of mice with DSS-induced colitis. In addition, the miR-217-5p target gene *hnf1b* was decreased after administration of DSS. After treatment with rSj16, the expression of miR-217-5p was decreased and the expression of *hnf1b* was increased compared with the DSS-treated group (Figure 3A and B). Pearson's correlation coefficient analysis showed a negative correlation between miR-217-5p and HNF1B in in colon tissue of mice ($r = -0.3463$, $P < 0.05$) (Figure 3C). Western blot results also indicated that HNF1B was decreased after DSS treatment, and was increased after rSj16 treatment compared with DSS-treated group (Figure 3D and E).

In order to verify whether miR-217-5p regulates the expression of HNF1B, we generated a luciferase reporter plasmid contained 3'- UTR of HNF1B and located on both sides of the binding site of miR-217-5p. Relative luciferase activity of the reporter containing the predicted miR-217-5p binding sites for 3'UTR of HNF1B mRNA transcript was significantly reduced when co-transfected with miR-217-5p mimic compared with a control mimic (Figure 3F). Studies have shown that miRNA-217-5p is closely related to apoptosis[24,31]. Etoposide (an apoptosis inducer) was used to induce the apoptosis of MODE-K, and qPCR results showed that increased *miRNA-217-5p* expression, and decreased miR-217-5p target gene *hnf1b* expression in the process of apoptosis. However, rSj16 may inhibit the expression of miR-217-5p, and increase the expression of *hnf1b* (Figure 3G and H).

rSj16 anti-apoptotic action via regulation of miR-217-5p/HNF1B axis

We further verified the role of miR-217-5p in the process of apoptosis, and the mechanism of rSj16 in regulating apoptosis. Western blot showed that when Etoposide was used in combination with miR-217-5p mimic on MODE-K cells, the expression of cleaved-Caspase-3 and Bax was increased, and Bcl-2 was decreased compared with only Etoposide treatment, and the expression of HNF1B was significantly reduced. These results indicate that miR-217-5p acts as a pro-apoptotic in colon epithelial cells and down-regulates the target gene *hnf1b*. In addition, the expression of cleaved-Caspase-3 and Bax was decreased, while Bcl-2 and HNF1B were increased in mice treated with Etoposide + miR-217-5p + rSj16 compared with Etoposide + miR-217-5p (Figure 4A and B). TUNEL staining of MODE-K after treatment of Etoposide, Etoposide + miR-217-5p, and Etoposide + miR-217-5p + rSj16, showed that the number of TUNEL positive cells increased with Etoposide + miR-217-5p and decreased after

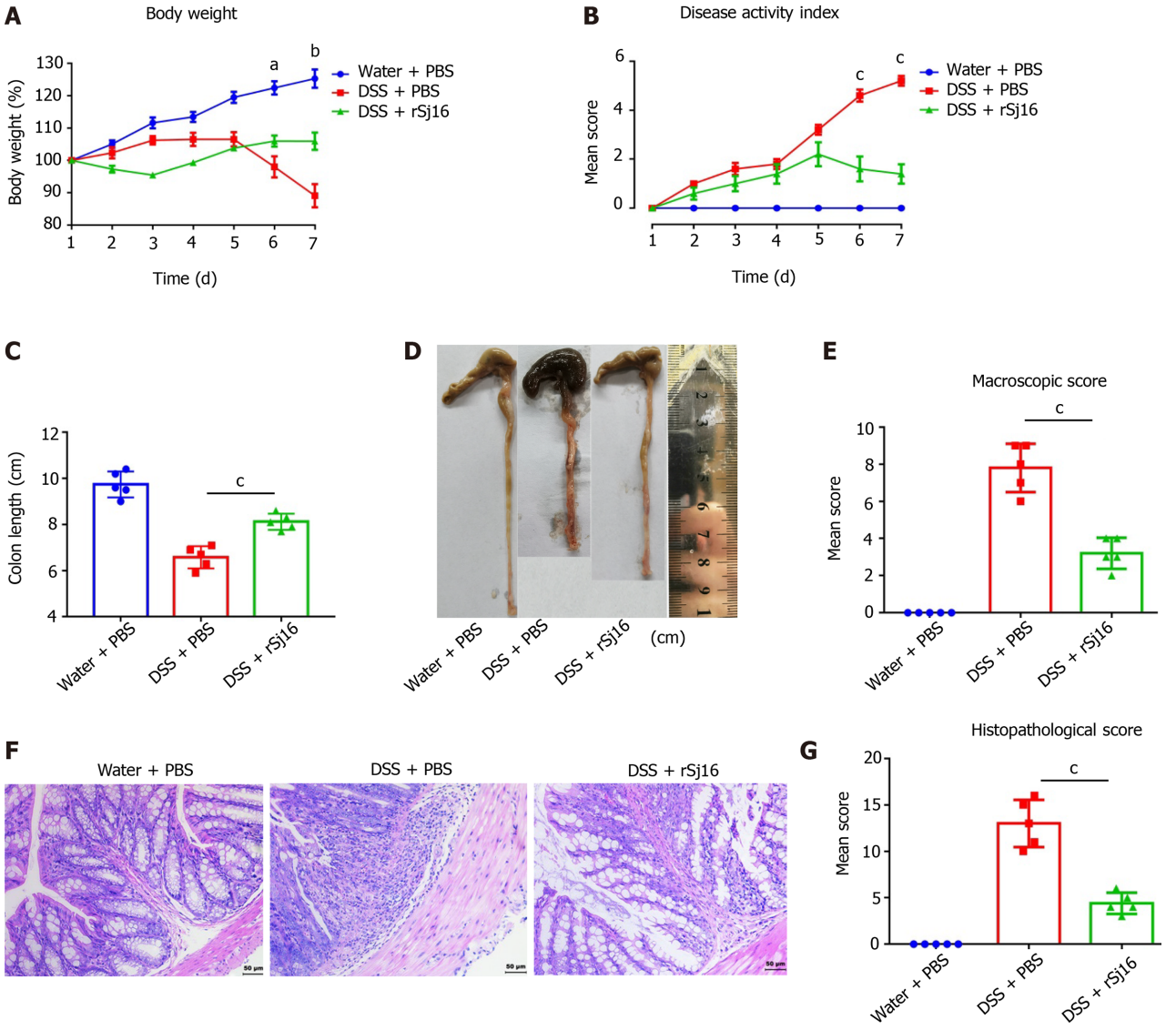

Figure 1 rSj16 protects against acute dextran sulfate sodium-induced colitis. A: Daily changes in body weight [dextran sulfate sodium (DSS) + rSj16 *vs* DSS + PBS]; B: Changes in DAI (DSS + rSj16 *vs* DSS + PBS); C and D: Colon lengths were measured and recorded; E: Macroscopic appearance of the colons; F: The histopathological changes in the colons were examined by H&E staining (20×); G: Histopathological scores of the colons were determined. DSS: Dextran Sulfate Sodium Salt. Statistical analysis was performed using one-way ANOVA. Data are presented as means ± SD; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

treatment with rSj16 (Figure 4C and D). Flow cytometry results also showed that miR-217-5p could obviously promote MODE -K apoptosis. However, rSj16 could significantly inhibit MODE -K apoptosis induced by Etoposide and miR-217-5p. (Figure 4E and F).

DISCUSSION

IBD encompasses Crohn's disease, ulcerative colitis and IBD-unclassified. Although newer treatments have increased the chances of remission, most IBD patients cannot maintain remission, and death is not an infrequent outcome of IBD[32,33]. Therefore, it is very important to explore the pathogenesis of IBD and to find effective therapeutic targets. We have found that rSj16 (a 16-kDa secreted protein of *Schistosoma japonicum*) has protective effects on DSS-induced mouse colitis. Body weight loss was alleviated in mice with colitis after treatment with rSj16. DAI (evaluated based on weight loss, diarrhea, and bleeding) also alleviated in colitis mice after treatment with rSj16. The results of colon length, mean colon macroscopic scores (assessed by hyperemia, wall thickening, ulceration, inflammation extension, and damage), H&E, and histopathological scores (based on extent of inflammation, neutrophil and lympho-histiocyte infiltration, crypt damage, crypt abscess formation, sub-mucosal edema, goblet cell

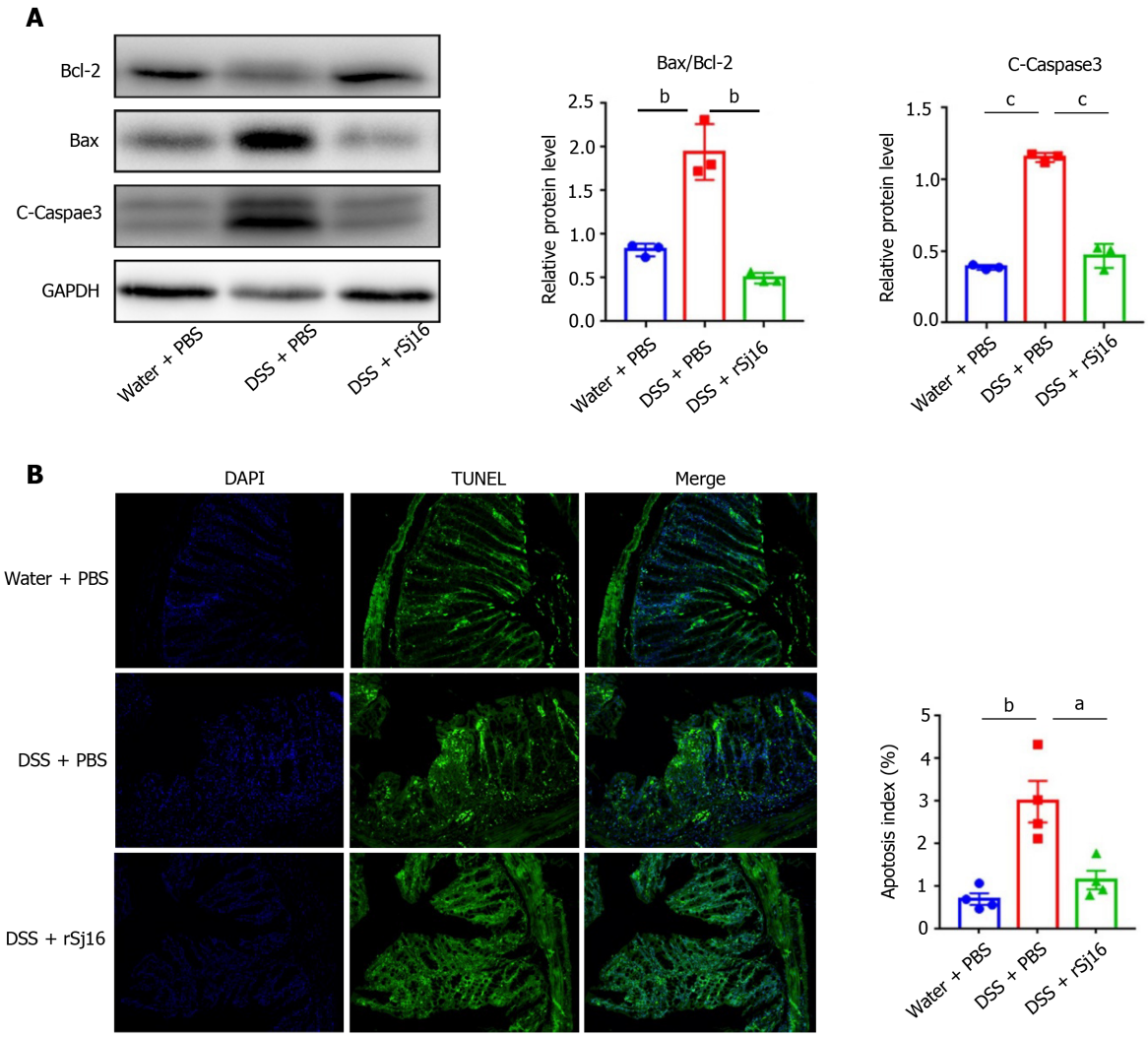

Figure 2 rSj16 inhibits dextran sulfate sodium induced apoptosis of colon epithelial cells. A: Western blot analysis for the expression of apoptosis relative proteins, including Bcl-2, Bax and cleaved-Caspase3; B: The apoptosis of colon tissue of mice treated with dextran sulfate sodium (DSS) + PBS and DSS + rSj16 was detected by TUNEL assay (20×), TUNEL positive cells were apoptotic cells, the number of TUNEL positive cells was quantified. TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling. Statistical analysis was performed using one-way ANOVA (A) and Kruskal–Wallis test (non-parametric) (B). Data are presented as means ± SD; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

loss, and reactive epithelial hyperplasia displayed) indicate that rSj16 protects against acute DSS-induced colitis.

Apoptosis is an important factor in the pathogenesis of colitis. DSS has been shown to initially cause damage in the colon by inhibition of proliferation and induction of apoptosis[34]. In the present study, we found significant apoptosis of colon epithelial cells after DSS administration in mice, and inhibition of the DSS-induced apoptosis after administration of rSj16. Therefore, we hypothesized that rSj16 alleviates DSS-induced colitis, in part by regulating apoptosis.

In recent years, miRNAs have become the key biomarkers and novel therapeutic targets in IBD[16,35]. MiR-217-5p plays dual roles in regulating cell survival and apoptosis. Flum *et al* reported that miR-217-5p could induce apoptosis by regulating multiple target genes involved in the ERK-MAPK signaling pathway including PRKCI, BAG3, ITGAV, and MAPK1[24]. Gao *et al* indicated that upregulation of miR-217-5p significantly inhibited TGF-β1-induced proliferation, migration, extracellular matrix (ECM) deposition, and promoted apoptosis in airway smooth muscle cells[36]. However, Yi *et al* indicated that upregulation of miR-217-5p improved cell viability and attenuated cell apoptosis in SH-SY5Y cells subjected to oxygen–glucose deprivation/reperfusion[37]. The specific regulatory mechanism between miR-217-5p and apoptosis needs to be further studied. In our study, miRNA miR-217-5p was expressed at a high level in IBD mice colon tissues, and was decreased significantly following treatment with rSj16. After inducing MODE-K apoptosis, miR-217-5p expression was significantly increased, after rSj16 treatment, miR-217-5p expression

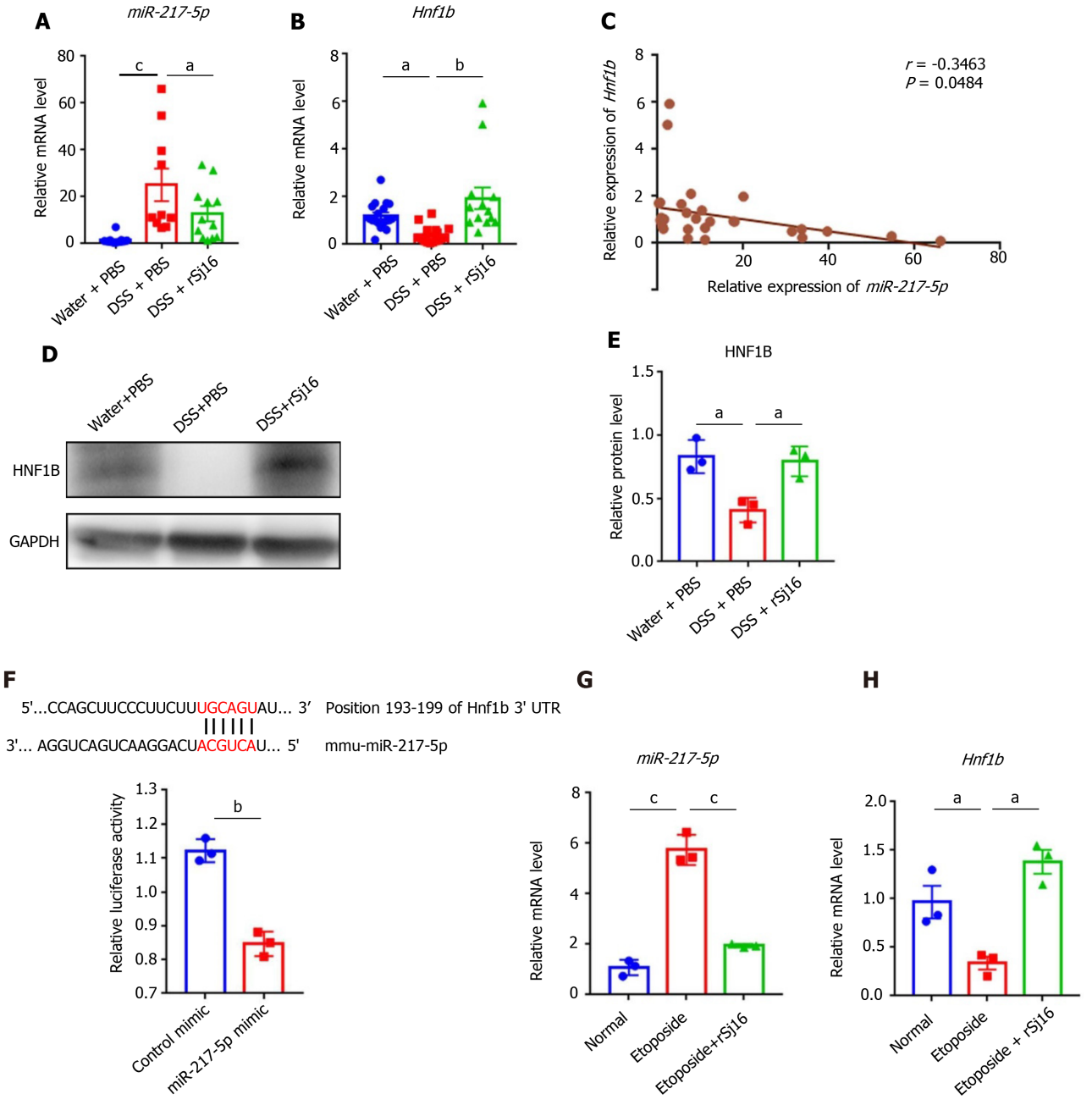

Figure 3 rSj16 can inhibit the expression of miR-217-5p in the colon of mice with dextran sulfate sodium-induced inflammatory bowel disease. A and B: Relative RNA expression of miR-217-5p and *hnf1b* in colon tissue of mice; data were normalized to levels detected in colon tissue of mice after treatment with Water and PBS (control) group; C: Pearson's correlation coefficient analysis showed a negative correlation between miR-217-5p and HNF1B in colon tissue of mice (r = -0.3463, P < 0.05); D and E: Western blot was used to detect the expression of HNF1B in protein levels; F: The wild-type HNF1B -3'- untranslated region (UTR) was cloned into psi-CHECK-2 to predict the binding site of miR-217-5p in the 3'-UTR of *hnf1b* gene. Dual-luciferase reporter assay was performed on HEK 293Tcells transfected with HNF1B UTR reporter plasmid together with miR-217-5p mimic or control mimic; G and H□MODE-K cells were treated with Etoposide or Etoposide + rSj16. The expression of *miR-217-5p* and *hnf1b* were determined using quantitative PCR. HNF1B: Hepatic nuclear factor-1beta. Statistical analysis was performed using one-way ANOVA (B and E) and Kruskal–Wallis test (non-parametric) (A), and unpaired two-sample *t*-test (F, G and H). Data are presented as means ± SD; ^{a}P < 0.05, ^{b}P < 0.01, ^{c}P < 0.001.

was significantly reduced. Therefore, we hypothesized that miR-217-5p is involved in the protective effects of rSj16 on colitis. Bcl-2, caspase-3, and Bax play key roles in cell apoptosis[38]. Caspase-3 is a marker of apoptosis because its activity is required for major apoptosis-related morphological and biochemical events, and its activation and function are regulated by the Bcl-2 family of proteins, among other molecules[39]. In the present study, after overexpression of miR-217-5p in MODE-K cells, cleaved-Caspase-3 and Bax expression were increased, but Bcl-2 was reduced, suggesting that miR-217-5p plays a pro-apoptotic role in MODE-K cells. After rSj16 treatment, the miR-217-5p, cleaved- Caspase-3 and Bax expression were decreased, but Bcl-2 was

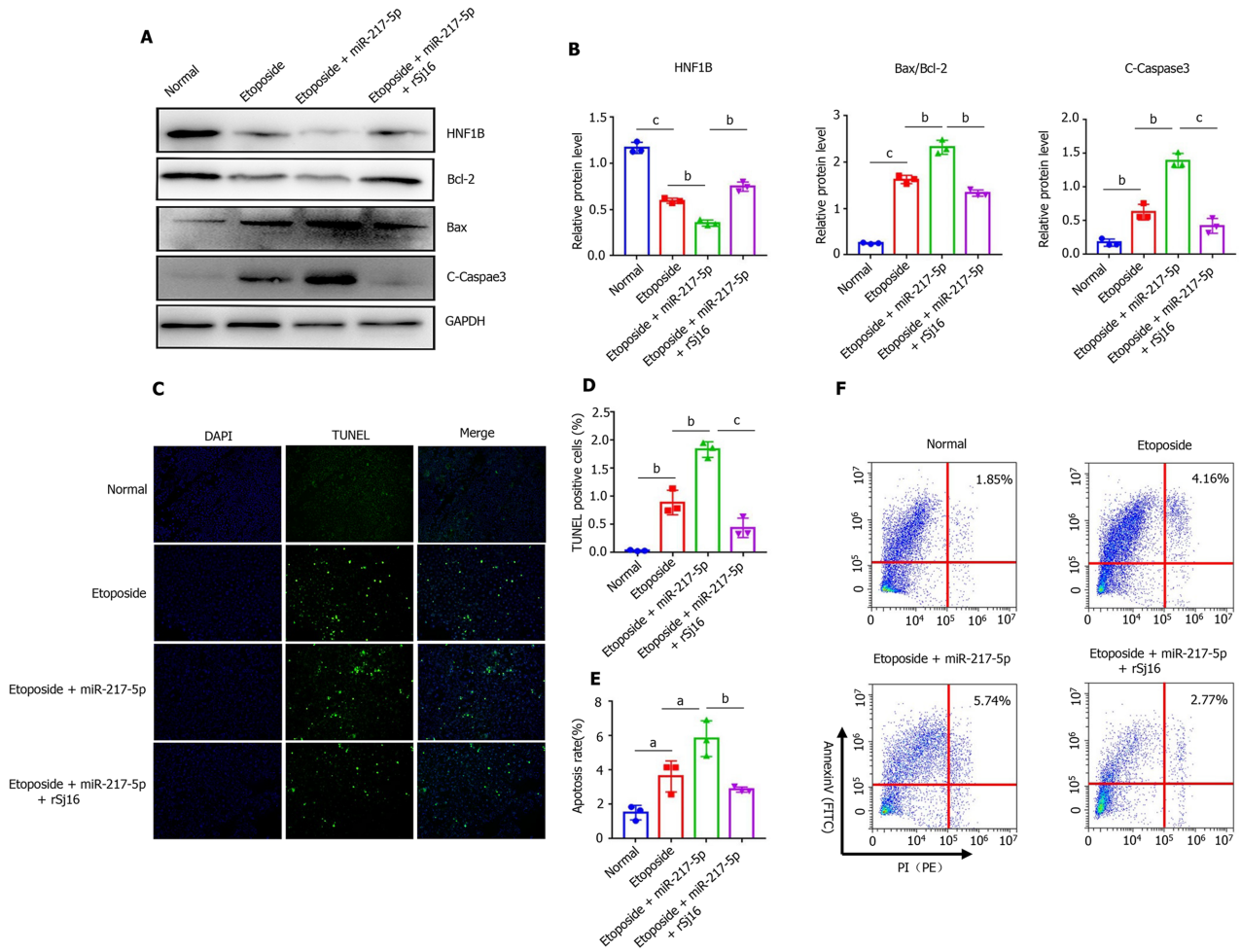

Figure 4 rSj16 have anti-apoptotic action by regulating the miR-217-5p/HNF1B axis. A and B: MODE-K cells were treated with Etoposide, Etoposide + miR-217-5p, and Etoposide + miR-217-5p + rSj16. The expression of apoptosis relative proteins, including Bcl-2, Bax and cleaved-Caspase3 was analyzed by Western blotting; C and D: The apoptosis of MODE-K cells was detected by TUNEL assay after treatment with Etoposide + miR-217-5p, and Etoposide + miR-217-5p + rSj16 (10×), TUNEL positive cells were apoptotic cells, the number of TUNEL positive cells was quantified; E and F: Flow cytometry analysis of MODE-K cells treated with Etoposide + miR-217-5p, and Etoposide + miR-217-5p + rSj16. Statistical analysis was performed using one-way ANOVA. PI: propidium iodide. Data are presented as means ± SD; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

increased, indicating that rSj16 could reduce the apoptosis. Results showed that miR-217-5p aggravated MODE-K cells apoptosis and rSj16 could significantly inhibit the apoptosis by inhibiting miRNA-217-5p expression.

MiRNAs exert pro-apoptotic functions by regulating the expression of target genes [40]. *hnf1b* acts as an oncogene in various tumors, is overexpressed in human prostate cancer and could promote tumor cell proliferation[41]. Early deletion of HNF1B results in a decrease in the number of pancreatic multipotent progenitor cells due to reduced proliferation and increased apoptosis[42]. In our study, we found that *hnf1b* is the direct target gene of miR-217-5p. In the present study, we found that DSS may induce apoptosis of colon epithelial cells, with increased expression of miR-217-5p and decreased expression of its target gene *hnf1b*. We speculated that miR-217-5p/HNF1B was involved in DSS-induced apoptosis of colon epithelial cells. Subsequently, we induced apoptosis and overexpression of miR-217-5p in MODE-K cells. After treatment with rSj16, the expression of miR-217-5p in tissues and MODE-K cells was decreased, the expression of its target gene *hnf1b* was increased, and the apoptosis of MODE-K cells significantly reduced. The results suggested that miR-217-5p exerted pro-apoptotic functions by regulating expression of the target gene *hnf1b*.

As for the limitations of the study, because rSj16 affects the progress of the disease through multiple pathways, we only explore one of them, suggesting that miR-217-5p/HNF1B axis could be used as a potential target for the treatment of enteritis. In addition, rSj16 may attenuate IBD through other pathways which we didn't make a comprehensive exposition, it is still worth exploring. Next, we will conduct a more comprehensive study on the treatment of IBD with rSj16, to provide more possibilities for the development of colitis drugs.

CONCLUSION

In conclusion, rSj16 attenuates IBD in mice by regulating the miR-217-5p/ HNF1B axis to reduce colon epithelial cell apoptosis. These data indicated that miR-217-5p and HNF1B may be potential biomarkers to improve the accuracy of IBD diagnosis and treatment, and that rSj16 may have potential for clinical drug development.

ARTICLE HIGHLIGHTS

Research background

IBD encompasses Crohn's disease, ulcerative colitis and IBD-unclassified. Although newer treatments have increased the chances of remission, most IBD patients cannot maintain remission, and death is not an infrequent outcome of IBD. Therefore, it is very important to explore the pathogenesis of IBD and to find effective therapeutic targets.

Research motivation

Exploring the pathogenesis of IBD and to find effective therapeutic targets.

Research objectives

To apoptosis and its mechanism.

Research methods

In-vivo, after DSS inducing colitis. The severity of colitis was assessed. WB was used to detect the changes of apoptosis-related genes in colon tissues. *In-vitro*, WB, Qpcr and tunel was used to detect the changes of apoptosis.

Research results

rSj16 attenuates clinical activity in DSS-induced colitis mice. rSj16 could reduce the expression of miR-217-5p in MODE-K cells. rSj16 could regulate the apoptosis of MODE-K cells.

Research conclusions

rSj16 attenuates IBD in mice by regulating the miR-217-5p/ HNF1B axis.

Research perspectives

miR-217-5p and HNF1B may be potential biomarkers to improve the accuracy of IBD diagnosis and treatment, and that rSj16 may have potential for clinical drug development.

REFERENCES

1 **Molodecky NA**, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW, Kaplan GG. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; **142**: 46-54.e42; quiz e30 [PMID: 22001864 DOI: 10.1053/j.gastro.2011.10.001]

2 **Kaplan GG**. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 720-727 [PMID: 26323879 DOI: 10.1038/nrgastro.2015.150]

3 **Liu Y**, Ye Q, Liu YL, Kang J, Chen Y, Dong WG. *Schistosoma japonicum* attenuates dextran sodium sulfate-induced colitis in mice *via* reduction of endoplasmic reticulum stress. *World J Gastroenterol* 2017; **23**: 5700-5712 [PMID: 28883695 DOI: 10.3748/wjg.v23.i31.5700]

4 **Hasby EA**, Hasby Saad MA, Shohieb Z, El Noby K. FoxP3 + T regulatory cells and immunomodulation after Schistosoma mansoni egg antigen immunization in experimental model of inflammatory bowel disease. *Cell Immunol* 2015; **295**: 67-76 [DOI: 10.1016/j.cellimm.2015.02.013]

5 **Floudas A**, Aviello G, Schwartz C, Jeffery IB, O'Toole PW, Fallon PG. Schistosoma mansoni Worm Infection Regulates the Intestinal Microbiota and Susceptibility to Colitis. *Infect Immun* 2019; **87** [PMID: 31138616 DOI: 10.1128/IAI.00275-19]

6 **Moreels TG**, Nieuwendijk RJ, De Man JG, De Winter BY, Herman AG, Van Marck EA, Pelckmans PA. Concurrent infection with Schistosoma mansoni attenuates inflammation induced changes in colonic morphology, cytokine levels, and smooth muscle contractility of trinitrobenzene sulphonic acid induced colitis in rats. *Gut* 2004; **53**: 99-107 [PMID: 14684583 DOI: 10.1136/gut.53.1.99]

7 **Wang L**, Yu Z, Wan S, Wu F, Chen W, Zhang B, Lin D, Liu J, Xie H, Sun X, Wu Z. Exosomes

Derived from Dendritic Cells Treated with *Schistosoma japonicum* Soluble Egg Antigen Attenuate DSS-Induced Colitis. *Front Pharmacol* 2017; **8**: 651 [PMID: 28959207 DOI: 10.3389/fphar.2017.00651]

8 **Wang L**, Xie H, Xu L, Liao Q, Wan S, Yu Z, Lin D, Zhang B, Lv Z, Wu Z, Sun X. rSj16 Protects against DSS-Induced Colitis by Inhibiting the PPAR-α Signaling Pathway. *Theranostics* 2017; **7**: 3446-3460 [PMID: 28912887 DOI: 10.7150/thno.20359]

9 **Kiesslich R**, Duckworth CA, Moussata D, Gloeckner A, Lim LG, Goetz M, Pritchard DM, Galle PR, Neurath MF, Watson AJ. Local barrier dysfunction identified by confocal laser endomicroscopy predicts relapse in inflammatory bowel disease. *Gut* 2012; **61**: 1146-1153 [PMID: 22115910 DOI: 10.1136/gutjnl-2011-300695]

10 **Qiu W**, Wu B, Wang X, Buchanan ME, Regueiro MD, Hartman DJ, Schoen RE, Yu J, Zhang L. PUMA-mediated intestinal epithelial apoptosis contributes to ulcerative colitis in humans and mice. *J Clin Invest* 2011; **121**: 1722-1732 [PMID: 21490394 DOI: 10.1172/JCI42917]

11 **Günther C**, Neumann H, Neurath MF, Becker C. Apoptosis, necrosis and necroptosis: cell death regulation in the intestinal epithelium. *Gut* 2013; **62**: 1062-1071 [PMID: 22689519 DOI: 10.1136/gutjnl-2011-301364]

12 **Lin W**, Ma C, Su F, Jiang Y, Lai R, Zhang T, Sun K, Fan L, Cai Z, Li Z, Huang H, Li J, Wang X. Raf kinase inhibitor protein mediates intestinal epithelial cell apoptosis and promotes IBDs in humans and mice. *Gut* 2017; **66**: 597-610 [PMID: 26801887 DOI: 10.1136/gutjnl-2015-310096]

13 **Kalla R**, Ventham NT, Kennedy NA, Quintana JF, Nimmo ER, Buck AH, Satsangi J. MicroRNAs: new players in IBD. *Gut* 2015; **64**: 504-517 [PMID: 25475103 DOI: 10.1136/gutjnl-2014-307891]

14 **Di Leva G**, Garofalo M, Croce CM. MicroRNAs in cancer. *Annu Rev Pathol* 2014; **9**: 287-314 [PMID: 24079833 DOI: 10.1146/annurev-pathol-012513-104715]

15 **Shin VY**, Chu KM. MiRNA as potential biomarkers and therapeutic targets for gastric cancer. *World J Gastroenterol* 2014; **20**: 10432-10439 [PMID: 25132759 DOI: 10.3748/wjg.v20.i30.10432]

16 **He C**, Shi Y, Wu R, Sun M, Fang L, Wu W, Liu C, Tang M, Li Z, Wang P, Cong Y, Liu Z. miR-301a promotes intestinal mucosal inflammation through induction of IL-17A and TNF-α in IBD. *Gut* 2016; **65**: 1938-1950 [PMID: 26338824 DOI: 10.1136/gutjnl-2015-309389]

17 **Tian Y**, Xu J, Li Y, Zhao R, Du S, Lv C, Wu W, Liu R, Sheng X, Song Y, Bi X, Li G, Li M, Wu X, Lou P, You H, Cui W, Sun J, Shuai J, Ren F, Zhang B, Guo M, Hou X, Wu K, Xue L, Zhang H, Plikus MV, Cong Y, Lengner CJ, Liu Z, Yu Z. MicroRNA-31 Reduces Inflammatory Signaling and Promotes Regeneration in Colon Epithelium, and Delivery of Mimics in Microspheres Reduces Colitis in Mice. *Gastroenterology* 2019; **156**: 2281-2296.e6 [PMID: 30779922 DOI: 10.1053/j.gastro.2019.02.023]

18 **Neudecker V**, Haneklaus M, Jensen O, Khailova L, Masterson JC, Tye H, Biette K, Jedlicka P, Brodsky KS, Gerich ME, Mack M, Robertson AAB, Cooper MA, Furuta GT, Dinarello CA, O'Neill LA, Eltzschig HK, Masters SL, McNamee EN. Myeloid-derived miR-223 regulates intestinal inflammation *via* repression of the NLRP3 inflammasome. *J Exp Med* 2017; **214**: 1737-1752 [PMID: 28487310 DOI: 10.1084/jem.20160462]

19 **Li M**, Zhang S, Qiu Y, He Y, Chen B, Mao R, Cui Y, Zeng Z, Chen M. Upregulation of miR-665 promotes apoptosis and colitis in inflammatory bowel disease by repressing the endoplasmic reticulum stress components XBP1 and ORMDL3. *Cell Death Dis* 2017; **8**: e2699 [PMID: 28333149 DOI: 10.1038/cddis.2017.76]

20 **Shamran H**, Singh NP, Zumbrun EE, Murphy A, Taub DD, Mishra MK, Price RL, Chatterjee S, Nagarkatti M, Nagarkatti PS, Singh UP. Fatty acid amide hydrolase (FAAH) blockade ameliorates experimental colitis by altering microRNA expression and suppressing inflammation. *Brain Behav Immun* 2017; **59**: 10-20 [PMID: 27327245 DOI: 10.1016/j.bbi.2016.06.008]

21 **Lussier CR**, Brial F, Roy SA, Langlois MJ, Verdu EF, Rivard N, Perreault N, Boudreau F. Loss of hepatocyte-nuclear-factor-1alpha impacts on adult mouse intestinal epithelial cell growth and cell lineages differentiation. *PLoS One* 2010; **5**: e12378 [PMID: 20808783 DOI: 10.1371/journal.pone.0012378]

22 **Holton NW**, Singhal M, Kumar A, Ticho AL, Manzella CR, Malhotra P, Jarava D, Saksena S, Dudeja PK, Alrefai WA, Gill RK. Hepatocyte nuclear factor-4α regulates expression of the serotonin transporter in intestinal epithelial cells. *Am J Physiol Cell Physiol* 2020; **318**: C1294-C1304 [PMID: 32348179 DOI: 10.1152/ajpcell.00477.2019]

23 **Zheng J**, Liu X, Xue Y, Gong W, Ma J, Xi Z, Que Z, Liu Y. TTBK2 circular RNA promotes glioma malignancy by regulating miR-217/HNF1β/Derlin-1 pathway. *J Hematol Oncol* 2017; **10**: 52 [PMID: 28219405 DOI: 10.1186/s13045-017-0422-2]

24 **Flum M**, Kleemann M, Schneider H, Weis B, Fischer S, Handrick R, Otte K. miR-217-5p induces apoptosis by directly targeting PRKCI, BAG3, ITGAV and MAPK1 in colorectal cancer cells. *J Cell Commun Signal* 2018; **12**: 451-466 [DOI: 10.1007/s12079-017-0410-x]

25 **Zheng M**, Karki R, Vogel P, Kanneganti TD. Caspase-6 Is a Key Regulator of Innate Immunity, Inflammasome Activation, and Host Defense. *Cell* 2020; **181**: 674-687.e13 [PMID: 32298652 DOI: 10.1016/j.cell.2020.03.040]

26 **Wang L**, Liao Y, Yang R, Yu Z, Zhang L, Zhu Z, Wu X, Shen J, Liu J, Xu L, Wu Z, Sun X. Sja-miR-71a in *Schistosome* egg-derived extracellular vesicles suppresses liver fibrosis caused by schistosomiasis *via* targeting semaphorin 4D. *J Extracell Vesicles* 2020; **9**: 1785738 [PMID: 32944173 DOI: 10.1080/20013078.2020.1785738]

27 **Di Sabatino A**, Ciccocioppo R, Luinetti O, Ricevuti L, Morera R, Cifone MG, Solcia E, Corazza GR.

Increased enterocyte apoptosis in inflamed areas of Crohn's disease. *Dis Colon Rectum* 2003; **46**: 1498-1507 [PMID: 14605569 DOI: 10.1007/s10350-004-6802-z]

28 **Zhang J**, Xu M, Zhou W, Li D, Zhang H, Chen Y, Ning L, Zhang Y, Li S, Yu M, Zeng H, Cen L, Zhou T, Zhou X, Lu C, Yu C, Li Y, Sun J, Kong X, Shen Z. Deficiency in the anti-apoptotic protein DJ-1 promotes intestinal epithelial cell apoptosis and aggravates inflammatory bowel disease *via* p53. *J Biol Chem* 2020; **295**: 4237-4251 [PMID: 32075910 DOI: 10.1074/jbc.RA119.010143]

29 **Liu Y**, Peng J, Sun T, Li N, Zhang L, Ren J, Yuan H, Kan S, Pan Q, Li X, Ding Y, Jiang M, Cong X, Tan M, Ma Y, Fu D, Cai S, Xiao Y, Wang X, Qin J. Epithelial EZH2 serves as an epigenetic determinant in experimental colitis by inhibiting TNFα-mediated inflammation and apoptosis. *Proc Natl Acad Sci U S A* 2017; **114**: E3796-E3805 [PMID: 28439030 DOI: 10.1073/pnas.1700909114]

30 **Qi Y**, Zhang K, Li P, Wu Z. Down-regulating miR-217-5p Protects Cardiomyocytes against Ischemia/Reperfusion Injury by Restoring Mitochondrial Function *via* Targeting SIRT1. *Inflammation* 2021; **44**: 383-396 [PMID: 33064238 DOI: 10.1007/s10753-020-01343-5]

31 **Gong X**, Zhu Z. Long Noncoding RNA *HOTAIR* Contributes to Progression in Hepatocellular Carcinoma by Sponging *miR-217-5p*. *Cancer Biother Radiopharm* 2020; **35**: 387-396 [PMID: 32315535 DOI: 10.1089/cbr.2019.3070]

32 **Olén O**, Askling J, Sachs MC, Neovius M, Smedby KE, Ekbom A, Ludvigsson JF. Mortality in adult-onset and elderly-onset IBD: a nationwide register-based cohort study 1964-2014. *Gut* 2020; **69**: 453-461 [PMID: 31092591 DOI: 10.1136/gutjnl-2018-317572]

33 **Ng SC**, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, Panaccione R, Ghosh S, Wu JCY, Chan FKL, Sung JJY, Kaplan GG. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* 2017; **390**: 2769-2778 [PMID: 29050646 DOI: 10.1016/S0140-6736(17)32448-0]

34 **Renes IB**, Verburg M, Van Nispen DJ, Taminiau JA, Büller HA, Dekker J, Einerhand AW. Epithelial proliferation, cell death, and gene expression in experimental colitis: alterations in carbonic anhydrase I, mucin MUC2, and trefoil factor 3 expression. *Int J Colorectal Dis* 2002; **17**: 317-326 [PMID: 12172925 DOI: 10.1007/s00384-002-0409-4]

35 **Park JH**, Peyrin-Biroulet L, Eisenhut M, Shin JI. IBD immunopathogenesis: A comprehensive review of inflammatory molecules. *Autoimmun Rev* 2017; **16**: 416-426 [PMID: 28212924 DOI: 10.1016/j.autrev.2017.02.013]

36 **Gao Y**, Wang B, Luo H, Zhang Q, Xu M. miR-217 represses TGF-β1-induced airway smooth muscle cell proliferation and migration through targeting ZEB1. *Biomed Pharmacother* 2018; **108**: 27-35 [PMID: 30212709 DOI: 10.1016/j.biopha.2018.09.030]

37 **Yi Z**, Shi Y, Zhao P, Xu Y, Pan P. Overexpression of miR-217-5p protects against oxygen-glucose deprivation/reperfusion-induced neuronal injury *via* inhibition of PTEN. *Hum Cell* 2020; **33**: 1026-1035 [PMID: 32683553 DOI: 10.1007/s13577-020-00396-w]

38 **Moon DO**, Park SY, Heo MS, Kim KC, Park C, Ko WS, Choi YH, Kim GY. Key regulators in bee venom-induced apoptosis are Bcl-2 and caspase-3 in human leukemic U937 cells through downregulation of ERK and Akt. *Int Immunopharmacol* 2006; **6**: 1796-1807 [PMID: 17052670 DOI: 10.1016/j.intimp.2006.07.027]

39 **Kuo WT**, Shen L, Zuo L, Shashikanth N, Ong MLDM, Wu L, Zha J, Edelblum KL, Wang Y, Nilsen SP, Turner JR. Inflammation-induced Occludin Downregulation Limits Epithelial Apoptosis by Suppressing Caspase-3 Expression. *Gastroenterology* 2019; **157**: 1323-1337 [PMID: 31401143 DOI: 10.1053/j.gastro.2019.07.058]

40 **Xu P**, Wu Q, Lu D, Yu J, Rao Y, Kou Z, Fang G, Liu W, Han H. A systematic study of critical miRNAs on cells proliferation and apoptosis by the shortest path. *BMC Bioinformatics* 2020; **21**: 396 [DOI: 10.1186/s12859-020-03732-x]

41 **Wang J**, He C, Gao P, Wang S, Lv R, Zhou H, Zhou Q, Zhang K, Sun J, Fan C, Ding G, Lan F. HNF1B-mediated repression of SLUG is suppressed by EZH2 in aggressive prostate cancer. *Oncogene* 2020; **39**: 1335-1346 [PMID: 31636385 DOI: 10.1038/s41388-019-1065-2]

42 **De Vas MG**, Kopp JL, Heliot C, Sander M, Cereghini S, Haumaitre C. Hnf1b controls pancreas morphogenesis and the generation of Ngn3 + endocrine progenitors. *Development* 2015; **142**: 871-882 [PMID: 25715395 DOI: 10.1242/dev.110759]

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2021 December 14; 27(46): 7995-8009

DOI: 10.3748/wjg.v27.i46.7995

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Digestive system involvement and clinical outcomes among COVID-19 patients: A retrospective cohort study from Qatar

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Author contributions: Khan MU and Mushtaq K conceived and designed the study and performed data analysis, literature review, and manuscript writing; Alsoub DH, Iqbal P, Ata F, Chaudhry HS, Iqbal F, Balaraju G, Maslamani MAA, Varughese B, Ejji KA, Kaabi SA, and Kamel YM performed data collection, data analysis, manuscript writing, and literature review; Singh R reviewed the statistical part of the manuscript; Butt AA performed the literature review and revised the final manuscript; all authors verified the final version of the study.

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Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 virus most commonly presents with respiratory symptoms. While gastrointestinal (GI) manifestations either at presentation or during hospitalization are also common, their impact on clinical outcomes is

Institutional review board statement: The study was approved by the Medical Research Center of Hamad Medical Corporation (MRC-01-20-631).

Informed consent statement: Due to the retrospective design of the study, the requirement of informed consent was waived by the Institutional Review Board.

Conflict-of-interest statement: All authors and study participants declare no potential conflicting interests related to this paper.

Data sharing statement: The authors confirm that the data supporting the findings of this study are available within the article.

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

Supported by Hamad Medical Corporation, No. MRC-01-20-631.

Country/Territory of origin: Qatar

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

controversial. Some studies have described worse outcomes in COVID-19 patients with GI symptoms, while others have shown either no association or a protective effect. There is a need for consistent standards to describe GI symptoms in COVID-19 patients and to assess their effect on clinical outcomes, including mortality and disease severity.

AIM

To investigate the prevalence of GI symptoms in hospitalized COVID-19 patients and their correlation with disease severity and clinical outcomes.

METHODS

We retrospectively reviewed 601 consecutive adult COVID-19 patients requiring hospitalization between May 1-15, 2020. GI symptoms were recorded at admission and during hospitalization. Demographic, clinical, laboratory, and treatment data were retrieved. Clinical outcomes included all-cause mortality, disease severity at presentation, need for intensive care unit (ICU) admission, development of acute respiratory distress syndrome, and need for mechanical ventilation. Multivariate logistic regression model was used to identify independent predictors of the adverse outcomes.

RESULTS

The prevalence of any GI symptom at admission was 27.1% and during hospitalization was 19.8%. The most common symptoms were nausea (98 patients), diarrhea (76 patients), vomiting (73 patients), and epigastric pain or discomfort (69 patients). There was no difference in the mortality between the two groups (6.21% *vs* 5.5%, $P = 0.7$). Patients with GI symptoms were more likely to have severe disease at presentation (33.13% *vs* 22.5%, $P < 0.001$) and prolonged hospital stay (15 d *vs* 14 d, $P = 0.04$). There was no difference in other clinical outcomes, including ICU admission, development of acute respiratory distress syndrome, or need for mechanical ventilation. Drugs associated with the development of GI symptoms during hospitalization were ribavirin (diarrhea 26.37% $P < 0.001$, anorexia 17.58%, $P = 0.02$), hydroxychloroquine (vomiting 28.52%, $P = 0.009$) and lopinavir/ritonavir (nausea 32.65% $P = 0.049$, vomiting 31.47% $P = 0.004$, and epigastric pain 12.65% $P = 0.048$). In the multivariate regression analysis, age > 65 years was associated with increased mortality risk [odds ratio (OR) 7.53, confidence interval (CI): 3.09-18.29, $P < 0.001$], ICU admission (OR: 1.79, CI: 1.13-2.83, $P = 0.012$), and need for mechanical ventilation (OR: 1.89, CI:1.94-2.99, $P = 0.007$). Hypertension was an independent risk factor for ICU admission (OR: 1.82, CI:1.17-2.84, $P = 0.008$) and need for mechanical ventilation (OR: 1.66, CI: 1.05-2.62, $P = 0.028$).

CONCLUSION

Patients with GI symptoms are more likely to have severe disease at presentation; however, mortality and disease progression is not different between the two groups.

Key Words: COVID-19; Gastrointestinal manifestations; Mortality; Intensive care unit admission; Mechanical ventilation; Disease severity

Core Tip: There is a high prevalence of gastrointestinal symptoms in coronavirus disease 2019 patients both at presentation and during hospitalization. Drugs are associated with the development of gastrointestinal symptoms during hospitalization. The presence of gastrointestinal symptoms in coronavirus disease 2019 patients is associated with disease severity at presentation but is not a predictor of mortality or disease progression.

Citation: Khan MU, Mushtaq K, Alsoub DH, Iqbal P, Ata F, Chaudhry HS, Iqbal F, Balaraju G, Maslamani MAA, Varughese B, Singh R, Ejji KA, Kaabi SA, Kamel YM, Butt AA. Digestive

Received: April 28, 2021
Peer-review started: April 28, 2021
First decision: June 13, 2021
Revised: June 29, 2021
Accepted: November 28, 2021
Article in press: November 28, 2021
Published online: December 14, 2021

P-Reviewer: Karna ST
S-Editor: Liu M
L-Editor: Filipodia
P-Editor: Liu M

system involvement and clinical outcomes among COVID-19 patients: A retrospective cohort study from Qatar. *World J Gastroenterol* 2021; 27(46): 7995-8009
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/7995.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.7995

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection presents most commonly as a respiratory illness with symptoms including fever, cough, and shortness of breath. Disease severity ranges from mild disease requiring no intervention to severe illness requiring intensive care unit (ICU) admission and mechanical ventilation[1,2]. While most studies have focused on respiratory manifestations of coronavirus disease 2019 (COVID-19), extra-respiratory manifestations have also been described, including gastrointestinal (GI) symptoms and liver enzyme abnormalities[3,4]. The prevalence of GI symptoms in COVID-19 patients ranges from 7% to 15%[5,6]. Fecal shedding of SARS-CoV-2 has been reported in 40.5%-48.1% of the patients[7,8]. The significant variation in the proportion of patients with GI symptoms among different studies might be related to geographical region[5] and whether symptoms were reported on admission or during hospitalization[3,9].

Diarrhea, nausea, vomiting, and abdominal pain are the most frequently reported GI symptoms[5,8]. The association between GI symptoms and adverse outcomes in patients with SARS-CoV-2 infection is controversial[8,10-13]. Some studies have shown an inverse correlation between GI symptoms and adverse outcomes, including mortality[10,11,14], while others have shown a direct correlation of GI symptoms with disease severity and adverse outcomes[8,12]. Still others, including a recent meta-analysis, have shown that GI symptoms bear no association with adverse outcomes or mortality[13,15]. Studies are needed with consistent standards for describing GI symptoms and distinguishing between GI symptoms on admission *vs* symptoms that develop during the hospital stay to determine whether GI symptoms have correlation to disease severity.

The aim of our study was to evaluate the prevalence of GI symptoms in COVID-19 patients at admission and during hospitalization and their association with adverse outcomes, including mortality.

MATERIALS AND METHODS

Study design and participants

We conducted a retrospective cohort study investigating the epidemiological and clinical characteristics and outcomes among consecutive adult patients with SARS-CoV-2 infection who were admitted to one of the dedicated COVID-19 hospitals in the state of Qatar between May 1-15, 2020. SARS-CoV-2 infection was diagnosed by real-time polymerase chain reaction assays Cobas SARS-CoV-2 Test (Roche Diagnostics, Rotkreuz, Switzerland) or TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Waltham, MA, United States) on nasopharyngeal and throat swabs.

The severity of COVID-19 was defined according to the World Health Organization guidelines and categorized into five groups[16] (see Supplementary material, Appendix). Demographic, clinical, laboratory, treatment, and outcome data were retrieved from the electronic medical records. These included a complete blood count, renal function, electrolytes, coagulation profile, liver function tests, and other biochemical markers including creatine kinase, lactate dehydrogenase, C-reactive protein, troponin-T, serum lipase, amylase, procalcitonin, and ferritin. Microbiological investigations, including blood, respiratory, fecal, and urine cultures, were reviewed. Radiologic assessments included chest radiography on admission and subsequent chest computed tomography or abdomen ultrasound according to the patient's clinical care needs. X-ray findings were recorded from the medical records and by examining the films.

Outcomes and definitions

The primary outcome was all-cause mortality during the hospitalization. Secondary outcomes included disease severity at admission, disease progression defined by admission to the ICU, development of acute respiratory distress syndrome, and need

for mechanical ventilation. Other outcomes included the development of septic shock and length of hospital stay. All outcomes were compared between those with and without GI symptoms at admission.

GI symptoms were defined by the presence of at least one of the following symptoms: nausea, vomiting, diarrhea, epigastric pain or discomfort, acid reflux, anorexia, or GI bleeding. GI symptoms were recorded on admission and during hospitalization to determine the influence of medical therapy and other external factors. Diarrhea was defined as the passing of loose stools > three times per day with a negative stool culture for routine bacterial pathogens. Diarrhea that developed during hospitalization was recorded only after recording negative stool culture and absence of *Clostridium difficile* infection. Liver enzyme abnormalities were classified into normal, borderline (< 2 × upper limit of normal), mild impairment (2–5 × elevation), moderate (5–10 × elevation), and severe (> 10 × upper limit of normal).

Travel history in the 3 mo before the presentation and exposure to a confirmed case were recorded. Acute respiratory distress syndrome and shock were defined per the World Health Organization guidelines for COVID-19.

Study oversight

The study was approved by the Medical Research Center of Hamad Medical Corporation (MRC-01-20-631). Due to the retrospective design of the study, the requirement of informed consent was waived, and Institutional Review Board exemption was granted.

Statistical analysis

We summarized continuous variables using mean (standard deviation) and median (interquartile range) for normal and non-normally distributed data, respectively. Categorical variables were expressed as number (%) and compared using the Pearson's χ^2 test or Fisher's exact test, as indicated. Univariate logistic regression analysis was used to identify the risk factors for adverse outcomes. All variables with a P value of < 0.10 from univariate analysis were included in a multivariate logistic regression model with the forward method to identify independent predictors of the adverse outcomes. No adjustment for multiple testing was performed. A two-sided P value of < 0.05 was considered statistically significant. Statistical analyses were performed using Stata Statistical Software Stata/IC 16.1 (StataCorp LLC, College Station, TX, United States).

RESULTS

Demographic and epidemiological characteristics

We identified 601 adult patients hospitalized with confirmed SARS-CoV-2 infection during the study period. The mean age was 46.20 ± 13.66 years, and 85.4% were males. The clinical characteristics at presentation are shown in Table 1. Fever (79.7%), cough (75.7%), and shortness of breath (50.2%) were the most common presenting symptoms. Overall, 163 (27.1%) had at least one GI symptom at presentation. Patients without GI symptoms were more likely to have a cough (77.8% *vs* 68.7%, $P = 0.02$), while patients with GI symptoms had more fatigue (48.0% *vs* 19.0%, $P < 0.001$) and myalgias (38.7% *vs* 27.0%, $P = 0.007$). The patients with GI symptoms had a significantly longer duration of symptoms before the presentation (4.81 ± 2.51 *vs* 4.04 ± 2.51, $P = 0.002$) compared to patients without GI symptoms. There was no difference between the two groups regarding exposure to a sick contact, family clustering, and travel outside the country.

Patients with GI symptoms were more likely to have underlying chronic liver disease (1.2% *vs* 0.0%, $P < 0.001$), malignancy (2.5% *vs* 1.8%, $P < 0.001$), and immunosuppression (4.3% *vs* 3.0%, $P < 0.001$) but were less likely to have chronic lung conditions (4.9% *vs* 6.1%, $P < 0.001$). Severe disease at presentation was more frequent in the patients with GI symptoms compared with those without GI symptoms (33.1% *vs* 22.5%, $P < 0.001$). There was no statistically significant difference in the rest of the general demographics or other epidemiological parameters between the two groups. Of those 163 (27.1%) patients reporting at least one GI symptom at admission, the most common symptoms were nausea (98 patients), diarrhea (76 patients), vomiting (73 patients), and epigastric pain or discomfort (69 patients) (Table 2).

Laboratory and radiological abnormalities

The laboratory parameters of the study participants are presented in Table 3. Alanine

Table 1 Demographic and epidemiological characteristics and presenting symptoms of coronavirus disease 2019 patients with and without gastrointestinal symptoms, *n* (%)

Characteristics	Overall (*n* = 601)	With GI (*n* = 163)	Without GI (*n* = 438)	*P* value
Age (yr)	46.20 13.66	46.45 13.76	46.12 13.64	0.728
Sex, male	513 (85.4)	136 (83.4)	377 (85.9)	0.515
BMI (kg/m^2)	27.55 (24.90-31.00)	27.49 (24.40-30.60)	27.55 (24.90-31.00)	0.811
Duration of symptoms (d)	4.24 2.53	4.81 2.51	4.04 2.51	0.002[a]
Nationality				0.765
Qatar	67 (11.1)	17 (25.4)	50 (74.6)	
India	127 (21.1)	27 (21.3)	100 (78.7)	
Nepal	89 (14.8)	23 (25.8)	66 (74.2)	
Bangladesh	105 (17.5)	33 (31.4)	72 (68.6)	
Pakistan	48 (8.0)	14 (29.2)	43 (70.8)	
Philippines	56 (9.3)	18 (32.1)	38 (67.9)	
Arab countries	60 (9.9)	16 (26.7)	44 (73.3)	
Others	49 (8.15)	15 (30.6)	34 (69.4)	
Presenting symptoms				
Fever	478 (79.7)	130 (79.7)	348 (79.1)	0.147
Cough	455 (75.7)	112 (68.7)	343 (77.8)	0.016[a]
Sputum	75 (12.5)	23 (14.1)	52 (11.8)	0.483
Shortness of breath	303 (50.2)	84 (51.5)	219 (49.7)	0.412
Sore throat	146 (24.3)	37 (22.7)	109 (24.7)	0.747
Nasal obstruction	51 (8.5)	18 (11.0)	33 (7.5)	0.359
Fatigue	163 (27.1)	79 (48.5)	84 (19.1)	< 0.001[a]
Myalgia	182 (30.3)	63 (38.7)	119 (27.0)	0.007[a]
Anosmia	303 (50.2)	86 (52.8)	217 (49.5)	0.424
Exposure history				
Smoking	62 (10.1)	24 (14.9)	38 (8.7)	0.199
Ex-smoker	7 (1.2)	3 (1.9)	4 (0.9)	
Alcohol	44 (7.3)	9 (5.6)	35 (8.3)	0.28
Travel history	43 (7.2)	9 (5.6)	34 (7.8)	0.456
Sick contact	142 (23.6)	34 (20.9)	108 (24.6)	0.533
Family cluster	27 (4.6)	10 (6.3)	17 (3.4)	0.477
Pre-existing conditions				
No. of comorbid conditions				0.39
0	261 (43.4)	72 (43.6)	189 (43.0)	
1-2	203 (33.8)	53 (32.1)	150 (32.2)	
> 2	137 (22.8)	38 (24.9)	99 (22.1)	
Diabetes mellitus	242 (40.3)	72 (44.2)	170 (38.6)	0.327
Hypertension	209 (34.6)	52 (31.9)	157 (35.6)	0.536
Coronary artery disease	46 (7.7)	6 (3.7)	40 (9.1)	0.622
Chronic kidney disease	47 (7.8)	12 (7.4)	35 (8.0)	0.882
Chronic liver disease	6 (1.0)	2 (1.2)	4 (0.9)	< 0.001[a]

Malignancy	12 (2.0)	4 (2.5)	8 (1.8)	< 0.001[a]
Lung disease	35 (5.8)	8 (4.9)	27 (6.1)	< 0.001[a]
Immunosuppression	20 (3.3)	7 (4.3)	13 (3.0)	< 0.001[a]
Disease severity at admission				0.0024[a]
Asymptomatic	48 (8.0)	2 (1.2)	46 (10.5)	
Mild	109 (18.1)	32 (19.6)	77 (17.7)	
Moderate	291 (48.4)	75 (46.0)	216 (49.1)	
Severe	70 (11.6)	26 (15.9)	44 (10.0)	
Critical	83 (13.8)	28 (17.2)	55 (12.5)	
Severe–non severe	153(25.5)	54 (33.1)	99 (22.5)	< 0.001[a]

[a]Statistically significant *P* value.
BMI: Body mass index; GI: Gastrointestinal.

Table 2 Frequency of gastrointestinal symptoms in coronavirus disease 2019 patients at admission and during hospital stay, *n* (%)

GI symptoms at admission (*n* = 601)	Frequency	During hospital stay (*n* = 438)	Frequency
Diarrhea	76 (12.6)	Diarrhea	42 (7.0)
Nausea	98 (16.2)	Nausea	94 (15.6)
Vomiting	73 (12.1)	Vomiting	87 (14.5)
Epigastric pain	69 (11.4)	Epigastric pain	22 (3.6)
GERD	6 (1.0)	GERD	2 (0.3)
Anorexia	66 (10.9)	Anorexia	26 (4.3)
GI bleeding	4 (0.7)	GI bleeding	6 (1.0)
Any GI symptoms	163 (27.1)	Any GI symptoms	119 (19.8)
Any nausea, vomiting, diarrhea	140 (19.0)		

GI: Gastrointestinal; GERD: Gastro-esophageal reflux disease.

aminotransferase levels were significantly higher in patients with GI symptoms (P = 0.04). Overall, the proportion of patients with any liver test abnormality was higher in patients with GI symptoms, but the difference did not approach statistical significance.

All but 2 patients had a chest x-ray at presentation. A normal chest x-ray was observed in 23.5% of the patients, and bilateral lung infiltrates (54.6%) were the most common radiological abnormality at presentation.

Treatment and outcomes

The drug treatment received by the patients is summarized in Table 4. Hydroxychloroquine (HCQ) (90.45%) and azithromycin (90.12%) were the most common drugs administered to the patients followed by broad-spectrum antibiotics other than azithromycin (82.60%). The drug treatment did not differ between the two groups except for broad-spectrum antibiotics, with patients having GI symptoms receiving more antibiotics (92.6% *vs* 78.9%, P = 0.000). We also divided the treatment regimen into six groups based on the different combinations of administered drugs. Group 1 containing hydroxychloroquine plus azithromycin was the most frequently administered treatment combination. COVID-19 patients with GI symptoms received significantly more treatment combination 1 HCQ + azithromycin (92.0% *vs* 85.4%, P = 0.039) and combination 2 HCQ + azithromycin + oseltamivir + antibiotics (78.5% *vs* 69.2%, P = 0.024).

Overall, 34 patients (5.7%) died. Mortality was not different between patients with and without GI symptoms (6.2% *vs* 5.5%, P = 0.741). In addition, 260 (43.6%), 185 (30.8%), and 130 (21.6%) patients needed ICU stay, mechanical ventilation, or developed shock, respectively. There was no statistically significant difference

Table 3 Laboratory and radiological findings of coronavirus disease 2019 patients with and without gastrointestinal symptoms, *n* (%)

Parameters	Total	With GI symptoms (*n* = 163)	Without GI symptoms (*n* = 438)	*P* value
Hematological parameters				
Hemoglobin gm/dL	14.0 (12.7-15.0)	13.9 (12.7-15.0)	14.0 (12.7-15.1)	0.590
Hematocrit	42.1 (39.0-45.2)	42.0 (38.7-45.0)	42.3 (39.1-45.3)	0.010
WBC (10^3/L)	6.3 (4.9-8.6)	6.2 (4.6-8.6)	6.4 (5.0-8.5)	0.595
Neutrophils (10^3/L)	4.3 (3.1-6.6)	4.4 (2.9-6.5)	4.3 (3.1-6.6)	0.617
Lymphocytes (10^3/L)	1.2 (0.8-1.6)	1.1 (0.8-1.6)	1.2 (0.8-1.6)	0.987
Eosinophils (10^3/L)	0 (0-0.02)	0 (0-0)	0 (0-0.05)	0.643
Monocytes (10^3/L)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.919
Platelets (10^3/L)	211 (169-261)	202 (155-257)	215 (170-261)	0.961
Coagulation function				
INR	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	0.486
Blood biochemistry				
BUN (mmol/L)	4.0 (3.0-5.3)	3.8 (2.4-5.2)	4.1 (3.1-5.3)	0.758
Creatinine (mol/L)	85 (70-102)	86 (69-101)	84 (70-103)	0.407
Sodium (mmol/L)	136 (133-138)	135 (132-137)	136 (133-138)	0.225
ALT (U/L)	32 (22-51)	34 (22-56)	32 (22-49)	0.043[a]
AST (U/L)	37 (25-59)	42 (28-69)	36 (24-55)	0.116
ALK-P (U/L)	70 (57-88)	71 (58-88)	70 (57-89)	0.199
Bilirubin (mol/L)	9 (6-12)	8 (7-12)	9 (6-12)	0.438
Albumin (gm/L)	36 (31-39)	35 (30-38)	36 (32-39)	0.058
Glucose (mmol/L)	6.6 (5.4-9.0)	6.7 (5.5-9.0)	6.6 (5.4-9.0)	0.708
Lactate (mmol/L)	1.3 (1.0-1.8)	1.4 (1.1-1.9)	1.2 (1.1-1.8)	0.297
CK (U/L)	218 (72-481)	147 (84-518)	237 (68-477)	0.455
Amylase (U/L)	48 (25-124)	47 (23-119)	47 (28-109)	0.604
Lipase (U/L)	55 (35-129)	56 (35-152)	54 (36-103)	0.648
Troponin-T (ng/L)	10 (6-26)	10 (6-17)	11 (6-26)	0.296
LDH (U/L)	436 (305-559)	446 (337-578)	435 (302-547)	0.167
Infection-related biomarkers				
CRP (mg/L)	55.3 (16.0-113.7)	55.5 (24.9-113.7)	55.3 (13.2-113.7)	0.614
Procalcitonin (ng/ml)	0.21 (0.10-0.68)	0.21 (0.10-0.50)	0.21 (0.10-0.70)	0.789
Ferritin (g/L)	659 (327-1229)	805 (450-1475)	618 (289-1154)	0.561
Liver injury at admission				0.059
None	272 (45.87)	65 (40.37)	207 (47.92)	
Abnormality < 2 × ULN	218 (36.76)	61 (37.89)	157 (36.34)	
Mild	85 (14.33)	30 (18.63)	55 (12.73)	
Moderate	15 (2.53)	5 (3.11)	10 (2.31)	
Severe	2 (0.34)	0 (0.00)	2 (0.46)	
Liver injury during hospitalization				0.302
None	107 (19.60)	26 (16.56)	81 (20.88)	
Abnormality < 2 × ULN	163 (29.91)	51 (32.48)	112 (28.87)	

Mild 2-5 ×	171 (31.40)	44 (28.03)	127 (32.73)	
Moderate 5-10 ×	63 (11.56)	21 (13.38)	42 (10.82)	
Severe > 10 ×	41 (7.52)	15 (9.55)	26 (6.70)	
Radiological findings				
X-ray chest				0.286
Not done	2 (0.3)	0 (0.0)	2 (0.5)	
Normal	141 (23.5)	35 (21.6)	107 (24.3)	
Unilateral PNA	100 (16.6)	24 (14.8)	76 (17.2)	
Bilateral PNA	328 (54.6)	96 (59.3)	232 (52.6)	
Ground glass	29 (4.8)	7 (4.3)	22 (5.0)	

WBC: White blood cells; BUN: Blood urea nitrogen; INR: International normalized ratio; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase, ALK-P: Alkaline phosphatase; CK: Creatine Kinase; LDH: Lactate dehydrogenase; CRP: C-reactive protein; ULN: Upper limit of normal; PNA: Pneumonia; GI: Gastrointestinal.

between the two groups. Seven (1.2%), 29 (4.8%), and 48 (8.0%) patients developed acute liver failure, renal failure requiring renal replacement therapy, and multiorgan failure, respectively, with no significant difference between the two groups. Patients with GI symptoms had a longer total hospital length of stay compared with patients without GI symptoms (15 d *vs* 14 d, $P = 0.036$).

GI symptoms during hospitalization

An additional 119 patients (19.8%) developed GI symptoms during hospitalization. Nausea (15.64%) and vomiting (14.48%) were the most commonly reported GI symptoms, followed by diarrhea (6.99%) and anorexia (4.32%) (Table 2). Regarding the treatment administered, use of HCQ was associated with vomiting (28.52%, $P = 0.009$), ribavirin use was associated with diarrhea (26.37%, $P < 0.001$) and anorexia (17.58%, $P = 0.02$), while the use of lopinavir/ritonavir was independently related to the development of nausea (32.65%, $P = 0.05$), vomiting (31.47%, $P = 0.004$), and epigastric pain (12.65%, $P = 0.05$) (Table 5).

The frequency of GI symptoms in different treatment combinations is shown in the Supplementary Table 1, along with significant *P* values. Specifically, treatment group 3 had more chances of developing nausea and vomiting, while treatment groups 5 and 6 had a significant association with anorexia.

Prediction of risk factors for severe/critical COVID-19 and adverse outcomes

In the multivariate regression analysis, age > 65 years was the only significant factor associated with increased mortality risk [adjusted odds ratio (aOR) 7.53, confidence interval (CI): 3.09-18.29, $P < 0.001$]. For disease severity at presentation, presence of GI symptoms (aOR: 1.66, CI: 1.09-2.52, $P = 0.02$), diabetes (aOR: 1.92, CI: 1.28-2.87, $P = 0.002$), hypertension (aOR: 1.68, CI: 1.08-2.60, $P = 0.02$), and smoking (aOR: 1.62, CI: 1.01-2.63, $P = 0.05$) were independent predictors in multivariate regression. Risk factors for ICU admission included age > 65 years (aOR: 1.79, CI: 1.13-2.83, $P = 0.012$), male sex (aOR: 1.82, CI: 1.05-3.15, $P = 0.033$) fever at admission (aOR: 2.14, CI: 1.24-3.69, $P = 0.006$), shortness of breath (aOR: 2.90, CI: 1.99-4.24, $P < 0.001$), and hypertension (aOR: 1.82, CI: 1.17-2.84, $P = 0.008$). Risk factors for mechanical ventilation included age > 65 (aOR: 1.89, CI: 1.94-2.99, $P = 0.007$), male sex (aOR: 1.88, CI: 1.02-3.45, $P = 0.043$), vomiting (aOR: 2.03, CI: 1.10-3.75, $P = 0.023$), fever (aOR: 3.16, CI: 1.63-6.09, $P < 0.001$), shortness of breath (aOR: 2.36, CI: 1.57-3.55, $P < 0.001$), and hypertension (aOR: 1.66, CI: 1.05-2.62, $P = 0.028$) (Table 6). The univariate analysis of risk factors for severe coronavirus disease 2019 at presentation and clinical outcomes is shown in Supplementary Table 2.

DISCUSSION

COVID-19 disease caused by the novel coronavirus SARS-CoV-2 started in China in December 2019 and soon became pandemic, causing unprecedented global public health challenges. COVID-19 mainly presents with respiratory symptoms; however, GI

Table 4 Clinical outcomes and treatment in coronavirus disease 2019 patients with and without gastrointestinal symptoms, *n* (%)

	Total	With GI symptoms *n* = 163	Without GI symptoms *n* = 438	*P* value
Outcomes				
ICU admission	260 (43.55)	78 (48.45)	182 (41.74)	0.143
ARDS	206 (34.30)	61 (38.12)	145 (33.30)	0.268
Shock	130 (21.60)	40 (25.00)	90 (20.64)	0.254
MOF	48 (8.00)	15 (9.32)	33 (7.59)	0.491
ALF	7 (1.20)	1 (0.62)	6 (1.38)	0.447
MV	185 (30.80)	55 (34.80)	130 (29.90)	0.252
ECMO	4 (0.70)	1 (0.62)	3 (0.69)	0.929
CRRT	29 (4.80)	6 (3.73)	23 (5.28)	0.610
Death	34 (5.70)	10 (6.21)	24 (5.50)	0.741
LOS (d)	15 (8-21)	15 (10-22)	14 (7-21)	0.036[a]
Treatment				
Azithromycin	538 (90.12)	151 (93.80)	387 (88.80)	0.068
HCQ	540 (90.45)	150 (93.20)	390 (89.40)	0.170
Chloroquine	38 (6.30)	7 (4.35)	31 (7.11)	0.388
Antibiotics	493 (82.6)	149 (92.6)	344 (78.9)	< 0.001[a]
Steroids	248 (41.61)	67 (41.60)	181 (41.60)	0.831
IFN	62 (10.30)	21 (13.04)	41 (9.43)	0.368
RBV	91 (15.10)	31 (19.25)	60 (13.80)	0.097
Tocilizumab	236 (39.50)	66 (40.99)	170 (38.99)	0.657
Lopinavir/ritonavir	340 (56.6)	96 (59.6)	244 (56.0)	0.422
Oseltamivir	510 (85.43)	139 (86.30)	371 (85.09)	0.702
Darunavir	48 (8.0)	14 (8.7)	34 (7.8)	0.782
Treatment groups				
Group 1	524 (87.2)	150 (92.0)	374 (85.4)	0.039[a]
Group 2	431 (71.7)	128 (78.5)	303 (69.2)	0.025[a]
Group 3	307 (51.1)	88 (54.0)	219 (50.0)	0.409
Group 4	181 (30.1)	50 (30.7)	131 (29.9)	0.920
Group 5	61 (10.1)	22 (13.5)	39 (8.9)	0.128
Group 6	56 (9.3)	20 (12.3)	36 (8.2)	0.155

[a]Statistically significant *P* value.

Group 1: Hydroxychloroquine + Azithromycin; Group 2: Hydroxychloroquine + Azithromycin + Oseltamivir + Antibiotics; Group 3: Hydroxychloroquine + Azithromycin + Oseltamivir + Antibiotics + Lopinavir/ritonavir; Group 4: Hydroxychloroquine + Azithromycin + Oseltamivir + Antibiotics + Lopinavir/ritonavir + Steroids; Group 5: Hydroxychloroquine + Azithromycin + Oseltamivir + Antibiotics + Lopinavir/ritonavir + Steroids + Interferon/ribavirin; Group 6: Hydroxychloroquine + Azithromycin + Oseltamivir + Antibiotics + Lopinavir/ritonavir + Steroids + Interferon/ribavirin + Tocilizumab.

ARDS: Acute respiratory distress syndrome; MOF: Multiorgan failure; ALF: Acute liver failure; MV: Mechanical ventilation; ECMO: Extra-corporal membrane oxygenation; CRRT: Continuous renal replacement therapy; LOS: Length of stay; HCQ: Hydroxychloroquine; IFN: Interferon; RBV: Ribavirin; GI: Gastrointestinal; ICU: Intensive care unit.

manifestations were quickly recognized as frequent presenting symptoms. While numerous studies have reported GI symptoms in COVID-19 patients, the criteria of GI symptoms have been variable and inconsistent[3,4,11,17,18]. Furthermore, some studies reported symptoms at presentation only while others described anytime during illness.

Table 5 Association of gastrointestinal symptoms with individual drugs, *n* (%)

	Azithromycin (*n* = 538)	HCQ (*n* = 540)	Antibiotics (*n* = 493)	Steroids (*n* = 248)	RBV (*n* = 91)	Tocilizumab (*n* = 236)	L/r (*n* = 340)	Oseltamivir (*n* = 510)
Diarrhea	78 (14.50)	79 (14.63)	70 (14.20)	37 (14.92)	24 (26.37)*P* < 0.001[a]	35 (14.83)	44 (12.94)	75 (14.71)
Nausea	160 (29.74)	165 (30.60)	147 (29.82)	74 (29.84)	26 (8.57)	66 (27.97)	111 (32.65)*P* = 0.049 [a]	153 (30.00)
Vomiting	148 (27.51)	154 (28.52)*P* = 0.009 [a]	135 (27.38)	68 (27.42)	22 (24.18)	64 (27.12)	107 (31.47)*P* = 0.004[a]	140 (27.45)
Epigastric pain	57 (10.59)	60 (11.11)	54 (10.95)	29 (11.69)	13 (14.29)	24 (10.17)	43 (12.65)*P* = 0.048[a]	55 (10.78)
Anorexia	55 (10.22)	58 (10.74)	50 (10.14)	30 (12.10)	16 (17.58)*P* = 0.022[a]	28 (11.86)	41 (12.06)	56 (10.98)
Any GI symptoms	208 (38.66)	209 (38.70)	191 (38.74)	95 (38.31)	41 (45.05)	92 (38.98)	138 (40.59)	194 (38.04)

[a]Statistically significant *P* value.

HCQ: Hydroxychloroquine; RBV: Ribavirin; L/r: Lopinavir/ritonavir; GI: Gastrointestinal

In our cohort, 27% of the patients had at least one GI symptom at presentation. Our cohort has a similar prevalence of symptoms as reported in the different meta-analyses [5,6,12,15], and slight variation can be attributed to different geographic locations and varied ethnic backgrounds and patients' perceptions of the importance of symptoms. However, our study differentiated clearly between GI symptoms at presentation and those developing during hospitalization. GI symptoms at the time of presentation are more likely attributable to COVID-19 as most patients were not taking any medications before the hospitalization. In contrast, GI symptoms developing during hospitalization may be multifactorial, including nosocomial infection, drug-related side effects, or progression of COVID-19.

Numerous mechanisms have been proposed for the development of GI symptoms in COVID-19 patients. The entry of the SARS-CoV-2 virus into host cells depends on the interaction of the virus spike protein with the receptor angiotensin-converting enzyme 2 and priming of the spike protein by host cell transmembrane serine protease 2[19,20]. Angiotensin-converting enzyme 2 and transmembrane serine protease 2 have been reported to be coexpressed in the GI tract, including esophageal upper epithelial and gland cells and absorptive enterocytes from the ileum and colon[19,20]. These enterocytes can be damaged, resulting in malabsorption and intestinal secretion abnormalities due to coronavirus or rotavirus infection[21,22]. It is, therefore, possible that GI manifestations in patients with COVID-19 might be associated with direct infection of enterocytes with the SARS-CoV-2 virus[19]. Elevated levels of fecal calprotectin, an inflammatory marker secreted by infiltrated neutrophils, in the fecal samples of COVID-19 patients with diarrhea also support this hypothesis[20].

Gut dysbiosis has been proposed as another mechanism to explain GI symptoms in COVID-19 patients. It is characterized by an increase in the opportunistic pathogens and reduction of beneficial commensals and correlates with COVID-19 severity and fecal levels of SARS-CoV-2[23]. It has been shown that the gut microbial signature of COVID-19 patients is different from healthy controls[24]. Although the clinical significance of these findings is still uncertain, it is possible that gut microbiota composition plays a role in modulating the systemic immune response.

The two primary modes of transmission for SARS-CoV-2 are respiratory droplets and direct contact[25], while the possibility of aerosol transmission has been suggested as well[25]. The GI tract has recently been proposed as an alternative transmission route for SARS-CoV-2 infection in a non-human primate model[26], raising the possibility of potential fecal-oral spread of the disease in humans as well. However, our study did not show any significant correlation of GI symptoms in family clusters, thus arguing against the potential fecal-oral transmission of the virus. Studies have reported the SARS-CoV-2 RNA in fecal samples or rectal swabs and fecal shedding of the virus continues even after clearance of respiratory samples[8,27-29]. The risk of transmission secondary to this prolonged shedding is unknown and warrants further studies.

Table 6 Multivariate analysis of risk factors for severe coronavirus disease 2019 at presentation and clinical outcomes, *n* (%)

Risk factors	Multivariate analysis	
	Adjusted odds ratio	*P* value
	Death	
Age > 65 yr	7.53 (3.09-18.29)	< 0.001
	Disease severity at presentation	
Hypertension	1.68 (1.08-2.60)	0.021
Diabetes mellitus	1.92 (1.28-2.87)	0.002
GI symptoms	1.66 (1.09-2.52)	0.017
Smoking	1.62 (1.01-2.63)	0.049
	ICU admission	
Age > 65 yr	1.79 (1.13-2.83)	0.012
Sex	1.82 (1.05-3.15)	0.033
Fever	2.14 (1.24-3.69)	0.006
Shortness of breath	2.90 (1.99-4.24)	< 0.001
Hypertension	1.82 (1.17-2.84)	0.008
	Mechanical ventilation	
Age > 65 yr	1.89 (1.94-2.99)	0.007
Sex	1.88 (1.02-3.45)	0.043
Fever	3.16 (1.63-6.09)	0.001
Shortness of breath	2.36 (1.57-3.55)	< 0.001
Hypertension	1.66 (1.05-2.62)	0.028
Vomiting	2.03 (1.10-3.75)	0.023

GI: Gastrointestinal; ICU: Intensive care unit.

COVID-19 patients with GI symptoms were more likely to have a longer duration of symptoms before diagnosis, more likely to have fatigue and myalgias, and less likely to have a cough. Lack of typical COVID symptoms (cough) and presence of atypical symptoms (GI symptoms) may result in delayed recognition and diagnosis of COVID-19 patients. Our findings are in keeping with those reported earlier[6,14]. The increased prevalence of myalgias and fatigue has been previously reported as well[9] and may be a reflection of the increased inflammatory burden in these patients. Patients with GI symptoms were more likely to have underlying malignancy and chronic liver disease. This finding has also been reported in the literature[30] and warrants careful evaluation of GI symptoms in cancer patients.

The severity of disease at presentation seen in COVID-19 patients with GI symptoms could be related to increased inflammatory activity in the intestines contributing to the systemic inflammatory response and cytokine syndrome[22]. Cytokine release syndrome is considered a leading cause of severe pneumonia and even death during COVID-19 disease[31]. Higher plasma levels of both proinflammatory and anti-inflammatory cytokines interleukin (IL)-2, IL-6, IL-7, IL-10, IL-18, granulocyte-macrophage colony-stimulating factor, C-C motif chemokine ligand 2 (also known as MCP1), tumor necrosis factor, and macrophage inflammatory protein 1 α have been detected in patients with severe disease compared to those with moderate disease[31,32]. The intestine produces high levels of IL-6 normally involved in crypt homeostasis[33] and can potentially contribute to the increased systemic IL-6 concentrations seen in COVID-19 patients with severe disease[32]. Similarly, the intestinal release of another proinflammatory cytokine, IL-18, can also contribute to disease severity and GI manifestations[34,35]. One recent study has shown downregulation of essential inflammatory genes in the small intestine and relative absence of inflammatory response in COVID-19 patients with GI symptoms[36]. These patients also had reduced levels of inflammatory proteins in circulation and reduced disease severity

and mortality, suggesting that gut inflammatory response has a potential role in modulating systemic immune reaction[36].

The new-onset GI symptoms during the hospitalization were most likely related to the use of several repurposed drugs, including hydroxychloroquine, ribavirin, and lopinavir/ritonavir. The GI side effects of these drugs have been reported in the literature and among COVID-19 patients in different clinical studies[37-39]. It must be noted that most of these therapies were administered during the first wave due to the lack of robust clinical evidence. Ribavirin, lopinavir/ritonavir, and hydroxychloroquine have not shown significant efficacy over standard care/placebo in COVID-19 patients[40,41]. This, coupled with potential adverse effects, warrant against the routine use of these medicines for COVID-19 treatment.

Our study has several limitations. It was a retrospective design, and reporting bias might affect the accurate estimates. We did not evaluate the mechanism and pathogenesis of GI symptoms in our patients. We also did not report the fecal viral load as there was no validated test available during the study period.

The strengths of our study include large sample size, a multi-ethnic population representing a real-world cohort, well-defined inclusion criteria, clear definition of GI symptoms, and distinction of GI symptoms at admission from those developing during the hospitalization. We had clearly defined outcomes and estimated the severity of the disease at presentation and later disease progression. We used multivariate regression to identify the independent risk factors.

CONCLUSION

In this current retrospective cohort study conducted in Qatar on a population with diverse ethnic backgrounds, we found a high prevalence (27.1%) of at least one GI symptom at presentation among COVID-19 patients requiring hospitalization. The mortality and disease progression was not different in patients with or without GI symptoms at presentation. However, patients with GI symptoms were more likely to have the severe disease at presentation and longer length of stay in the hospital. Additionally, one-quarter of the patients developed new GI symptoms during hospital admission. The most common culprit drugs associated with new GI symptoms development were lopinavir/ritonavir, ribavirin, and hydroxychloroquine. The lack of efficacy of several repurposed drugs for COVID-19 coupled with side effect profile warrants against their routine use in clinical practice. Further studies are needed to elucidate the mechanism and importance of GI manifestations in COVID-19 patients. Long-term sequelae of GI manifestations in COVID-19 patients remains unknown and needs to be studied.

ARTICLE HIGHLIGHTS

Research background

Gastrointestinal (GI) manifestations are present in 7%-15% of the patients with coronavirus disease 2019 (COVID-19). The association of GI manifestations with adverse clinical outcomes remains controversial, with some studies suggesting protective effects while others have reported adverse outcomes.

Research motivation

Previous studies reporting the association of GI symptoms with clinical outcomes in COVID-19 patients varied in determining the timing of symptoms development. We planned this study to clearly define GI symptoms in COVID-19 patients and distinguish between GI symptoms on admission and symptoms that develop during the hospital stay. We wanted to determine if there is any correlation of GI symptoms with disease severity and adverse clinical outcomes.

Research objectives

We aimed to determine the prevalence of GI symptoms in COVID-19 patients at admission and during hospitalization. We also aimed to study the correlation of GI symptoms with all-cause mortality, and disease severity at admission and disease progression during hospitalization defined by admission to the intensive care unit, development of acute respiratory distress syndrome, and need for mechanical ventilation.

Research methods

We conducted a retrospective cohort study investigating the epidemiological and clinical characteristics and outcomes among 601 consecutive adult patients with SARS-CoV-2 infection who were admitted to one of the dedicated COVID-19 hospitals in the state of Qatar between May 1-15, 2020. Clinical characteristics, laboratory parameters, treatment data, and disease outcome, including mortality, were compared between patients with and without GI symptoms. A multivariate logistic regression model with the forward method to identify independent predictors of the adverse outcomes.

Research results

The prevalence of any GI symptom at admission was 27.1% and during hospitalization was 19.8%. Nausea, vomiting, and diarrhea were the most common GI symptoms on presentation. There was no difference in mortality between the two groups (6.21% *vs* 5.50%, $P = 0.7$). However, patients with GI symptoms were more likely to have severe disease at presentation (33.13% *vs* 22.50%, $P < 0.001$) and prolonged hospital stay (15 d *vs* 14 d, $P = 0.04$). Age > 65 years was the single risk factor associated with increased mortality on multivariate regression analysis.

Research conclusions

Patients with GI symptoms are more likely to have severe disease at presentation. However, there is no difference in mortality between patients with and without GI symptoms.

Research perspectives

Future studies are needed to elucidate the mechanism of GI symptoms development in COVID-19 patients. Long-term effects and follow-up of COVID-19 patients with GI symptoms are needed.

REFERENCES

1 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

2 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

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

4 **Jin X**, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, Hao SR, Jia HY, Cai H, Zhang XL, Yu GD, Xu KJ, Wang XY, Gu JQ, Zhang SY, Ye CY, Jin CL, Lu YF, Yu X, Yu XP, Huang JR, Xu KL, Ni Q, Yu CB, Zhu B, Li YT, Liu J, Zhao H, Zhang X, Yu L, Guo YZ, Su JW, Tao JJ, Lang GJ, Wu XX, Wu WR, Qv TT, Xiang DR, Yi P, Shi D, Chen Y, Ren Y, Qiu YQ, Li LJ, Sheng J, Yang Y. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020; **69**: 1002-1009 [PMID: 32213556 DOI: 10.1136/gutjnl-2020-320926]

5 **Sultan S**, Altayar O, Siddique SM, Davitkov P, Feuerstein JD, Lim JK, Falck-Ytter Y, El-Serag HB; AGA Institute. AGA Institute Rapid Review of the Gastrointestinal and Liver Manifestations of COVID-19, Meta-Analysis of International Data, and Recommendations for the Consultative Management of Patients with COVID-19. *Gastroenterology* 2020; **159**: 320-334. e27 [PMID: 32407808 DOI: 10.1053/j.gastro.2020.05.001]

6 **Mao R**, Qiu Y, He JS, Tan JY, Li XH, Liang J, Shen J, Zhu LR, Chen Y, Iacucci M, Ng SC, Ghosh S, Chen MH. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020; **5**: 667-678 [PMID: 32405603 DOI: 10.1016/S2468-1253(20)30126-6]

7 **Parasa S**, Desai M, Thoguluva Chandrasekar V, Patel HK, Kennedy KF, Roesch T, Spadaccini M, Colombo M, Gabbiadini R, Artifon ELA, Repici A, Sharma P. Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019: A Systematic Review and Meta-analysis. *JAMA Netw Open* 2020; **3**: e2011335 [PMID: 32525549 DOI: 10.1001/jamanetworkopen.2020.11335]

8 **Cheung KS**, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, Ng YY, Chu MY, Chung TWH, Tam

AR, Yip CCY, Leung KH, Fung AY, Zhang RR, Lin Y, Cheng HM, Zhang AJX, To KKW, Chan KH, Yuen KY, Leung WK. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis. *Gastroenterology* 2020; **159**: 81-95 [PMID: 32251668 DOI: 10.1053/j.gastro.2020.03.065]

9 **Redd WD**, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Thompson CC, Shen L, Chan WW. Prevalence and Characteristics of Gastrointestinal Symptoms in Patients With Severe Acute Respiratory Syndrome Coronavirus 2 Infection in the United States: A Multicenter Cohort Study. *Gastroenterology* 2020; **159**: 765-767. e2 [PMID: 32333911 DOI: 10.1053/j.gastro.2020.04.045]

10 **Hajifathalian K**, Krisko T, Mehta A, Kumar S, Schwartz R, Fortune B, Sharaiha RZ; WCM-GI research group. Gastrointestinal and Hepatic Manifestations of 2019 Novel Coronavirus Disease in a Large Cohort of Infected Patients From New York: Clinical Implications. *Gastroenterology* 2020; **159** : 1137-1140. e2 [PMID: 32389667 DOI: 10.1053/j.gastro.2020.05.010]

11 **Aghemo A**, Piovani D, Parigi TL, Brunetta E, Pugliese N, Vespa E, Omodei PD, Preatoni P, Lleo A, Repici A, Voza A, Cecconi M, Malesci A, Bonovas S, Danese S; Humanitas COVID-19 Task Force. COVID-19 Digestive System Involvement and Clinical Outcomes in a Large Academic Hospital in Milan, Italy. *Clin Gastroenterol Hepatol* 2020; **18**: 2366-2368. e3 [PMID: 32437870 DOI: 10.1016/j.cgh.2020.05.011]

12 **Wan Y**, Li J, Shen L, Zou Y, Hou L, Zhu L, Faden HS, Tang Z, Shi M, Jiao N, Li Y, Cheng S, Huang Y, Wu D, Xu Z, Pan L, Zhu J, Yan G, Zhu R, Lan P. Enteric involvement in hospitalised patients with COVID-19 outside Wuhan. *Lancet Gastroenterol Hepatol* 2020; **5**: 534-535 [PMID: 32304638 DOI: 10.1016/S2468-1253(20)30118-7]

13 **Liu J**, Cui M, Yang T, Yao P. Correlation between gastrointestinal symptoms and disease severity in patients with COVID-19: a systematic review and meta-analysis. *BMJ Open Gastroenterol* 2020; **7**: e000437 [PMID: 32665397 DOI: 10.1136/bmjgast-2020-000437]

14 **Nobel YR**, Phipps M, Zucker J, Lebwohl B, Wang TC, Sobieszczyk ME, Freedberg DE. Gastrointestinal Symptoms and Coronavirus Disease 2019: A Case-Control Study From the United States. *Gastroenterology* 2020; **159**: 373-375. e2 [PMID: 32294477 DOI: 10.1053/j.gastro.2020.04.017]

15 **Shehab M**, Alrashed F, Shuaibi S, Alajmi D, Barkun A. Gastroenterological and hepatic manifestations of patients with COVID-19, prevalence, mortality by country, and intensive care admission rate: systematic review and meta-analysis. *BMJ Open Gastroenterol* 2021; **8**: e000571 [PMID: 33664052 DOI: 10.1136/bmjgast-2020-000571]

16 **World Health Organization**. COVID-19 Clinical management: living guidance. [cited 25 January 2021]. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1

17 **Zhou F**, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**: 1054-1062 [PMID: 32171076 DOI: 10.1016/S0140-6736(20)30566-3]

18 **Chen A**, Agarwal A, Ravindran N, To C, Zhang T, Thuluvath PJ. Are Gastrointestinal Symptoms Specific for Coronavirus 2019 Infection? *Gastroenterology* 2020; **159**: 1161-1163.e2 [PMID: 32422209 DOI: 10.1053/j.gastro.2020.05.036]

19 **Zhan GF**, Wang Y, Yang N, Luo AL, Li SY. Digestive system involvement of infections with SARS-CoV-2 and other coronaviruses: Clinical manifestations and potential mechanisms. *World J Gastroenterol* 2021; **27**: 561-575 [PMID: 33642829 DOI: 10.3748/wjg.v27.i7.561]

20 **Effenberger M**, Grabherr F, Mayr L, Schwaerzler J, Nairz M, Seifert M, Hilbe R, Seiwald S, Scholl-Buergi S, Fritsche G, Bellmann-Weiler R, Weiss G, Müller T, Adolph TE, Tilg H. Faecal calprotectin indicates intestinal inflammation in COVID-19. *Gut* 2020; **69**: 1543-1544 [PMID: 32312790 DOI: 10.1136/gutjnl-2020-321388]

21 **Crawford SE**, Ramani S, Tate JE, Parashar UD, Svensson L, Hagbom M, Franco MA, Greenberg HB, O'Ryan M, Kang G, Desselberger U, Estes MK. Rotavirus infection. *Nat Rev Dis Primers* 2017; **3**: 17083 [PMID: 29119972 DOI: 10.1038/nrdp.2017.83]

22 **Glass RI**, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med* 2009; **361**: 1776-1785 [PMID: 19864676 DOI: 10.1056/NEJMra0804575]

23 **Zuo T**, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology* 2020; **159**: 944-955. e8 [PMID: 32442562 DOI: 10.1053/j.gastro.2020.05.048]

24 **Gu S**, Chen Y, Wu Z, Gao H, Lv L, Guo F, Zhang X, Luo R, Huang C, Lu H, Zheng B, Zhang J, Yan R, Zhang H, Jiang H, Xu Q, Guo J, Gong Y, Tang L, Li L. Alterations of the Gut Microbiota in Patients With Coronavirus Disease 2019 or H1N1 Influenza. *Clin Infect Dis* 2020; **71**: 2669-2678 [PMID: 32497191 DOI: 10.1093/cid/ciaa709]

25 **Liu Y**, Ning Z, Chen Y, Guo M, Liu Y, Gali NK, Sun L, Duan Y, Cai J, Westerdahl D, Liu X, Xu K, Ho KF, Kan H, Fu Q, Lan K. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* 2020; **582**: 557-560 [PMID: 32340022 DOI: 10.1038/s41586-020-2271-3]

26 **Jiao L**, Li H, Xu J, Yang M, Ma C, Li J, Zhao S, Wang H, Yang Y, Yu W, Wang J, Yang J, Long H, Gao J, Ding K, Wu D, Kuang D, Zhao Y, Liu J, Lu S, Liu H, Peng X. The Gastrointestinal Tract Is an Alternative Route for SARS-CoV-2 Infection in a Nonhuman Primate Model. *Gastroenterology* 2021; **160**: 1647-1661 [PMID: 33307034 DOI: 10.1053/j.gastro.2020.12.001]

27 **Wu Y**, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X,

Mishra N, Lu J, Shan H, Jiang G, Huang X. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol Hepatol* 2020; **5**: 434-435 [PMID: 32199469 DOI: 10.1016/S2468-1253(20)30083-2]

28 **Zheng S**, Fan J, Yu F, Feng B, Lou B, Zou Q, Xie G, Lin S, Wang R, Yang X, Chen W, Wang Q, Zhang D, Liu Y, Gong R, Ma Z, Lu S, Xiao Y, Gu Y, Zhang J, Yao H, Xu K, Lu X, Wei G, Zhou J, Fang Q, Cai H, Qiu Y, Sheng J, Chen Y, Liang T. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ* 2020; **369**: m1443 [PMID: 32317267 DOI: 10.1136/bmj.m1443]

29 **Han C**, Duan C, Zhang S, Spiegel B, Shi H, Wang W, Zhang L, Lin R, Liu J, Ding Z, Hou X. Digestive Symptoms in COVID-19 Patients With Mild Disease Severity: Clinical Presentation, Stool Viral RNA Testing, and Outcomes. *Am J Gastroenterol* 2020; **115**: 916-923 [PMID: 32301761 DOI: 10.14309/ajg.0000000000000664]

30 **Grover S**, Redd WD, Zhou JC, Nije C, Wong D, Hathorn KE, McCarty TR, Bazarbashi AN, Shen L, Chan WW. High Prevalence of Gastrointestinal Manifestations of COVID-19 Infection in Hospitalized Patients With Cancer. *J Clin Gastroenterol* 2021; **55**: 84-87 [PMID: 33116066 DOI: 10.1097/MCG.0000000000001462]

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

32 **Lucas C**, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, Ellingson MK, Mao T, Oh JE, Israelow B, Takahashi T, Tokuyama M, Lu P, Venkataraman A, Park A, Mohanty S, Wang H, Wyllie AL, Vogels CBF, Earnest R, Lapidus S, Ott IM, Moore AJ, Muenker MC, Fournier JB, Campbell M, Odio CD, Casanovas-Massana A; Yale IMPACT Team, Herbst R, Shaw AC, Medzhitov R, Schulz WL, Grubaugh ND, Dela Cruz C, Farhadian S, Ko AI, Omer SB, Iwasaki A. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 2020; **584**: 463-469 [PMID: 32717743 DOI: 10.1038/s41586-020-2588-y]

33 **Jeffery V**, Goldson AJ, Dainty JR, Chieppa M, Sobolewski A. IL-6 Signaling Regulates Small Intestinal Crypt Homeostasis. *J Immunol* 2017; **199**: 304-311 [PMID: 28550196 DOI: 10.4049/jimmunol.1600960]

34 **Guo M**, Tao W, Flavell RA, Zhu S. Potential intestinal infection and faecal-oral transmission of SARS-CoV-2. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 269-283 [PMID: 33589829 DOI: 10.1038/s41575-021-00416-6]

35 **Tao W**, Zhang G, Wang X, Guo M, Zeng W, Xu Z, Cao D, Pan A, Wang Y, Zhang K, Ma X, Chen Z, Jin T, Liu L, Weng J, Zhu S. Analysis of the intestinal microbiota in COVID-19 patients and its correlation with the inflammatory factor IL-18. *Med Microecol* 2020; **5**: 100023 [PMID: 34173452 DOI: 10.1016/j.medmic.2020.100023]

36 **Livanos AE**, Jha D, Cossarini F, Gonzalez-Reiche AS, Tokuyama M, Aydillo T, Parigi TL, Ladinsky MS, Ramos I, Dunleavy K, Lee B, Dixon RE, Chen ST, Martinez-Delgado G, Nagula S, Bruce EA, Ko HM, Glicksberg BS, Nadkarni G, Pujadas E, Reidy J, Naymagon S, Grinspan A, Ahmad J, Tankelevich M, Bram Y, Gordon R, Sharma K, Houldsworth J, Britton GJ, Chen-Liaw A, Spindler MP, Plitt T, Wang P, Cerutti A, Faith JJ, Colombel JF, Kenigsberg E, Argmann C, Merad M, Gnjatic S, Harpaz N, Danese S, Cordon-Cardo C, Rahman A, Schwartz RE, Kumta NA, Aghemo A, Bjorkman PJ, Petralia F, van Bakel H, Garcia-Sastre A, Mehandru S. Intestinal Host Response to SARS-CoV-2 Infection and COVID-19 Outcomes in Patients With Gastrointestinal Symptoms. *Gastroenterology* 2021; **160**: 2435-2450. e34 [PMID: 33676971 DOI: 10.1053/j.gastro.2021.02.056]

37 **Bansal P**, Goyal A, Cusick A 4th, Lahan S, Dhaliwal HS, Bhyan P, Bhattad PB, Aslam F, Ranka S, Dalia T, Chhabra L, Sanghavi D, Sonani B, Davis JM 3rd. Hydroxychloroquine: a comprehensive review and its controversial role in coronavirus disease 2019. *Ann Med* 2021; **53**: 117-134 [PMID: 33095083 DOI: 10.1080/07853890.2020.1839959]

38 **Hung IF**, Lung KC, Tso EY, Liu R, Chung TW, Chu MY, Ng YY, Lo J, Chan J, Tam AR, Shum HP, Chan V, Wu AK, Sin KM, Leung WS, Law WL, Lung DC, Sin S, Yeung P, Yip CC, Zhang RR, Fung AY, Yan EY, Leung KH, Ip JD, Chu AW, Chan WM, Ng AC, Lee R, Fung K, Yeung A, Wu TC, Chan JW, Yan WW, Chan JF, Lie AK, Tsang OT, Cheng VC, Que TL, Lau CS, Chan KH, To KK, Yuen KY. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet* 2020; **395**: 1695-1704 [PMID: 32401715 DOI: 10.1016/S0140-6736(20)31042-4]

39 **Sung H**, Chang M, Saab S. Management of Hepatitis C Antiviral Therapy Adverse Effects. *Curr Hepat Rep* 2011; **10**: 33-40 [PMID: 21423320 DOI: 10.1007/s11901-010-0078-7]

40 **Welte T**, Ambrose LJ, Sibbring GC, Sheikh S, Müllerová H, Sabir I. Current evidence for COVID-19 therapies: a systematic literature review. *Eur Respir Rev* 2021; **30** [PMID: 33731328 DOI: 10.1183/16000617.0384-2020]

41 **Li H**, Xiong N, Li C, Gong Y, Liu L, Yang H, Tan X, Jiang N, Zong Q, Wang J, Lu Z, Yin X. Efficacy of ribavirin and interferon-α therapy for hospitalized patients with COVID-19: A multicenter, retrospective cohort study. *Int J Infect Dis* 2021; **104**: 641-648 [PMID: 33515771 DOI: 10.1016/j.ijid.2021.01.055]

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World J Gastroenterol 2021 December 14; 27(46): 8010-8030

DOI: 10.3748/wjg.v27.i46.8010

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Life prognosis of sentinel node navigation surgery for early-stage gastric cancer: Outcome of lymphatic basin dissection

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Author contributions: Kinami S was responsible for the scientific conception of the study and writing of the manuscript; Kinami S, Nakamura N, Miyashita T, Kitakata H, Fushida S, Fujimura T, and Ito T contributed to the surgery and data collection; Iida Y was responsible for the statistical analysis; Takamura H and Inaki N contributed to the drafting, editing, and critical revision of the manuscript; and all authors contributed to the approval of the final version of the manuscript.

Institutional review board statement: This study was approved by the ethics committee of Kanazawa University Hospital and Kanazawa Medical University (Trial Number R093, M288). ICG mapping was approved by the ethics committee of Kanazawa Medical University (Trial Number

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Abstract

BACKGROUND

Lymphatic basin dissection is a sentinel node biopsy method that is specific for gastric cancer. In this method, the dyed lymphatic system is dissected *en bloc*, and sentinel nodes are identified at the back table (*ex vivo*). Even with lymphatic basin dissection, blood flow to the residual stomach can be preserved, and function-preserving curative gastrectomy can be performed. The oncological safety of function-preserving curative gastrectomy combined with lymphatic basin dissection has not yet been fully investigated. We hypothesized that the oncological safety of sentinel node navigation surgery (SNNS) is not inferior to that of the guidelines.

AIM

To investigate the life prognosis of SNNS for gastric cancer in comparison with guidelines surgery.

METHODS

This was a retrospective cohort study. Patients were selected from gastric cancer

M404).

Informed consent statement: All patients provided written informed consent for surgery and the use of their data. Regarding data use in the retrospective study, the patients were given the opportunity to opt out of the study at any time.

Conflict-of-interest statement: The authors declare no conflicts of interest related to the publication of this study.

Data sharing statement: No additional data are available.

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

Country/Territory of origin: Japan

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

patients who underwent sentinel node biopsy from April 1999 to March 2016. Patients from April 1999 to August 2008 were from the Department of Surgery II, Kanazawa University Hospital, and patients from August 2009 to March 2016 were from the Department of Surgical Oncology, Kanazawa Medical University Hospital. Patients who were diagnosed with gastric cancer, which was preoperatively diagnosed as superficial type (type 0), 5 cm or less in length, clinical T1-2 and node negative, and underwent various gastrectomies guided by sentinel node navigation were retrospectively collected. The overall survival (OS) and relapse-free survival (RFS) of these patients (SNNS group) were investigated. Patients with gastric cancer of the same stage and who underwent guidelines gastrectomy with standard nodal dissection were also selected as the control group.

RESULTS

A total of 239 patients in the SNNS group and 423 patients in the control group were included. Pathological nodal metastasis was observed in 10.5% and 10.4% of the SNNS and control groups, respectively. The diagnostic abilities of sentinel node biopsy were 84% and 98.6% for sensitivity and accuracy, respectively. In the SNNS group, 81.6% of patients underwent modified gastrectomy or function-preserving curative gastrectomy with lymphatic basin dissection, in which the extent of nodal dissection was further reduced compared to the guidelines. The OS rate in the SNNS group was 96.8% at 5 years and was significantly better than 91.3% in the control group (P = 0.0014). The RFS rates were equal in both groups. After propensity score matching, there were 231 patients in both groups, and the cumulative recurrence rate was 0.43% at 5 years in the SNNS group and 1.30% in the control group, which was not statistically different.

CONCLUSION

The oncological safety of patients who undergo gastrectomy guided by sentinel node navigation is not inferior to that of the guidelines surgery.

Key Words: Early gastric cancer; Sentinel node biopsy; Function preserving surgery; Lymph node dissection; Gastrectomy; Lymphatic basin dissection

Core Tip: The oncological safety of 239 patients with early-stage gastric cancer who underwent sentinel node navigation surgery was investigated. In total, 81.6% of patients underwent modified gastrectomy or function-preserving curative gastrectomy with lymphatic basin dissection, and the extent of nodal dissection was reduced compared to the guidelines. The overall survival rate at 5 years was significantly better, and the cumulative recurrence rate was equal to that of the control group in original data sets and propensity score-matched comparisons. The oncological safety of patients undergoing gastrectomy guided by sentinel node navigation is not inferior to that of the guidelines surgery.

Citation: Kinami S, Nakamura N, Miyashita T, Kitakata H, Fushida S, Fujimura T, Iida Y, Inaki N, Ito T, Takamura H. Life prognosis of sentinel node navigation surgery for early-stage gastric cancer: Outcome of lymphatic basin dissection. *World J Gastroenterol* 2021; 27(46): 8010-8030
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/8010.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.8010

INTRODUCTION

The basic treatment for early gastric cancer not indicated for endoscopic submucosal dissection (ESD) is gastrectomy with lymph node dissection[1,2]. The range of prophylactic lymphadenectomy is determined in the greatest common denominator based on past data of lymph node metastasis, because most metastases to regional lymph nodes

Received: May 20, 2021
Peer-review started: May 20, 2021
First decision: June 22, 2021
Revised: June 28, 2021
Accepted: November 29, 2021
Article in press: November 29, 2021
Published online: December 14, 2021

P-Reviewer: Shi Y
S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

in early gastric cancer cannot be determined without pathological specimens. The Japanese Gastric Cancer Treatment Guidelines[3] recommends D1 + and D1 as the range of nodal dissection for cT1N0 cancer. D1 + requires sacrificial resection of most of the feeding arteries, resulting in the need for extensive gastrectomy. However, patients with nodal metastasis account for only approximately 20% of surgical patients with early gastric cancer. Excessive gastrectomy is performed in 80% of patients with early gastric cancer[4].

The preoperative diagnosis of lymph node metastasis is limited[5-11]. If lymph node metastasis can be diagnosed intraoperatively and node-negative patients can be distinguished, excessive dissection and extensive gastrectomy can be avoided. Currently, the most effective method for diagnosing lymph node metastasis is sentinel lymph node biopsy[12-27].

The sentinel lymph nodes of gastric cancer can be identified by administering a tracer with lymph-palatability to the submucosa using a gastroscopic injection needle and regarding the tracer-taking lymph nodes as sentinel nodes[14,15,24-26]. However, intraoperative pathological diagnosis of lymph node metastasis remains difficult[28]. Genetic diagnosis[29-33] is still in the research phase and, at present, we have to rely on intraoperative rapid frozen section diagnosis, but this method is accompanied by false negatives. Unlike breast cancer, reoperation for additional nodal dissection or additional radiation therapy is not acceptable in the case of gastric cancer. Therefore, a certain range of nodal dissection is necessary even in patients who are node negative by sentinel node biopsy. In view of these trends, Miwa[34] proposed lymphatic basin dissection, which is a sentinel node biopsy method specific for gastric cancer. In dye-based sentinel node biopsy, the lymphatic system specific to gastric cancer is stained by a dye tracer that is administered to the stomach and drains into the lymphatic system. The lymphatic system is then dissected *en bloc* and sentinel nodes are identified at the back table (*ex vivo*) in this method. This method not only reduces the difficulty of sentinel node biopsy, but also serves to a certain extent as backup dissection to cover false negatives of rapid intraoperative diagnosis. Even with lymphatic basin dissection, blood flow to the residual stomach can be preserved and function-preserving curative gastrectomy can be performed instead of extensive gastrectomy (Figure 1)[4,35].

Lymphatic basin dissection has been evaluated as a certain sentinel lymph node biopsy for gastric cancer[4,15,36]. However, the oncological safety of function-preserving curative gastrectomy combined with lymphatic basin dissection has not yet been fully investigated. In this study, we investigated the life prognosis of patients who underwent sentinel node navigation surgery (SNNS) for gastric cancer in comparison with standard surgery.

A prospective nation-wide study is currently undergoing in Japan to verify the oncological safety of the tailor-made surgical strategy guided by sentinel node navigation[37]. However, it is not a comparative study, and a control group has not been set due to difficulty in clinical circumference. In contrast, standard surgery performed at our facility complies with the Japanese guidelines has been performed as the routine medical treatment simultaneously and in parallel with the clinical trial of SNNS by the first author, which made it possible for us to compare the prognoses retrospectively. Therefore, we conducted this retrospective comparative study on patients who underwent SNNS and those who underwent the standard surgery performed as per the guidelines. The sentinel node biopsy is a diagnostic method for lymph node metastasis, and its applicability is determined based on the preoperative findings. To reproduce the findings of the prospective study, we selected patients with preoperative findings that were the same as those with indications for SNNS, and verified them using propensity score matching.

MATERIALS AND METHODS

This was a retrospective cohort study. Patients were selected from gastric cancer patients who underwent sentinel node biopsy by the first author (SK) from April 1999 to March 2016. The inclusion criteria were as follows: Age between 20 and 85 years; American Society of Anesthesiologists physical status (ASA-PS) 1-2 and tolerance to general anesthesia and gastrectomy; superficial type (type 0); preoperative diagnosis of 5 cm or less in length; preoperative diagnosis of T1 or T2 (clinical T1-2); node-negative preoperative diagnosis by X-computed tomography (CT); preoperative confirmation of adenocarcinoma by endoscopic biopsy; and reliable medical records. Conversely, patients with synchronous multiple advanced cancers in other organs,

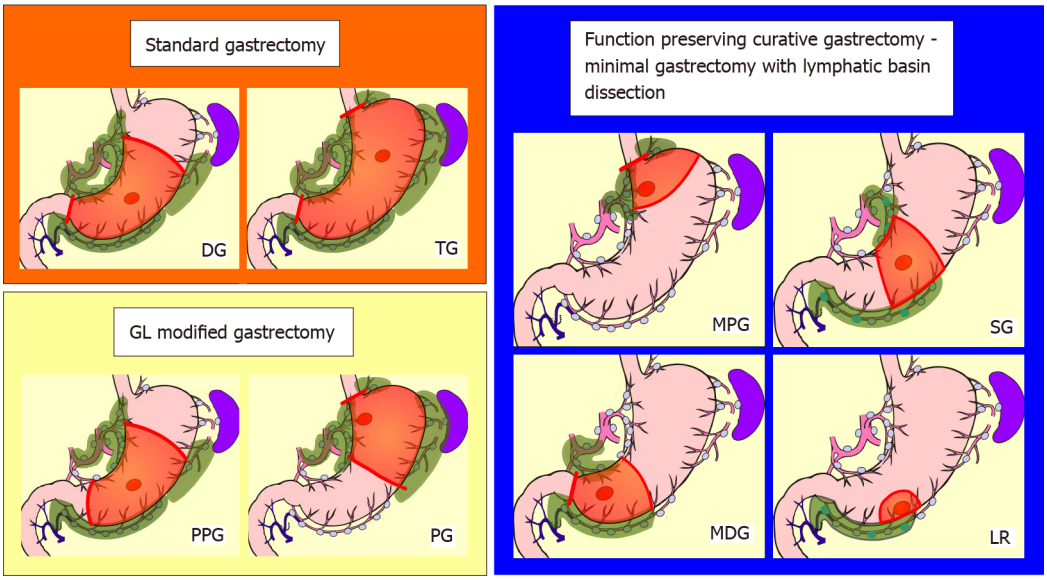

Figure 1 Schemas of standard gastrectomy, modified gastrectomy due to guidelines, and the function-preserving curative gastrectomy with lymphatic basin dissection. The red circle indicates the tumor, the green colored area indicates the extent of lymph node dissection, and the orange area indicates the extent of gastrectomy. The extent of nodal dissection in standard gastrectomy and modified gastrectomy according to the guidelines was D1 +. In contrast, the extent of nodal dissection in lymphatic basin dissection was defined as D0. GL: Japanese gastric cancer treatment guidelines; DG: Distal gastrectomy; TG: Total gastrectomy; PPG: Pylorus-preserving gastrectomy; PG: Proximal gastrectomy; MPG: Mini-proximal gastrectomy; SG: Segmental gastrectomy; MDG: Mini-distal gastrectomy; LR: Local resection.

with severe comorbidities, and those with an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 3 or higher were excluded. Patients from April 1999 to August 2008 were from the Department of Surgery II, Kanazawa University Hospital, and patients from August 2009 to March 2016 were from the Department of Surgical Oncology, Kanazawa Medical University Hospital.

For patients in the control group, early gastric cancer patients who underwent gastrectomy without sentinel node biopsy were extracted at the same time in the Department of Surgery II, Kanazawa University Hospital, and Department of Surgical Oncology, Kanazawa Medical University Hospital. The inclusion and exclusion criteria were the same as those of patients with sentinel node biopsy. In these patients, the standard surgeries in accordance with the Japanese guidelines[3] were mainly applied without mapping. The choice between mapping and non-mapping patients was mainly determined by the surgeon in charge. However, at both Kanazawa University Hospital and Kanazawa Medical University Hospital, a limited number of surgeons with the same treatment strategies and the same surgical skills were in charge of the gastrectomies.

The sentinel node biopsy methods used at Kanazawa University Hospital were the blue dye method, RI colloid method, and the combination method of blue dye and RI colloid. The dye tracers were patent blue or Lymphazurin, and the RI colloid tracers were ^{99m}Tc-tin colloid or ^{99m}Tc-phytate, which were endoscopically administered into the submucosal layer at four points around the tumor. The RI colloid was administered at 0.5 mL per site the day before surgery, and the blue dye was administered intraoperatively at 0.2 mL per site. The lymphatic basins were defined as the lymphatic system that was stained within 20 min after dye injection. The blue nodes were defined as nodes stained blue, and hot nodes were defined as nodes with radioactivity of more than 10 counts per second by using the gamma probe (Navigator GPS, Tyco Health Care, Mansfield, United States), and these were regarded as the sentinel nodes[14,36].

The indocyanine green (ICG) fluorescence method was used in Kanazawa Medical University Hospital[26]. ICG was adjusted to 50 μg/mL and endoscopically administered at 0.5 mL per site to the submucosal layer at four points around the tumor the day before surgery. Intraoperatively, ICG fluorescence was observed using a photodynamic eye (PDE, Hamamatsu Photonics, Shizuoka, Japan). The lymphatic basins were defined as the lymphatic system that was detected with fluorescent lymphatics, and the obvious fluorescent nodes were regarded as sentinel nodes. According to a previous report[36], lymphatic basins were integrated into the five lymphatic areas, except for the lymphatic flow to the left paracardial lymph node (No.

2 Lymph node, #2). Each of these is called the lymphatic compartment and is classified into five basins: The left gastric artery basin (*l*-GA); right gastric artery basin (*r*-GA); left gastroepiploic artery basin; right gastroepiploic artery basin; and the posterior gastric artery basin (*p*-GA) (Figure 2A). Classifying the lymphatic flow to #2 is challenging because of the multidirectional flow to *l*-GA and No. 19 ahead, and the lymphatic flow to *p*-GA nearby. Therefore, it was excluded from the lymphatic compartment classification and handled separately.

Patients who underwent sentinel node biopsy were divided into two groups: The feasibility phase group and the clinical application phase group. For patients in the former group, sentinel node biopsy was performed to evaluate the diagnostic ability of nodal metastasis; therefore, standard gastrectomy with nodal dissection was performed, and sentinel node identification was also performed postoperatively on the resected specimen. In contrast, in the clinical application phase, function-preserving curative gastrectomy was performed using sentinel node biopsy as a guide[4,26]. First, sentinel node mapping was performed, followed by lymphatic basin dissection, *ex vivo* identification and biopsy of the sentinel nodes, and intraoperative rapid pathology. If the sentinel nodes were diagnosed as metastasis at rapid diagnosis, standard gastrectomy with nodal dissection up to D2 was performed; if the sentinel nodes were diagnosed as node negative, the extent of gastrectomy was reduced and function-preserving curative gastrectomy, such as local resection (LR), segmental gastrectomy (SG), or proximal gastrectomy (PG) was performed according to the preserved blood flow (Figure 2B)[4]. This surgical strategy is generally called SNNS.

The patients were divided into two groups. Patients in the clinical application phase of sentinel node biopsy were designated as the study group (SNNS group). Patients who did not undergo sentinel node biopsy and those in the feasibility phase of sentinel node biopsy were defined as the control group. The control group consisted of patients who underwent guidelines gastrectomy, while the SNNS group consisted of patients who underwent tailor-made gastrectomy guided by sentinel node biopsy (Figure 3).

In this study, we examined and compared the prognosis of patients between the two groups. The prognosis of the patients at Kanazawa University Hospital was investigated in 2013, and that of Kanazawa Medical University Hospital was investigated in 2021. The prognosis was examined up to 10 years after initial gastrectomy, and the investigations included alive or dead, cause of death, presence or absence of recurrence, and the presence of newly detected metachronous multiple gastric cancer (MMGC) in the remnant stomach. Therefore, in this study, the prognosis up to 5 years was generally accurate, but some patients were censored because they did not reach 10 years after surgery at the time of investigation. The causes of death other than gastric cancer recurrence were divided into other cancer deaths (including MMGC) and non-cancer deaths from other diseases. The date of the confirmation of gastric cancer recurrence was also investigated. For cancers found in the remnant stomach, we distinguished between local recurrence and MMGC, and the latter was not judged as gastric cancer recurrence because of its favorable prognosis. In this study, overall survival (OS) treated all-cause mortality as an event, and relapse-free survival (RFS) treated gastric cancer recurrence as an event. All descriptions were described in accordance with the 15th edition of the Japanese classification of gastric carcinoma[38]. In this article, distal gastrectomy (DG) and total gastrectomy (TG) were defined as standard gastrectomy, pylorus-preserving gastrectomy (PPG), and PG were defined as guidelines-modified gastrectomy, and mini-DG (MDG), mini-PG (MPG), SG, and LR were defined as function-preserving curative gastrectomy (Figure 1)[4]. The diagnosis of lymph node metastases was determined by hematoxylin and eosin staining of the permanent slide at the maximum plane. The tumor cells were considered to be metastatic regardless of the size of metastatic foci, so both isolated tumor cells and micrometastases were also considered metastases. The results of immunohistochemical staining and genetic diagnosis were not considered in this study.

The chi-square test was used to compare the background factors of each group. Survival rates were compared by drawing survival curves using the Kaplan-Meier method and certified by using the log-rank test. Multivariate analysis of factors affecting survival was performed using Cox proportional hazards regression with a stepwise variable selection method. The Gray test was used to compare the cumulative incidence of recurrence, incidence of MMGC, other cancer-related deaths, and non-cancer deaths from other diseases, and Fine-Gray proportional hazards regression was used for multivariate analysis. In addition to these comparisons, propensity score matching was performed to adjust for differences in background factors between the two groups. Propensity scores were calculated for the two groups by logistic regression analysis using age, sex, location, circumference, long axis of tumor, macroscopic type, preoperative diagnosis of depth of invasion, and preoperative

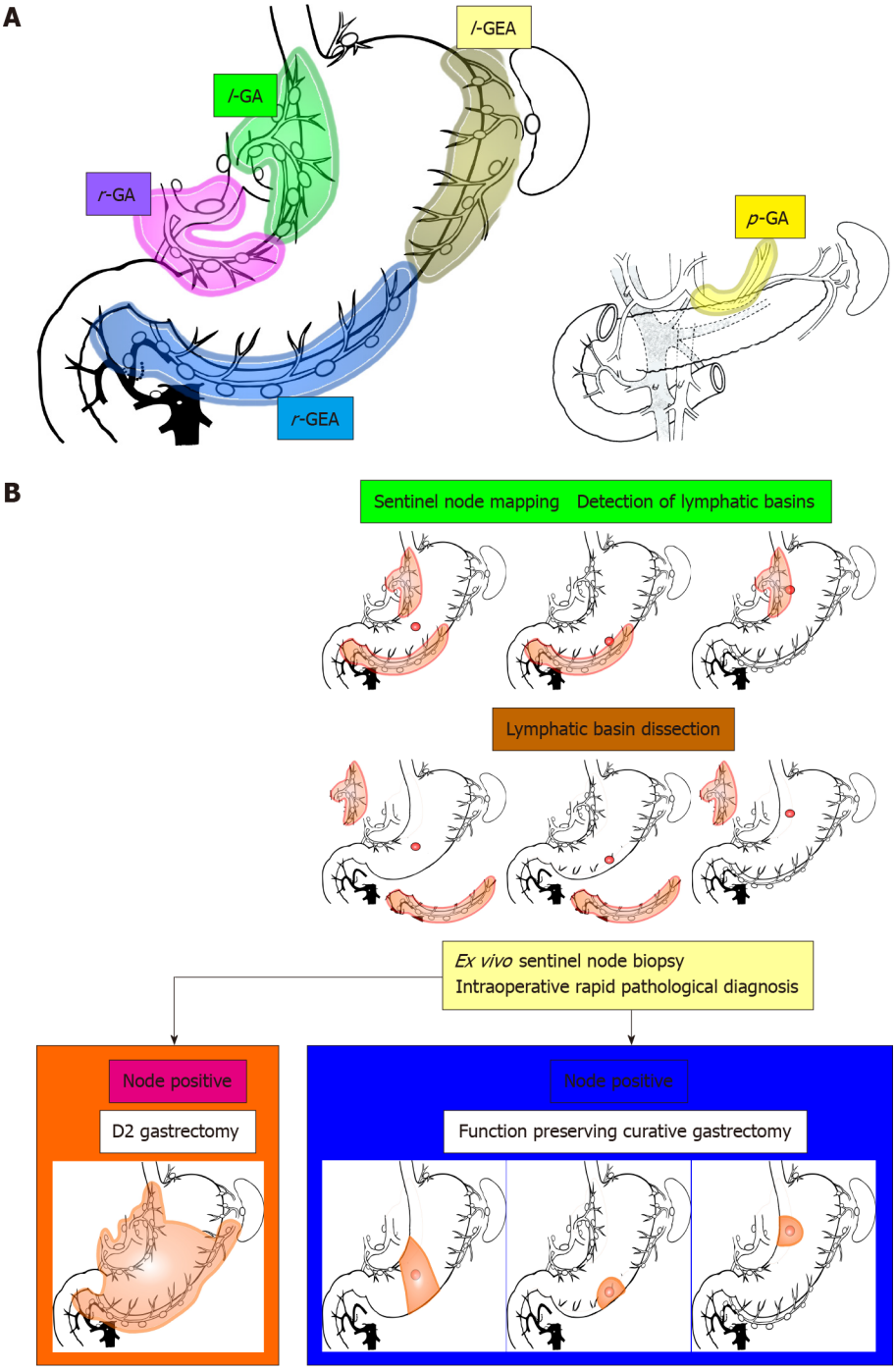

Figure 2 Lymphatic basins, lymphatic compartments, and the strategy of sentinel node navigation surgery. A: The lymphatic basins were defined as the lymphatic system that was detected with dyed or fluorescent lymphatics. The lymphatic basins were integrated into the five lymphatic areas. Each of these was called the lymphatic compartment and was classified into five basins; B: Algorithm for sentinel node navigation surgery for early gastric cancer. First, sentinel node mapping was performed, followed by lymphatic basin dissection, *ex vivo* identification and biopsy of the sentinel nodes, and intraoperative rapid pathology. If the sentinel nodes were diagnosed as metastasis at rapid diagnosis, standard gastrectomy with nodal dissection up to D2 was performed; if the sentinel nodes were diagnosed as node negative, the extent of gastrectomy was reduced and function-preserving curative gastrectomy, such as segmental gastrectomy or local resection, was applied. *l*-GA: Left gastric artery basin; *r*-GA: Right gastric artery basin; *l*-GEA: Left gastroepiploic artery basin; *r*-GEA: Right gastroepiploic artery basin; *p*-GA: Posterior gastric artery basin.

pathological diagnosis as variables. These variables were selected from among the factors that could affect the life prognosis and could be known preoperatively. To adjust for the covariates and estimate the causal effects, we used the nearest neighbor matching method with greedy matching and one-to-one matching with non-restorative extraction. The caliper of the propensity score was calculated by multiplying the standard deviation of the recommended propensity score estimated value by 0.2, after logit conversion. The balance between the groups was evaluated using the standardized difference score.

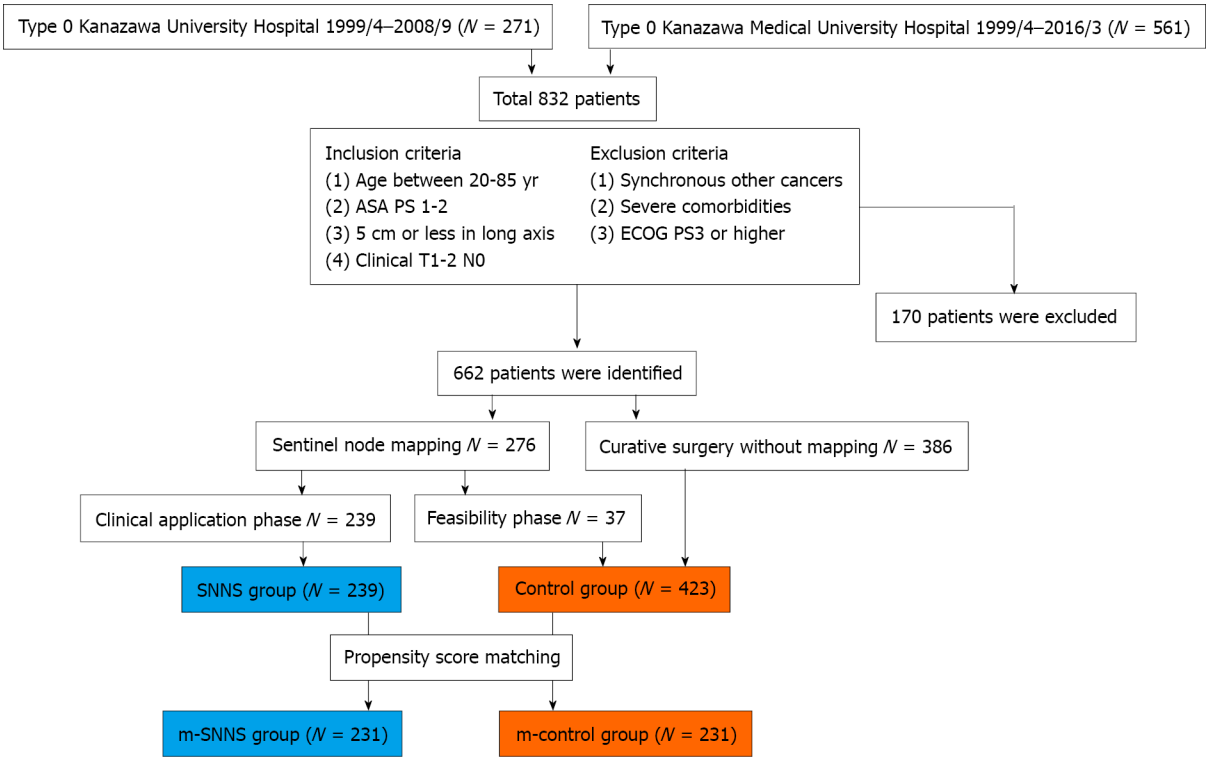

Figure 3 Summary of enrolled patients. The control group consisted of patients who underwent guidelines gastrectomy with standard lymph node dissection, while the sentinel node navigation surgery (SNNS) group consisted of patients who underwent tailor-made gastrectomy guided by sentinel node biopsy. SNNS: Sentinel node navigation surgery; m-SNNS: Propensity score-matched sentinel node navigation surgery; m-control: Propensity score-matched control; ECOG: Eastern Cooperative Oncology Group; ASA PS: American Society of Anesthesiologists physical status.

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). EZR is a modified version of R Commander designed to add statistical functions that are frequently used in biostatistics[39]. All statistical methods used in this study were reviewed by Yasuo Iida, Department of Mathematics, Division of General Education, Kanazawa Medical University.

This study was approved by the ethics committee of Kanazawa University Hospital and Kanazawa Medical University (Trial Number R093, M288) and registered with the University Hospital Medical Information Network Clinical Trials Registry (trial number UMIN000010154 and UMIN000023828). ICG mapping was approved by the ethics committee of Kanazawa Medical University (Trial Number M404 and jRCTs041180006 https://jrct.niph.go.jp/Latest-detail/iRCTs041180006).

This study was conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent for surgery and use of their data. Regarding data use in the retrospective study, the patients were allowed to opt out of the study at any time.

RESULTS

Characteristics of the patients

A total of 276 patients with sentinel node mapping and 386 patients who underwent sur-gery without mapping were collected. Of the sentinel lymph node mapping patients, 37 were in the feasibility phase and 239 were in the clinical application phase. Therefore, there were 239 patients in the SNNS group and 423 patients in the control group (Figure 3). The patient profiles are presented in Table 1. There were differences in age and histological type between the two groups. In the control group, 67.6% of the patients underwent standard surgery (TG, 5.4%; DG, 62.2%), and 26.7% of patients underwent guidelines-modified gastrectomy (PG, 12.1%; PPG, 14.6%). In contrast, only 18.4% of the patients in the SNNS group underwent standard surgery, 14.2% underwent modified gastrectomy, and 67.4% underwent function-preserving curative

Table 1 Patient characteristics

	n	SNNS *n* = 239	Control *n* = 423	*P* value
Age	Median (range)	64 (28-85)	67 (27-85)	0.004
Sex	Male:Female	157:82	289:134	0.491
Location	U:M:L	35:130:74	78:195:150	0.116
Circumference	Less:Ant:Gre:Post	107:37:48:47	201:78:72:72	0.492
Macroscopic type	Elevated:Depressed	62:177	108:315	0.926
Clinical T status (cT)	1a:1b:2	100:111:28	171:192:60	0.678
Clinical N status (cN)	0:1:2-3	239:0:0	423:0:0	1.000
Pathological diagnosis	DF:UDF	130:109	289:134	< 0.001
Sentinel node mapping	BD:RI:CM:ICG:None	39:6:135:59:0	2:1:13:21:386	< 0.001
Surgical procedure	TG:DG:PG:PPG; SG:MDG:MPG:LR	3:41:24:10; 84:33:6:38	23:263:51:62; 8:4:1:11	< 0.001
Nodal dissection	D0:D1(1 +):D2	174:42:23	45:191:187	< 0.001
Long axis (mm)	Median (range)	22 (2-65)	25 (4-87)	0.265
Pathological T (pT)	1a:1b:2:3-4	129:92:10:8	218:145:39:21	0.065
Pathological N (pN)	0:1:2-3	214:13:12	379:34:10	0.072
Recurrent cases		1	8	

SNNS: Sentinel node navigation surgery group; DF: Differentiated type; UDF: Undifferentiated type; BD: Blue dye mapping; RI: Radioisotope colloid mapping; CM: Dye and RI combination mapping; ICG: Indocyanine green fluorescence mapping; TG: Total gastrectomy; DG: Distal gastrectomy; PG: Proximal gastrectomy; PPG: Pylorus-preserving gastrectomy; SG: Segmental gastrectomy; MDG: Mini-distal gastrectomy; MPG: Mini-proximal gastrectomy; LR: Local resection.

gastrectomy (SG, 35.1%; MDG, 13.8%; MPG, 2.5%; LR, 15.9%), in which the extent of resection was reduced further than that recommended by the guidelines.

All patients in this study were preoperatively diagnosed as node negative by X-CT, but pathological nodal metastasis was observed in 10.5% (25 patients) in the SNNS group and 10.4% in the control group. Table 2 lists the 25 patients in the SNNS group.

Recurrence of gastric cancer and results of sentinel node biopsy

Two patients in the control group died after surgery (hospital death); one was due to aspiration pneumonia and the other was due to peritonitis from idiopathic colon perforation. In contrast, no in-hospital deaths were observed in the SNNS group. Gastric cancer recurrence was observed in one patient in the SNNS group and eight patients in the control group. The recurrent patient in the SNNS group is displayed as No. 16 in Table 2. He was diagnosed as node-positive intraoperatively by sentinel node biopsy, and DG D2 was performed. Although postoperative adjuvant chemotherapy with S1 was administered, the patient died of lymph node metastasis 53.2 mo later. The type of recurrence in eight patients in the control group were four of lymph node recurrence, two of liver metastasis, one of lung metastasis, and one patient of local recurrence.

Of the 276 patients with sentinel node mapping, 37 patients in the feasibility phase had no lymph node metastasis. In contrast, of the 239 patients in the clinical application phase, 25 patients had lymph node metastasis (Table 2). Of these 25 patients, 21 (No. 1-21) were diagnosed as positive for metastasis intraoperatively by sentinel node biopsy, and 4 (No. 22-25) were false negative. The diagnostic ability of sentinel node biopsy in this study was calculated to be 84% (21/25) for sensitivity, 100% for specificity, 100% for positive predictive value, 98.4% (251/255) for negative predictive value, and 98.6% (272/276) for accuracy. The reasons for false negatives were misdiagnosis of frozen section diagnosis in three patients (No. 22-24) and macroscopic lymph node metastasis, which was not able to take up tracer in one patient (No. 25). The diagnosis of metastasis in the later patient was easy due to intraoperative findings. Twenty-one patients who were diagnosed as node-positive by sentinel node biopsy during surgery underwent standard gastrectomy with D1 + or D2. On the other hand, two of the false-negative patients with rapid diagnosis underwent SG but were followed up without additional dissection. One patient died of pancreatic cancer (No.

Table 2 List of 25 patients of lymph node metastasis in the sentinel node navigation surgery group

No.	LOC	MAC	LA	cT	cN	sN	MP	LB	INDSN	OP	D	PD	pT	MS	NS	MLB	MOB	NNS	MRSC	PROG[1]
1	M	0 IIa + IIc	20	1b	0	0	ICG	*l*-GA	TP	DG	2	tub2	sm2	#3 #7	4	#7	-	1	82.3	Alive
2	M	0 IIc	25	1b	0	0	CM	*l*-GA	TP	DG	2	por1	sm2	#3	1	-	-	0	71.4	Alive
3	U	0 IIc	25	1b	0	0	ICG	*l*-GA	TP	PG	1 +	tub2	mp	#1 #3	2	#1	-	1	56.1	Trauma
4	M	0 IIa + IIc	40	2	0	0	ICG	*l*-GA	TP	DG	2	tub2	ss	#3	1	-	-	0	63.7	Alive
5	M	0 IIc + IIb	40	1b	0	0	ICG	*l*-GA	TP	DG	2	tub2	mp	#3	1	-	-	0	80.4	Alive
6	U	0 IIc	45	1b	0	0	CM	*l*-GA	TP	PG	1 +	sig	m	#1 #7	2	-	-	0	69.2	Alive
7	L	0 IIc + IIb	45	2	0	1	CM	*l*-GA	TP	DG	2	por2	ss	#3	2	#3	-	1	67.7	Alive
8	L	0 IIc + III	40	2	0	2	ICG	*r*-GEA	TP	DG	2	tub2	mp	#4d	2	#4d #6	#7	5	72.1	Alive
9	L	0 IIa	55	2	0	1	ICG	*l*-GA, *r*-GA	TP	DG	1 +	tub1	sm1	#5	1	-	-	0	67.7	Alive
10	L	0 IIa + IIc	20	1b	0	0	ICG	*l*-GA, *r*-GEA	TP	DG	2	tub2	sm2	#3 #4d #6	4	#6	-	1	62.0	Alive
11	M	0 IIc + III	25	2	0	0	BD	*l*-GA, *r*-GEA	TP	DG	2	por2	ss	#4d	2	-	-	0	120.0	Alive
12	M	0 IIa + IIc	25	1b	0	0	ICG	*l*-GA, *r*-GEA	TP	DG	1 +	tub2	sm2	#3 #7 #4d	4	-	-	0	59.5	CVD
13	M	0 I	32	1b	0	0	CM	*l*-GA, *r*-GEA	TP	TG	2	tub2	sm2	#3 #4d	5	-	-	0	64.7	Alive
14	M	0 IIc + IIb	37	1b	0	0	CM	*l*-GA, *r*-GEA	TP	DG	2	por2	m	#3	3	-	-	0	61.9	Alive
15	M	0 I	55	1a	0	0	ICG	*l*-GA, *r*-GEA	TP	DG	2	por2	mp	#4d	1	#3 #4d	-	4	69.8	Alive
16	M	0 IIc + III	30	2	0	1	BD	*l*-GA, *r*-GEA	TP	DG	2	por2	se	#1 #3 #4d	3	#4d	-	2	53.2	LNR
17	U	0 IIa + IIc	11	1a	0	0	CM	*l*-GA, *l*-GEA, *p*-GA	TP	PG	1 +	por1	sm2	#11d	1	#7	-	1	84.3	Alive
18	U	0 IIc	33	1b	0	0	CM	*l*-GA, *l*-GEA, *p*-GA	TP	PG	1 +	tub2	mp	#1	1	-	-	0	62.9	Alive
19	U	0 IIc	55	2	0	0	CM	*l*-GA, *r*-GEA, *r*-GEA	TP	TG	2	por2	sm2	#1 #3 #4d #10	4	#1	-	1	65.2	Alive
20	M	0 IIc	40	2	0	0	ICG	*l*-GA, *r*-GA, *r*-GEA	TP	DG	1+	tub2	sm1	#3	1	-	-	0	89.6	Alive
21	L	0 IIa + IIc	24	1b	0	2	CM	*l*-GA, *r*-GA, *r*-GEA	TP	DG	2	por1	sm2	#8a	1	#3	-	1	72.6	Alive
22	M	0 IIc	20	1b	0	0	ICG	*l*-GA, *r*-GEA	FN (FD)	SG	0	por2	sm2	(#4d)	1	#4d	-	1	61.2	Alive
23	M	0 IIc + III	23	2	0	0	CM	*l*-GA, *r*-GEA	FN (FD)	SG	0	tub2	mp	(#3)	1	#3	-	2	75.8	PK
24	L	0 IIc	45	1b	0	0	RI	*l*-GA, *r*-GA, *r*-GEA	FN (FD)	DG	2	por2	m	(#5)	1	-	-	0	66.8	Alive

25	L	0 I	25	1b	0	1	CM	*l*-GA, *r*-GA, *r*-GEA	FN (LM)	DG	2	tub2	sm2	-	0	#4d	-	1	63.6	Alive

[1]"PROG" column indicates whether the patients are alive at the time of recent survival confirmation, recurrent status of gastric cancer, or the cause of death.

SNNS: Sentinel node navigation surgery; LOC: Location; MAC: Macroscopic type; LA: Size of long axis (mm); cT: Clinical T status; cN: Clinical N status; sN: Surgical N status; MP; Mapping procedures; ICG: Indocyanine green fluorescence mapping; CM: Combination mapping; BD: Blue dye mapping; RI: Radioactive colloid mapping; LB: Distributions of lymphatic basins; *l*-GA: Left gastric artery basin; *r*-GA: Right gastric artery basin; *r*-GEA: Right gastroepiploic artery basin; *l*-GEA: Left gastroepiploic artery basin; *p*-GA: Posterior gastric artery basin; INDSN: Intraoperative nodal diagnosis by sentinel node biopsy; TP: True positive diagnosis for nodal metastasis; FN (FD): False-negative diagnosis because of frozen section diagnosis; FN (LM): False negative because of obvious macroscopic nodal metastasis; OP: Surgical procedures; DG: Distal gastrectomy; PG: Proximal gastrectomy; TG: Total gastrectomy; SG: Segmental gastrectomy; D: Degree of nodal dissection; PD: Dominant pathological diagnosis; pT: Pathological T status; MS: Metastastic stations of sentinel nodes; NS: Numerical numbers of metastatic sentinel nodes; MLB: Metastatic stations of not sentinel nodes inside the lymphatic basins; MOB: Metastatic stations of not sentinel nodes outside the basins; NNS: Numerical numbers of metastatic nodes of not sentinel nodes; MRSC: Months to recent survival confirmation; PROG: Prognosis, recurrent status or cause of death; CVD: Cerebrovascular disease; LNR: Lymph nodal recurrence; PK: Pancreas cancer.

23), while the other survived for 5 years without recurrence (No. 22). As for the remaining two patients, DG D2 was performed because one had macroscopic lymph node metastasis (No. 25) and the other was suspected from intraoperative findings to be advanced gastric cancer with serosal exposure (No. 24). However, No. 24 was pathologically a mucosal cancer. These patients survived for five years without recurrence.

Therefore, there were no recurrences in the 218 patients diagnosed as node negative by sentinel node biopsy (214 true negative + four false negative). Of these 218 patients, only 11 underwent standard surgery (DG or TG with D1 + or D2). A total of 190 patients underwent modified gastrectomy or function-preserving curative gastrectomy with reduction of the resection area, and 17 underwent gastrectomy with reduction of the nodal dissection.

Of the 25 patients with nodal metastasis in the SNNS group, 11 had metastasis to only the sentinel nodes, 12 had non-sentinel metastatic nodes other than the sentinel nodes, but they remained within the lymphatic basin, and one was a false-negative patient with macroscopic metastasis as described above, with only one metastatic node. Only one patient had a metastatic node outside the lymphatic basin (No. 8). In this patient, macroscopic metastasis was found intraoperatively, and the final pathological diagnosis was fT2(MP)N3a (#4d, 6, 7). The patient was alive 6 years after surgery without any sign of recurrence.

MMGC of the remnant stomach

After surgery, the residual stomach was followed up with periodic endoscopic examinations, and MMGCs were found in 21 patients. Table 3 shows a list of interval times until the diagnosis of MMGC and treatment details. Of the 21 patients, 5 were in the SNNS group and 16 were in the control group. Four patients in the SNNS group (80%) and eight in the control group (50%) underwent ESD; therefore, their remnant stomachs were preserved. In contrast, five patients in the control group required TG, and one patient was unresectable. The cumulative incidence of MMGC is shown in Figure 4, and there was no difference in the incidence of MMGC between the two groups.

Life prognosis of patients in the SNNS group

The OS of all the patients in this study is shown in Figure 5A. The 5-year survival rates were 92.7% and the 10-year survival rate was 83.2%, respectively. The results of univariate and multivariate analyses for factors affecting OS are shown in Table 4, which shows that OS was affected by age, sex, macroscopic type, size, and pathological nodal status, as well as by the SNNS group. The OS of the SNNS group was significantly better than that of the control group (Figure 5B).

RFS in the SNNS group was 99.6% at both 5 and 10 years, and the RFS in the control group was 98.1% at both 5 and 10 years. Since there were a small number of recurrent patients and these recurrences competed with other cancer deaths and non-cancer deaths from other diseases, the evaluation of RFS was difficult and should be examined by cumulative incidence. Figure 6 shows a graph of the cumulative incidence, including other cancer deaths and non-cancer deaths from other diseases. The cumulative incidence of non-cancer deaths from other diseases was lower in the SNNS group than in the control group, and a significant difference was observed in

Table 3 Profiles of metachronous multiple remnant gastric cancer patients

No.	Group	ISP	Treatment	MTMC	Curability[1]	MRSC	PROG[2]
1	SNNS	SG	DG	50.5	Curative	75.6	Pneumonia
2	SNNS	SG	ESD	60.2	Curative	114.9	Alive
3	SNNS	SG	ESD	22.9	Curative	79.9	Alive
4	SNNS	MPG	ESD	13.6	Curative	76.3	Alive
5	SNNS	LR	ESD	30.7	Curative	73.1	Alive
6	SNNS	DG	TG	43.6	Curative	62.9	Alive
7	Control	DG	TG	19.5	Curative	63.6	Alive
8	Control	DG	TG	17.2	Curative	97.4	Alive
9	Control	DG	ESD	44.3	Curative	55.5	AID
10	Control	DG	ESD	220.4	Curative	240.0	Alive
11	Control	DG	ESD	40.6	Curative	120.0	Alive
12	Control	PG	UR	74.3	UR	120.0	Alive
13	Control	PG	TG	76.8	Curative	120.0	Alive
14	Control	PG	ESD	18.5	Curative	120.0	Alive
15	Control	PG	ESD	28.2	Curative	120.0	Alive
16	Control	PPG	TG	50.8	Curative	120.0	Alive
17	Control	PPG	DG	85.0	Curative	116.2	Alive
18	Control	PPG	ESD	77.1	Curative	118.5	Alive
19	Control	PPG	ESD	22.0	Curative	98.2	Alive
20	Control	LR	DG	7.6	Cure	42.8	CVD
21	Control	LR	ESD	64.2	Cure	85.7	Alive

[1]"Curability" column indicates whether treatment for metachronous multiple remnant gastric cancers was curative or not. All but one unresectable patient could be resected radically, and there were no recurrences of metachronous gastric cancer. One unresectable patient was alive with metachronous cancer 10 years after the initial surgery.

[2]"PROG" column indicates whether the patients are alive at the time of recent survival confirmation, or the cause of death.

ISP: Initial surgical procedure; SG: Segmental gastrectomy; MPG: Mini-proximal gastrectomy; LR: Local resection; DG: Distal gastrectomy; PG: Proximal gastrectomy; PPG: Pylorus-preserving gastrectomy; ESD: Endoscopic submucosal dissection; TG: Total gastrectomy; UR: Unresectable; MTMC: Months to treat metachronous gastric cancer; MRSC: Months to recent survival confirmation; PROG: Prognosis or cause of death; AID: Autoimmune disease; CVD: Cerebrovascular disease; SNNS: Sentinel node navigation surgery.

the Gray test. Table 5 shows the results of the multivariate analysis using the Fine-Gray proportional hazard regression test. Age and the SNNS group were independent factors significantly affecting non-cancer deaths from other diseases, while age and macroscopic type were factors that significantly affected other cancer deaths, and pN was the only factor affecting gastric cancer recurrence.

Evaluation of life prognosis by propensity score matching

In the SNNS group, the gastric cancer recurrences might be comparable, and the number of non-cancer deaths from other diseases might be less than that in the control group. However, caution should be exercised when interpreting the results because of the significant difference in age distribution between the two groups. We re-examined the comparison of life prognosis using the propensity score matching method. Propensity score matching was performed for the two groups using the preoperatively recognizable items of age, sex, tumor location, macroscopic type, preoperative T factor, and pathological diagnosis. We added the long axis of the tumor to the items because size is an important factor affecting prognosis. The characteristics of the two groups after propensity score matching (m-SNNS and m-control groups) are shown in Table 6. There were 231 patients in both groups, and the backgrounds of the two groups became uniform. The distributions of the other factors were also examined after matching. There was no significant difference in pathological depth of invasion or

Table 4 Univariate and multivariate analysis for the factors affected to the overall survival

Factors	*vs*	Univariate[1] (Log-rank *P*)	Multivariate[2]		
			Hazard ratio	95%CI	*P* value
Age		< 0.0001	1.092	1.064-1.120	< 0.0001
Sex	Male:Female	0.0009	1.815	1.099-2.998	0.0199
Location		0.0978			
Circumference		0.301			
Macroscopic type	Elevated:Depressed	< 0.0001	1.678	1.131-2.490	0.0101
Clinical T status		0.632			
Pathological type	Diff.:Undiff.	0.0001			
Long axis (mm)		< 00001	0.9815	0.965-0.998	0.0287
Pathological N status		0.0033	1.785	1.288-2.473	0.0005
SNNS	SNNS:Control	0.0014	0.4892	0.298-0.802	0.0046

[1]The log-rank test was used for the univariate analysis of overall survival.
[2]The Cox proportional hazards regression model was used for multivariate analysis of overall survival.
SNNS: Sentinel node navigation surgery; diff.: Differentiated; Undiff.: Undifferentiated; CI: Confidential interval.

Table 5 Multivariate analysis for the factors affected to the cumulative incidence of causes of death or recurrences

Factors	Non-cancer deaths			Other cancers			Gastric cancer recurrence		
	HR	95%CI	*P* value	HR	95%CI	*P* value	HR	95%CI	*P* value
Age	1.101	1.064-1.140	< 0.0001	1.064	1.026-1.103	0.0008	0.992	0.894-1.101	0.88
Sex	1.533	0.823-2.855	0.18	2.126	0.863-5.239	0.1	3.213	0.592-17.43	0.18
Location	1.259	0.890-1.782	0.19	0.9636	0.637-1.457	0.86	1.472	0.482-4.502	0.5
Circumference	1.037	0.798-1.349	0.78	1.184	0.875-1.602	0.27	1.943	0.732-5.158	0.18
Macroscopic type	1.303	0.752-2.256	0.35	2.322	1.241-4.346	0.0084	1.083	0.136-8.636	0.94
Clinical T status	0.9053	0.584-1.403	0.66	0.8587	0.481-1.535	0.61	1.542	0.662-3.595	0.32
Pathological type	1.509	0.762-2.990	0.24	0.5908	0.211-1.657	0.32	1.27	0.165-9.786	0.82
Long axis	0.9802	0.958-1.003	0.086	0.9874	0.958-1.017	0.4	0.9468	0.867-1.034	0.22
Pathological N status	1.263	0.683-2.337	0.46	1.353	0.751-2.440	0.31	5.252	2.043-13.50	0.00058
SNNS	0.4438	0.230-0.855	0.015	0.7224	0.338-1.542	0.4	0.1859	0.030-1.166	0.072

SNNS: Sentinel node navigation surgery; HR: Hazard ratio; CI: Confidence interval.

pathological nodal status, although there was a natural difference in the distribution of sentinel node mapping and surgical techniques, and there were three cases of recurrence in the control group compared to one case in the SNNS group.

Figure 7 shows a graph of OS and the cumulative incidence of death or recurrence after matching. OS in the SNNS group was significantly better than that in the control group. The cumulative recurrence rate in the SNNS group was 0.43% at both 5 and 10 years, and in the control group was 1.30% at both 5 and 10 years, which was not statistically different. In contrast, the cumulative incidence of non-cancer deaths from other diseases was 2.6% at 5 years and 8.6% at 10 years in the SNNS group, and 5.7% at 5 years and 15.5% at 10 years in the control group. In the SNNS group, the cumulative incidence of non-cancer deaths from other diseases tended to be lower than that in the control group (P = 0.089).

Accuracy of preoperative diagnosis

Although all patients were preoperatively diagnosed with a long axis of 5 cm or less,

Table 6 Patient characteristics after propensity score matching

	n	m-SNNS *n* = 231	m-control *n* = 231	*P* value
Age	Median (range)	64 (29-85)	64 (27-85)	0.473
Sex	Male:Female	152:79	147:84	0.697
Location	U:M:L	34:125:72	37:119:75	0.843
Circumference	Less:Ant:Gre:Post	106:37:45:43	103:40:46:42	0.980
Macroscopic type	Elevated:Depressed	57:174	57:174	1.000
Clinical T status (cT)	1a:1b:2	98:105:28	88:108:35	0.528
Clinical N status (cN)	0:1:2-3	231:0:0	231:0:0	1.000
Pathological diagnosis	DF:UDF	130:101	126:105	0.779
Long axis (mm)	Median (range)	23 (2-65)	25 (4-87)	0.547
Sentinel node mapping	BD:RI:CM:ICG:None	38:5:132:56:0	1:1:8:15:206	< 0.001
Surgical procedure	TG:DG:PG:PPG; SG:MDG:MPG:LR	3:40:23:10; 80:31:6:37	14:147:25:32; 4:4:0:5	< 0.001
Nodal dissection	D0:D1(1+):D2	169:39:23	23:97:111	< 0.001
Pathological T (pT)	1a:1b:2:3-4	125:89:10:7	126:72:20:13	0.075
Pathological N (pN)	0:1:2-3	206:13:12	213:13:5	0.251
Recurrent cases		1	3	

m-SNNS: Matched sentinel node navigation surgery group; m-control: Matched control group; DF: Differentiated type; UDF: Undifferentiated type; BD: Blue dye mapping; RI: Radioisotope colloid mapping; CM: Dye and RI combination mapping; ICG: Indocyanine green fluorescence mapping; TG: Total gastrectomy; DG: Distal gastrectomy; PG: Proximal gastrectomy; PPG: Pylorus-preserving gastrectomy; SG: Segmental gastrectomy; MDG: Mini-distal gastrectomy; MPG: Mini-proximal gastrectomy; LR: Local resection.

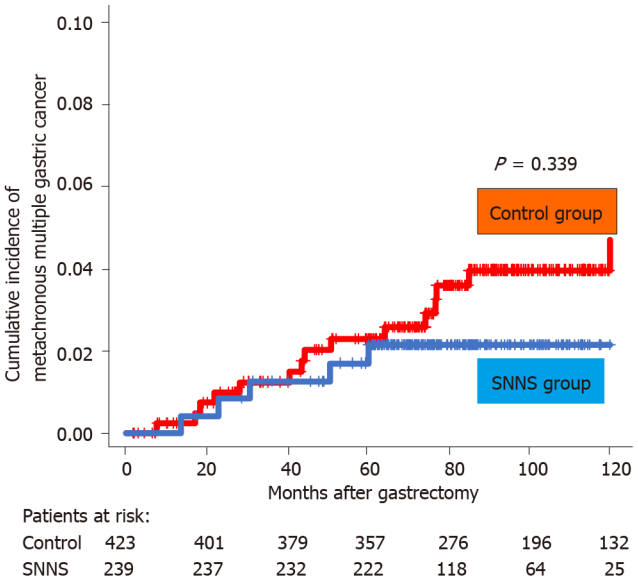

Figure 4 Cumulative incidence of metachronous multiple gastric cancer in the remnant stomach. There was no difference in the incidence of metachronous multiple gastric cancer between the SNNS and control groups. SNNS: sentinel node navigation surgery.

19 patients had a pathological diagnosis larger than 5 cm: Eight patients (3.3%) in the SNNS group and 11 (2.6%) in the control group. All 19 patients had a preoperative diagnosis of sN0, but four had pN1 and two had pN2. There were no recurrences in these 19 patients.

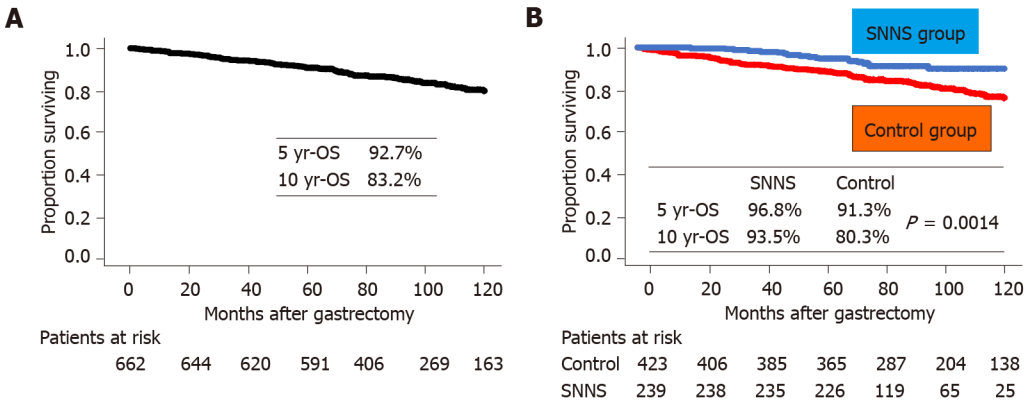

Figure 5 Overall survival curve. A: The overall survival of all patients; B: Comparison of overall survival between the sentinel node navigation surgery (SNNS) and control groups. The overall survival of the SNNS group was significantly better than the control group. SNNS: Sentinel node navigation surgery; OS: Overall survival.

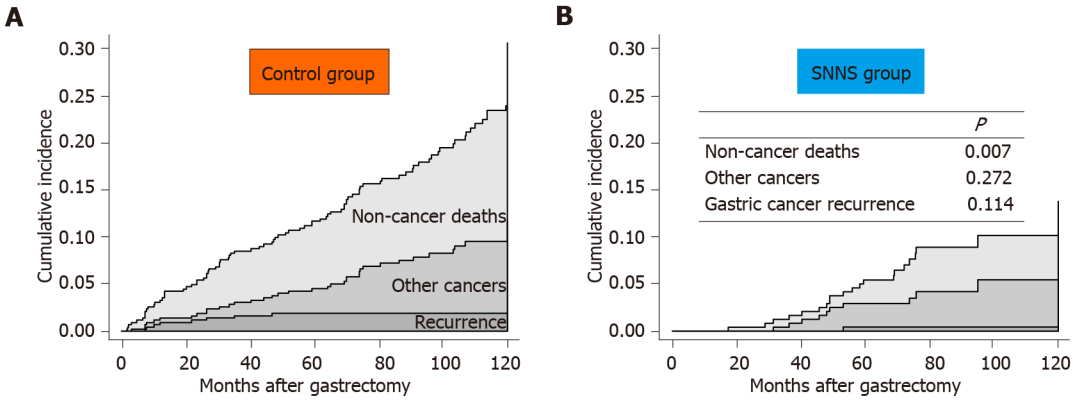

Figure 6 Cumulative incidence of gastric cancer recurrence or reason for death. A: Control group; B: Sentinel node navigation surgery (SNNS) group. The cumulative incidence of gastric cancer recurrence and other cancer-related deaths was almost equal between the two groups; in contrast, that of non-cancer deaths from other diseases was lower in the SNNS group than in the control group. SNNS: Sentinel node navigation surgery.

DISCUSSION

In both original data sets and propensity score-matched comparisons, the OS rate and RFS rate of patients who underwent gastrectomy guided by sentinel node navigation were not inferior to those of standard gastrectomy. In addition, there was no difference in the cumulative incidence of MMGC between the two groups.

Postgastrectomy syndrome (PGS) is a serious drawback after curative gastrectomy for gastric cancer[40-45], and occurs in a certain percentage of patients after standard gastrectomy. Currently, the most common approach for early gastric cancer is laparoscopic gastrectomy (D1 +) worldwide, especially in East Asian countries. Nevertheless, the occurrence rate of PGS and the quality of life (QoL) of patients after laparoscopic gastrectomy after 1 year is similar to that of patients after open gastrectomy[46-51]. To alleviate this, SNNS is a promising treatment strategy for function-preserving curative gastrectomy[4,14-27]. It has been reported that the PGS and QoL of function-preserving curative gastrectomy were less than those of standard gastrectomy[52-61]. However, there are two concerns that must be addressed before SNNS can be applied in clinical practice. One is that reducing the extent of nodal dissection may compromise curability. Another concern is whether preserving a large portion of the stomach will have any disadvantages, especially for an increase in the number of MMGCs of the remnant stomach.

In this study, we investigated the treatment outcome of SNNS from the viewpoint of life prognosis in comparison with the guidelines surgical strategy. In both the original data sets and propensity score-matched comparisons, the OS and RFS of the SNNS group were not inferior to those of the control group. This result supports the hypothesis that the oncological safety of the SNNS group is not inferior to that of the guidelines. Since this is a retrospective study, it is difficult to judge whether the life

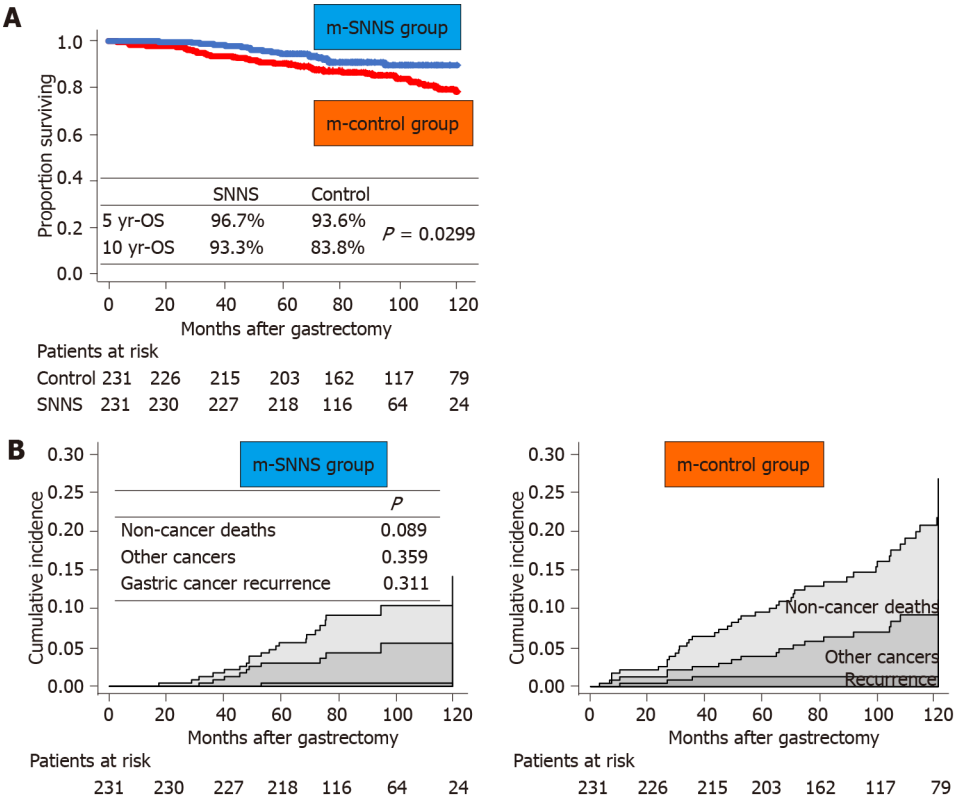

Figure 7 Comparisons of overall survival and cumulative incidence between the two groups after propensity score matching. A: Overall survival curves; B: Cumulative incidence curves of gastric cancer recurrence or the reason for death. The overall survival of the sentinel node navigation surgery (SNNS) group was significantly better than that of the control group. The cumulative recurrence of non-cancer deaths from other diseases in the SNNS group tended to be lower than that in the control group. SNNS: Sentinel node navigation surgery; m-SNNS: Propensity score-matched sentinel node navigation surgery; m-control: Propensity score-matched control; OS: Overall survival.

prognosis of the SNNS group is equivalent to that of the control group based on our results. A prospective non-inferiority trial is needed to make this scientific judgment. A prospective study is currently ongoing by the Japanese Society for SNNS[37]. In the protocol of this study, the expected 5-year recurrence-free survival rate was set at 98%, the non-inferiority margin was set at 10%, and the expected number of patients with sentinel node navigation was set to 225. The number of patients in the SNNS group in our study was 239 and, even after propensity score matching, 231 patients exceeded the number of patients in this prospective study. The result of life prognosis of the SNNS group in our study was one recurrent patient and 99.6% of RFS at both 5 and 10 years, comparable to conventional surgery. Extrapolating from these results, it seems that the curability of the SNNS could be proved to some extent. In addition, in the multivariate analysis, the only significant factor affecting gastric cancer recurrence was pN status, not SNNS grouping. In other words, the concern that reducing the extent of dissection may compromise curative outcomes would be unfounded.

The OS of the SNNS group was better than that of the control group in both comparisons of the original data sets and propensity score-matched groups. There was little difference in RFS between the two groups, and there was no significant difference in other cancer deaths. It was considered that the reason for this difference in OS would be the non-cancer deaths from other diseases. In multivariate analysis, the significant factors affecting non-cancer deaths were age and SNNS grouping. In the prospensity score-matched comparison, age was adjusted between the two groups, and a significantly better trend for non-cancer deaths was observed in the SNNS group. There is a possibility that keeping the gastrectomy area small leads to the maintenance of food volume, dietary habits, and nutritional status and has the effect of suppressing non-cancer death. However, this idea tends to be too advanced, and it may be reasonable to interpret that the survival outcome of patients with SNNS is not inferior to that of standard surgery.

In this study, we distinguished between MMGCs and local recurrence of gastric cancer. One patient with local recurrence of the oral stump was observed in the control group, whereas no local recurrence was observed in the SNNS group. This recurrent

patient was unresectable, underwent chemoradiotherapy, and died due to distant metastasis. Meanwhile, MMGC in the remnant stomach was observed in six patients in the SNNS group and 15 in the control group. One of these patients was unresectable and died after 10 years. However, all other MMGC patients were curatively resectable by gastrectomy or ESD, and there were no recurrent deaths from MMGC during the study period. Although it is sometimes difficult to distinguish between local recurrence and MMGC, we distinguished these two situations because of the favorable outcome of MMGC. A randomized prospective clinical trial of SNNS for gastric cancer was conducted in South Korea[27,62-65], and an interim analysis was recently reported at the American Society of Clinical Oncology (ASCO) annual meeting[63]. They reported that they failed to prove the non-inferiority of RFS in the SNNS group, but they did not strictly distinguish between MMGC and local recurrence. The MMGC and local recurrence should be clearly distinguished.

There was no difference in the cumulative incidence of MMGC[66-69] between the two groups in this study. Therefore, it was speculated that there is not much concern for whether MMGCs increase as the area of the remnant gastric mucosa increases. However, we cannot conclude with this result that there is no need to worry about the increased risk of MMGC in SNNS. Yaguchi *et al*[70] followed the prognosis of 50 SNNS cases and reported that MMGC occurred in 8% of cases. Kinami *et al*[71] conducted a national questionnaire survey and reported that the risk of MMGC increases as the area of the remnant stomach increases. The reason for this discrepancy between the present study and previous reports is unclear. Considering the natural history of early gastric cancer, most MMGC cases may have been caused by misdiagnosis at the time of initial endoscopy. The patients in the SNNS group had more detailed endoscopy than those in the control group to exclude multiple gastric cancers, which may be related to selection bias. However, in the study by Kinami *et al*[71], many MMGCs in surgeries with a large remaining gastric mucosal area were resected by ESD, and it was concluded that there is no need to hesitate to perform function-preserving surgery because of the increased risk of MMGC. The results of the present study also suggest that there is no need to forgo the adoption of SNNS due to concerns about MMGC.

Through this study, the problems of SNNS became apparent, that is, the preoperative diagnostic ability. The precise diagnosis of early gastric cancer is difficult, not only in the depth of invasion but also in the lateral margin. All patients had a preoperative diagnosis of ≤ 5 cm along the long axis; however, 2.8% of the patients were found to have more than 5 cm in the postoperative specimens, including one patient of 87 mm. Six (31.6%) patients > 50 mm had lymph node metastasis. Misdiagnosis of size not only entails a positive margin, but also increases the possibility of lymph node metastasis. It was suggested that the accuracy of preoperative diagnosis, especially accurate extent diagnosis, must be ensured in order to safely perform SNNS.

Standard surgical treatment for early gastric cancer is standard gastrectomy D1 +[1-4]. However, 72.8% of patients in the SNNS group had D0. All SNNS patients underwent lymphatic basin dissection. This result may be interpreted as follows: Early gastric cancer patients do not necessarily require nodal dissection up to D1 +; and in the patients who were node negative, the reduction of the dissection area to the lymphatic basin did not affect the prognosis. On the other hand, 96% (24/25) of nodal metastatic patients in the SNNS group had metastases only within the lymphatic basin; the patient who had nodal metastases that was spread outside the basin was the only one with advanced gastric cancer with macroscopic metastases that could be easily diagnosed intraoperatively. On the other hand, one patient in the SNNS group had nodal recurrence despite being judged to be positive for metastasis during surgery and changed to D2, and recurrence may not have been avoided even if standard treatment was applied initially. Considering these facts, it may be possible to reduce the extent of nodal dissection to only the lymphatic basin for all patients with cT1N0 less than 5 cm in the future.

This study has some limitations. This was a retrospective study. It is possible that there was a selection bias in the SNNS group. Another problem is that the study was conducted over a long period of time. The diagnostic and therapeutic techniques have advanced during this period, and this may have affected the prognosis of patients and the incidence of MMGC. In addition, there were no QoL data of the SNNS group in this study. A nationwide multicenter prospective study is essential to correctly determine the prognosis, rate of non-cancer deaths from other diseases, and QoL assessment data. The results of a Japanese study[37] are awaited.

CONCLUSION

In both original data sets and propensity score-matched comparisons, OS and RFS of patients who underwent gastrectomy guided by sentinel node navigation were not inferior to those of standard gastrectomy. In addition, there was no difference in the cumulative incidence of MMGC between the two groups. The oncological safety of SNNS is not inferior to that of the guidelines. This study also indicates the possibility of reducing the extent of nodal dissection to only the lymphatic basin for all patients with cT1N0 less than 5 cm in the future.

ARTICLE HIGHLIGHTS

Research background

If early gastric cancer patients who are negative for lymph node metastasis can be diagnosed intraoperatively, excessive nodal dissection and extensive gastrectomy can be avoided. Currently, the most effective method for diagnosing lymph node metastasis is sentinel node biopsy. Lymphatic basin dissection is a sentinel node biopsy method that is specific for gastric cancer. The dyed lymphatic system was dissected *en bloc* and sentinel nodes were identified at the back table (*ex vivo*) using this method. This method not only reduces the difficulty of sentinel node biopsy, but also serves to a certain extent as backup dissection. Even with lymphatic basin dissection, blood flow to the residual stomach can be preserved and function-preserving curative gastrectomy can be performed, such as segmental gastrectomy and local resection.

Research motivation

The oncological safety of function-preserving curative gastrectomy combined with lymphatic basin dissection has not yet been fully investigated.

Research objectives

This study aimed to investigate the life prognosis of patients with early gastric cancer who underwent sentinel node navigation surgery (SNNS) in comparison with standard guideline surgery.

Research methods

Gastric cancer patients were retrospectively collected. The inclusion criteria were as follows: Superficial type (type 0); preoperative diagnosis of 5 cm or less in length; clinical T1-2; and node-negative on X-computed tomography. The patients underwent SNNS. First, sentinel node mapping was performed, followed by lymphatic basin dissection and rapid intraoperative pathology. If the sentinel nodes were diagnosed as metastasic at rapid diagnosis, standard gastrectomy with nodal dissection up to D2 was performed; if the sentinel nodes were diagnosed as node-negative, the extent of gastrectomy was reduced, and function-preserving curative gastrectomy was performed. The life prognosis and cumulative incidence of metachronous multiple gastric cancer (MMGC) were investigated. Patients with the same inclusion criteria and who underwent standard gastrectomy and guideline lymph node dissection with or without sentinel node biopsy were selected as the control group.

Research results

There were 239 patients in the SNNS group and 423 patients in the control group. All patients were diagnosed as node-negative preoperatively, but pathological nodal metastasis was observed in 10.5% of patients in the SNNS group and 10.4% in the control group. The diagnostic ability of sentinel node biopsy in this study was 84% and 98.6% for sensitivity and accuracy, respectively. In the SNNS group, 18.4% of patients underwent standard surgery, 14.2% had modified gastrectomy, and 67.4% had function-preserving curative gastrectomy, in which the extent of resection was further reduced than that recommended by the guidelines. The overall survival (OS) rate in the SNNS group was 96.8% at 5 years and was significantly better than 91.3% in the control group ($P = 0.0014$). The relapse-free survival (RFS) rate in the SNNS group was 99.6% at 5 years and 98.1% in the control group. After propensity score matching, there were 231 patients in both groups, and the OS in the SNNS group remained significantly better than that in the control group ($P = 0.030$). The cumulative recurrence rate in the SNNS group was 0.43% in 5 years and 1.30% in the control group, which was not statistically different. There was no difference in the incidence of

MMGC between the SNNS group (1.7% at 5-years) and the control group (2.3% at 5-years).

Research conclusions

In both original data sets and propensity score-matched comparisons, the OS rate and RFS rate of patients who underwent gastrectomy guided by sentinel node navigation were not inferior to those of standard gastrectomy. In addition, there was no difference in the cumulative incidence of MMGC between the two groups.

Research perspectives

The oncological safety of sentinel node navigation surgery for early-stage gastric cancer is not inferior to that of the guideline. This study also indicates the possibility of reducing the extent of nodal dissection to only the lymphatic basin for all patients with cT1N0 less than 5 cm in the future.

ACKNOWLEDGEMENTS

The authors are most grateful to Dr. Koichi Miwa, an ex-professor at Kanazawa University. We would also like to thank Dr. Takeo Kosaka, an ex-professor at Kanazawa Medical University.

REFERENCES

1 **Tanaka N**, Katai H, Taniguchi H, Saka M, Morita S, Fukunaga T, Shimoda T. Surgical treatment for early gastric cancer (in Japanese with English abstract). Stomach Intest 2009; 44: 700-706
2 **Kumagai K**, and Sano T. Part III Lymph node metastasis: Clinical significance Gastric Cancer. In: Natsugoe S editor. Lymph Node Metastasis in Gastrointestinal Cancer. Singapore: Springer Nature; 2019: p267-282
3 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2018 (5th edition). *Gastric Cancer* 2021; **24**: 1-21 [PMID: 32060757 DOI: 10.1007/s10120-020-01042-y]
4 **Kinami S**, Nakamura N, Tomita Y, Miyata T, Fujita H, Ueda N, Kosaka T. Precision surgical approach with lymph-node dissection in early gastric cancer. *World J Gastroenterol* 2019; **25**: 1640-1652 [PMID: 31011251 DOI: 10.3748/wjg.v25.i14.1640]
5 **Kwee RM**, Kwee TC. Imaging in assessing lymph node status in gastric cancer. *Gastric Cancer* 2009; **12**: 6-22 [PMID: 19390927 DOI: 10.1007/s10120-008-0492-5]
6 **Noda N**, Sasako M, Yamaguchi N, Nakanishi Y. Ignoring small lymph nodes can be a major cause of staging error in gastric cancer. *Br J Surg* 1998; **85**: 831-834 [PMID: 9667718 DOI: 10.1046/j.1365-2168.1998.00691.x]
7 **Luo M**, Lv Y, Guo X, Song H, Su G, Chen B. Value and impact factors of multidetector computed tomography in diagnosis of preoperative lymph node metastasis in gastric cancer: A PRISMA-compliant systematic review and meta-analysis. *Medicine (Baltimore)* 2017; **96**: e7769 [PMID: 28816957 DOI: 10.1097/MD.0000000000007769]
8 **Kubota K**, Suzuki A, Shiozaki H, Wada T, Kyosaka T, Kishida A. Accuracy of Multidetector-Row Computed Tomography in the Preoperative Diagnosis of Lymph Node Metastasis in Patients with Gastric Cancer. *Gastrointest Tumors* 2017; **3**: 163-170 [PMID: 28611983 DOI: 10.1159/000454923]
9 **Mocellin S**, Marchet A, Nitti D. EUS for the staging of gastric cancer: a meta-analysis. *Gastrointest Endosc* 2011; **73**: 1122-1134 [PMID: 21444080 DOI: 10.1016/j.gie.2011.01.030]
10 **Mocellin S**, Pasquali S. Diagnostic accuracy of endoscopic ultrasonography (EUS) for the preoperative locoregional staging of primary gastric cancer. *Cochrane Database Syst Rev* 2015; CD009944 [PMID: 25914908 DOI: 10.1002/14651858.CD009944.pub2]
11 **Yang QM**, Kawamura T, Itoh H, Bando E, Nemoto M, Akamoto S, Furukawa H, Yonemura Y. Is PET-CT suitable for predicting lymph node status for gastric cancer? *Hepatogastroenterology* 2008; **55**: 782-785 [PMID: 18613454]
12 **Morton DL**, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, Foshag LJ, Cochran AJ. Technical details of intraoperative lymphatic mapping for early stage melanoma. *Arch Surg* 1992; **127**: 392-399 [PMID: 1558490 DOI: 10.1001/archsurg.1992.01420040034005]
13 **Veronesi U**, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, Intra M, Veronesi P, Robertson C, Maisonneuve P, Renne G, De Cicco C, De Lucia F, Gennari R. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med* 2003; **349**: 546-553 [PMID: 12904519 DOI: 10.1056/NEJMoa012782]
14 **Miwa K**, Kinami S, Taniguchi K, Fushida S, Fujimura T, Nonomura A. Mapping sentinel nodes in patients with early-stage gastric carcinoma. *Br J Surg* 2003; **90**: 178-182 [PMID: 12555293 DOI: 10.1002/bjs.4031]
15 **Kitagawa Y**, Takeuchi H, Takagi Y, Natsugoe S, Terashima M, Murakami N, Fujimura T, Tsujimoto

H, Hayashi H, Yoshimizu N, Takagane A, Mohri Y, Nabeshima K, Uenosono Y, Kinami S, Sakamoto J, Morita S, Aikou T, Miwa K, Kitajima M. Sentinel node mapping for gastric cancer: a prospective multicenter trial in Japan. *J Clin Oncol* 2013; **31**: 3704-3710 [PMID: 24019550 DOI: 10.1200/JCO.2013.50.3789]

16 **Hiratsuka M**, Miyashiro I, Ishikawa O, Furukawa H, Motomura K, Ohigashi H, Kameyama M, Sasaki Y, Kabuto T, Ishiguro S, Imaoka S, Koyama H. Application of sentinel node biopsy to gastric cancer surgery. *Surgery* 2001; **129**: 335-340 [PMID: 11231462 DOI: 10.1067/msy.2001.111699]

17 **Ichikura T**, Morita D, Uchida T, Okura E, Majima T, Ogawa T, Mochizuki H. Sentinel node concept in gastric carcinoma. *World J Surg* 2002; **26**: 318-322 [PMID: 11865368 DOI: 10.1007/s00268-001-0226-x]

18 **Carlini M**, Carboni F, Petric M, Santoro R, Guadagni F, Marandino F, Castelli M, Santoro E. Sentinel node in gastric cancer surgery. *J Exp Clin Cancer Res* 2002; **21**: 469-473 [PMID: 12636091]

19 **Kitagawa Y**, Fujii H, Mukai M, Kubota T, Otani Y, Kitajima M. Radio-guided sentinel node detection for gastric cancer. *Br J Surg* 2002; **89**: 604-608 [PMID: 11972551 DOI: 10.1046/j.1365-2168.2002.02065.x]

20 **Hayashi H**, Ochiai T, Mori M, Karube T, Suzuki T, Gunji Y, Hori S, Akutsu N, Matsubara H, Shimada H. Sentinel lymph node mapping for gastric cancer using a dual procedure with dye- and gamma probe-guided techniques. *J Am Coll Surg* 2003; **196**: 68-74 [PMID: 12517553 DOI: 10.1016/s1072-7515(02)01594-6]

21 **Nimura H**, Narimiya N, Mitsumori N, Yamazaki Y, Yanaga K, Urashima M. Infrared ray electronic endoscopy combined with indocyanine green injection for detection of sentinel nodes of patients with gastric cancer. *Br J Surg* 2004; **91**: 575-579 [PMID: 15122608 DOI: 10.1002/bjs.4470]

22 **Song X**, Wang L, Chen W, Pan T, Zhu H, Xu J, Jin M, Finley RK 3rd, Wu J. Lymphatic mapping and sentinel node biopsy in gastric cancer. *Am J Surg* 2004; **187**: 270-273 [PMID: 14769318 DOI: 10.1016/j.amjsurg.2003.11.013]

23 **Ishizaki M**, Kurita A, Kubo Y, Takashima S, Nishina T, Nishimura E. Evaluation of sentinel node identification with isosulfan blue in gastric cancer. *Eur J Surg Oncol* 2006; **32**: 191-196 [PMID: 16412604 DOI: 10.1016/j.ejso.2005.11.013]

24 **Kusano M**, Tajima Y, Yamazaki K, Kato M, Watanabe M, Miwa M. Sentinel node mapping guided by indocyanine green fluorescence imaging: a new method for sentinel node navigation surgery in gastrointestinal cancer. *Dig Surg* 2008; **25**: 103-108 [PMID: 18379188 DOI: 10.1159/000121905]

25 **Yoshida M**, Kubota K, Kuroda J, Ohta K, Nakamura T, Saito J, Kobayashi M, Sato T, Beck Y, Kitagawa Y, Kitajima M. Indocyanine green injection for detecting sentinel nodes using color fluorescence camera in the laparoscopy-assisted gastrectomy. *J Gastroenterol Hepatol* 2012; **27** Suppl 3: 29-33 [PMID: 22486868 DOI: 10.1111/j.1440-1746.2012.07067.x]

26 **Kinami S**, Oonishi T, Fujita J, Tomita Y, Funaki H, Fujita H, Nakano Y, Ueda N, Kosaka T. Optimal settings and accuracy of indocyanine green fluorescence imaging for sentinel node biopsy in early gastric cancer. *Oncol Lett* 2016; **11**: 4055-4062 [PMID: 27313740 DOI: 10.3892/ol.2016.4492]

27 **Park JY**, Kim YW, Ryu KW, Nam BH, Lee YJ, Jeong SH, Park JH, Hur H, Han SU, Min JS, An JY, Hyung WJ, Cho GS, Jeong GA, Jeong O, Park YK, Jung MR, Yoon HM, Eom BW. Assessment of laparoscopic stomach preserving surgery with sentinel basin dissection versus standard gastrectomy with lymphadenectomy in early gastric cancer-A multicenter randomized phase III clinical trial (SENORITA trial) protocol. *BMC Cancer* 2016; **16**: 340 [PMID: 27246120 DOI: 10.1186/s12885-016-2336-8]

28 **Takeuchi H**, Kitagawa Y. Sentinel node and mechanism of lymphatic metastasis. *Ann Vasc Dis* 2012; **5**: 249-257 [PMID: 23555522 DOI: 10.3400/avd.ra.12.00033]

29 **Takeuchi H**, Ueda M, Oyama T, Shimizu Y, Kitagawa Y. Molecular diagnosis and translymphatic chemotherapy targeting sentinel lymph nodes of patients with early gastrointestinal cancers. *Digestion* 2010; **82**: 187-191 [PMID: 20588032 DOI: 10.1159/000309464]

30 **Yaguchi Y**, Sugasawa H, Tsujimoto H, Takata H, Nakabayashi K, Ichikura T, Ono S, Hiraki S, Sakamoto N, Horio T, Kumano I, Otomo Y, Mochizuki H, Yamamoto J, Hase K. One-step nucleic acid amplification (OSNA) for the application of sentinel node concept in gastric cancer. *Ann Surg Oncol* 2011; **18**: 2289-2296 [PMID: 21301968 DOI: 10.1245/s10434-011-1591-9]

31 **Yanagita S**, Natsugoe S, Uenosono Y, Kozono T, Ehi K, Arigami T, Arima H, Ishigami S, Aikou T. Sentinel node micrometastases have high proliferative potential in gastric cancer. *J Surg Res* 2008; **145**: 238-243 [PMID: 17603078 DOI: 10.1016/j.jss.2007.04.037]

32 **Arigami T**, Uenosono Y, Yanagita S, Nakajo A, Ishigami S, Okumura H, Kijima Y, Ueno S, Natsugoe S. Clinical significance of lymph node micrometastasis in gastric cancer. *Ann Surg Oncol* 2013; **20**: 515-521 [PMID: 22546997 DOI: 10.1245/s10434-012-2355-x]

33 **Zhou Y**, Zhang GJ, Wang J, Zheng KY, Fu W. Current status of lymph node micrometastasis in gastric cancer. *Oncotarget* 2017; **8**: 51963-51969 [PMID: 28881703 DOI: 10.18632/oncotarget.17495]

34 **Miwa K**. [Sentinel node concept and its application for cancer surgery]. *Nihon Geka Gakkai Zasshi* 2000; **101**: 307-310 [PMID: 10773998]

35 **Hiki N**, Nunobe S, Kubota T, Jiang X. Function-preserving gastrectomy for early gastric cancer. *Ann Surg Oncol* 2013; **20**: 2683-2692 [PMID: 23504120 DOI: 10.1245/s10434-013-2931-8]

36 **Kinami S**, Fujimura T, Ojima E, Fushida S, Ojima T, Funaki H, Fujita H, Takamura H, Ninomiya I, Nishimura G, Kayahara M, Ohta T, Yoh Z. PTD classification: proposal for a new classification of gastric cancer location based on physiological lymphatic flow. *Int J Clin Oncol* 2008; **13**: 320-329

[PMID: 18704632 DOI: 10.1007/s10147-007-0755-x]
37 **Kamiya S**, Takeuchi H, Fukuda K, Kawakubo H, Takahashi N, Mitsumori N, Terashima M, Tsujimoto H, Kinami S, Natsugoe S, Ohi M, Kadoya S, Fushida S, Hayashi H, Nabeshima K, Sakamoto J, Matsuda S, Mayanagi S, Irino T, Sato Y, Kitagawa Y. A multicenter non-randomized phase III study of sentinel node navigation surgery for early gastric cancer. *Jpn J Clin Oncol* 2021; **51**: 305-309 [PMID: 33017014 DOI: 10.1093/jjco/hyaa179]
38 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma. 15th ed. Tokyo: Kanehara Shuppan 2018; 2017
39 **Kanda Y**. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452-458 [PMID: 23208313 DOI: 10.1038/bmt.2012.244]
40 **Bolton JS**, Conway WC 2nd. Postgastrectomy syndromes. *Surg Clin North Am* 2011; **91**: 1105-1122 [PMID: 21889032 DOI: 10.1016/j.suc.2011.07.001]
41 **Carvajal SH**, Mulvihill SJ. Postgastrectomy syndromes: dumping and diarrhea. *Gastroenterol Clin North Am* 1994; **23**: 261-279 [PMID: 8070912]
42 **Eagon JC**, Miedema BW, Kelly KA. Postgastrectomy syndromes. *Surg Clin North Am* 1992; **72**: 445-465 [PMID: 1549803 DOI: 10.1016/s0039-6109(16)45689-6]
43 **Davis JL**, Ripley RT. Postgastrectomy Syndromes and Nutritional Considerations Following Gastric Surgery. *Surg Clin North Am* 2017; **97**: 277-293 [PMID: 28325187 DOI: 10.1016/j.suc.2016.11.005]
44 **Sawyers JL**. Management of postgastrectomy syndromes. *Am J Surg* 1990; **159**: 8-14 [PMID: 2294803 DOI: 10.1016/s0002-9610(05)80600-2]
45 **Cooperman AM**. Postgastrectomy syndromes. *Surg Annu* 1981; **13**: 139-161 [PMID: 7025276]
46 **Terashima M**, Tanabe K, Yoshida M, Kawahira H, Inada T, Okabe H, Urushihara T, Kawashima Y, Fukushima N, Nakada K. Postgastrectomy Syndrome Assessment Scale (PGSAS)-45 and changes in body weight are useful tools for evaluation of reconstruction methods following distal gastrectomy. *Ann Surg Oncol* 2014; **21** Suppl 3: S370-S378 [PMID: 24590434 DOI: 10.1245/s10434-014-3583-z]
47 **Misawa K**, Fujiwara M, Ando M, Ito S, Mochizuki Y, Ito Y, Onishi E, Ishigure K, Morioka Y, Takase T, Watanabe T, Yamamura Y, Morita S, Kodera Y. Long-term quality of life after laparoscopic distal gastrectomy for early gastric cancer: results of a prospective multi-institutional comparative trial. *Gastric Cancer* 2015; **18**: 417-425 [PMID: 24801197 DOI: 10.1007/s10120-014-0374-y]
48 **Ikenaga N**, Nishihara K, Iwashita T, Suehara N, Mitsuyama S. Long-term quality of life after laparoscopically assisted distal gastrectomy for gastric cancer. *J Laparoendosc Adv Surg Tech A* 2006; **16**: 119-123 [PMID: 16646700 DOI: 10.1089/lap.2006.16.119]
49 **Yasuda K**, Shiraishi N, Etoh T, Shiromizu A, Inomata M, Kitano S. Long-term quality of life after laparoscopy-assisted distal gastrectomy for gastric cancer. *Surg Endosc* 2007; **21**: 2150-2153 [PMID: 17479329 DOI: 10.1007/s00464-007-9322-9]
50 **Best LM**, Mughal M, Gurusamy KS. Laparoscopic versus open gastrectomy for gastric cancer. *Cochrane Database Syst Rev* 2016; **3**: CD011389 [PMID: 27030300 DOI: 10.1002/14651858.CD011389.pub2]
51 **Kobayashi D**, Kodera Y, Fujiwara M, Koike M, Nakayama G, Nakao A. Assessment of quality of life after gastrectomy using EORTC QLQ-C30 and STO22. *World J Surg* 2011; **35**: 357-364 [PMID: 21104250 DOI: 10.1007/s00268-010-0860-2]
52 **Isozaki H**, Matsumoto S, Murakami S, Takama T, Sho T, Ishihara K, Sakai K, Takeda M, Nakada K, Fujiwara T. Diminished Gastric Resection Preserves Better Quality of Life in Patients with Early Gastric Cancer. *Acta Med Okayama* 2016; **70**: 119-130 [PMID: 27094837 DOI: 10.18926/AMO/54191]
53 **Okubo K**, Arigami T, Matsushita D, Sasaki K, Kijima T, Noda M, Uenosono Y, Yanagita S, Ishigami S, Maemura K, Natsugoe S. Evaluation of postoperative quality of life by PGSAS-45 following local gastrectomy based on the sentinel lymph node concept in early gastric cancer. *Gastric Cancer* 2020; **23**: 746-753 [PMID: 32086650 DOI: 10.1007/s10120-020-01047-7]
54 **Katai H**. Function-preserving surgery for gastric cancer. *Int J Clin Oncol* 2006; **11**: 357-366 [PMID: 17058133 DOI: 10.1007/s10147-006-0613-2]
55 **Nunobe S**, Hiki N. Function-preserving surgery for gastric cancer: current status and future perspectives. *Transl Gastroenterol Hepatol* 2017; **2**: 77 [PMID: 29034350 DOI: 10.21037/tgh.2017.09.07]
56 **Seto Y**, Yamaguchi H, Shimoyama S, Shimizu N, Aoki F, Kaminishi M. Results of local resection with regional lymphadenectomy for early gastric cancer. *Am J Surg* 2001; **182**: 498-501 [PMID: 11754858 DOI: 10.1016/s0002-9610(01)00747-4]
57 **Hosoda K**, Yamashita K, Sakuramoto S, Katada N, Moriya H, Mieno H, Watanabe M. Postoperative quality of life after laparoscopy-assisted pylorus-preserving gastrectomy compared With laparoscopy-assisted distal gastrectomy: A cross-sectional postal questionnaire survey. *Am J Surg* 2017; **213**: 763-770 [PMID: 27751530 DOI: 10.1016/j.amjsurg.2016.09.041]
58 **Pu YW**, Gong W, Wu YY, Chen Q, He TF, Xing CG. Proximal gastrectomy versus total gastrectomy for proximal gastric carcinoma. A meta-analysis on postoperative complications, 5-year survival, and recurrence rate. *Saudi Med J* 2013; **34**: 1223-1228 [PMID: 24343461]
59 **Huang C**, Yu F, Zhao G, Xia X. Postoperative quality of life after laparoscopy-assisted pylorus-preserving gastrectomy compared with laparoscopy-assisted distal gastrectomy for early gastric cancer. *J Gastroenterol Hepatol* 2020; **35**: 1712-1719 [PMID: 31945189 DOI: 10.1111/jgh.14985]
60 **Isozaki H**, Okajima K, Momura E, Ichinona T, Fujii K, Izumi N, Takeda Y. Postoperative evaluation

of pylorus-preserving gastrectomy for early gastric cancer. *Br J Surg* 1996; **83**: 266-269 [PMID: 8689185]

61 **Xiao XM**, Gaol C, Yin W, Yu WH, Qi F, Liu T. Pylorus-Preserving versus Distal Subtotal Gastrectomy for Surgical Treatment of Early Gastric Cancer: A Meta-Analysis. *Hepatogastroenterology* 2014; **61**: 870-879 [PMID: 26176089]

62 **An JY**, Min JS, Hur H, Lee YJ, Cho GS, Park YK, Jung MR, Park JH, Hyung WJ, Jeong SH, Kim YW, Yoon HM, Eom BW, Kook MC, Han MR, Nam BH, Ryu KW; SEntinel Node ORIented Tailored Approach (SENORITA) Study Group. Laparoscopic sentinel node navigation surgery versus laparoscopic gastrectomy with lymph node dissection for early gastric cancer: short-term outcomes of a multicentre randomized controlled trial (SENORITA). *Br J Surg* 2020; **107**: 1429-1439 [PMID: 32492186 DOI: 10.1002/bjs.11655]

63 **Ryu KW**, Kim YW, Min JS, An JY, Yoon HM, Eom BW, Hur H, Lee YJ, Cho GS, Park Y, Jung MR, Park JH, Hyung WJ, Jeong S-H, Han M, Nam BH. Laparoscopic sentinel node navigation surgery vs laparoscopic standard gastrectomy with lymph node dissection in early gastric cancer: Final three-year survival results of multicenter randomized controlled phase III trial (SENORITA trial). *J Clin Oncol* 2020; **38**: 4510 [DOI: 10.1200/JCO.2020.38.15_suppl.4510]

64 **An JY**, Min JS, Lee YJ, Jeong SH, Hur H, Han SU, Hyung WJ, Cho GS, Jeong GA, Jeong O, Park YK, Jung MR, Park JY, Kim YW, Yoon HM, Eom BW, Ryu KW; Sentinel Node Oriented Tailored Approach (SENORITA) Study Group. Safety of Laparoscopic Sentinel Basin Dissection in Patients with Gastric Cancer: an Analysis from the SENORITA Prospective Multicenter Quality Control Trial. *J Gastric Cancer* 2018; **18**: 30-36 [PMID: 29629218 DOI: 10.5230/jgc.2018.18.e6]

65 **Park JY**, Kook MC, Eom BW, Yoon HM, Kim SJ, Rho JY, Kim SK, Kim YI, Cho SJ, Lee JY, Kim CG, Choi IJ, Kim YW, Ryu KW. Practical intraoperative pathologic evaluation of sentinel lymph nodes during sentinel node navigation surgery in gastric cancer patients - Proposal of the pathologic protocol for the upcoming SENORITA trial. *Surg Oncol* 2016; **25**: 139-146 [PMID: 27566014 DOI: 10.1016/j.suronc.2016.05.004]

66 **Tanigawa N**, Nomura E, Lee SW, Kaminishi M, Sugiyama M, Aikou T, Kitajima M; Society for the Study of Postoperative Morbidity after Gastrectomy. Current state of gastric stump carcinoma in Japan: based on the results of a nationwide survey. *World J Surg* 2010; **34**: 1540-1547 [PMID: 20182716 DOI: 10.1007/s00268-010-0505-5]

67 **Takeno S**, Hashimoto T, Maki K, Shibata R, Shiwaku H, Yamana I, Yamashita R, Yamashita Y. Gastric cancer arising from the remnant stomach after distal gastrectomy: a review. *World J Gastroenterol* 2014; **20**: 13734-13740 [PMID: 25320511 DOI: 10.3748/wjg.v20.i38.13734]

68 **Ohira M**, Toyokawa T, Sakurai K, Kubo N, Tanaka H, Muguruma K, Yashiro M, Onoda N, Hirakawa K. Current status in remnant gastric cancer after distal gastrectomy. *World J Gastroenterol* 2016; **22**: 2424-2433 [PMID: 26937131 DOI: 10.3748/wjg.v22.i8.2424]

69 **Hosokawa O**, Kaizaki Y, Watanabe K, Hattori M, Douden K, Hayashi H, Maeda S. Endoscopic surveillance for gastric remnant cancer after early cancer surgery. *Endoscopy* 2002; **34**: 469-473 [PMID: 12048630 DOI: 10.1055/s-2002-32007]

70 **Yaguchi Y**, Tsujimoto H, Hiraki S, Ito N, Nomura S, Horiguchi H, Kumano I, Einama T, Okamoto K, Noro T, Kajiwara Y, Shinto E, Aosasa S, Ichikura T, Ueno H. Long-term outcome following sentinel node navigation surgery for cT1 gastric cancer. *Mol Clin Oncol* 2019; **10**: 615-618 [PMID: 31031977 DOI: 10.3892/mco.2019.1833]

71 **Kinami S**, Aizawa M, Yamashita H, Kumagai K, Kamiya S, Toda M, Takahata T, Fujisaki M, Miyamoto H, Kusanagi H, Kobayashi K, Washio M, Hosoda K, Kosaka T. The incidences of metachronous multiple gastric cancer after various types of gastrectomy: analysis of data from a nationwide Japanese survey. *Gastric Cancer* 2021; **24**: 22-30 [PMID: 32780194 DOI: 10.1007/s10120-020-01104-1]

World Journal of Gastroenterology

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World J Gastroenterol 2021 December 14; 27(46): 8031-8032

DOI: 10.3748/wjg.v27.i46.8031

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CORRECTION

Erratum: Author's research-fund support notation correction. Mass forming chronic pancreatitis mimicking pancreatic cystic neoplasm: A case report *World J Gastroenterology* 2018; Jan 14; 24 (2): (297-302)

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Author contributions: Jee KN designed the report, collected the patient's clinical data, drafted the article and reviewed the manuscript, and approved the final manuscript as submitted.

Conflict-of-interest statement: The author has no conflicts of interest to disclose.

Supported by Dankook University Research Fund, No. R201600314.

Country/Territory of origin: South Korea

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

Correction to "Mass forming chronic pancreatitis mimicking pancreatic cystic neoplasm: A case report" *World J Gastroenterology* 2018; 24 (2): 297-302. This article had accidentally omitted the fact of research-fund support notation. It should be added as supported by Dankook University Research Fund (R201600314).

Key Words: Correction; Supported by; Research fund

Core Tip: This manuscript is a research-fund support notation correction for Mass forming chronic pancreatitis mimicking pancreatic cystic neoplasm: A case report *World J Gastroenterology* 2018; Jan 14; 24 (2): (297-302).

Citation: Jee KN. Erratum: Author's research-fund support notation correction. Mass forming chronic pancreatitis mimicking pancreatic cystic neoplasm: A case report *World J Gastroenterology* 2018; Jan 14; 24 (2): (297-302). *World J Gastroenterol* 2021; 27(46): 8031-8032

URL: https://www.wjgnet.com/1007-9327/full/v27/i46/8031.htm

DOI: https://dx.doi.org/10.3748/wjg.v27.i46.8031

Received: September 7, 2021
Peer-review started: September 7, 2021
First decision: September 29, 2021
Revised: September 30, 2021
Accepted: November 29, 2021
Article in press: November 29, 2021
Published online: December 14, 2021

P-Reviewer: Cao ZF, Herraiz JL, Peng JY
S-Editor: Wu YXJ
L-Editor: A
P-Editor: Wu YXJ

TO THE EDITOR

Correction to "Mass forming chronic pancreatitis mimicking pancreatic cystic neoplasm: A case report" *World J Gastroenterology* 2018; 24 (2): 297-302. This article had accidentally omitted the fact of research-fund support notation. It should be added as supported by Dankook University Research Fund (R201600314).

REFERENCES

1 **Jee KN**. Mass forming chronic pancreatitis mimicking pancreatic cystic neoplasm: A case report. *World J Gastroenterol* 2018; **24**: 297-302 [PMID: 29375215 DOI: 10.3748/wjg.v24.i2.297]

World Journal of Gastroenterology

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World J Gastroenterol 2021 December 14; 27(46): 8033-8039

DOI: 10.3748/wjg.v27.i46.8033

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LETTER TO THE EDITOR

Surveillance strategies for precancerous gastric conditions after *Helicobacter pylori* eradication: There is still need for a tailored approach

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Author contributions: Shahini E conducted study conceptualization, drafting the manuscript, data collecting, and curation; Maida M supervised; Shahini E and Maida M edited paper, critically reviewed the data entries for important intellectual content, checked for completeness of information; and both authors approved the final draft.

Conflict-of-interest statement: All the authors have no conflict of interest related to the manuscript.

Country/Territory of origin: Italy

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

Prevailing evidence declares that *Helicobacter pylori* (*H. pylori*) eradication therapy could shift precancerous gastric conditions (PGC) and positively confines gastric cancer (GC) risk during long-term endoscopic follow-up. Nonetheless, there is a yet unsolved controversy regarding the best-individualized surveillance strategies following *H. pylori* eradication, based on malignant risk stratification. This last dispute is due to the uncertainty of contemporary evidence and the role of *H. pylori* inflammatory changes in underestimating PGC at the index endoscopy. However, the current state of the art suggests that it is reasonable that high-quality endoscopy with histological assessment for the most accurate diagnosis of PGC may be delayed in selected high-risk patients without alarm signs for malignancy, following the eradication of *H. pylori*. Notwithstanding, these aspects need to be further examined in the next future to establish and optimize the most beneficial and cost-effective strategies for recognizing and managing *H. pylori*-positive patients with PGC in the short- and long-term follow-up. Accordingly, additional studies are yet required to sharpen the hazard stratification of patients with the greatest chance of GC evolution, also recognizing the evolving racial, ethnic, immigration factors and the necessity of novel biomarkers to limit GC development or accomplish a diagnosis of malignancy at an early stage.

Key Words: *Helicobacter pylori*; Endoscopic surveillance; Atrophic gastritis; Intestinal metaplasia; Dysplasia; Gastric cancer

Received: September 4, 2021
Peer-review started: September 4, 2021
First decision: October 2, 2021
Revised: October 3, 2021
Accepted: November 25, 2021
Article in press: November 25, 2021
Published online: December 14, 2021

P-Reviewer: An T, Jin CH, Katayama Y
S-Editor: Wang JJ
L-Editor: A
P-Editor: Wang JJ

Core Tip: Prevailing evidence affirms that *Helicobacter pylori* (*H. pylori*) eradication therapy could shift precancerous gastric conditions and positively confines gastric cancer risk during long-term endoscopic follow-up. Nonetheless, there is a yet unsolved dispute concerning the most useful individualized surveillance strategies following *H. pylori* eradication, based on malignant risk stratification. These aspects should be examined in the next future to establish and optimize the most cost-effective strategies for recognizing and managing *H. pylori*-positive patients with precancerous gastric conditions in the short- and long-term follow-up. Accordingly, new studies are required to sharpen the hazard stratification of patients with the greatest chance of progressing into gastric cancer.

Citation: Shahini E, Maida M. Surveillance strategies for precancerous gastric conditions after *Helicobacter pylori* eradication: There is still need for a tailored approach. *World J Gastroenterol* 2021; 27(46): 8033-8039
URL: https://www.wjgnet.com/1007-9327/full/v27/i46/8033.htm
DOI: https://dx.doi.org/10.3748/wjg.v27.i46.8033

TO THE EDITOR

We read with great interest the review of Weng *et al*[1], pointing out the most recent literature supporting the impact of *Helicobacter pylori* (*H. pylori*) on the gastric mucosa alterations. Specifically, the authors assumed that, despite some controversy, current evidence suggests that *H. pylori* eradication treatment could reverse atrophic gastritis (AG) and intestinal metaplasia (IM) and favorably limits the appearance of gastric cancer (GC), particularly in long-term surveillance[1].

However, there is still unresolved debate regarding the best-individualized follow-up strategies, based on malignant risk stratification, due to uncertainty of current evidence and the role of *H. pylori* inflammatory changes in underestimating IM extension and dysplastic lesions at the index endoscopy (Table 1)[2-12].

In a recent article focused on the crucial role of high-resolution endoscopy with narrow-band imaging (NBI) for the optimal detection of IM, Dinis-Ribeiro M *et al*[13] criticized the recent U.S. guidelines that discourage short-interval endoscopic surveillance of patients with IM[14]. They supported and elaborated on the rationale behind the suggested 3-year-interval endoscopic surveillance of high-risk subjects with more extensive IM[13,14], for detecting early gastric neoplasia that, due to dismal prognosis of GC and increased aging of the population, can improve patient's survival [14]. Additionally, they stated that "*The majority of patients with gastric IM, those who during high-quality endoscopy were shown to have IM of limited severity and extent, confined to the antrum, and have a negative family history for GC do not require surveillance*"[13]. Notwithstanding, maybe this affirmation seems to neglect genetic/epigenetic/racial factors, personal habits and underlying comorbidity roles (*i.e.*, alcohol consumption, smoking, autoimmune and metabolic diseases) that can hold distinctive malignant potential, theoretically affecting subsequent endoscopic surveillance.

Notably, a recent prospective cohort study[12], including 85 Italian patients with *H. pylori*-related active gastritis, undergoing upper gastrointestinal endoscopy 6 mo following eradication therapy, demonstrated that high-resolution endoscopy with NBI doubled the rate of identifying histological low-grade dysplasia (LGD) missed at pre-treatment endoscopy, in a high-risk subgroup which had extensive atrophy and IM at baseline. In over 40% of patients, visible gastric lesions with LGD were found following *H. pylori* eradication was not identified at their first pre-treatment endoscopy, thus suggesting that inflammatory changes associated with active *H. pylori* infection hinder the correct detection of gastric LGD lesions[12].

Of interest, in cases of indefinite gastric dysplasia, or with "not visible" dysplasia diagnosed randomly throughout the stomach without endoscopic evidence of visible lesions, the prevailing guidelines recommend a necessary endoscopic reassessment using high-resolution endoscopy with NBI to rule out dysplasia on missed visible lesions[12,15].

Moreover, some authors consider high-resolution surveillance endoscopy with NBI as "*sufficient for a diagnosis of extensive IM or premalignant stomach even without biopsy sampling*"[13]. There is an established association between the endoscopic grading of

Table 1 Characteristics of patients included in the eleven selected studies applying endoscopic surveillance shorter than two years for the evaluation of precancerous gastric conditions following *Helicobacter pylori* eradication

	van der Hulst RW *et al*[2], 1997	Tucci A *et al* [3], 1998	Sung JJ *et al* [4], 2000	Annibale B *et al* [5], 2000	Ohkusa T *et al*[6], 2001	Oda Y *et al*[7], 2004	Annibale B *et al* [8], 2002	Yamada T *et al*[9], 2003	Iacopini F *et al* [10], 2003	Wambura C *et al* [11], 2004	Panarese *et al*[12], 2020
Study	Prospective	Retrospective	Prospective, randomized, placebo controlled trial	Observational, prospective study	Single-blind, uncontrolled prospective trial	Retrospective	Retrospective	Retrospective	Observational, prospective study	Observational, prospective study	Observational, prospective study
Country	Netherlands	Italy	China	Italy	Japan	Japan	Italy	Japan	Italy	Japan	Italy
Mean age, yr	49.2	50	51 (Median)	48.7	54	51	46 (Median)	52.6	55	51.2	56.1
Male, %	54	50	49.5	14.3	73	89.8	22.5	64.4	75	74.7	37.6
Overlap AAG	NA	0	NA	48.6	NA	NA	55	NA	NA	NA	26.3
Mean follow-up, mo	12	12	12	6-12	12-15	1-2	6-12	22	12	12	6
Total, *n*	106	10	226	25	115	59	40	87	40	107	85
Resolution of gastric acute/chronic inflammation in the antrum *n* (%)	S	10/10 (100)	S	25/25 (100)	NA	S	S	S	S	S	81/85 (95.3)
Resolution of gastric acute/chronic inflammation in the corpus, *n* (%)	S	NA	S	25/25 (100)	NA	S	S	S	S	S	81/85 (85.3)
Resolution of gastric acute/chronic inflammation in the fundus *n* (%)	NA	10/10 (100)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Improvement of AG in the antrum, *n* (%)	NS	NS	NS	NS	34/38 (89)	NS	NS	NS	NS	S	NS
Improvement of AG in the corpus, *n* (%)	NS	NA	NS	NS	34/38 (89)	NS	8/40 (20) AG reversed	S	NA	S	NS

Improvement of AG in the fundus, *n* (%)	NA	S	NA	NA	NA	NA	NA	S	NA	NA	NA
Improvement of IM in the antrum, *n* (%)	NS	S	S	NS	28/46 (61)	NS	NS	NS	NS	NS	NS
Improvement of IM in the corpus, *n* (%)	NS	NA	NS	NS	28/46 (61)	NS	NS	NS	NA	S	NS
Improvement of IM in the fundus, *n* (%)	NA	NS	NA	NA	NA	NA	NA	NA	NA	S	NA
ECL pattern regression, *n* (%)	NA	NA	NA	8/15 (53.3) patients with AG in the body (12 mo after curing *H. pylori*)	NA	NA	NA	NA	NA	NA	36/39 (92.3)
LGD regression (or progression), *n* (%)	NA	NA	NA	1/1 (100) regression in a patient with AG in the body (12 mo after curing *H. pylori*)	NA	NA	NA	NA	NA	NA	The proportion of patients with histological diagnosis of LGD on random biopsies did not significantly change after *H. pylori* eradication [15 (17.6) *vs* 9 (10.6)]; the detection of LGD on visible lesions significantly increased after *H. pylori* eradication [0 (0) *vs* 19 (22.3)]
Conclusions	The usefulness of *H. pylori* eradication to regress precancerous lesions following 12 mo follow-up is uncertain	The natural history of AG can be modified by the eradication of *H.pylori*	At 12 mo, *H. pylori* eradication can block the histological progression of gastric mucosa alterations	*H. pylori* infection may be cured in patients with AG in the body with a partial reversing of its adverse outcomes on acid secretion and body ECL cell hyperplasia	After successful *H. pylori* eradication, precancerous lesions improved in most patients	After *H. pylori* eradication, neutrophil infiltration in the gastric mucosa improved relatively soon, while AG and IM did not display such tendency	In patients with AG of the body and *H. pylori* infection, the assessment of histological data after eradication is essential. In patients with maintaining body atrophy after *H. pylori* elimination, there is no association with the reversal of body atrophy, even at long-term surveillance	AG in the corpus can be improved after 12 mo following *H. pylori* eradication	*H. pylori* positive patients with AG, the overall oxidative damage of the gastric mucosa is more severe than that in *H. pylori* positive patients with nonatrophic gastritis	Eradication of *H. pylori* may decrease the risk of GC, due to the importance of *H. pylori* infection in the contributory role of gastritis in COX-2 expression and the dissociation between the processes of regression in gastritis and the reduction in COX-2	HR-WLE with NBI can be more reliable in diagnosing LGD on visible lesions after *H. pylori* elimination, presumably due to the removal of the underlying confounding effects of inflammatory and mucosal lymphoproliferative changes induced by *H. pylori* chronically active infection. Aged patients and those with autoimmune diseases (especially AAG) could be at higher risk for *H. pylori* persistent infection

AAG: Autoimmune gastritis; AG: Atrophic gastritis; IM: Intestinal metaplasia; ECL: Enterochromaffin-like cell; LGD: Low-grade dysplasia; *H. pylori*: *Helicobacter pylori*; NA: Not available; NS: Not significant; S: Significant improvement;

HR-WLE: High-resolution wight light endoscopy; NBI: Narrow band imaging.

gastric intestinal-metaplasia (EGGIM) and operative link on gastritis/intestinal-metaplasia assessment (OLGIM) stages in the assessment of the presence/extent of IM [12,13], and EGGIM stages ≥ 5 with OLGIM III/IV predicts early GC risk[12,13], although its reproducibility needs to be further confirmed in larger prospective studies as also expressed in the U.S. guidelines[14].

Nevertheless, even if feasible as a surveillance program in specialized referral centers, this strategy may not be widely applicable in endoscopy units that do not have access to such technologies. A targeted bioptic mapping seems more adequate for identifying mucosal gastric areas at risk of malignant transformation[12], despite the existing risk of overestimating OLGIM in patients with mild/focal IM. Concomitant *H. pylori*-related gastritis may limit the accuracy of EGGIM classification at the time of the initial endoscopy.

Advanced histological atrophy stages, even after *H. pylori* eradication, carry the highest risk for developing gastric neoplasia[12-15]. Nevertheless, recent long-term cohort studies from Eastern countries reported late development of GC during 5-14 years monitoring also in patients with none/mild gastric atrophy or antral IM, irrespectively of *H. pylori* eradication[12,16,17].

Notably, even with a high-resolution endoscope, if morphological changes do not appear, genetic and epigenetic changes in epithelial cells cannot be detected[18]. Specifically, epigenetic alterations (*i.e.*, aberrant DNA methylation), accumulate in cancers and also in normal-appearing tissues surrounding cancers[18]. Indeed, cross-sectional studies prove that aberrant methylation levels in normal tissues may be associated with cancer risk, particularly in chronic inflammation-associated cancers. Additionally, the relationship between miR-124a-3 DNA methylation abnormalities and similar trends for EMX1 and NKX6-1, have been judged extremely relevant predictors of developing authentic metachronous GCs[18].

Therefore, it is reasonable that high-quality endoscopy with histological assessment for the most accurate diagnosis of PGC[12] may be delayed, in selected high-risk patients who are symptomatic but have no alarm hallmarks for malignancy, after eradication of *H. pylori* diagnosed according to prior results of non-invasive tests had been achieved and serological autoimmunity biomarkers had been performed (*e.g.*, autoimmune AG-AAG), rather than applying the prevailing guidelines suggestion of operating targeted biopsies at initial endoscopy for histological estimation and determination of *H. pylori* status[12]. Such an approach is likely to enhance the PGC detection rate, especially for dysplastic lesions, reducing the confounding effect of *H. pylori*-related gastritis or AAG, and complies with the European guidelines[15], which recommend immediate high-quality endoscopy after the diagnosis of dysplasia without endoscopically visible lesions[12,15].

Therefore, we believe that further large prospective multicenter studies are still needed to identify additional risk factors of gastric malignancy development.

Moreover, multiple and evolving racial, ethnic, and immigration factors, may affect the risk of gastric neoplasia[19,20], calling also for the necessity of novel biomarkers for tailoring surveillance strategies to different patients.

These aspects should be considered in the next future to better define and optimize cost-effective strategies for identifying and managing *H. pylori*-positive patients with PGC in the short- and long-term follow-up.

REFERENCES

1 **Weng CY**, Xu JL, Sun SP, Wang KJ, Lv B. *Helicobacter pylori* eradication: Exploring its impacts on the gastric mucosa. *World J Gastroenterol* 2021; **27**: 5152-5170 [PMID: 34497441 DOI: 10.3748/wjg.v27.i31.5152]

2 **van der Hulst RW**, van der Ende A, Dekker FW, Ten Kate FJ, Weel JF, Keller JJ, Kruizinga SP, Dankert J, Tytgat GN. Effect of Helicobacter pylori eradication on gastritis in relation to cagA: a prospective 1-year follow-up study. *Gastroenterology* 1997; **113**: 25-30 [PMID: 9207258 DOI: 10.1016/s0016-5085(97)70076-3]

3 **Tucci A**, Poli L, Tosetti C, Biasco G, Grigioni W, Varoli O, Mazzoni C, Paparo GF, Stanghellini V, Caletti G. Reversal of fundic atrophy after eradication of Helicobacter pylori. *Am J Gastroenterol* 1998; **93**: 1425-1431 [PMID: 9732919 DOI: 10.1111/j.1572-0241.1998.00454.x]

4 **Sung JJ**, Lin SR, Ching JY, Zhou LY, To KF, Wang RT, Leung WK, Ng EK, Lau JY, Lee YT, Yeung CK, Chao W, Chung SC. Atrophy and intestinal metaplasia one year after cure of H. pylori infection: a prospective, randomized study. *Gastroenterology* 2000; **119**: 7-14 [PMID: 10889149 DOI: 10.1053/gast.2000.8550]

5 **Annibale B**, Aprile MR, D'ambra G, Caruana P, Bordi C, Delle Fave G. Cure of Helicobacter pylori infection in atrophic body gastritis patients does not improve mucosal atrophy but reduces hypergastrinemia and its related effects on body ECL-cell hyperplasia. *Aliment Pharmacol Ther* 2000; **14**: 625-634 [PMID: 10792127 DOI: 10.1046/j.1365-2036.2000.00752.x]

6 **Ohkusa T**, Fujiki K, Takashimizu I, Kumagai J, Tanizawa T, Eishi Y, Yokoyama T, Watanabe M. Improvement in atrophic gastritis and intestinal metaplasia in patients in whom Helicobacter pylori was eradicated. *Ann Intern Med* 2001; **134**: 380-386 [PMID: 11242498 DOI: 10.7326/0003-4819-134-5-200103060-00010]

7 **Oda Y**, Miwa J, Kaise M, Matsubara Y, Hatahara T, Ohta Y. Five-year follow-up study on histological and endoscopic alterations in the gastric mucosa after Helicobacter pylori eradication. *Dig Endosc* 2004; **16**: 213-218 [DOI: 10.1111/j.1443-1661.2004.00360.x]

8 **Annibale B**, Di Giulio E, Caruana P, Lahner E, Capurso G, Bordi C, Delle Fave G. The long-term effects of cure of Helicobacter pylori infection on patients with atrophic body gastritis. *Aliment Pharmacol Ther* 2002; **16**: 1723-1731 [PMID: 12269964 DOI: 10.1046/j.1365-2036.2002.01336.x]

9 **Yamada T**, Miwa H, Fujino T, Hirai S, Yokoyama T, Sato N. Improvement of gastric atrophy after Helicobacter pylori eradication therapy. *J Clin Gastroenterol* 2003; **36**: 405-410 [PMID: 12702982 DOI: 10.1097/00004836-200305000-00009]

10 **Iacopini F**, Consolazio A, Bosco D, Marcheggiano A, Bella A, Pica R, Paoluzi OA, Crispino P, Rivera M, Mottolese M, Nardi F, Paoluzi P. Oxidative damage of the gastric mucosa in Helicobacter pylori positive chronic atrophic and nonatrophic gastritis, before and after eradication. *Helicobacter* 2003; **8**: 503-512 [PMID: 14535997 DOI: 10.1046/j.1523-5378.2003.00172.x]

11 **Wambura C**, Aoyama N, Shirasaka D, Kuroda K, Maekawa S, Ebara S, Watanabe Y, Tamura T, Kasuga M. Influence of gastritis on cyclooxygenase-2 expression before and after eradication of Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 2004; **16**: 969-979 [PMID: 15371919 DOI: 10.1097/00042737-200410000-00004]

12 **Panarese A**, Galatola G, Armentano R, Pimentel-Nunes P, Ierardi E, Caruso ML, Pesce F, Lenti MV, Palmitessa V, Coletta S, Shahini E. *Helicobacter pylori*-induced inflammation masks the underlying presence of low-grade dysplasia on gastric lesions. *World J Gastroenterol* 2020; **26**: 3834-3850 [PMID: 32774061 DOI: 10.3748/wjg.v26.i26.3834]

13 **Dinis-Ribeiro M**, Kuipers EJ. How to Manage a Patient With Gastric Intestinal Metaplasia: An International Perspective. *Gastroenterology* 2020; **158**: 1534-1537 [PMID: 31926996 DOI: 10.1053/j.gastro.2020.01.008]

14 **Matysiak-Budnik T**, Camargo MC, Piazuelo MB, Leja M. Recent Guidelines on the Management of Patients with Gastric Atrophy: Common Points and Controversies. *Dig Dis Sci* 2020; **65**: 1899-1903 [PMID: 32356261 DOI: 10.1007/s10620-020-06272-9]

15 **Pimentel-Nunes P**, Libânio D, Marcos-Pinto R, Areia M, Leja M, Esposito G, Garrido M, Kikuste I, Megraud F, Matysiak-Budnik T, Annibale B, Dumonceau JM, Barros R, Fléjou JF, Carneiro F, van Hooft JE, Kuipers EJ, Dinis-Ribeiro M. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. *Endoscopy* 2019; **51**: 365-388 [PMID: 30841008 DOI: 10.1055/a-0859-1883]

16 **Rugge M**, Meggio A, Pravadelli C, Barbareschi M, Fassan M, Gentilini M, Zorzi M, Pretis G, Graham DY, Genta RM. Gastritis staging in the endoscopic follow-up for the secondary prevention of gastric cancer: a 5-year prospective study of 1755 patients. *Gut* 2019; **68**: 11-17 [PMID: 29306868 DOI: 10.1136/gutjnl-2017-314600]

17 **Quach DT**, Hiyama T, Gotoda T. Do subjects with mild or moderate atrophic gastritis or intestinal metaplasia confined to the antrum benefit from gastric cancer surveillance? *Gut* 2020; **69**: 968-969 [PMID: 31030192 DOI: 10.1136/gutjnl-2019-318720]

18 **Asada K**, Nakajima T, Shimazu T, Yamamichi N, Maekita T, Yokoi C, Oda I, Ando T, Yoshida T, Nanjo S, Fujishiro M, Gotoda T, Ichinose M, Ushijima T. Demonstration of the usefulness of epigenetic cancer risk prediction by a multicentre prospective cohort study. *Gut* 2015; **64**: 388-396 [PMID: 25379950 DOI: 10.1136/gutjnl-2014-307094]

19 **Kim GH**, Liang PS, Bang SJ, Hwang JH. Screening and surveillance for gastric cancer in the United States: Is it needed? *Gastrointest Endosc* 2016; **84**: 18-28 [PMID: 26940296 DOI: 10.1016/j.gie.2016.02.028]

20 **Huang RJ**, Ende AR, Singla A, Higa JT, Choi AY, Lee AB, Whang SG, Gravelle K, D'Andrea S, Bang SJ, Schmidt RA, Yeh MM, Hwang JH. Prevalence, risk factors, and surveillance patterns for gastric intestinal metaplasia among patients undergoing upper endoscopy with biopsy. *Gastrointest Endosc* 2020; **91**: 70-77.e1 [PMID: 31425693 DOI: 10.1016/j.gie.2019.07.038]

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