# Recent Advances in Histopathology




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# Chapter 3

# Hepatic manifestations of COVID-19: A clinico-pathological review

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## INTRODUCTION

The novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the infectious disease coronavirus disease 2019 (COVID-19) and the ongoing worldwide pandemic. As reported by the World Health Organization (WHO), globally as of the 13th of February 2022, there have been 404,910,528 confirmed cases of COVID-19, including 5,783,776 deaths [1]. Most infected people develop mild to moderate illnesses with fever, cough, fatigue and loss of taste or smell and recover without hospitalisation. Pulmonary complications have been most associated with increased mortality and morbidity. However, gastrointestinal symptoms like diarrhoea, nausea, vomiting, and abdominal pain have also been well documented and may often precede respiratory symptoms [2].

Coronavirus disease 2019 is a systemic disease that targets multiple organs, including the digestive tract and hepatobiliary system. The SARS-CoV-2 virus was detected in stool samples of over 50% of infected hospitalised patients [3]. A number of studies have shown that liver injury is generally manifested in the form of mild to moderate elevation of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) during the early stage of COVID-19. Severe cases requiring prolonged hospitalisation were more likely

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to show severe liver injury compared to milder cases [4]. Expression of the angiotensin-converting enzyme-2 (ACE-2) receptors in vascular endothelial cells, smooth muscle cells, cholangiocytes, and the cells of the stomach, small bowel, and colon is the most likely reason for the occurrence of gastrointestinal symptoms, including hepatobiliary manifestations [5]. Chai et al used single-cell RNAseq and reported that in healthy livers, ACE-2 receptor expression levels in the cholangiocytes are similar to that of alveolar cells in the lungs [6]. In addition, hepatocellular expression of ACE-2 receptors is about 20-fold lower than in cholangiocytes. Bioinformatics analyses from a single-cell transcriptome database called "Single Cell Portal" also support the higher expression of ACE-2 receptor expression in cholangiocytes compared to hepatocytes [7]. Interestingly, upregulation of ACE-2 receptor expression has been reported in hepatocytes cultured under hypoxic conditions. This finding may have profound relevance since preexisting liver injury and presence of micro-thrombi appear to exacerbate SARS-CoV-2 hepatic tropism and COVID-19 complications [8]. Other mechanisms of concomitant liver injury in COVID-19, beyond direct viral infection of hepatic cells, have been explored in numerous studies, including thrombotic complications, sepsis, drug/toxin induced liver injury (DILI), and virus-induced altered cellular metabolism.

Liver injury in COVID-19 has been documented by evaluation of both serological studies and clinical manifestations. In a published series by Ortiz et al 14.8–53% of COVID-19 patients presented with evidence of liver injury [9]. Of the 12,882 hospitalised patients studied in this systematic review, 41.1% had elevated AST and 29.1% ALT [9]. These elevations correlated with unfavourable outcomes. Clinical signs and symptoms include jaundice, hepatomegaly, and ascites, in addition to general respiratory symptoms (i.e. cough, sputum production, hemoptysis, and hyperventilation) [10].

Several studies have also suggested that monitoring clinical symptoms and laboratory markers may offer key insights into the degree of liver injury in COVID-19. According to Spearman et al between 10% and 58% of hospitalised COVID-19 patients had elevated bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). AST levels were typically higher than ALT [11]. Spearman et al also reported a positive association between elevated AST and mortality [11]. Chen et al studied the clinical characteristics of 274 patients, of which 113 (41%) died due to complications of COVID-19, had elevated serum level of AST > 40 IU/L (median AST was 45 (31–67) for patients who died as compared to a median AST of 25 IU/L (20.0–33.3) for patients who recovered) [12]. These findings were also corroborated by Lei et al who studied a retrospective cohort of 5,771 patients in Hubei Province of China. Patients with elevated AST between 40 U/L and 120 U/L had a 4.81-fold increased mortality while patients with AST over 120 U/L had a 14.87-fold increased mortality after adjusting for age, gender, and co-morbidities [9]. An association of severity of liver injury with outcome of COVID was also reported in a retrospective study ($n$ = 3,381) by Phipps et al who reported that patients with ALT more than five-fold the upper limit of normal had an increased mortality rate [13].

According to Spearman et al predictors of liver injury and elevated transaminases also include age, male gender, BMI, diabetes mellitus, medications, and inflammatory markers [11]. Moreover, Kukla et al reported that elevated AST was more often associated with patients who experienced gastrointestinal symptoms, including nausea, vomiting, and diarrhoea [14]. These patients also had significantly higher fevers, fatigue, dyspnoea, and headache.

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Importantly, liver injury can occur in patients without preexisting liver injury and can be exacerbated in individuals with liver disease (LD). A multi-centre network study performed by Singh et al compared the impact of preexisting LD in COVID-19 patient outcomes [15]. Among 2,780 patients with COVID-19 across 34 healthcare organisation in the United States, 250 (9%) patients with preexisting liver disease were included in the LD group. In this group, 1.8% were diagnosed with cirrhosis and 42% fatty LD or non-alcoholic steatohepatitis (NASH) or chronic liver disease (CLD). The remaining patients ($n$ = 2,530) were in the nonLD group. The outcomes of patients in these two groups were compared. Regardless of preexisting LD, the majority of patients manifested elevated liver enzyme activity (i.e. AST, ALT, GGT, bilirubin, and ALP). However, patients in the LD group, predominantly patients with cirrhosis, had increased risk for mortality. The relative risk (RR) of mortality in cirrhosis was (RR: 4.6) and in CLD (RR: 3.0) compared to non-CLD patients. The risk of mortality was also higher in the LD group (12% vs. 4% in CLD compared to non-CLD group) [15]. Overall, patients with cirrhosis (with compensated or decompensated LD), who develop COVID-19 have poorer outcomes and higher rates of 30-day mortality [16]. Additionally, patients with cirrhosis and COVID-19 may decompensate, also called acute-on-chronic liver failure. Iavarone et al reported a 30-day mortality rate of 34% for patients with cirrhosis and acute-on-chronic liver failure [16]. Patients with NAFLD/NASH are at an increased risk of more severe COVID-19 [17]. Patients with co-morbidities (i.e. hypertension, diabetes, and cardiovascular disease), which encompassed a large proportion of patients in the LD group, had poorer outcomes. While patients with preexisting LD may be predisposed to higher risk of hospitalisation and mortality, COVID-19 can induce concomitant liver injury even in patients without preexisting liver disease.

Liver failure in severely ill patients has been commonly described in the context of COVID-19. The mechanism by which COVID-19 exacerbates existing LD has not yet been elucidated [11]. It also remains unclear how COVID-19 causes concomitant liver injury or end-stage LD. As a result, specialists in hepatology and hepatopathology have been actively involved in the care of patients with COVID-19. Data regarding histopathology was first obtained from autopsy studies in the earliest months of the pandemic, but additional biopsy data have become available.

In this review, we will present studies of liver biopsies and, in rare cases, explanted livers in patients undergoing liver transplantation. Attention will be given to histopathology and mechanisms for findings in several categories of concomitant liver injury in COVID-19: autopsy findings, COVID-19 associated hepatitis, drug-induced liver injury, SARS-CoV-2 associated cholangiopathy, sepsis, thrombosis and endothelial injury, and steatosis. We will conclude on recent findings from vaccine-associated hepatitis.

## AUTOPSY FINDINGS

The earliest histopathologic findings from the liver in the setting of COVID-19 were presented in studies of autopsies. The first two reports consisted of postmortem liver biopsy specimens (LBx). In a 51-year-old patient who died from respiratory complications of COVID-19, their LBx showed moderate micro-vesicular steatosis with mild lobular and portal activity [18]. Another study included four patients, in which their LBx had non-specific findings such as mild sinusoidal dilatation, nuclear glycogen accumulation in hepatocytes, and focal macro-vesicular steatosis [19]. One of these cases was a patient with a prior diagnosis of chronic lymphocytic leukaemia, and atypical lymphomatous infiltrates were

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found in the portal tracts. One of these cases had a known history of end-stage LD and cirrhosis of unknown cause. One case showed patchy hepatic necrosis in the periportal and centrilobular areas suggesting acute hepatitis.

More data were provided by Lagana et al in a series of liver histopathology from 40 autopsies of patients (29 male and 11 female) who died of complications due to COVID-19. Excluding perimortem changes of congestion and ischaemia, their main findings were hepatic steatosis, mostly macrovascular, in 75% of the cases, lobular necroinflammation in 50% of the cases, individual hepatocyte injury in 25% of the cases, and portal inflammation in 50% of the cases with a subset of these (6 of 20) that did not have concomitant lobular inflammation. PCR tests were used to detect the virus in the liver, and the authors reported that 55% of patients who died due to complications of COVID-19 had detectable virus in the liver. The detectable virus levels measured in the liver were extremely low, reflecting circulating virus and not necessarily direct hepatic infection [20].

A series of limited autopsies from the New York University (NYU) Langone – Long Island Hospital in seven patients, four females and three males, highlighted the presence of mild, macro-vesicular steatosis without features of steatohepatitis. One case had advanced staged liver fibrosis (cirrhosis) secondary to chronic alcohol use. Numerous platelet-fibrin micro-thrombi were found using immunohistochemical reactivity to CD61 (i.e. platelet glycoprotein IIIa, a megakaryocytic and platelet marker) in the pulmonary, renal, and cardiac micro-vasculature, irrespective of anticoagulation therapy. Micro-thrombi were also found in hepatic sinusoids in six of the seven cases. In two of the seven cases, an ischaemic type of hepatic necrosis was associated with the micro-thrombi formation. Portal veins in these two cases were associated with large platelet aggregates [21]. In addition, zone 3 necrosis with a hepatic vein thrombosis was also reported in one of these cases.

A separate report on 17 autopsy livers by Rapkeiwicz et al further highlighted the widespread presence of platelet-fibrin micro-thrombi. Platelet micro-thrombi were localised primarily in the sinusoids, central veins, and the portal veins in 12 of 17 cases (70%). The mean number of megakaryocytes/10 HPF in the liver was 1.08 ± 0.15 in patients with COVID-19 compared to patients without COVID-19 ($p < 0.01$). In addition, an increased number of histiocytes immuno-reactive to CD-68 were noted in five of the 17 cases. These vacuolated histiocytes were also positive for the SARS-CoV-2 virus by immuno-histochemical stain [22]. The presence of steatosis, lobular inflammation, and mild portal tract inflammation were similar to the findings reported in prior studies [22].

In addition, Bryce et al examined 93 postmortem livers, of which 12 were grossly cirrhotic, nine were "nutmeg" congested, one with multiple cysts, and the remainder grossly unremarkable. Histological examination of the livers showed ischaemic coagulative necrosis of zone 3 in 12% of the cases (11 out of 92 livers) with two livers showing changes reminiscent of ischaemia-reperfusion injury and associated with neutrophilic infiltrate. About 30% of the cases (28 of 92) showed steatohepatitis, of which eight cases showed advanced stage fibrosis histologically. About 40% of the cases (37 of 92) showed portal venules associated with early organising thrombi. Organising thrombi, confirmed by the positive immuno-reactivity to CD61, was also seen in the terminal hepatic venules in 21% of the cases (20 of 92). In addition, about 44.5% of the cases (41 of 92) had features suggestive of congestive hepatopathy and venous outflow obstruction associated with Kupffer cells and ingested red cells. This was considered indicative of hemophagocytosis [23]. Portal and lobular lymphocytic infiltrate comprising CD4 T-lymphocytes accompanied by a variable degree of portal vein endotheliitis with mild-to-moderate portal fibrosis was reported

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from wedge liver samples of 48 autopsy patients who died due to complications of SARS-CoV-2 infections in Italy [24]. However, no histological abnormalities were reported in the intrahepatic biliary tree. In addition, vascular abnormalities in the form of increased number of dilated portal veins with herniation into the periportal parenchyma and wall fibrosis were reported. Activated Kupffer cells containing necrotic debris were abundant.

Fowkes et al provided a meta-analysis of data gathered from preprints and published articles to compare the postmortem findings of the SARS-CoV-2 virus ($n = 241$ patients) and the SARS-CoV-1 virus ($n = 91$ patients) [25]. Focal hepatocyte degeneration and necrosis, mild portal and periportal lymphocytic inflammation, rare lobular lymphocytic inflammation, steatosis, and lymphocytic endotheliitis were reported in both types of SARS infections. However, hepatic necrosis and/or portal inflammation was highly associated with SARS-CoV-1 patients, and a higher proportion of patients with SARS-CoV-2 infection had thrombi and/or lobular inflammation. But, this feature was not found to be statistically significant. Examples of micro-thrombi in the sinusoids and few portal veins immunostained by CD61, diffuse panlobular steatosis with mild steatohepatitis, and patchy pericellular fibrosis by trichrome stain are shown in **Figure 3.1**.

Many findings in these autopsy series are nonspecific and do not seem to define a SARS-CoV-2 related or initiated specific liver injury pattern. There is no set of findings that would be diagnostic of a liver-specific COVID-19 injury. Additionally, autopsies often represent the sickest of individuals who died from COVID-19. Lastly, since many autopsies were performed in a hospital setting, there may be overlapping mechanisms of injury from those related to the primary infection, the body's response to the infection, and unsuccessful medical interventions.

# LIVER BIOPSY SPECIMEN AND MECHANISMS OF LIVER INJURY IN COVID-19

## Drug-induced liver injury

Experimental use of off-label, prophylactic, and therapeutic drugs may be responsible for drug-induced liver injury (DILI). For example, Weber et al reported liver injury in a patient after administering antivirals [4]. A 65-year-old man with no prior history of LD was

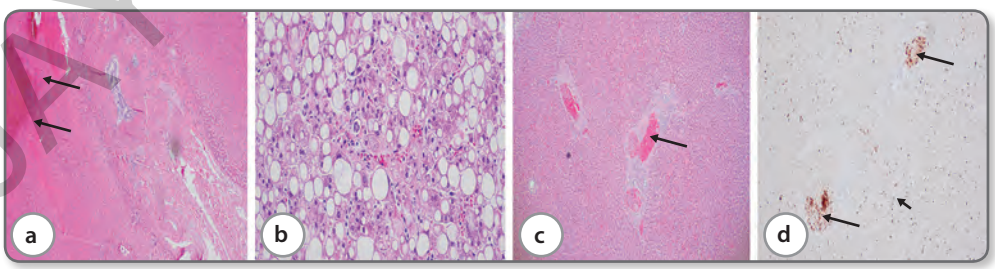



**Figure 3.1** Liver from autopsies of patients who died of complications of COVID-19. (a) Area of haemorrhage (arrows) associated with extensive hepatocyte loss indicates ischaemic injury in a 46-year-old man (H&E, x100). (b) Severe, diffuse panlobular steatosis and steatohepatitis in a 22-year-old female (H&E, x200). (c) Thrombi in the portal venules (arrows) (H&E, x100) and (d) CD61 immunohistochemical stain (DAB, brown) highlights the platelet-fibrin thrombi (arrow) and sinusoidal platelet aggregates (short arrow) in an 89-year-old male (x100).

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administered ritonavir and lopinavir with interferon-β after developing respiratory failure. However, the drugs were discontinued after persistently elevated ALT, creatinine, and bilirubin. The patient progressed to end-stage liver failure and died of multi-organ failure.

The mechanisms of DILI in COVID-19 are not fully understood. Several hypotheses may pertain, may act independently, or overlap in the same patient. For example, according to Xu et al DILI may be related to moderate micro-vesicular hepatocyte steatosis due to drug impairment of cellular metabolism [26,27]. A second hypothesis is the down-regulation of cytochromes P450 in hepatocytes, resulting in impaired pharmacokinetics and increased cellular inflammation. Inflammation resulting from synergistic drug may also cause drug hepatotoxicity. A third hypothesis from Griffin et al suggested that protease inhibitor drugs interfere with bile acid efflux from hepatocytes, inducing hepatotoxicity [27,28]. Mechanisms of DILI are increasingly cited in literature to understand the cellular processes causing liver damage.

This paragraph summarises therapeutic treatments used to treat COVID-19 that may be relevant to DILI. Molnupiravir reduced the risk of hospitalisation and death from COVID-19 [29]. A randomised controlled trial of the drug showed molnupiravir was well tolerated at doses of 300, 600 and 800 mg without reported hepatotoxicity [30]. Pfizer's Paxlovid has also been reported effective without hepatotoxicity [31,32]. COVID-19 monoclonal antibodies are also under evaluation with no evidence of apparent liver injury currently, according to LiverTox [33]. Remdesivir (RDV) has been associated with hypoalbuminaemia, increased bilirubin, acute hepatic failure and hepatitis. According to LiverTox database, RDV has a likelihood score of D or possible uncommon cause of clinically apparent liver injury [34,35]. No studies on humans treated with RDV have been published currently. Tocilizumab (TCZ) has a greater association with hepatotoxicity and is categorised C, or probable rare cause of clinically apparent liver injury in the LiverTox database [34]. TCZ may be associated with severe liver injury and jaundice [27,29]. Interestingly, there were reports showing that TCZ may improve adverse liver effects caused by lopinavir/ritonavir [27]. The verdict on TCZ safety and DILI still remain unclear. HCQ also caused a 10-fold increase in liver enzyme activity [27,36], and favipiravir also elevated liver enzyme activity [31]. Reported histopathological changes from DILI include steatosis, portal fibrosis, lymphocytic infiltrates and ductular reaction, lobular cholestasis, acute hepatocyte necrosis, and central vein thrombosis [37]. Other common antibacterial drugs such as azithromycin and fluoroquinolones were used to treat patients with COVID-19, and these were already known to have hepatotoxic effects.

Ortiz et al highlighted the importance of careful monitoring and documentation of DILI in COVID-19 patients [9]. Additionally, while an increased AST/ALT ratio may serve as a key indicator of liver injury, it may not be sufficient in predicting the progression of DILI because of varying experimental treatment regimens and clinical hepatic manifestation.

## COVID-19 ASSOCIATED HEPATITIS

COVID-19 may cause acute hepatitis in patients with no predisposing cause (i.e. no preexisting LD and no acetaminophen or antibiotic intake), a perplexing but a commonly observed manifestation of COVID-19 [38].

In a case report, liver biopsies were performed on a liver transplanted successfully from a COVID-19 positive donor to a female infant with cirrhosis, portal hypertension, and biliary atresia, status post failed Kasai procedure [39]. A biopsy of the donor's liver was

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taken 7 days post-operation documented the histopathology from this transplanted liver, an acute hepatitis pattern of injury attributed to SARS-CoV-2 infection. A moderate degree of acute hepatitis featuring prominent clusters of "crumbling" apoptotic hepatocytes and lymphohistiocytic inflammation of sinusoidal and central vein endothelium was noted. Interestingly, micro- and macro-vesicular steatosis were also found, which could be related to the donor's documented NAFLD history. A later section will discuss COVID-19 related steatosis. Mitotic cells were present, but not to a large degree. Lastly, endotheliitis of the sinusoids and central veins was also found with prominent Kupffer cells that the authors interpreted as reflecting an acute hepatitic injury rather than endophlebitis or acute rejection. In summary, the salient histopathological finding from the donor's LBx was prominent azonal hepatocyte apoptosis, suggestive of COVID-19 associated hepatitis [39].

Notably, liver impairment and histopathology has previously been identified in the coronavirus family (i.e. SARS-CoV in 2003). Liver biopsies of patients with SARS also presented with acidophilic bodies, hepatocyte ballooning, and mild lobular activity. Pertaining to SARS-CoV-2, increased mitosis, apoptosis, and ballooned hepatocytes also suggest that the virus may be binding to cholangiocytes and hepatocytes to dysregulate liver function. Ultra-structural examination of SARS-CoV-2-infected hepatocytes showed coronavirus particles, characterised by spike structures, and localised in the cytoplasm of these hepatocytes, associated with prominent mitochondrial swelling, dilatation of endoplasmic reticulum, and decrease in glycogen granule [40]. Studies of liver organoids and animal models have shown that the virus can infect and replicate in hepatocytes, indicating that abnormalities in the liver may be a direct result of the virus in the hepatocytes [41]. Again, however, DILI cannot always be firmly excluded [42].

## COVID-19 CHOLANGIOPATHY

Cholangiopathy, or cholestatic injury of the biliary tract, is a complication of severe COVID-19. The aetiology may be associated with hypoxic liver injury and the clinical and histological findings reveal similarities with secondary sclerosing cholangitis in critically ill patients (SSC-CIP). SSC-CIP-like patterns of progressive cholestatic liver injury were reported in patients with COVID-19 and acute respiratory distress syndrome (ARDS) [43]. First identified in 2001 by Scheppach et al and currently with only 250 total cases, SSC-CIP has been treated in the ICU due to associations with severe infection, trauma, burns, and postcardiothoracic surgery [44,45]. In addition to elevated liver enzyme activity seen in both post-COVID-19 cholangiopathy and SSC-CIP, ALP is also significantly elevated, and imaging shows biliary tract inflammation and strictures. In SSC-CIP, magnetic resonance cholangiopancreatography (MRCP) of severely ill patients reveals focal strictures in intrahepatic bile ducts with intraluminal sludge and casts. Biopsies reveal disrupted architecture of the liver parenchymal and portal tracts. In post-COVID-19 cholangiopathy, imaging also showed beading of the intrahepatic biliary ducts. The findings of post-COVID-19 cholangiopathy are similar to those in SSC-CIP, which, if prognoses parallel, may imply serious concerns for long-term morbidity. The pathogenesis remains unclear, and diagnoses are made with endoscopic retrograde cholangio-pancreatography (ERCP) and biopsy [46].

In a retrospective study at NYU Langone Health hospitals, the clinical, laboratory, radiological, and histological findings all seemed to be consistent with biliary duct and hepatic injury [46]. In LBx, chronic large duct obstruction with portal expansion due

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to fibrosis and ductular reaction was found. Salient findings from ERCP were beaded appearance of intrahepatic ducts, dilatation of the biliary tree, and multi-focal strictures. In fact, ERCP results showed worsening intrahepatic biliary dilatation in some patients at 3, 4, then 7 months post-COVID. In summary, the biopsies from this study confirmed cholangitis and impaired biliary flow as a cause of COVID-19 cholangiopathy [46,47].

An index case of COVID-19 cholangiopathy in a 60-year-old male who underwent a transplantation is highlighted in **Figure 3.2.**

His LBx prior to transplant showed features of acute large duct obstruction with focal bile duct injury, suggestive of secondary sclerosing cholangitis. Portal tracts were expanded by oedema and ductular reactions with admixed neutrophils. Focal bile infarcts were also noted. In the absence of significant scarring (using trichrome and reticulin stains) these changes are most suggestive of acute obstruction, though extensive hepatocyte keratin-7 staining raises the possibility of chronicity. Immunohistochemical stain for cytokeratin-19 highlighted the marked duct injury, but definitive duct loss is not present in this patients native liver (**Figure 3.3**), corroborating the findings of acute large duct obstruction with focal bile duct injury, suggestive of SSC. Mild steatosis compatible with fatty LD was also noted but steatohepatitis and steatofibrosis were absent. Focal perivenular drop out was suggestive of ischaemic injury, and edge of bile infarct was also entertained in the differential diagnosis.

One hypothesis is that the virus may directly cause cholangiocyte injury by direct infection via the ACE-2 receptors [6,7]. On the other hand, the underlying pathophysiology of cholangiopathy in COVID-19 may be related to bile duct ischaemia, or ischaemic cholangiopathy [48]. Ischaemic cholangiopathy is necrosis and irreversible intrahepatic biliary destruction due to poor perfusion. Unlike hepatocytes that have a dual blood supply, intrahepatic biliary epithelium is more prone to ischaemic injury since these cells have only a singular, arterial blood supply. Disrupted arterial flow results in biliary ischaemia, progressive fibrosis, and inflammation. Percutaneous liver biopsies showed these histological features. There was extensive cholangiocyte injury, re-generative changes, apoptosis, and necrosis. Moderate portal and periportal fibrosis with bridging fibrosis from portals to adjacent portals and central veins were also found. Lastly, endothelial cells of hepatic arteries in the portal tract showed swelling with luminal narrowing and obliteration. As such, hepatic and cholangiocyte injury was found with biliary obstruction and micro-vascular changes [49].

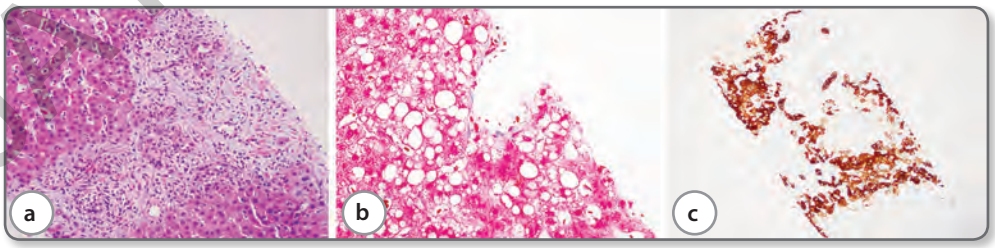



**Figure 3.2** Needle liver biopsy specimen with features of COVID-19 cholangiopathy. (a) Features of acute and chronic large duct obstruction include portal oedema and fibrosis and ductular reactions at the portal-parenchymal interface (H&E, x10). (b) Prominent steatosis is present indicating concomitant fatty liver disease, though without steatohepatitis or steatofibrosis (H&E, x40). (c) Keratin 7 immunohistochemical stain highlights aberrant hepatocyte staining through much of the lobule as is typically seen in chronic cholestatic liver injury (x10).

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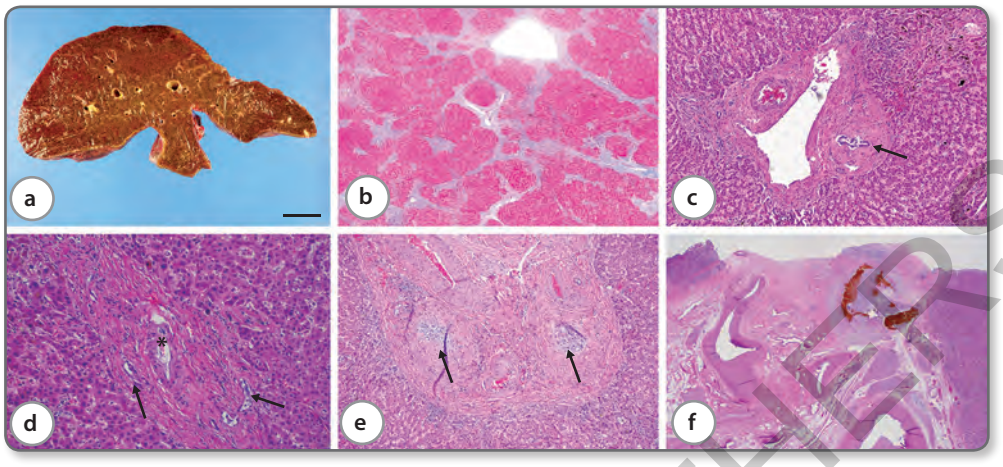



**Figure 3.3** Native liver explant with COVID-19 cholangiopathy. Images are from the same patient as in Figure 3.2. (a) The liver was large at 3,510 g and measuring 33.0 x 24.0 x 10.0 cm. Cut surface shows nodularity compatible with advanced stage biliary disease. (Bar = 3 cm). (b) Collagen stain (blue) shows a typical biliary pattern of scarring with odd, "jigsaw puzzle" shaped regions of parenchyma (Trichrome, x2). (c) A portal tract with an artery to the left of the portal vein and an atrophic bile duct (arrow) with circumferential fibrosis to the right (H&E, x20). (d) A fibrotic portal tract with minute bile duct remnants (arrows) on either side of a larger hepatic artery (*) (H&E, x40). (e) Fibrotic portal tract with replacement of bile ducts by scar and inflammation (arrows) (H&E, x10). (f) Large intrahepatic portal tract with a bile duct containing bile concretions and luminal abscess (H&E, x4).

Another cause may be toxic bile secretion [48]. Under physiological states, chloride-bicarbonate anion exchanger 2 (AE2) on cholangiocytes create an alkaline environment that protects the cell from bile acids. However, ischaemia disrupts the body's defence mechanism and cell membrane transporters and channels cannot protect cholangiocytes from toxic bile salts. Systemic hyperinflammation and high levels of pro-inflammatory cytokines also enhance the development of toxic bile, which together cause cholangiocyte necrosis [44,50]. Early MRCP findings showed accumulation of biliary casts from intrahepatic biliary filling defects that progressed to diffuse biliary structuring, dilatations, and beading.

## SEPSIS

Liver injury in COVID-19 has also been suspected to be related to sepsis, a frequent occurrence in severe COVID-19. Systemic inflammation upregulates proinflammatory cytokines [i.e. interleukin (IL)1B, IL1RA, IL7, IL8, IL9, IL10, basic fibroblast growth factor (FGF), granulocyte colony stimulating factor (G-CSF), granulocyte-monocyte colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), interferon gamma-induced protein (IP)10, monocyte chemo-attractant protein (MCP)1, macrophage inflammatory protein (MIP)1A, MIP 1b, platelet-derived growth factor (PDGF), and tumour necrosis factor (TNF)-α], angiogenesis (VEGF), macrophages, and the innate and adaptive (T-helper cell activation) immune response. Along with hypoxia, these conditions exacerbate ischaemia and increase reactive oxygen species (ROS) that can cause significant organ damage [10].

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The liver plays a critical role in defence against sepsis, acting as the second line of defence after intestinal bacteria have penetrated the gut epithelial barrier. A pre-COVID review by Strand et al of liver involvement in sepsis showed that sepsis was especially problematic in patients with CLD [51]. Sepsis can trigger acute-on-chronic liver failure, which is a syndrome with high short-term mortality. During sepsis, the liver integrates inflammatory signals, specifically type 1 immunity, to activate phagocytosis, helper T cells, IFN-γ, and IL-2 cytokines. This inflammatory activation aids in clearing systemic bacterial infection but can also cause tremendous organ damage. One such damage reported is potentially end-stage liver failure [51].

Leucocytes and innate immune cells are also critical players in the face of viral sepsis. As part of the first line of defence, neutrophils release extracellular fiber networks called neutrophil extracellular traps (NETs) to trap and kill microbes. However, due to the sticky nature of NETs, blood flow to the liver and biliary system is reduced and may become obstructed, resulting in ischaemia. The lack of perfusion to the hepatobiliary system can result in complications such as cholestasis and SSC-CIP. These were already cited as common complications of septic infection in severely ill patients with COVID-19. While leucocytes are part of an essential defence response, sustained activation of them can add fuel to the thrombo-inflammatory fire.

A prominent example is the association of hypercoagulable states and inflammation with occlusion of the extrahepatic portal vein [52] The portal vein will dilate while hepatic lobular architecture remains normal and little to no periportal and lobular fibrosis is seen on biopsy [53]. These hepatic circulatory disorders have the potential to manifest in patients with COVID-19 because of the high prevalence of sepsis and hypercoagulable states [54,55] In a systematic review, Kheyrandish et al reported portal vein thrombosis ($n = 40$) due to COVID-19 and COVID-19 vaccines. In this study, the median age of presentation was 41 years with a predilection among males (66.7%). However, in portal vein thrombosis following vaccination, the median age of cases was 45% and 79% were females. The average number of days until the onset of the symptoms was 8.3 days. The authors hypothesised that the increase risk of portal vein thrombosis in COVID-19 is likely dependent on the upregulation of ACE-2 receptors in cholangiocytes and the cascade of proinflammatory cytokines [56,57]. Liver pathology in sepsis includes steatosis, cholangiolitis and intrahepatic cholestasis, periportal inflammation, and the presence of acidophil bodies [58]. In addition to portal vein thrombosis, presence of mesenteric, splenic vein and other unusual splanchnic vein thrombosis including Budd–Chiari syndrome has also been reported in systematic reviews [59].

In conclusion, sepsis has the potential to complicate and exacerbate LD, cirrhosis, and increase mortality [51]. Newer COVID-19 studies also share the hypothesis that sepsis disrupts normal liver perfusion and homeostasis, which can contribute to progressive liver injury even in patients without preexisting liver pathology [60,61].

## ENDOTHELIOPATHY, THROMBOSIS, AND VASCULAR INJURY

The SARS-CoV-2 virus can also exacerbate organ failure and liver injury via micro-thrombi formation and large vessel thrombosis. Emerging research has demonstrated significant D-dimer elevations along with other biomarkers indicating a hypercoagulable and

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proinflammatory state in COVID-19 patients with severe disease (defined as requiring mechanical ventilation, ICU admission, and increased mortality rate). These biomarkers have shown that COVID-19 may be associated with an increased risk of micro-vascular, venous, and arterial thrombotic complications.

In addition to elevated D-dimer levels, abnormal biomarkers indicative of a prothrombotic state include elevated fibrinogen and C-reactive protein (CRP), which have correlated with increased severity of infection and mortality. Zhang et al studied 135 hospitalised COVID-19 patients in Wuhan, China. Up to 43.2% of patients with high levels of D-dimer (>0.4) had more severe presentations (i.e. respiratory rate > 30 breaths per minute, $Spo_2$ < 93%, and an oxygenation index < 300 mmHg) [62]. Additionally, Lippi et al reviewed four of five studies that reported significant D-dimer elevation, supporting a positive association between the severity of COVID-19 and hypercoagulation. Huang et al reported D-dimer levels increased five-fold in COVID-19 patients with severe disease (41 patients) [63]. Tang et al reported D-dimer elevation by 3.5-fold in patients with severe disease (183 patients) [63,64]. Wang et al also reported D-dimer elevation by 2.5-fold in patients with severe disease (139 patients) [2,63]. And Zhou et al reported a D-dimer level increase by 9-fold (191 patients) [63,65].

Liver histopathology from COVID-19 patients without prior history of LD or liver failure during hospitalisation is suggestive of major vascular changes. Liver wedge biopsies from 48 patients showed focal ($n$ = 14), multifocal ($n$ = 11), and diffuse ($n$ = 4) portal vein parietal fibrosis (phlebosclerosis). Herniated portal vein in periportal parenchyma was noted focally in 18 patients while multifocal (25–75% of portal tract) and diffuse (defined as >75% of the portal tract involvement) were seen in 18 patients. Periportal abnormal vessels were noted focally in 27 patients and extensively in 21 patients. Portal fibrosis was reported in 29 patients and presence of incomplete fibrous septa in eight patients. Mild to moderate lobular inflammation was noted in half the patents ($n$ = 24) and many of the patients ($n$ = 32) showed mild portal inflammation. Notably, partial ($n$ = 24) and complete ($n$ = 11) portal vascular thrombosis and incomplete ($n$ = 7) and complete ($n$ = 6) sinusoidal thrombosis were also seen [24].

Diaz et al found a high prevalence of vascular thrombosis in deceased COVID-19 patients. The histopathological autopsy findings from the systematic review (18 studies from seven countries) include congestion of hepatic sinuses, vascular thrombosis, fibrosis, Kupffer cell proliferation or hyperplasia, portal inflammation, lobular inflammation, venous outflow obstruction, phlebosclerosis of the portal vein, herniated portal vein, periportal abnormal vessels, haemophagocytosis, and necrosis [66].

There are multiple mechanisms and pathways by which the SARS-CoV-2 virus promotes a prothrombotic state [67]. One of the main mechanisms postulated to cause vascular endothelial injury is viral hijacking of ACE-2 receptors that are present in blood vessels, the heart, the kidneys, and the intestinal cells, in addition to the lung epithelium. Once the virus is internalised via the ACE-2 receptor, the receptor is concurrently down-regulated while up-regulating ACE. This shifts the balance of the renin-angiotensin-aldosterone system (RAAS) pathway, favouring increased angiotensin II levels. Angiotensin II acts on the angiotensin-1 receptor (AT1R) on endothelial cells to promote micro-vascular thrombosis, coagulopathy, hypofibrinolysis, and proinflammatory processes. Additionally, increased angiotensin II levels and ACE mediate the breakdown of bradykinin into inactive peptides, resulting in lower levels of bradykinin. In normal physiology, bradykinin would increase tissue plasminogen activator (tPA) levels, which causes vasodilation, fibrinolysis,

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and inhibits a prothrombotic state. Alternatively, virus-mediated ACE-2 down-regulation and RAAS dysfunction causes endothelial dysfunction. This has many deleterious effects, including hypercoagulability, hyperinflammation, and hypoperfusion.

A positive association between pro-inflammatory cytokine levels and loss of endothelial integrity and hypercoagulation has also been documented [68]. Hypoxia, resulting from pneumonia, may further induce and activate thrombosis. Activation of hypoxia-inducible transcription factors (HIFs) indicates a cellular response to hypoxia. Additionally, endothelial tissue factors and platelets are recruited while anticoagulants are down-regulated. Platelets support coagulation, recruit leucocytes, facilitate neutrophils' release of NETs, all augmenting thrombin generation.

McConnell et al hypothesised that pro-inflammatory cytokine interleukin-6 (IL-6) drives endotheliopathy, to induce a pro-inflammatory and procoagulant state, that may contribute to liver injury [69]. Severely ill patients with COVID-19 exhibit significantly elevated IL-6, factor VIII, fibrinogen, D-dimer, von Willebrand factor (vWF) activity and antigen, soluble thrombomodulin and liver enzyme activity. The constituents of the coagulation cascade have also been associated with increased morbidity and mortality. McConnell et al postulate that aberrant IL-6 signalling in liver sinusoidal endothelial cells (LSECs) may cause endotheliopathy and liver injury that has been widely observed in many severely ill patients [69]. IL-6 induces trans-signalling of Janus kinase (JAK) and STAT activation. They demonstrated that JAK1-STAT1/3 signalling is essential for inducing procoagulant and proinflammatory LSEC phenotypes by blocking JAK/STAT induction with inhibitors ruxolitinib and filgotinib. It was also shown that IL-6 alone was sufficient to produce the proinflammatory phenotype. Following, inhibiting JAK/STAT signalling resulted in decreased factor VIII along with the expression of other proinflammatory factors (*IL-6, CXC1/2, ICAM1, P-selectin, and E-selectin*). COVID-19 may significantly elevate IL-6 levels, which drives coagulation and thrombosis. This has significant implications for liver function. Increased hepatic vWF expression and increased platelet levels mediate neutrophil recruitment and fibrinogenesis in the liver.

These findings suggest that inhibiting IL-6 signalling and controlling proinflammatory up-regulation in patients with COVID-19 may prevent liver injury and other organ damage. Notably, endotheliopathy is endothelial dysfunction that is not only in the liver but also throughout other parts of the body [69,70]. Global endotheliopathy may broaden the importance of IL-6 inhibition to treat severely ill patients.

In conclusion, emerging literature demonstrates a strong association between COVID-19 and the RAAS pathway, coagulation, and cytokine and immune system modulation. Histological evidence of widespread presence of platelet-fibrin rich micro-thrombi in the sinusoids, central vein and portal vein corroborate the above hypothesis [22]. Our case series of COVID-19 cholangiopathy demonstrated widespread presence of platelets and exosomes in small sinusoidal aggregates in the absence of intravascular thrombi. A better understanding of the COVID-19-associated pro-inflammatory phenotype is important for treating thrombotic complications in patients. This is important for all patients and some studies have considered that the virus-induced pro-thrombotic phenotype may cause greater harm to patients with preexisting cardiovascular risk factors, and acute myocardial injury may result from aberrant ACE-2 receptor signalling [68]. Anticoagulants (i.e. heparin, FXII inhibitors, fibrinolytic drugs, nafamostat, and dipyridamole) and inhibitors of pro-inflammatory signalling are currently under consideration as potential effective treatments for COVID-19.

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ISBN 978-1-78779-172-5

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