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Title: SARS-COV-2 induced liver injury: Incidence, risk factors, impact on COVID-19 severity and prognosis in different population groups

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ABSTRACT

Liver is unlikely the key organ driving mortality in COVID-19 however, liver function tests (LFTs) abnormalities are widely observed mostly in moderate and severe cases. According to this review, the overall prevalence of abnormal LFTs in COVID-19 patients ranges from 2.5% to 96.8% worldwide. The geographical variability in the prevalence of underlying diseases is the determinant for the observed discrepancies between East and West. Multifactorial mechanisms are implicated in COVID-19-induced liver injury. Among them, hypercytokinemia with "bystander hepatitis", cytokine storm syndrome with subsequent oxidative stress and endotheliopathy, hypercoagulable state and immuno-thromboinflammation are the most determinant mechanisms leading to tissue injury. Liver hypoxia may also contribute under specific conditions, while direct hepatocyte injury is an emerging mechanism. Except for initially observed SARS-CoV-2 tropism for cholangiocytes, more recent cumulative data show SARS-CoV-2 virions within hepatocytes and sinusoidal endothelial cells using electron microscopy (EM). The best evidence for hepatocellular invasion by the virus is the identification of replicating SARS-CoV-2 RNA, S protein RNA and viral nucleocapsid protein within hepatocytes using in-situ hybridization and immunostaining with observed intrahepatic presence of SARS-CoV-2 by EM and by in-situ hybridization. New data mostly derived from imaging findings indicate possible long-term sequelae for the liver months after recovery, suggesting a post-COVID-19 persistent live injury.

Key words

COVID-19; liver injury; cytokine storm; endotheliopathy; immuno-thromboinflammation; direct hepatocyte injury

Core tip

Following respiratory system, liver is the second most involved organ in COVID-19. Besides the well-observed cholangiocyte tropism, typical SARS-CoV-2 lesions indicated by ultrastructural and histological evidence, identification of replicating SARS-CoV-2, S and nucleocapsid proteins RNAs within hepatocytes, as well as intrahepatic virus observation by electron microscopy and in-situ hybridization, converge to the conclusion that SARS-CoV-2 may also be hepatotropic. Most prevalent mechanisms of COVID-19-related liver injury are hypercytokinemia with "bystander hepatitis", cytokine storm syndrome with subsequent oxidative stress, endotheliopathy and immuno-thromboinflammation. Depending on the grade of their abnormalities, increased serum AST, (mostly peak) ALT, ALP, TBIL, inflammatory markers (CRP, ferritin, IL-6, IL-10) and decreased albumin levels are independent discriminators of COVID-19 severity and mortality. Age, male gender, CLD, liver cirrhosis, obesity, diabetes, and NAFLD are independent prognostic factors of unfavorable COVID-19 outcomes.

INTRODUCTION

SARS-CoV-2 RNA determined by quantitative rt-PCR is widely spread outside the respiratory tract, including the liver[1]. Regardless of pre-existing chronic liver disease (CLD), COVID-19-induced liver injury (LI) is mainly reflected by hypertransaminasemia, elevations of gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) (less frequently), and hypoalbuminemia[2-8], with the later being a negative acute phase reactant

rather than manifestation of liver failure and is one of the most prevalent abnormalities. COVID-19-induced LI is secondary than primary[9,10], mostly mild, transitory and self-limiting[11], it does not impact the majority of patients[12], and is common in absence of CLD[13]. In asymptomatic/subclinical cases randomly diagnosed by CT scans a mild increase in transaminases(8.8%) is observed[14]. LI definition varies among values just above the upper limit of normal (ULN)[15,16] up to 2–5xULN[17,18]. Substantial transaminases increases are linked to unfavorable outcomes, such as death, invasive mechanical ventilation (IMV), and intensive care unit (ICU) admission[7,18-22]. The prognostic relevance of higher LFTs may result from a more vigorous host immunological and inflammatory response to infection[12], particularly in younger individuals[12,23]. The pattern of LI is typically hepatocellular rather than cholestatic[24]. Severe liver injury (SLI) defined as ALT elevations>10–15xULN with or without jaundice, occurs in 2% of COVID-19[25], while acute liver failure (ALF) without underlying CLD is extremely rare and is typically associated with severe pneumonia and multiple organ dysfunction syndrome (MODS)[26]. SARS-CoV-2-induced ALF has been described in case reports[21].

PATHOPHYSIOLOGY OF SARS-CoV-2 INFECTION

The SARS-CoV-2 spike (S) protein is recognized by the angiotensin converting enzyme-2 (ACE2), whilst the androgen-induced transmembrane serine protease-2 (TMPRSS2) and paired basic amino acid cleaving enzyme (FURIN) are necessary for cell tropism and entry[27,28]. ACE2 cleaves the vasoconstrictor peptide angiotensin II to vasodilator angiotensin I[29]. S protein interacts with ACE2[30] and with an identified co-receptor neuropilin-1[31]. FURIN evades immune surveillance thus promoting transmission[28]. SARS-CoV-2 binding to ACE2 causes inflammation, oxidative stress, and pro-apoptotic reactions, ultimately leading to LI[30]. According to single-cell RNA sequencing studies of healthy livers,

cholangiocytes exhibit the highest expression of ACE-2, with modest expression found in hepatocytes, sinusoidal endothelial cells, and resident Kupffer cells[32]. Luminal immunohistochemical staining for ACE2 is observed in the bile ducts[33]. A few hepatocytes co-express both TMPRSS2 and FURIN[34,35]. Liver ductal organoids that express ACE2 and TMPRSS2 have been shown to recapitulate SARS-CoV-2 infection[36], whereas liver organoids generated from pluripotent stem cells also express ACE2 and allow SARS-CoV-2 pseudoparticle entry[36,37]. A small population of TROP2+ liver epithelial progenitors express both ACE2 and TMPRSS2. In healthy livers *vs* cirrhotics, 1.8/10.000 cells *vs* 10.6/10.000 expressed ACE2 and 97.2/10,000 *vs* 216/10,000 expressed TMPRSS2 representing a significant ($p< 0.001$) increase in the number of TMPRSS2+ cells in cirrhotics[38]. In untreated HBV infected livers, only 1.4/10,000 and 48.3/10,000 cells expressed ACE2 and TMPRSS2 respectively, significantly fewer than both healthy and cirrhotics[38]. ACE2 expression is 30 times higher in HCV-related cirrhosis than in healthy liver[39]. As ACE2 has been identified as an interferon-inducible gene[40,41], LI and inflammation may therefore enhance SARS-CoV-2 hepatotropism by modifying viral receptor expression, which is consistent with the damage to the respiratory epithelia[41]. In non-infected individuals with obesity and NASH, ACE2 and TMPRSS2 liver mRNA co-expression is likewise upregulated[42]. NAFLD and cirrhotic livers have much higher TMPRSS2+ progenitor cells indicating a susceptibility to SARS-CoV-2, findings consistent with the sc-RNA-seq results[38]. Given the recognized link between obesity and NAFLD[43], the finding of a larger abundance of TMPRSS2+ progenitor cells in NAFLD livers may offer a potential explanation for why obese people experience more severeCOVID-19[38].

RATIONALE AND MECHANISMS OF LIVER INJURY

The variability in prevalence and severity of LI among COVID-19 patients suggests that the mechanisms of LI are multifactorial (Figure 1).

Direct Liver Injury

Many studies propose that SARS-CoV-2 hepatotropism and its direct liver function impairment is implicated in COVID-19-induced LI, while only a few speculate that definite evidence is lacking[44,45]. Liver progenitor cells, particularly those destined to become cholangiocytes, contain ACE2[46], in addition to virus isolation in bile[47] imply a direct invasion by SARS-CoV-2. Its infection triggers cell apoptosis factors resulting in cholangiocyte death[36] by lysis and/or by inducing necrosis and apoptosis[48-50]. SARS-CoV-2 virions have been seen within hepatocytes and sinusoidal endothelial cells using electron microscopy (EM)[51]. The best evidence is the identification of replicating SARS-CoV-2 RNA, S, and nucleocapsid proteins RNA within hepatocytes using in-situ hybridization and immunostaining[51,52]. In hepatocytes and sinusoidal endothelial cells, SARS-CoV-2 virions have been seen using electron microscopy (EM)[51]. The strongest supporting data were found employing in-situ hybridization and immunostaining to identify replicating SARS-CoV-2 RNA, S, and nucleocapsid proteins RNA within hepatocytes[51,52].Viral genomic RNA was also identified in postmortem COVID-19 liver examinations[53,54], with observed intrahepatic presence of SARS-CoV-2 by EM and by in-situ hybridization[55-57], and viral replication within hepatocytes[58,59], thus reinforcing the role of direct SARS-CoV-2 hepatocyte injury. SARS-CoV-2 particles without membrane-bound vesicles were found in the hepatocyte cytoplasm of COVID-19 patients with aberrant LFTs[51], which is additional proof.

Liver hypoxia

Hypoxia can cause hepatocytes inflammatory cells infiltration, lipid accumulation, an increase in reactive oxygen species (ROS), and death[60,61]. ROS peroxidation products act as a second messenger amplifying the release of multiple cytokines[62]. In COVID-19, hypoxia and CSS are considered as risk factors for LFT abnormalities[63]. Hypoxic hepatitis features (e.g.

centrilobular necrosis) are widely shown in postmortem liver biopsies[64]. In severe COVID-19, IMV, positive end-expiratory pressure, and/or vasopressor support negatively impact hepatic perfusion by lowering cardiac output, raising hepatic vascular resistance, and increasing portal vein pressure, which obstructs venous drainage, leading to acute liver injury (ALI) and/or cholestasis[58,65,66]. Gut ischemic injury on the other hand, results in intestinal endotoxinaemia and activation of the sympathetic nervous and adrenocortical systems furthermore contributing to LI[58,66]. Additionally, Kupffer cells can stimulate cytokines due to ischemia[67], while mitochondrial damage by SARS-CoV-2 results in AST release[68-70]. Direct interaction of mitochondrial proteins with the virus nonstructural protein 5 provides a probable reason for the AST-dominant liver profile[71]. In addition, unlike the hepatic preponderance of ALT, zone 3 of the hepatic acini containing higher AST concentrations is more susceptible to hypoxic injury[72]. In COVID-19 aminotransferases elevations, typically mild, are incompatible with very high AST/ALT elevations of primary hypoxic hepatitis[73]. Secondary hypoxic LI owing to the presence of acute respiratory distress syndrome (ARDS) as well as to an overactive inflammatory response to SARS-CoV-2 and MODS[73] might be implicated. In addition, cell iron overload might play a role, and hepcidinmimetic action of S protein may induce ferroportin blockage[25].

Cytokine Storm Syndrome (CSS)

In order to maintain homeostasis, the body activates the immunological defense system and the oxidative stress response with the release of many cytokines when activated by endogenous or external stimuli like viruses[74]. SevereCOVID-19 exhibits a distinct immunological dysregulation with two essential characteristics: lymphocyte dysregulation with lymphopenia and overproduction of pro-inflammatory cytokines by monocytes[75,76]. The relationship between lymphopenia and CSS in COVID-19 pathogenesis was described in previous coronaviruses outbreaks[77,78]. Severe hypercytokinemia results in a cascade of actions leading to tissue (especially liver) damage and

MODS[79]. Lymphopenia, decreased CD4+, early and persistent elevation of cytokines (TNF-a, IL-2,-6,-7,-10,-18, granulocyte-colony stimulating factor, IFN-γ, ferritin, IP-10, MCP-1, and macrophage inflammatory-protein-1a, chemokines), lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, D-dimer and of coagulopathy markers (thrombopoietin), are independent risk factors for SLI (Table 2), and are linked to unfavorable outcomes[23,37,50,59,80-87]. CSS in severe COVID-19 is also associated with reduced CD8+, CD3+ and CD4+ T-cells[88,89]. Depletion of circulating CD8+ T-cells, the main determinant of LI in viral infections (influenza, measles, and SARS), reflects their trapping in the liver[90,91]. The syndrome known as "bystander hepatitis," which is frequently seen in systemic viral infections[92] and in COVID-19[93], is caused by circulating cytokines activating hepatic immune cells without compromising liver function. By activating Kupffer cells in the absence of viral antigens in the liver, viral-specific CD8+ T-cells that are confined to locations outside the liver may cause T-cell-mediated hepatitis[94].Also, T-cells depletion cannot control the viral infection, leading to macrophage activation and more secondary inflammatory reactions[95,96]. In severe situations, the SARS-CoV-2 virus may cause a hyperinflammatory disease known as macrophage activation syndrome (MAS) or secondary hemophagocytic lymphohistiocytosis (HLH)[75,76]. This syndrome is characterized by CSS, cytopenias, disseminated intravascular coagulation (DIC) and MODS. The pathogenesis of cytokine-driven hyperinflammatory disorders is heavily dependent on IL-6 signaling[76], that strongly correlates with elevated transaminases[88]. Inflammasome, a complex intracellular protein that SARS-CoV-2 may produce, helps promote caspase-1's autocatalytic activation (apoptosis/pyrolysis) and the exudation of pro-inflammatory cytokines[97], triggering the expression of other genes involved in the immune process[98], therefore resulting in MODS[87]. However, patients with mild COVID-19 may experience LFT abnormalities regardless of their inflammatory condition, probably because the unique inflammation brought on by SARS-CoV-2 is more likely to do so than inflammation brought on by other pathogens[99].

Endotheliopathy– Hypercoagulable state– immuno-thromboinflammation

COVID-19 is considered to affect the endothelium, one of the largest organs in the human body[82]. SARS-CoV-2 may worsen microcirculation and encourage thrombus formation, tissue oedema, and organ ischemia by encouraging endothelial cell damage in the arteries, veins, arterioles, capillaries, and venules of all major organs[87,100-101]. Hepatic artery branches in the portal tract with endothelial enlargement and luminal constriction, as well as portal vein endophlebitis, and endotheliitis (leukocyte attachment to the vascular wall) with thrombotic material[102-105], are pathology findings indicative of endotheliopathy in COVID-19-related LI. The observed network of sinusoids decorated by CD34 suggests abnormal hepatic blood circulation[102]. In deceased patients with elevated ALT, significantly higher fibrinogen, factors VIII and II activity, and platelet marker CD61 liver staining was morphologically shown, in accordance with their serum levels (fibrinogen, D-dimer, von Willebrand factor (vWF) activity and antigen, and CRP[45,46,106,107]), thus resembling a microangiopathy thrombotic state[86,106,107]. Additionally, vWF-positive areas correlate with CD61-positive areas[60,171] and with intralobular neutrophil infiltration suggesting a link between the procoagulant state and liver inflammation[45,107]. Endotheliopathy, vWf expression on cell surfaces, and platelet adhesion are all mediated by IL-6 trans-signaling in liver sinusoidal endothelial cells (LSECs), which also plays a role in LSECs inflammation and activation of coagulation therefore being involved in COVID-19-related LI[45,107,108]. As LSECs are endothelial cells and do not express IL-6Ra, trans-signaling is thought to be the main method of IL-6 signaling to LSECs[109,110]. The Janus kinase-signal transducer and activator of transcription (JAK/STAT) pathway may also be used to promote IL-6 trans-signaling[45,107], which is essential for inducing a procoagulant and proinflammatory LSECs phenotype[111,112]. Activated neutrophils may generate neutrophil extracellular traps[113]. Decreased ADAMTS13 levels, another typical finding in severe COVID-19[114,117,118] can induce increased

platelet-endothelial interaction[111,115-118], while DIC may also result from CSS in critical/fatal COVID-19[119,120]. A significant imbalance between inhibitors and activators of fibrinolysis is also demonstrated. Reduced action of endogenous anticoagulants (antithrombin, tissue factor (TF) pathway inhibitor (TFPI), and proteins C and S[121]) is a hallmark of hemostasis dysregulation. As the pulmonary inflammation worsens, hypofibrinolysis is caused by the consumption of plasminogen, high levels of plasminogen activator inhibitor-1 (PAI-1) and a decrease in tissue plasminogen activator, which prolongs the prothrombotic state[122,123].The pathological state involving platelet hyper-reactivity, hypercoagulability and hypofibrinolysis during COVID-19 is named "immuno-thromboinflammation"[123]. As platelets express both ACE2 and TMPRSS2 on their surface[124], it is intriguing that SARS-CoV-2 can attach to them directly and activate them, causing the release of clotting factors and inflammatory chemicals. Endothelial injury triggers the release of TF in circulation which may be also derived from macrophage/monocyte cells as a consequence of MAS[125]. When PAI-1 is overproduced, it binds to TLR4 on macrophages and triggers the release of cytokines and chemokines[126], which in turn promotes inflammation, steatosis, and microvascular thrombosis[127]. SARS-CoV-2 binds to the ACE2 receptor on tissues inceasing Ang II levels, favoring PAI-1 and TF expression thus promoting hypercoagulability and impairing fibrinolysis[115]. Extensive pericyte activation during LI contributes to the recruitment of inflammatory cells, and their conversion into cells that resemble myofibroblasts results in the creation of extracellular matrix proteins and the ensuing fibrosis of the vessel wall[128].

Oxidative stress

The expression of antioxidant proteins is regulated by nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor that is triggered by oxidative stress[129,130,131]. One of the most significant mechanisms of antioxidant and anti-inflammatory element signaling is the complex KEAP1-NRF2-ARE[132].

The NRF2 antioxidant pathway is suppressed in COVID-19, while infected cells show a GSK-3 (conserved serine/threonine kinase) activity that degrades NRF2[133]. NRF2 interacts with NF-kB, a proinflammatory signal transduction pathway[134] driving the initial proinflammatory response[135], to reciprocally regulate redox metabolism[136-139]. When NRF2 activity reaches its maximum level, NF-κB activation is inhibited. In response to viral infection or other stimuli, inhibitor kappa B (IB) is phosphorylated, which releases and translocates NF-B to the nucleus, causing inflammatory cascades and the generation of inflammatory mediators[140]. Via the TNFR1-NF-B signaling axis, TNF- may activate NF-B[141], and NF-kB in turn enhances the release of inflammatory cytokines[135]. This creates a vicious loop that feeds CSS and exacerbates LI[142-144]. Oxidative stress is mostly caused by ROS. The NF-B pathway is activated by COVID-19, ARDS, and sepsis as they cause tissue ischemia and ROS production[145]. In the early stages of COVID-19, ACE2 is the most critical component, whereas the IL-6-STAT3 axis is crucial in the late stages and in CSS[146]. Indeed, both NF-κB and STAT3 pathways are activated in COVID-19 promoting inflammation by activating the IL-6 amplifier[147]. NRF2 promotes Glutathione (GSH) synthesis[148], and participates in the tricarboxylic acid cycle by regulating the production of NADPH, a key co-factor of antioxidant reactions[149,150]. NRF2 inhibits liver fibrosis and promotes liver regeneration[151-153] therefore protecting liver cells in viral hepatitis[154], drug-induced liver injury (DILI)[155-157], cholestasis[144,158,159], and NAFLD[160,161], by reducing gluconeogenesis and fat deposition, restoring insulin resistance, and boosting the anti-inflammatory and antioxidant effects[162].

Drug-induced liver injury

The large use of antiviral drugs may contribute to COVID-19-related LI especially in individuals with increased baseline ALT[163]. The pooled incidence of DILI in COVID-19 is reported 25.4%[6]. In DILI, AST usually peaks before ALT, a biochemical pattern also observed in severe COVID-19.

In some cases, observed microvascular steatosis and mild hepatic inflammation are consistent with DILI[20,49]. In remdesivir-treated patients, 23%-35% show increased LFTs[164,165] indicating hepatotoxicity, while 2–3% required treatment discontinuation[164]. Lopinavir/ritonavir incidence of DILI is 37.2%[9] with a significant increased risk (OR=4.44) for severity[20]. Medicines with possible antiviral effects should only be administered on patients who have risk factors for severe illness[82] and early in the course of the disease[166]. In tocilizumab-treated patients, 15%-51% presented a transitory but not significant hypertransaminasemia between 9-13 days, some of which showed surprisingly higher mortality[167,168]. The liver's IL-6-mediated endotheliopathy should be improved by treatment with the JAK inhibitor baricitinib. A significant disadvantage of all those treatments those treatment clinical trials is the frequent exclusion of patients with AST/ALT>5xULN[169]. Moreover, immunosuppressive drugs, such as tocilizumab, tofacitinib, and dexamethasone, can potentially induce LI via HBV reactivation in patients with occult infections[170,171], therefore antiviral prophylaxis should be administered. Dexamethasone may ameliorate endothelial injury[172] by dampening of endothelial IL-6 production[173]. The most typical contributors to DILI in the general population, antibiotics and nonsteroidal anti-inflammatory medications, may also cause LI, while acetaminophen can cause alterations in aminotransferases even at therapeutic doses[174].

Gut Microbiome

In COVID-19, dysbiosis of the gut microbiota may have a significant impact on the clinical outcome of patients with comorbid conditions such diabetes, hypertension, and obesity[176] and may lead to liver damage[175]. For example, older people often have less variety in their gut microbiota, and COVID-19 is more severe in this age group, supporting the possibility that microbes play a role in outcomes[177]. Hepatic dysfunction brought on by sepsis may result from disruption of the gut microbiota and a breach of the gut-mucosal

barrier[178]. Moreover, the diversity of the gut microbiota influences how the host immune system responds[178]. It is hypothesized that changes in the gut-liver axis may contribute to the severity of COVID-19 seen in cirrhotics. Cirrhosis is characterized by changes to intestinal permeability, gut microbiota composition, and function[179].

INCIDENCE

The earliest available epidemiological data of COVID-19 patients came from China. Abnormal LFTs were first reported in a cohort from Wuhan, China[9], making liver the most frequently damaged outside of the respiratory system. It's interesting to note that Wuhan, the COVID-19 epicenter, had a substantially greater incidence of elevated aminotransferases than the surrounding areas (21% vs. 10%)[18,63] possibly because of higher SARS-CoV-2 doses exposure in Wuhan[80]. Western populations show abnormal LFTs more clearly than Eastern populations (Figure 2). The timing of LFT determination during disease course, different definitions, but mostly, the geographical variability in the prevalence of underlying diseases are the determinants for the observed discrepancies[18]. With respect to worldwide published data the overall prevalence of abnormal LFTs ranges from 2.5% to 96.8% (Table 1), while SLI accounts for 4.94%-21.8% of COVID-19 patients[7,20,22,23,38,80,180]. Patients with SLI are younger and more likely to be male[23]. Younger patients may exhibit a more robust immune response to infection, causing LI and determining its degree[23,38]. Aminotransferases are higher in severe COVID-19 cases, in accordance with the 2002-2004 SARS outbreak[181]. Concerning cholestatic enzymes, elevated GGT and ALP range between 15%-47.3% and 4%-58.5% respectively (Table 1). GGT, a surrogate marker for increased oxidative stress and chronic inflammation[182], usually increases in severe cases[58,59] implying cholangiocyte injury[183,184]. The GGT elevation without accompanied by ALP elevation[59] may also develop in DILI more frequently than obstruction. ALP elevation is rare, usually <2xULN[185], and is mostly

observed in MODS or death from COVID-19[186]. The joint trajectory of GGT, ALP, and bilirubin points towards a cholestatic LI seen in impaired survival[187]. The prevalence of TBIL elevations ranges between 3.1% and 52.1%. Concerning longitudinal changes, LFTs become more frequently, and more severely deranged during hospitalization[20,188,189]. The median time to peak AST levels is 3 days after admission, normalizing within 4.4 days[190]. ALT elevations peak between 4-17 hospital day[188]. In deceased patients, ALT levels are normal in the first week but subsequently rise rapidly along with AST at the third week. In survivors, slightly elevated ALT levels occur at 2-3 weeks after symptoms onset when AST levels might remain normal[191]. A biphasic pattern with early aminotransferase onset, culminating around days 10-15 of hospitalization, and then gradual normalization accompanied by rising ALP is also suggested[187,304]. ALI (ALT>3xULN) occurs between 17-18.5 day after symptoms onset[28,192]. AST is diffusely represented in many tissues while ALT is considered liver-specific[193]. Greater AST levels may be related to mitochondrial damage or damage to other organs[194]. In the liver, ALT is only found in the cellular cytoplasm[72,195] whereas AST is both cytosolic (20%) and mitochondrial (80%) localized, and is in higher concentrations in Zone 3 of the hepatic acinus therefore ischemic or toxic damage to this zone may result in greater AST elevations.

UNDERLYING DISEASES, SEVERITY, PROGNOSIS

Underlying Diseases

The median age of COVID-19-induced LI patients ranges between 51.5-56 years with male predominance[65,191]. The incidence of hypertension, diabetes, and coronary heart disease ranges between 23.08%-31,8%, 11.54%-15.3%, and 7.8%-11.54%, respectively[192]. Age, male sex, hypertension, and diabetes are negatively correlated with SLI in COVID-19[23]. The association of LI with hypertension and poorer prognosis is more significant in the absence of pre-existing CLD[38]. CLD prevalence varies widely with Chinese studies being

reported between 1.4% -15.3%[9,184,196], lower than in Western countries (5% - 37.6%)[197,198]).

Severity

Risks of severity for specific LFTs indices are shown in Figure 3. Aminotransferases levels>5xULN correlate to mortality[23,192], while incidence of LI is higher in ICU than non-ICU patients (61.5% *vs* 25.0%)[199]. Elevated LFTs on admission show a 3-fold greater risk of severe disease and 3.5-fold risk for mortality[6,200]. After adjustment, patients with LI are at a 9-fold greater risk of severe COVID-19[20], and at a 7.5-fold risk for mortality[201]. A few studies however, failed to show such an association with severity/mortality[202-205], disease progression[17], and ICU admission[202,206]. These differences could be attributed to the nature of the studies that were included and the various outcomes definitions[207,208]. It seems that LI portends the need for ICU care and IMV[209]. CLD is also associated with severity (OR/RR=1.48, 1.70)[208,210-213], and mortality (OR/RR=1.08, 2.65)[208,212,214] while in a few studies such an association was not observed[6,207,215-219] (Table 2). Age, male gender, higher body mass index (BMI), corticosteroids, antifungals, lymphocyte decrease, neutrophil increase, and CLD are factors positively associated with ALT/AST increase[6,18,20,192,194,220]. ALP levels are tightly associated with male gender, antifungals, neutrophil count increase, and CLD. Antifungals, antivirals, systemic corticosteroids, and platelet count reduction are positively correlated with increased TBIL levels.

Within five weeks, patients with SLI are significantly more likely to have been intubated, to require renal replacement therapy, or to decease compared to moderate/mild LI[23]. LFTs elevations during hospitalization correlate well with the severity of inflammatory indexes (CRP, procalcitonin, ferritin, LDH, GGT, lactate, and D-dimer)[16,200,221]. In essence, LFTs can be used as a surrogate for the monitoring of inflammation. ALT levels correlate well with the CSS inflammatory markers[317,222-225] (Table 3). This

immunological response is consistent with that reported for other viral respiratory tract infections[226]. IL-6,-8, TNF-a are positively correlated with the increased AST, TBIL, and ALP, therefore, cytokines contribute to COVID-19-induced LI[65]. Significantly higher WBC and neutrophils, and lowered lymphocytes are observed in LI[65,227,228].

Prognostic factors

In mixed-effect Cox model adjusted for age, gender, and comorbidities patients with AST>3xULN compared to normal AST exhibited increased risks of death and IMV (19.27-fold and 116.72-fold, respectively)[191]. Risk of severity and all-cause mortality of LFTs abnormalities are shown in Figure 3. Patients with LI have a 4-fold higher rate of mortality, 7-fold higher rate of ICU admission, and 11-fold higher rate of intubation[20,229], while hepatocellular and cholestatic type LI increases the risk by 3-fold[20]. LI is suggested as an independent prognostic factor of COVID-19[200,211]. AST/ALT ratio>1 predicts mortality, severe pneumonia and ICU admission[221]. While hepatic steatosis is considered to have no impact on disease course, FIB-4 score<1.45 is a significant protective factor[230]. Vasopressor use (ischemia), and hyperinflammatory/hypercoagulable state are also independent predictors of death[190]. ALP peak value is a risk factor for in-hospital mortality[231]. All-cause adjusted mortality risk is 6-fold significantly increased in patients with an elevated TBil>2-5×ULN and 1.42-fold in patients with ALP>1-2×ULN[232]. Serum albumin is negatively associated with severity. Hyperglycaemia at admission is associated with severity/mortality[233]. In fully models adjusted for confounders, increasing age, non-white and non-black race, hypertension, overweight/obesity, kidney disease, cardiovascular disease, diabetes, cancer, and dementia, are independently associated with an increased risk of in-hospital mortality[232-234]. Higher state of inflammation is also significantly associated with mortality[232], while peak ferritin and IL-6 levels are associated with SLI (Table 2)[23].

COVID-19 IN DIFFERENT CLD POPULATIONS

Cirrhosis

In COVID-19 the presence of cirrhosis, mostly of decompensated, is an independent predictor of liver-related (OR=3.24) and overall complications, as well as mortality (aOR; 11.3-12.5)[232,235]. In patients with CLD, the 30-day cumulative overall mortality is higher in cirrhotics (RR=4.6)[236], with respiratory complications being the main cause of death[208,237-239]. In terms of CLD etiology, more frequent is viral (60.5%), followed by NAFLD (32.6%), alcohol-related liver disease (ArLD) (4.7%), and autoimmune hepatitis (2.3%). ArLD, NAFLD and hepatocellular carcinoma (HCC) but neither viral nor autoimmune hepatitis are associated with increased mortality[237,240-242]. Cirrhosis-associated immune dysfunction (CAID), is a condition that affects patients with CLD, particularly cirrhosis, who display a variety of immune dysfunctional mechanisms that enhance their vulnerability to infection and abnormal inflammatory responses[44]. The CAID phenotypes represent a continuum of dynamic events that shift from being primarily pro-inflammatory to being primarily immunodeficient. Reduced bacterial opsonization, phagocytosis, protein C activity, antigen T-lymphocyte dependent responses, vaccination responses, hypoalbuminemia, hypocomplementaemia, and intestinal dysbiosis are some of the characteristics of this condition[44,243,244]. CAID is also associated with increased serum levels of IL-1β,-6,-17,-18, TNFα, and IFN-γ[245], and predisposes to a variety of viral or fungal-related diseases[246]. Despite the increased risk of infection[247], cirrhotics exhibit a lower risk of acquiring SARS-CoV-2[209,248,249], whereas in large population studies patients with CLD are not over-represented[250]. In patients with CLD, a condition known as acute-on-chronic liver failure (ACLF) is characterized by abrupt hepatic decompensation and extrahepatic organ failures and is linked with a significant short-term mortality[251]. While being typically linked to bacterial infections, COVID-19, among other virsues may cause ACLF[251-253].The

incidence of ACLF in COVID-19 patients with CLD ranges from 10% to 50%[235,237], with a reported 65% mortality rate[237]. ACLF may also occur in compensated cirrhotics with severe COVID-19[254]. Also, as the severity of cirrhosis is assessed by the Child-Turcotte-Pugh (CTP) score, there is a stepwise rise in morbidity and mortality[237]. After adjusting for baseline characteristics, COVID-19-related mortality is significant across the CTP stages (aORs): A=1.90, B=4.14 and C=9.32[237], similar to non-COVID-19 hospitalized cirrhotics[235,238]. CTP score>9 at presentation independently predicts mortality, in accordance with MELD and Chronic Liver Failure Consortium (CLIF-C) scores[235,238,239]. In cirrhotics LI is evident at presentation (OR=6.2)[200]. The non-survivors cirrhotics have higher AST levels, AST/ALT ratio>1.4 (aHR=1.4), and a low R value that predicts mortality[235]. Development of liver decompensation during COVID-19 exhibits increased mortality compared to maintenance of hepatic compensation (63.2% *vs* 26.2%)[239].

Despite suffering higher mortality, those cirrhotics who survive the initial insult show re-admission/death rates at 90-days comparable to cirrhotics without COVID-19[254]. SARS-CoV-2 does not appear to accelerate the progression of liver disease beyond cirrhosis' normal course after acute infection. The interaction of severe COVID-19, pulmonary illness, and ACLF is probably mediated by CAID. Cirrhosis is linked to an increase in cytokine production and baseline endotoxinaemia, which may cause an increased inflammatory response to infection[44]. Hypercytokinemia, another characteristic of COVID-19, causes hepatocyte apoptosis and necrosis, which, in the presence of depleted liver reserves, may result in hepatic decompensation[244,255]. Therefore, cirrhosis and COVID-19 may have detrimental effects on each other. Cirrhosis decompensation in COVID-19 is characterized by deteriorating ascites, worsening of jaundice and hepatic encephalopathy more frequent than variceal bleeding or spontaneous bacterial peritonitis[235,240,241]. Despite compensated cirrhotics having greater problems, mortality among them and non-cirrhotics is comparable,

supporting the idea that there is adequate hepatic reserve for recovery. The more significant predictor of death in COVID-19[237] is hepatic decompensation as opposed to cirrhosis *per se*. The data indicate that non-traditionally hepatotropic infections, such as SARS-CoV-2, may directly precipitate ACLF in cirrhotic patients[256] as also observed in influenza[252]. The median PLT count and IFN-γ are significantly decreased in CLD than non-CLD patients[226,257], and cirrhotics are at higher risk for thrombotic events[105].

NAFLD

NAFLD has been advocated as a risk factor for severe COVID-19, it is present in the majority of COVID-19 patients with CLD worldwide, and shows longer viral shedding time[258]. Patients with NAFLD assessed by CT scan and with intermediate/high risk of fibrosis (FIB-4) have higher risk for severe COVID-19 (OR=2.95) *vs* patients without NAFLD fibrosis score (NFS), suggesting a pathogenetic role for advanced liver fibrosis in severe COVID-19 with worse outcomes[232,250-260]. Increasing FIB-4 or NFS values when included as continuous measures in multivariable regression, they are significantly associated with COVID-19 severity (aORs=1.90, and 2.57, respectively). NAFLD is epidemiologically associated with an increased risk of severe COVID-19[261,262], independently of BMI[263]. Hepatic steatosis is an interesting pathological characteristic that frequently occurs in COVID-19. It is possible that activation of coagulation, which is capable of causing hepatic steatosis in NAFLD, represents a unique mechanism connecting thrombosis and steatosis, two diseases that are both common in COVID-19[264]. Given that high PAI-1 has been linked to NAFLD and NASH[265,266], the involvement of PAI-1 in COVID-19 may be noteworthy. Lastly, obesity, a major risk factor for thrombosis owing to adipocytokine-mediated processes, increases inflammatory molecules, Ang II/Ang 1,7 imbalance, ROS-mediated endothelial dysfunction, and alterations in lipid/glucose metabolism[267-269]. Greater risk of severe COVID-19 is found in non-diabetics patients<60 years with NAFLD (aOR=4.07)[259,270]. Additionally, underlying diabetes with

NAFLD shows a 2-fold higher risk of severe COVID-19, much higher when LI is present (OR=6.4), or in obese NAFLD patients (aOR=6.32)[271]. NAFLD is associated with an increased risk of severity when it coexists with obesity[272], in non-diabetics[270], in younger patients[259], and in individuals with high hepatic fibrosis scores[260]. Although it is unknown how obesity and NAFLD might worsen COVID-19, comparable mechanisms are frequently present in both disorders comprising alterations in the immune response, macrophage activation, and (low-grade) inflammation[260,273,274]. Obesity-related chronic inflammation impairs macrophage activation via antigen presentation and pro-inflammatory cytokine synthesis[275]. Moreover, obesity reduces B- and T-cell responses, which leads to greater susceptibility and delayed clearance of viral infections[275,276]. The advancement of COVID-19 is favored in NAFLD patients when hepatic macrophage polarization changes from M1 (which promotes inflammation) to M2 (which suppresses inflammation)[58,274,277]. The polarization states of macrophages may be unbalanced, influencing the host's inflammatory or tolerance response to SARS-CoV-2 signals provided via the gut-liver axis. ACE2 expression is elevated in CLD/NASH patients, and cytokine secretion is enhanced in association with COVID-19[42]. Conversely, in a few studies NASH was not associated with severe disease[278]. It is also demonstrated that COVID-19 patients exhibit increased serum levels of MCP-1, which exacerbates steatohepatitis[279]. Age, gender, obesity, and other comorbidities are thought to be less important risk factors for COVID-19 than NAFLD. NAFLD progression may also be hastened by COVID-19.

Alcohol-related liver disease

The SECURE-Cirrhosis and COVID-Hep registries identified ArLD as a risk factor for COVID-19 mortality(aOR=1.79) related to advanced disease and CAID[237]. Increased mortality is also seen in alcohol-related cirrhosis[238,239]. Immune dysregulation, particularly changes in the gut-liver axis, is accentuated in ArLD, with increased endotoxinaemia and Kupffer cell

activation leading to proinflammatory cytokine transcription and superoxide production[44,178]. Moreover, alcohol impairs adaptive immunity, including both cell-mediated and humoral responses[179]. The inflammatory state caused by danger-associated molecular patterns is linked to ArLD leading to the production of pro-inflammatory cytokines[238,256]. It is postulated that CSS could aggravate the increased inflammatory process in ArLD, resulting in worse outcomes[119]. In the COVID-19 period, hospitalized patients with ArLD appear at more advanced disease stages, with acute decompensation, higher MELD scores, and greater rates of ICU and mortality[185].

Autoimmune hepatitits

Immunosuppressed patients exhibit higher SARS-CoV-2 viral titres, prolonged viral shedding but do not exhibit increased risk of complications[241-242,280,281]. Analysis of AIH *vs* non-CLD patients demonstrates increased risk of hospitalization, but equivalent risk of all other outcomes including death[281]. As a result, in stable patients, immunosuppression should not be lowered as a COVID-19 risk reduction strategy[11,282]. Immunosuppression may also reduce the risk of new-onset LI[242,281,282].

Viral hepatitis

Persons with chronic HBV or HCV infection who do not have cirrhosis are not more likely to get COVID-19 or have a worse outcome[9,59]. Conversely, patients with SARS-CoV-2 and HBV co-infection are prone to a worse prognosis[283] and tend to have 2.2-fold more severe COVID-19[284]. The in-hospital mortality in COVID-19 patients with HBV is 6.0%[22] with ACLF precipitation<1%, while LI prevalence in non-decompensated is comparable with non-CLD patients[22]. Significantly lower monocyte and WBC counts, higher levels of CD8+ T-cells and thrombocytopenia in HBV with COVID-19 are observed compared to COVID-19 alone[285]. The likelihood of HBV

reactivation during SARS-CoV-2 infection is mentioned, however the risk appears to be low[286]. Those with HCV who have COVID-19 are more susceptible to hospitalization, but not at a higher risk of death[287]. Because recently treated HCV patients were less prone to contracting with SARS-CoV-2, HCV antibodies may be indicative of "protection" against COVID-19[288].

Liver transplantation

Liver transplant (LT) recipients are more frequently diagnosed with COVID-19 than general population. This might be attributable in part to more careful surveillance and a lower viral testing criterion in LT recipients[289]. The hospitalization rate for COVID-19-positive LT recipients is 50–70%[290]. Immunocompromised individuals above the age of 60 are more prone to develop SARS-CoV-2 infection with protracted viral clearance[291,292]. LT recipients however, are not at increased risk of severity/death as compared to non-LT recipients[44,290]. LT can involve the donor-to-recipient transmission of SARS-CoV-2[293]. COVID-19 is linked to worse postoperative transplant outcomes, particularly in elderly and obese patients[294]. Most LT patients with COVID-19[295,209] should continue to receive systemic immunosuppression with stable dosages, except for immunomodulators[209], as mycophenolate treatment is considered an independent risk factor for severe COVID-19[289]. The case-fatality rates (17%-18%) are consistent with the expected mortality rates[296]. Those with underlying cancer may require particular consideration[297]. The mortality risk does not change between early (1year) and remote transplantation[296,297].The rate of graft dysfunction during COVID-19 infection is estimated 2.3%-5%[294,298]. The inflammatory reaction in COVID-19 solid organ transplant (SOT) patients is not more severe while IL-6 levels and incidence of ARDS are lower in SOT suggesting that immunosuppressive medication might limit the COVID-19 hyperinflammation[92].

Hepatocellular carcinoma

Data on HCC patients with COVID-19 infection are limited. COVID-19 in cancer patients is associated with poor outcomes especially if antitumor treatment was received within 14 days[299]. The all-cause mortality in the HCC subgroup is reported 52.4%, almost 7-fold higher than in patients without HCC[238]. COVID-19 patients with HCC may exhibit exacerbated progression with aggravation of existing liver disease[300].

SARS-COV-2 VACCINATION IN CLD

Because neither the adenoviral vector nor the mRNA vaccines contain live or attenuated virus, it is unlikely that vaccination poses a special safety risk for CLD patients. Vaccine trials of both Pfizer-BioNTech[301] and Moderna[302] demonstrated consistent efficacy among subgroups with coexisting conditions, but small numbers of CLD patients were included. It is unknown if SARS-CoV-2 vaccinations are as effective in cirrhotic/transplanted/immunocompromised CLD patients as they are in the general population, especially against quickly evolving viral variations. Preliminary findings suggest that transplant patients are safe[303]. Whilst historically there have been anxieties that vaccination in SOT recipients might develop alloimmunity and graft rejection, no clinical evidence support this concern[44]. LT recipients should be prioritized for immunization since the advantages exceed the risks[44].

LONG COVID-19 LIVER INJURY

Patients who recovered from COVID-19 and followed for months post-infection show increased risk of LFTs abnormalities, suggesting some possible long-term sequelae for the liver[304].The likelihood of persisting liver inflammation and fat deposition (MRI) following COVID-19[305] is discussed, as is the prospect of growing liver stiffness over time[306]. In a cohort, ninety

randomly selected participants were enrolled three to nine months post-COVID-19 infection and were compared to healthy individuals (negative anti-SARS-CoV-2 IgM/IgG). The patterns of LI were defined using multiparametric ultrasound (mpUS)[307]. MpUS examination of post-COVID-19 hepatic parenchyma demonstrated higher liver stiffness and steatosis (attenuation imaging-ATI) scores suggestive of LI compared to healthy controls. The most notable change is increased liver stiffness, as measured by greater shear-wave elastography values[307]. Metabolomic analysis of individuals three months after COVID-19 infection reveals higher taurine concentrations, which may be suggestive of LI[308]. Additionally, persistent viral protein and RNA infection of enterocytes[309] in intestinal biopsies for several months after infection renders the small intestine a reservoir of long-term viral replication[34]. Despite mechanisms of long-term LI remain speculative, the sustained endotheliopathy following COVID-19[310] suggests endothelial-mediated inflammation as a possible mechanism. A new entity, post-COVID-19-cholangiopathy, resembling secondary sclerosing cholangitis has also been described in critical COVID-19 cases and typically presents several weeks (mean 118 days) after COVID-19 diagnosis, implying direct LI from COVID-19[311-313].

IMAGING FINDINGS

Increased liver stiffness correlates well with increased ALT/GGT values indicating underlying hepatocellular/cholangiocellular damage on a biochemical level[308]. Patients with increased liver echogenicity and increased ATI values have increased risk for severity (8-fold, 5-fold, respectively). Increased BMI and CRP levels are also associated with hepatic steatosis (ORs=1.459, 1.387, respectively), while patients with higher steatosis scores present more severe disease[308]. Sonographic findings of ALI, including signs of acute hepatitis and vascular complications, appear in 37.3% of COVID-19

and in 48.7% of ICU COVID-19 patients[314]. CT scan findings are liver hypodensity (26%) and pericholecystic fat stranding (21%).

HISTOPATHOLOGICAL FINDINGS

Most frequently observed liver-related histopathological findings (Table 4) are associated with underlying comorbidities (e.g., NAFLD), rather than of SARS-CoV-2 itself. In initial pathology reports some authors cautioned about the observed "spiked virions", "corona-like" inclusions that could be artifacts of tissue autolysis or of an alternative origin (e.g., intrahepatic cholesterol crystals, lamellations, "crown-like" structures seen in NAFLD, multi-vesicular bodies, exosomes)[12,315,316]. Further investigations found evidence of apoptosis, an abundance of mitosis, mixed inflammatory infiltration in the portal region, and extensive bile duct damage. The hypothesis of direct cell damage was strengthened by identifiable viral particles, viral RNA in the liver, and hepatocytes, together with the intrahepatic detection of SARS-CoV-2 by EM and in-situ hybridization[55–57]. Hepatocytes' cytoplasm contained characteristic coronavirus particles with their spikes, according to utrastructural analysis[317]. While EM reveals mitochondrial enlargement and apotposis, in situ hybridization may identify SARS-CoV-2 in 68% of samples[36].Mitochondrial swelling, endoplasmic reticulum dilatation, and cell membrane dysfunction, document SARS-CoV-2 ability to replicate within hepatocytes[102,317]. Proteomic analysis of autopsy tissue showed mitochondrial dysfunction with dysregulated fatty acid oxidation and oxidative phosphorylation[68]. Biopsies from LT recipients who tested positive for COVID-19 revealed endotheliitis, bile duct damage, and mixed inflammatory portal infiltrates, which are findings of T-cell-mediated rejection[318]. Steatosis is predominant in obesity and overweight patients[102], and its high prevalence is attributed to population's baseline characteristics (severe COVID-19 and steatosis share common risk factors). DILI and CSS

may also contribute to the development of hepatic steatosis. Direct vascular damage, portal endotheliitis, portal vein herniation, terminal vessel dilations, and thrombosis with luminal ectasia are examples of acute vascular alterations. Chronic alterations in the portal and sinusoidal vessels, characterized by fibrous thickening of the vascular wall (thrombotic bodies), are sinusoidal inclusions positive for the platelet marker CD61[56,102,108,319]. Lobular ductal pathology is common showing the presence of moderate nuclear pleomorphism in cholangiocytes and canalicular cholestasis[108].

CONCLUSION

This review sheds light on issues raised by early COVID-19 studies concerning discrepancies in prevalence of LI and CLD, and the role of direct SARS-CoV-2 hepatocyte/cholangiocyte injury. The weighty implication of COVID-19-induced LI mechanisms comprising CSS, endotheliopathy/immuno-thromboinflammation, liver hypoxia, and oxidative stress with respect to histopathological and immunohistochemical findings is meticulously discussed. Finally, an emerging entity, long-COVID-19 persistent LI is also studied.

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