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Liver pathology in COVID-19 related death and the leading role of autopsy in the pandemic

Zanon M *et al*. Liver pathology in COVID-19 related death

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Abstract

BACKGROUND

Information about liver involvement in patients with coronavirus disease 2019 is currently fragmented.

AIM

To highlight the pathological changes found during the autopsy of severe acute respiratory syndrome coronavirus 2 positive patients.

METHODS

A systematic literature search on PubMed was carried out until June 21, 2022.

RESULTS

The review of the literature made it possible to highlight that pre-existing liver disease and elevation of liver enzyme is these patients is not common; liver enzymes elevations tends to be seen in those who are in critical conditions. Despite the poor expression of viral receptors in the liver, it seems that the virus is able to infect this organ and therefore

cause liver damage. Unfortunately, to date, the search for the virus inside the liver is not frequent (16% of the cases) and only a small number shows the presence of the virus. In most of the autopsy cases, macroscopic assessment is lacking, while microscopic evaluation of livers allowed to highlight the frequent presence of congestion (42.7%) and steatosis (41.6%). Less frequent is the finding of hepatic inflammation or necrosis (19%) and portal inflammation (18%). The presence of microthrombi, frequently found in the lungs, is infrequent in the liver, with only 12% of cases presenting thrombotic formations within the vascular tree.

CONCLUSION

To date, the greatest problem in interpreting these modifications remains that of associating the damage with the direct action of the virus, rather than with the inflammation or alterations induced by hypoxia and hypovolemia in patients undergoing oxygen therapy and decompensated.

Key Words: Liver; COVID-19; Autopsy; Immunohistochemistry; In situ hybridization; Immunofluorescence

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Core Tip: A literature review, about liver pathology in coronavirus disease 2019 (COVID-19) patients, demonstrated the presence of liver damage, which is represented mainly of congestion, steatosis, hepatic inflammation and necrosis and portal inflammation. The problem to date is whether the damage is COVID-19 related (meaning from direct virus damage/inflammatory related/systemic pathology related) or drug induced. However, this demonstration involves the need to be careful during drug treatment in patients with altered liver enzymes values to prevent further clinical worsening.

INTRODUCTION

The new disease coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been well studied in relation to pulmonary and cardiac histologic manifestations, but little is yet known regarding hepatic manifestations. COVID-19 has, in fact, to be considered a systemic infectious and inflammatory disease with histological changes also in other organs apart from its main target represented by the lungs. Liver involvement to date is recognized and defined as any liver damage occurring during the course of the disease or its treatment[1], meaning liver damage can be from direct cytotoxicity or inflammatory response or hypoxic/cardiovascular changes or be drug-induced[2-4]. SARS-CoV-2 Liver tropism is also well studied, with many authors demonstrating the presence of angiotensin converting enzyme 2 (ACE2) receptor and transmembrane serine protease 2 in liver, mainly expressed on cholangiocytes, with level of expression similar to those of alveolar cells, and only minimally on hepatocytes. No ACE2 expression was demonstrated on sinusoidal endothelium cells or Kupffer cells, apart from Wanner *et al*[5] that demonstrated through immunofluorescence a minimal expression of ACE2 on Kupffer cell and Pirisi *et al*[6] that demonstrated the presence of virus-like particles in endothelial cells of hepatic sinusoids. Curiously in patients with liver fibrosis/cirrhosis and in cases of hypoxia the expression of ACE2 is increased, thereby pre-existing liver injury or hypoxic conditions, common in patients with COVID-19, could favor SARS-CoV-2 liver tropism[4,7-9]. Liver infection could be also explained because of its immunological role and the proximity to the digestive organs, which exhibit a strong SARS-CoV-2-tropism, that could favor the entry of the virus through the portal system. Hepatic macrophages (mainly Kupffer cells) and sinusoidal endothelial cells have a key role in the activation of the immune response through pathogen recognition receptors, thus favoring virus entry[10].

The incidence of liver injury in COVID-19 patients is seen in 14%-53% cases[9,11,12] mainly demonstrated through abnormal liver function enzymes. In literature, only a small number of studies focus on liver damage and even less on histological changes in

patients who died with or from COVID-19. The purpose of this review is to summarize the results of studies in the literature and evaluate the biochemical and histological changes in the liver, demonstrating that the execution of autopsies is not obsolete, but represents a fundamental tool to create a bridge between clinical manifestations and cytological damage.

MATERIALS AND METHODS

A systematic literature search on PubMed was carried out until June 21, 2022. No time restrictions were applied. The review was conducted using MeSH terms, Boolean operators and free-text terms to broaden the research. Studies focusing on autopsies of COVID-19 deaths and in particular on liver pathology were initially searched using the terms "((COVID-19) AND (autopsy) AND ((death) OR (liver))" in title, abstract, and keywords. Study design included case reports, case series, retrospective and prospective studies. Reviews were excluded in order not to create duplication of data, but were analyzed to search for any studies not resulting from the search in the database. No unpublished or gray literature was searched. A total of 526 articles were found in the database. The evaluation of references during full text screening allowed the inclusion of further 7 studies. After evaluation of abstracts and full text, 46 articles were included because corresponding to the inclusion criteria. Data from each included study were extracted using Microsoft Excel spreadsheets, including information on authors, publishing years, nation, sample size, gender, age, type of autopsy, laboratory results, pre-existing liver disease, macroscopic and microscopic results, additional stainings, cause of death, medications and search of the virus in the liver (Table 1).

RESULTS

Demographics

A total of 11 case reports and 35 case series were analyzed, with a total of 994 autopsy cases of COVID-19 patients. Studies were from all over the world: one from Hungary, Romania, Japan, South Africa, United States in association with Brazil each, two from

Austria, Belgium, India, Iran and Turkey each. Three studies from the United Kingdom, four papers from Italy, 5 reports from Germany, Switzerland and China each and 9 studies from the United States. Sex was described in 882 cases, 54% (540) male and 35% (342) female. Age ranged from 18 to 102 years with a mean age of 53 years. Age distribution is summarized in Figure 1.

Liver disease

Pre-existing liver diseases were described in 61 (6%) cases encompassing: 28 cases of fatty liver disease, 19 cases of chronic liver disease, 11 cases of cirrhosis and 1 case each of hepatitis B and C. In 161 cases body mass index (BMI) was over 30 kg/m^2.

Laboratory findings

Laboratory values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were described in 350 cases with only 1 case described with values within the ranges of normality. The description of the laboratory values was rather different between the different studies, with only 5 studies (55 cases) reporting AST and ALT values at admission and 8 (64 cases) reporting the maximum values during hospitalization. Additional 4 reports for AST (51 cases) and 5 papers for ALT (61 cases) described the laboratory values without specifying the timing of the sampling. Data is summarized in Table 2. Abnormal AST and ALT values were described in additional 105 and 91 cases each.

Hospitalization and medications

For the subsequent analysis of the macro and microscopic findings, it was decided to evaluate whether the patients were hospitalized and whether drug therapies capable of causing liver alterations, such as antibiotics, antivirals and quinine, were administered. In 861 cases the place where the death took place was described. In 752 cases the patient was hospitalized and died in the hospital, 76 cases were found death in their home, 22 cases died in community settings and 11 cases were not hospitalized and were found

death in other circumstances such as car accidents and fall from height. In 133 cases a hospital stay or the place of death was not described. Medications administration was described in 201 cases, with 22 cases with only hydroxychloroquine administration. In 41 cases quinine was administered together with an antibiotic or antiviral, in 17 cases antibiotic and antiviral were given and in 56 only an antibiotic was administered. In 766 cases the administration of hepatotoxic drugs was not reported.

Type of autopsy

Autopsies were performed in all 994 cases, in 508 (51%) cases autopsies were complete, 2% (22) of the cases had a complete autopsy without the evaluation of the brain to avoid the risk of COVID-19 infection, in 38% (372) of the cases a core biopsy was performed, in 51 (5%) cases a partial autopsy was carried out and in 41 cases information about the type of autopsy performed was not reported.

Macroscopic result

Macroscopic results were described in only 265 (27%) cases. The most frequent finding, 79 cases, was the presence of congestion, followed by steatosis in 39 cases. A nutmeg or yellow aspect of the liver surface was seen in 16 cases, a fibrosis-indurated consistency in 6 cases and only 1 case showed the macroscopic presence of cancer. Lastly, 11 livers were described of increased size (hepatomegaly) and 10 livers as normal. For 144 patients the weight was reported; mean weight was 1805 g with a range from 520 to 3220 g.

Microscopic results

Microscopic results were described in 983 (99%) cases. The two most frequent findings were congestion, in 420 cases, and steatosis, in 409 cases. 4 cases were described as normal. All findings are described in Table 3.

Cause of death

Cause of death was reported for 440 (44%) cases. The most frequent cause of death was respiratory failure in COVID-19, seen in 355 (81%) cases, followed by multi organ failure in 33 cases, cardiovascular failure in 22 cases, pulmonary thrombosis in 11 cases and sepsis in 8 cases. The remaining 11 cases died respectively of hemorrhagic shock (3 cases), acute liver failure (2 cases), acute mesenteric ischemia (2 cases), bronchopneumonia (2 cases) and one case each of cardiac tamponade and multiple injuries.

Virus search

The research of the presence of SARS-CoV-2 was performed in only 162 (16%) cases. Of these 105 were tested by real-time reverse-transcription polymerase chain reaction (RT-PCR) and found positive in 53 cases, 34 cases were tested with immunohistochemistry (IHC) and found all negative, 28 were tested with in situ hybridization (ISH) and found negative in all cases and lastly 16 were tested with transmission electron microscopy and were found positive in 2 cases.

DISCUSSION

A total of 994 autopsy cases of COVID-19 patients with liver assessment were found in literature. As expected, more than half of the deceased were males and age distribution were highly variable, with a predominance of subjects in the age group 60-90 (71.1%).

Pre-exiting liver disease was rare (6%-literature data shows a frequency of 2%-11%), with only 16.2% of the cases presenting with obesity (BMI > 30 kg/m^2)[7]. Obesity, in association with diabetes and hypertension, is a prominent risk factor for severe disease and could predispose to nonalcoholic fatty liver disease (NAFLD), a metabolic syndrome which is known to suppress the pro-inflammation M1 macrophages favoring the progression of virus infection[2,8,11]. NAFLD seems to be identified with a higher prevalence in patients with severe COVID-19 and predisposes to higher liver enzymes at admission and at discharge[59]. To date the fact that pre-exiting liver disease is an independent risk factor for poor outcome is still debated, for some authors patients with liver diseases are not over represented in hospital casuistry[4,60-62], while for others the

presence of a pre-exiting illness is index of a greater probability of a bad outcome[7,63-65]. This does not count in the case of cirrhosis, seen in only 1% of this review, which is known to be an important predictor of mortality, with mortality rates of 31%[2,61]. It appears that in case of cirrhosis those who survive the first insult have a re-admission rate in hospital similar to those with cirrhosis, but without COVID-19, indicating that beyond the acute phase SARS-CoV-2 does not change the natural history of the disease[4]. There is currently few data regarding the mortality rate associated with alcohol liver disease as an independent risk factor, mainly related to the difficulties of correlating liver damage or elevation of liver enzymes to alcohol consumption. To date, it seems that alcohol liver disease increases the mortality risk of 1.8 fold[61].

Laboratory findings have not been collected in a homogeneous way, with 27 papers not reporting any data, 5 reporting AST and ALT values at admission, 8 reporting the maximum values during hospitalization and 4 reports for AST and 5 papers for ALT described the laboratory values without specifying the timing of the sampling. Abnormal values, without specifying the laboratory values, were described in 5 articles. From literature data it appears that liver enzymes abnormalities have a big range, occurring in 14%-76% of the cases[4,5,7,11,66]. This great range, as Marjot *et al*[4] pointed out, could be attributed to different limits of the definition of normal values. It is still debated whether elevated liver enzymes are associated with a greater risk of mortality, because patients with worst outcomes tend to be monitored in intensive care units, while those with mild symptoms are not strictly monitored. Thus, the use of abnormal laboratory findings at admission as a predictor for poor outcome is still not sure. Liver enzyme elevation mainly affects AST and ALT, indicating hepatocellular damage rather than cholestatic, despite a greater expression of ACE2 receptor in cholangiocytes[3]. As the study of Wong *et al*[67] pointed out, the odd ratio of elevated AST and ALT levels in COVID-19 patients is respectively 3.4 and 2.5.

Due to the presence of such fragmented laboratory data, it is difficult to draw conclusions about the trend of laboratory values during hospitalization, although some authors have found a tendency of increased values during hospitalization, in particular

in those in critical conditions[9,11,12,68,69]. Whether enzyme elevation is induced directly by the virus or because of the inflammation or congestion or medications is still not clear. Certainly, many of the drugs used in COVID-19 positive patients turn out to be hepatotoxic such as hydroxychloroquine and antivirals such as ritonavir, lopinavir and remdesivir[8,66]. The meta-analysis by Wong *et al*[67] and Cai *et al*[66] suggest that liver injury is higher in studies with high usage of Lopinavir/Ritonavir, despite their hepatotoxic role is still to be described in patients without pre-existing liver disease, while there was no evidence of a higher risk of liver injury for those treated with antibiotics, nonsteroidal anti-inflammatory drugs, ribavirin, herbal medications, and interferon.

The review of the literature highlighted the presence of a great discrepancy in the autopsy protocols, with only half of the autopsies performed as complete (full autopsies), while the other half as partial. Macroscopic evaluation of the liver was not frequent, while microscopic assessment was present in almost every case (99%). As expected, congestion and steatosis were the most frequent findings. The congestion can be traced back to the presence in these patients of cardiovascular dysfunction due to the massive inflammation and cytokine storm linked to the infection. The presence of steatosis needs a more complex analysis; lipid accumulation due to SARS-CoV-2 has to be differentiated from pre-existing modifications, typical of patient with metabolic syndrome. COVID-19 lipid accumulation can be explained because of the cytopathic effect of the coronavirus, which induces endoplasmatic stress and lipogenesis[2]. Transcriptomic profiling of COVID-19 patients by Wanner *et al*[5] demonstrated an upregulation of cellular processes involved in lipid/cholesterol synthesis. Furthermore, corticosteroid therapy, widely used in the treatment of COVID-19, is known to be associated with steatosis or glycogenosis[2].

Hepatic necrosis and inflammation can be multifactorial, that is induced from a cytopathic direct effect of the virus or because of inflammatory storm or hypoxic hepatitis or be drug induced. These hepatic changes are the third most frequent finding in liver autopsies of COVID-19 patients[70]. Differentiating the different causes from a pathological point of view is impossible, also in consideration of the fact that they can overlap one another. In addition, patients with pre-existing liver diseases, such as chronic

liver disease, have an increased risk of drug-induced hepatic damage, thus in those patients the use of hepatotoxic treatments should be weighted. Liver damage in critically ill patients is known and is linked to the so-called hypoxic hepatitis, which is caused by underlying cardiac dysfunction and respiratory failure that decreases the blood flow and oxygenation inducing cellular stress. Moreover, damage could be even mediated by reperfusion, which promotes the production of reactive oxygen species, leading to damage. This process can be highlighted in some cases as a picture of endothelitis[2,3,11]. Massive inflammation is common in COVID-19 patients and macrophage activation is evidenced by the presence of hemophagocytosis in liver tissue.

Unlike what reported by Marjot *et al*[4], the frequency of thrombotic phenomena of the hepatic vascular tree is lower, with 12% of cases instead of 29%. As Kleiner[70] noted, death could occur long after the acute phase of liver damage, thus the histological changes do not always represent a reliable image of what happened in acute, but are the result of damage and reparative modifications. Thus, to better understand the acute damage, it could be of help to perform a liver biopsy in patients with liver damage. Obviously, it is understandable that the execution of such an invasive examination is not a priority in the treatment of these patients, but it could be performed in those cases where the hepatic injury dominates the clinical picture.

Despite the presence of hepatic injury, the presence of SARS-CoV-2 in the liver has been sought infrequently (16% of the cases). Most of the studies have exploited the RT-PCR for the search of the viral genome, but only a few applied other techniques (IHC, ISH and transmission electron microscopy) to find in which cells the viral proteins were expressed (Figure 2 A and B). It is not surprising that using RT-PCR a greater number of cases resulted positive, because this type of analysis uses an homogenized tissue, which contains also vessels and immunity cells. However, the few available data allows us to confirm the fact that the virus can be found mainly in Kupffer cells, endothelial cells of centrolobulare veins and cholangiocytes (Figure 2 C and D). To note that Wanner *et al*[5] demonstrated that, when comparing the levels of SARS-CoV-2 RNA copies per cell

between airway samples and autopsy livers biopsies, the levels of RNA show similar ranges, but with lower median RNA in liver specimens.

CONCLUSION

Postmortem investigations remain the gold standard to investigate the effects of SARS-CoV-2 in the different organs and apparatus. It is well known that the absence of post mortem investigations in the first wave of the pandemic has failed to provide a valuable contribution to the correct management and treatment of patients. On the other hand, the execution of clinical and forensic autopsies has disclosed several important aspects of the disease, clarifying morphological and virologic features and promoting unexplored therapeutic approaches and new frontiers of research[71-74]. Despite the limited number of performed autopsies worldwide, to date there is no doubt that liver is a target for the virus, despite minimal viral receptor expression. However, liver damage is not always directly linked to the action of the virus, but can be secondary to inflammation or even simply caused by the therapy administered during hospitalization. Therefore, it is important to monitor patients who use hepatotoxic drugs, to avoid worsening of the liver functions, which can affect the outcome the patient.

ARTICLE HIGHLIGHTS

Research background

Hepatic histologic manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are far to be completely investigated. Many authors demostrated the presence of angiotensin converting enzyme 2 receptor in liver as well as transmembrane serine protease 2.

Research motivation

Liver injury was demonstrated in 14%-53% of cases of patients with SARS-CoV.2 infection. In the first wave of the pandemic few autopsies were performed and only few

authors can provide a wide casistic. Authors started to study the histologic manifestations of coronavirus disease 2019 (COVID-19) in lungs, heart and liver too.

Research objectives

The objectives of the study are to summarize the biochemical and histological changes in the liver and to promote the leading role of autopsy in the pandemic.

Research methods

Authors provide a systematic review focusing on autopsies studies of COVID-19 deaths and in particular on liver pathology.

Research results

Forty six articles were included because corresponding to the inclusion criteria with only 994 autopsy cases of COVID-19 patients. Congestion and steatosis were the main histopathological findings followed by hepatic necrosis, hepatic and portal inflammation and fibrosis. The most cause of death was respiratory failure, pulmonary thrombosis and sepsis. Acute liver failure was indicated as the cause of death in 2 cases.

Research conclusions

The review of the literature highlighted the presence of a great discrepancy in the autopsy protocols, with only half of the autopsies performed as complete (full autopsies), while the other half as partial. Macroscopic and microscopic evaluation of the liver was not always performed or described. Despite the presence of hepatic injury, the presence of SARS-CoV-2 in the liver has been sought infrequently (16% of the cases).

Research perspectives

Much more effort need to be addressed to completely investigate the role liver toxicity from COVID-19. Autopsy had a leading role during the pandemic and were important

understand the physiopathology of SARS-CoV-2 infection and should be always considered to improve scientific research.

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