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Increased Hepatic Expression of SARS-CoV-2 Entry Points and Proinflammatory Cytokines in Cirrhosis

It has been recently reported that patients with cirrhosis have significantly higher mortality following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection compared with those without.\(^1,2\) Specifically, it was demonstrated that mortality was greater in those with advanced cirrhosis (Child-Pugh B and C), and that from cirrhotic patients experiencing SARS-CoV-2 infection, close to half suffer acute decompensation including acute-on-chronic liver failure (ACLF).\(^2\) Unfortunately, the presence of hepatic decompensation at baseline has been shown to be an independent predictor of all-cause mortality in patients with coronavirus disease 2019 (COVID-19).\(^1\) Patients with decompensated cirrhosis contracting COVID-19 have a poor outcome, with an overall reported mortality of over 30%.\(^1\)

It is believed that the higher cytokine production and endotoxemia already present in patients with cirrhosis\(^3\) leads to an amplified inflammatory response following an infection and thus could put them at higher risk of adverse outcomes following SARS-CoV-2 infection.

COVID-19 is characterized by the release of proinflammatory cytokines such as interleukin (IL)-6, IL-8 and monocyte chemoattractant protein 1 (MCP-1), which have also been proposed as biomarkers of COVID-19 disease severity\(^4,5\) and at the same time are involved in the dysregulated immune responses during advanced cirrhosis. Whether it is the underlying chronic inflammation, the presence of an increased expression of SARS-CoV-2 entry sites in the liver of these patients, or a combined effect, what contributes to the observed increased mortality is not really known.

Based on this discussion, we sought to analyze the hepatic expression of ACE2, TMPRSS2, IL-6, IL-8, and MCP-1 in patients with cirrhosis to provide insight into a potential mechanism that predisposes them to severe COVID-19 and higher mortality.

Angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) messenger RNA (mRNA) expression was evaluated in 115 liver samples (79 cirrhotic patients and 36 controls) (see Supplementary Table 1 for detailed characteristics). We categorized cirrhotic patients by disease stage into compensated cirrhosis (n = 8), decompensated cirrhosis (n = 36), or ACLF (n = 35).

Liver mRNA of both ACE2 (P < .0001) and TMPRSS2 (P < .01) was significantly upregulated in patients with cirrhosis when compared with noncirrhotic controls (Figure 1A) and from those, decompensated patients had the highest expression (Figure 1B).

It is known that the virus depends on both ACE2 and TMPRSS2 to invade host cells\(^1\) and in the liver, ACE2 is expressed mainly by biliary epithelial cells.\(^7\) Patients with cirrhosis, especially in the most advanced stages, have increased proliferation of biliary epithelial cells in the form of ductular reactions.\(^8\) Our results indicate that both receptors are significantly increased in cirrhosis and even more in decompensated cirrhosis. Furthermore, we found a very good correlation between ACE2 and TMPRSS2 (r = 0.73, P < .0001) (Figure 1C). Thus, it is reasonable to speculate that patients with advanced cirrhosis might be at risk of an added viral load due to an augmented viral access to the hepatobiliary system mediated through these 2 receptors. In fact, autopsies from patients with SARS-CoV-2 infection have shown the presence of viral particles in the liver tissue accompanied by an important inflammatory infiltration with prominent bile duct damage.\(^9\)

Given that sex influences immune responses to viruses, we analyzed men and women separately. The hepatic expression of both proteins ACE2 and TMPRSS2 was not statistically different (data not shown), suggesting that if infected, cirrhotic patients would be at equally high risk of severe COVID-19 regardless of sex.

An intriguing part of COVID-19 and the liver involvement has been that the abnormalities observed in the liver biochemistries are mostly indicative of a hepatocellular pattern. It has been postulated that this could be multifactorial, including an immune-mediated inflammatory response.\(^10\)

To investigate if cirrhotic patients, besides the well-known underlying systemic inflammation,\(^3\) also have an underlying intrahepatic inflammation, we measured the mRNA hepatic expression of 3 well-known cytokines and chemokines associated with inflammation and recruitment of macrophages/neutrophils, IL-6, IL-8, and MCP-1.\(^4,5\) We found that mRNA expression of all 3 molecules was significantly upregulated in cirrhotic patients when compared with noncirrhotic controls (P < .0001) (Figure 1D), indicating that indeed patients with cirrhosis have an important underlying intrahepatic inflammatory state. Such cytokines could not only influence immune responses to viruses, but could also lead to inflammation and hepatocellular damage.
and perpetuate further inflammation in the liver, but also contribute through spillover to the systemic inflammation in these patients. In addition, we found significantly positive correlations between ACE2 and TMPRSS2 with each of those inflammatory markers (Supplementary Figure 1A and B).

When SARS-CoV-2 enters the liver of a cirrhotic patient facilitated by the high expression of ACE2 and TMPRSS2 and encounters this inflammatory environment, the likelihood of having an uncontrolled inflammatory response to it is very high. This could induce hepatocyte collateral damage, causing the very commonly observed increases of aspartate aminotransferase and alanine aminotransferase, and further aggravate the already present systemic inflammation in cirrhotic patients. And more importantly, this cascade could also lead to decompensation events and organ failure resulting in ACLF.

In conclusion, our results indicate that cirrhotic patients have a significant hepatic upregulation of ACE2 and TMPRSS2 in conjunction with an elevated proinflammatory state as assessed by the high liver expression of IL-6, IL-8, and MCP-1. This might explain in part why cirrhotic patients are predisposed to a severe COVID-19 outcome, and why, following SARS-COV-2 infection, hepatic decompensation and ACLF can ensue, which could contribute to the increased mortality observed in these patients.

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Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2021.08.053.

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Conflicts of Interest
The authors disclose no conflicts.

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Supplementary Materials And Methods

Patient Samples Collection

These analyses were carried out under the study approved by our institution’s research and ethics committee (GAS-2368-17-20) and conform to the provisions of the Declaration of Helsinki. Samples from a total of 79 patients with cirrhosis who underwent liver transplantation at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico, and 36 control liver donors are included. Patients were classified into compensated cirrhosis (n = 8) or decompensated cirrhosis (n = 36) according to the absence or presence of symptoms associated with portal hypertension including ascites, encephalopathy, or gastrointestinal bleeding, respectively. Additionally, patients were diagnosed with acute-on-chronic liver failure (n = 35) if during their clinical course until receiving a donor liver fulfilled criteria as established by the CANONIC (Chronic liver failure [CLIF] Acute-on-Chronic Liver Failure in Cirrhosis) study, which specifies the development of hepatic or extrahepatic organ failures (kidney, brain, coagulation, circulation, or lung) in the context of an episode of acute large ascites, encephalopathy, gastrointestinal hemorrhage, or bacterial infection. All compensated patients received liver transplant due to hepatocarcinoma mainly secondary to hepatitis C virus. Samples were collected at the time of the liver transplantation procedure from both the explanted cirrhotic liver and from the donor liver, preserved in RNAlater stabilization solution (Invitrogen, Carlsbad, CA) and stored at −70°C until further analysis. All patients gave written informed consent, and no donor organs were obtained from executed prisoners or other institutionalized people.

RNA Isolation and Gene Expression Analysis

Total RNA was extracted from the liver samples with the guanidine thiocyanate-phenol-chloroform method using TRIzol reagent (Invitrogen). Quality of the RNA was assessed by 1% formaldehyde agarose gel electrophoresis and quantification was performed on a Nano-Drop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Reverse transcription of the RNA was carried out with the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Indianapolis, IN), and the resultant complementary DNA was analyzed for gene expression analyses by quantitative real-time polymerase chain reaction with the LightCycler TaqMan Master mix (Roche Diagnostics, Indianapolis, IN) and a LightCycler 480 II system (Roche Diagnostics). Target gene expression was normalized with the ribosomal RNA reference gene 18s, which was then compared to that of a control sample following the comparative threshold cycle method (ΔΔCt). The latter belonged to an 18-year-old patient without liver dysfunction who died of traumatic brain injury and whose donor risk index was 1.25. All samples were tested in duplicate and final results are expressed as the fold change in gene expression levels given by 2-ΔΔCt. Sequences of the primers used in these analyses are the following:

| Primer  | Forward                      | Reverse                        |
|---------|------------------------------|--------------------------------|
| ACE2    | 5′- GTTCGATGCTATGAGGCAGT-3′ | 5′- TCAATATTAGCCAAGCAGCA-3′    |
| TMPRSS2 | 5′- GGGACGTGAGTTCTGC-3′      | 5′- CCGTACACTCTGTTGCG-3′       |
| IL-6    | 5′- GAAAGTGGCTATGCAGTGGAA-3′ | 5′- GAGGTAGCTACACTTCCAAGA-3′   |
| IL-8    | 5′- GAGCAGCTCCATAAGGCAACAA-3′| 5′- ATGGTCCTTCCGTTGCTG-3′      |
| MCP-1   | 5′- TCAAGAAGCTGAGGCTCCTC-3′  | 5′- GTGTGGGGCTCAAGATGG-3′      |
| 18s     | 5′- CGATGTGCTGTTTAGTGAGG-3′  | 5′- AGTTCGACCGTTCTCAGC-3′      |

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Supplementary Figure 1. Correlation between angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), and inflammatory markers interleukin (IL)-6, IL-8, and monocyte chemoattractant protein 1 (MCP-1) in patients with cirrhosis. Correlations between (A) ACE2 and (B) TMPRSS2 with IL-6 (left), IL-8 (middle), and MCP-1 (right) (Spearman’s coefficient).
## Supplementary Table 1. Anthropometric, Biochemical, and Clinical Characteristics of Cirrhotic Patients

|                      | CC Patients (n = 8) | DC Patients (n = 36) | ACLF Patients (n = 35) | P Value       |
|----------------------|---------------------|----------------------|------------------------|---------------|
| Male                 | 4 (50.0)            | 15 (41.7)            | 13 (37.1)              | .80           |
| Age, y               | 57.0 (53.25–60.5)   | 51.5 (41.0–58.75)    | 51.0 (39.0–57.0)       | .06           |
| Liver cirrhosis etiology |                    |                      |                        |               |
| Autoimmune           | 1 (12.5)            | 11 (30.6)            | 18 (51.4)              | .07           |
| HCV                  | 5 (62.5)            | 11 (30.6)            | 3 (8.6)                | <.01          |
| Alcoholic liver disease | 1 (12.5)         | 1 (2.8)              | 2 (5.7)                | .31           |
| NASH                 | 0 (0.0)             | 1 (2.8)              | 2 (5.7)                | .99           |
| Cryptogenic          | 1 (12.5)            | 8 (22.2)             | 6 (17.1)               | .92           |
| Other\(^a\)          | 0 (0.0)             | 4 (11.1)             | 4 (11.4)               | .99           |
| Pretransplantation clinical and laboratory data |                           |                      |                        |               |
| MELD-Na              | 11 ± 3\(^b\)\(^c\) | 18 ± 4\(^b\)\(^d\)   | 26 ± 6\(^c\)\(^d\)     | <.0001        |
| Encephalopathy       | 0 (0.0)\(^b\)\(^c\) | 18 (50.0)\(^b\)\(^d\) | 28 (80.0)\(^c\)\(^d\)  | <.0001        |
| Clinical ascites     | 0 (0.0)\(^b\)\(^c\) | 25 (69.4)\(^b\)\(^d\) | 34 (97.1)\(^c\)\(^d\)  | <.0001        |
| Total bilirubin, mg/dL | 1.14 (0.95–1.93)\(^b\)\(^c\) | 3.26 (2.13–5.10)\(^b\)\(^d\) | 10.62 (5.49–22.03)\(^c\)\(^d\) | <.0001 |
| Serum creatinine, mg/dL | 0.64 (0.55–0.70)\(^c\) | 0.69 (0.60–0.79)\(^f\) | 0.96 (0.78–1.32)\(^c\)\(^d\) | <.0001 |
| AST, U/L             | 59.0 (47.0–112.50)  | 70.0 (49.50–141.0)   | 64.0 (40.0–144.0)      | .75           |
| ALT, U/L             | 55.3 (31.0–90.50)   | 47.5 (32.50–85.15)   | 33.0 (22.0–74.0)       | .15           |
| INR                  | 1.2 (1.0–1.3)\(^c\) | 1.5 (1.3–1.6)        | 1.7 (1.3–2.2)\(^c\)    | <.001         |
| Leukocyte count (< 10^9/L) | 2.4 (2.0–3.9)   | 3.8 (3.1–5.9)        | 4.5 (2.9–5.6)          | .07           |

Values are n (%) or median (interquartile range).

ACLF, acute-on-chronic liver failure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CC, compensated cirrhosis; DC, decompensated cirrhosis; HCV, hepatitis C virus; INR, international normalized ratio; MELD-Na, Model for End-Stage Liver Disease–Sodium; NASH, nonalcoholic steatohepatitis.

\(^a\)Other etiologies include secondary biliary cirrhosis, drug-induced liver injury, and congenital liver diseases.

\(^b\)Statistical significance between groups CC and DC.

\(^c\)Statistical significance differences between groups CC and ACLF.

\(^d\)Statistical significance between groups DC and ACLF.