Reviewer 1

This is an interesting Retrospective cohort study, however with many limitations. I feel that i am missing the main findings of the study when I read the manuscript. What did you find? Elevated isolated AST is significantly associated with in hospital mortality. Why isolated? if it is liver injury why aren't other elevations present? you see an association to ferritin and to DM. So it might be the DM itself and NOT the liver injury behind the association. CK has not been analyzed which would solve the question as AST here can be of muscular origin especially in the absence of other liver marker elevations. Other acute phase reactant proteins have not been recorded like haptoglobin. Obesity and pre-existing NAFLD are not discussed. History of HBsAg is not given and this could be significant especially in the discussion of predisposition for immune mediated injuries= autoimmune hepatitis. I would consider to find additional information as per above in the patient files and re-write the study.

Dear reviewer,

Thanks for your interesting and scientific comments.

(Regarding your concern about isolated elevation of AST)

At admission, 54% of patients in our cohort had liver injury. AST levels were elevated in 32.5% (n=122), ALT levels were elevated in 36% (n=135), and both ALT and AST levels were increased in 23% (n=87). Among nonsurvivors, the pattern of liver injury was hepatocellular injury with an AST predominance. Both ALT and AST levels were elevated in 45% of nonsurvivors. An isolated elevation of AST levels was detected in 31% of nonsurvivors, while an isolated elevation of ALT levels was detected in 3%. Notably, 48% of nonsurvivors presented an AST/ALT ratio > 1.2, and serum total bilirubin levels were increased in 1.6% of patients in our study cohort.

In healthy individuals, plasma levels of ALT and AST represent the balance between normal turnover of hepatocytes by apoptosis and the clearance rates of these enzymes from hepatic sinusoids. Normally, ALT is present in the cytoplasm of

hepatocytes, whereas AST is present in the cytoplasm and mitochondria of hepatocytes. Although the ratio of hepatic AST/ALT is 2.5:1, the serum levels of AST and ALT are similar after hepatocyte turnover because the clearance rate of AST is two times faster than the clearance rate of ALT.

In individuals with hepatocellular injury, serum levels of AST and ALT reflect the time course of hepatic injury and prognosis of hepatic insult. Early hepatocyte injury results in the release of cytosolic AST and ALT. If hepatocyte injury is severe, mitochondrial damage will result in increased release of mitochondrial AST in serum. Therefore, the predominant increase in the admission AST levels in our cohort might reflect early and severe hepatocyte injury. Furthermore, SARS-CoV-2 may induce endothelial cell injury in the hepatic microcirculation and promote portal or sinusoidal microthrombosis. In individuals with ischaemic liver injury (due to microthrombosis), serum AST levels peak before ALT levels, a pattern that was observed in our cohort.

Regarding your concern about possible association between AST and creatinine kinase

In practice, an isolated and predominant elevation of AST levels indicates a nonhepatic source of AST, e.g., muscle, and haemolysis.

Myositis results in increased levels of AST and, to a lesser extent, ALT; however, the increased serum levels of muscular aminotransferases should be associated with increased serum levels of CK. In our cohort, no significant correlation between AST and CK levels at admission was observed. This finding suggests true hepatic injury as the main source of elevated AST levels.

Regarding Haptoglobin

Haemolysis results in increased levels of AST and unconjugated bilirubin. In our cohort, we identified 6 patients (normal haemoglobin levels) with *biphasic*

hyperbilirubinemia. ALT levels correlated with serum total bilirubin levels. Therefore, hyperbilirubinemia was due to liver injury and not haemolysis.

Regarding obesity and NAFLD

Among our cohort, 33% of patients were obese, perhaps with underdiagnosed nonalcoholic fatty liver disease. In addition, 20% of patients in our cohort were diagnosed with diabetes. Both diabetes mellitus and obesity increase serum levels of AST and ALT, but this change is more prominent for ALT than for AST.

(History of HBsAg is not given and this could be significant especially in the discussion of predisposition for immune mediated injuries= autoimmune hepatitis)

Chronic liver disease was one of our exclusion criteria. Moreover, *[in accordance with institutional clinical guidelines; patients with elevated aminotransferase levels were screened for markers of viral hepatitis and markers of autoimmune hepatitis]*. So, all participants in our study were screened for viral and autoimmune hepatitis at admission.

Ferritin and diabetes mellites

Our study provides evidence that serum ferritin levels were associated with all-cause in-hospital mortality. Of our cohort, 56% of patients presented elevated serum ferritin levels. Moreover, 97% of nonsurvivors had elevated serum ferritin levels. In addition, logistic regression analysis showed that the serum ferritin level was an independent risk biomarker for in-hospital mortality among patients with COVID-19. Furthermore, admission serum ferritin levels correlated with CRP levels. These results suggest that elevated serum ferritin levels at admission may reflect disease severity. Our findings are consistent with a recent report confirming that increased ferritin levels are associated with in-hospital mortality in patients with COVID-19.

Inflammatory cytokines stimulate hepatocytes and macrophages to release ferritin, which plays a vital role in many autoimmune diseases and inflammatory disorders. A vicious loop exists between ferritin and inflammatory cytokines, i.e., activated hepatocytes and macrophages release ferritin, which in turn stimulates the

production of various inflammatory cytokines. Serum ferritin is an inflammatory cytokine that indirectly stimulates proinflammatory pathways through the activation of the transcription factor nuclear factor kappa-B. Moreover, the heavy subunit of ferritin directly increases the mRNA expression of many inflammatory cytokines, such as IL-1, IL-6, TNF, and NOD-like receptor 3, indicating the proinflammatory properties of ferritin.

Patients with diabetes have elevated serum ferritin levels, and these patients are at increased risk of serious complications from COVID-19. Therefore, ferritin may be a key mediator of immune dysregulation that contributes to the cytokine storm in patients with diabetes mellitus and COVID-19.

Reviewer 2 and 3

Thanks for your interesting and scientific comments.