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DOCTORAL (PhD) THESIS

LIVER IMPAIRMENT AND HEMATOLOGICAL CHANGES DURING COVID-19 INFECTION IN PATIENTS WITH CHRONIC HEPATITIS C

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Keywords: COVID-19, SARS-CoV-2 infection, hepatitis C, liver impairment, hematological changes, mortality risk

1. INTRODUCTION

Hepatitis C is an infection transmitted by the hepatitis C virus that mainly affects the liver (HCV). Numerous studies published in recent years suggest that HCV infection may be either sudden or persistent. Although the infection is frequently asymptomatic, acute hepatitis C frequently manifests itself in mild anicteric forms. Still, the persistence of the virus in the liver can lead to progressive, fibrous liver damage and, eventually, to cirrhosis after many years of activity. Patients with cirrhosis may continue to develop liver failure or malignancy. Once the serological identification of hepatitis A and B became available, it became clear that viral hepatitis is not caused exclusively by these two viruses but by another clinical entity called Hepatitis nonA, nonB. The hepatitis C virus is a member of the Flavivirus family, a genus consisting of a diverse collection of RNA viruses. Certain structural and organizational characteristics distinguish it from Flaviviruses and Pestiviruses, with a structure similar to that of hepatitis G virus, yellow fever virus, and dengue fever virus. Hepatocytes and perhaps B lymphocytes are the natural targets of HCV. Even during the chronic phase of the infection, viral replication is very strong and is expected to produce more than 10 trillion virions each day. However, the replication rate is higher than that of HIV and HBV in vivo. Attempts to increase HCV levels in cultures have been ineffective.

Globally, the estimated prevalence of HCV infection is 2.2 percent, equating to approximately 1.3 billion people with HCV positive. It is estimated that approximately 130-170 million people worldwide are infected with hepatitis C. The disease affects approximately 3.9 million people in the United States. Romania represents about 10% of the approximately 12 million people infected with the hepatitis C virus throughout Europe. "The most common type in the United States is type 1. None is worse than the others, yet they all respond. Romania ranks first in Europe in terms of global cases of hepatitis C, according to World Health Organization prevalence data, and the fourth term of mortality associated with liver disease, with 44.5 deaths per 100,000 people, compared to the European average of 15 deaths per thousand inhabitants.

HCV infection is a major comorbidity, with immune implications and multisystem damage still incompletely known. In this regard, during the COVID-19 pandemic, certain studies have demonstrated the role of the SARS-CoV-2 virus in triggering and intensifying hepatotoxicity in patients with chronic liver disease, such as hepatitis C. Patients with chronic liver disease have been shown to be more prone to die from acute-chronic liver failure and respiratory failure when infected with COVID-19. Similarly, the researchers investigated the molecular mechanisms underlying coronavirus hepatotropism, which has previously been hypothesized as liver damage and inflammation, being able to increase SARS-CoV-2 hepatotropism by modulating viral receptor expression, given that the enzyme

Angiotensin 2 (ACE2) receptor has been previously identified as an interferon-inducible gene in human respiration. In addition, the SARS-CoV receptor-binding domain (RBD) attaches to ACE2 receptors, allowing the virus to enter specific cells while blocking the enzyme ACE2, which is normally a protective factor for the lungs. Due to the presence of ACE2 receptors in bile and liver epithelial cells, the liver appears to be particularly susceptible to SARS-CoV-2 infection.

Formerly called 2019-nCOV, the new coronavirus (SARS-CoV-2) is the causative agent of 2019 coronavirus disease or COVID-19 pneumonia, as the World Health Organization designated. The disease was first reported in December 2019, following an outbreak of pneumonia of unknown etiology in Wuhan, China. To date, no verified source of contamination has been found. The virus infects people of all ages and is transmitted through the air. Fatality rates for confirmed SARS-CoV-2 cases range from 8.8% in Mexico to 0.3% in the United Arab Emirates, averaging 2-3 percent globally. This rate may be affected by national demographics, region-specific characteristics, infection curve shapes, the health system, and preventive measures implemented by each country. However, pre-existing diseases, such as chronic hepatitis C, may still affect mortality.

The present study aimed to detect modifiable hematological parameters in chronic HCV infection as potential risk factors for mortality and severity of COVID-19 infection and to study hepatitis C in assessing the risk of death in patients with SARS-CoV-2 coinfection. Significant changes in blood parameters were observed in both study groups, with leukocyte counts, ALT, ASAT, alkaline phosphatase, LDH, Quick time, procalcitonin, PCR, and HCV viral load being significantly higher and platelet count significantly lower at patients with COVID-19 with active HCV infection. Thus, hematological changes involving elevated ALT, PCR, procalcitonin, and HCV viral load were shown to be significant independent risk factors for hepatic impairment and all-cause mortality. At the same time, chronic HCV infection has been observed as a significant predictor of mortality risk in patients with COVID-19.

2. PURPOSE OF THE STUDY

This paper aimed to identify relevant issues in the absence of previous research on COVID-19 in patients with hepatitis C. This paper also sought to provide current and clinically relevant perspectives by highlighting important hematological parameters in assessing the severity of infection. COVID-19 in patients with hepatitis C and assessing the risk of death of the same category by building a predictive score. In this way, these results may contribute to establishing new therapeutic approaches in patients with chronic hepatitis C and acute SARS-CoV-2 coinfection, to prevent significant mortality and morbidity.

According to these aspects, the **specific objectives of this paper** can be systematized as follows:

1. Use of existing statistical methods to analyze blood parameters routinely evaluated in the clinic to identify risk factors for mortality and severity of COVID-19 infection.

2. Evaluation of comorbidities by statistical techniques and other complementary analysis tests, which may allow a predictive score of mortality in patients with chronic hepatitis C and acute coinfection SARS-CoV-2.

FIRST STUDY: LIVER IMPAIRMENT AND HEMATOLOGICAL CHANGES DURING COVID-19 INFECTION IN PATIENTS WITH CHRONIC HEPATITIS C

We performed a retrospective cohort study to assess the risk of liver failure and all-cause mortality in patients with COVID-19 with active HCV infection. We focused on evaluating hematological changes and liver function abnormalities detected by routine laboratory tests in patients co-infected with HCV and SARS-CoV-2, as well as the relationship between severe COVID-19 infection and all-cause mortality. The clinical history, laboratory results, and the result of 126 patients admitted to our ward with established HCV infection and COVID-19 disease are discussed.

Of the 1057 HCV-infected patients who were followed in our clinic, 95 patients (75.4 percent) were confirmed with COVID-19, received sofosbuvir / velpatasvir treatment, or had a sustained virologic response (SVR), while the remaining 31 (24.6 percent) had active HCV replication. 88 (92.6%) of patients with inactive HCV infection had a mild to moderate type of COVID-19 infection, while seven (7.4%) had a severe form of the disease. 21 (67.7 percent) of patients with current HCV infection had a mild to moderate form of COVID-19 infection, while 10 (32.3 percent) had a severe form of COVID-19 infection. 19, facts illustrated in figure 1.

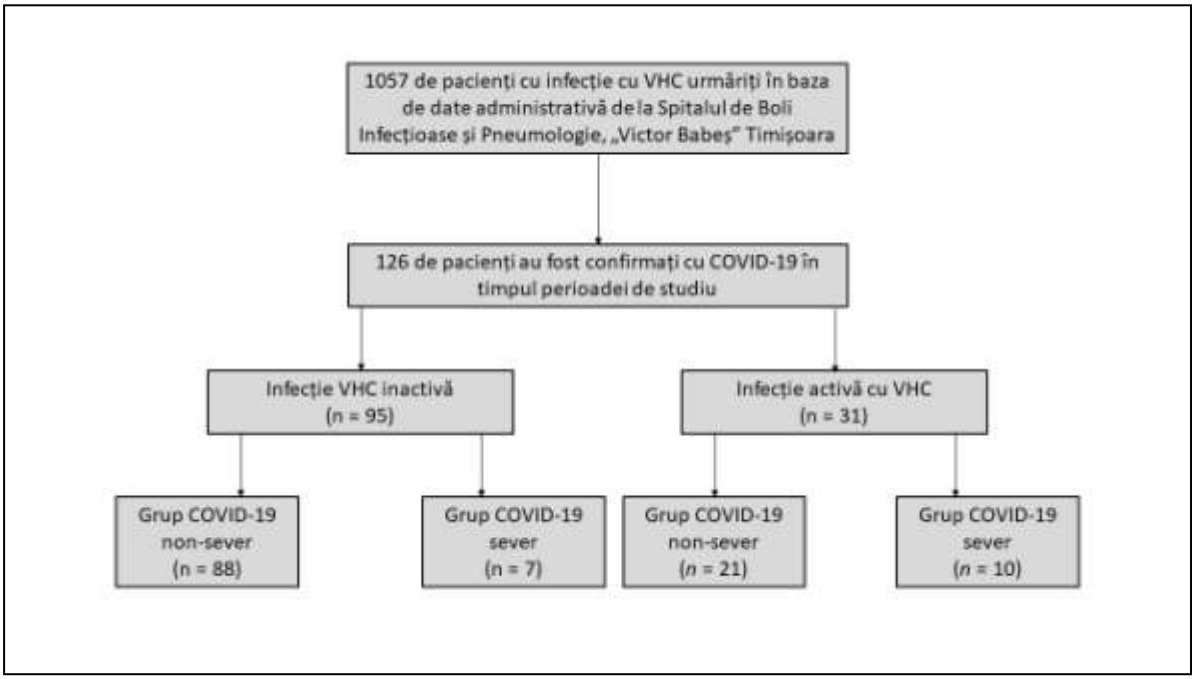


Figure1– Flowchart of the study cohort

Significant changes in blood parameters were observed in both study groups, with leukocyte counts, ALT, ASAT, alkaline phosphatase, LDH, quick time, procalcitonin, PCR, and HCV viral load being significantly higher and platelet count significantly lower in patients with COVID-19 and active HCV infection, as can be seen in Table 1.

Table 1 – Blood parameters depending on the SARS-CoV-2 infection

Blood parameters	Non-active HCV (n=95)	Active HCV (n=31)	P-value
WBC (x/ mm3)	11434 (4662)	14890 (4285)	<.0001
RBC (x/ mm3)	4455509 (519104)	4574418 (688314)	0.9100
Hb (g/dl)	13.2 (1.5)	13.3 (1.7)	0.4298
PLT (x/ mm3)	214541 (76778)	183327 (71225)	<.0001
ALT (U/L)	34.1 (44.8)	121.4 (89.7)	<.0001
AST (U/L)	22.8 (15.6)	104.5 (72.7)	<.0001
ALP (U/L)	41.4 (12.8)	66.2 (13.3)	<.0012
Albumin (g/dl)	4.5 (0.4)	4.1 (0.4)	0.4752
Total proteins (g/dl)	7.2 (2.0)	6.9 (1.8)	0.3821
Total bilirubin (g/dl)	1.1 (0.3)	1.2 (0.4)	0.0520
GGT (U/L)	12.2 (3.1)	13.7 (3.3)	0.0921
LDH (U/L)	255 (62.6)	320.5 (97.2)	<.0001
PT (seconds)	11.2 (0.9)	14.7 (2.4)	<.0001
Procalcitonin (ug/L)	0.2 (0.0)	0.5 (0.1)	<.0001
CRP (mg/L)	10.5 (1.2)	74.9 (15.6)	<.0001
HCV viral load (U/L*103)	0.011 (0.006-0.019)	103650 (43921)	<.0001

Risk variables for liver damage and all causes of fatality in patients with COVID-19 with chronic hepatitis C were determined using multivariate analysis. It was observed that age over 60 years increases the risk of mortality in COVID-19 infection by 2.51 times, male

sex by 2.36 times, pathological values of ALAT increase the risk of death by 3.17 times, increased procalcitonin is associated with a risk of 3.17 times more HCV viral load 2.46-fold, all independently associated with chronic hepatitis C liver failure. In addition, age greater than 60 years (OR = 8.27), male gender (OR = 1.66), elevated ALT (OR = 1.45), and HCV viral load (OR = 2.46) were all independently associated with all-cause mortality. These findings are described in Table 2.

Table 2 – Multivariate analysis of risk factors

Variable	Liver impairment		All-cause mortality	
	Adjusted OR	95% CI	Adjusted OR	95% CI
Age >60 years old	2.51	1.43-3.02	8.27	5.14-13.5
Sex, male	2.36	1.42-3.15	1.66	1.27-2.20
ALT	3.17	1.61-3.98	1.45	1.18-2.19
Procalcitonin	2.88	1.45-2.95	1.02	0.88-2.13
HCV viral load	8.72	5.28-11.3	2.46	1.17-3.56

Conclusions:

1. In patients co-infected with SARS-CoV-2, active HCV infection has been associated with more severe disease and an increase in death, with HCV viral load serving as an independent risk factor for all-cause mortality and liver failure.
2. In patients with COVID-19, the degree of hepatic impairment was correlated with adverse clinical outcome.
3. The active group with HCV had a substantially higher proportion of patients with severe COVID-19 than the group with inactive HCV.
4. In patients with COVID-19 and hepatic impairment, old age, male gender, elevated alanine aminotransferase levels, elevated C-reactive protein, procalcitonin, and HCV viral load were significant independent risk factors for hepatic impairment and death for all.
5. Further prospective studies,based on larger samples are needed to validate our findings.

SECOND STUDY:PREDICTIVE VALUE OF COMORBID CONDITIONS FOR COVID-19 MORTALITY

This research aims to develop and validate a simplified model for predicting mortality based on a spectrum of the most common diseases. The spectrum consists of 13 broad categories of pathologies most common in the general population, including chronic hepatitis C, the TRIPOD statement serving as a reference guide. The approach aims to improve the distribution of attention to high-risk individuals while allowing the triage of confirmed SARS-

CoV-2 infections in early hospitalization or home accommodation for those determined to have a low risk of mortality based on existing comorbidities. The paper details the creation and internal validation of the prognostic score in a real setting.

The development sample consisted of 510 individuals, of which 310 died as a result of the disease. The average age of non-survivors was 67, and 61.9 percent were men. The most common condition in this group was high blood pressure, which was seen in 113 people, followed by heart disease in 121 patients and diabetes in 106 patients. On the other hand, the survival group consisted of 91 (43.3%) men and 109 women, with a mean age of 49 years. None of these 200 patients had cancer or hemophilia. As with non-survivors, hypertension was the most common comorbidity, followed by diabetes in 37 patients and heart disease in 20, although chronic hepatitis C was one of the least common diseases in our research group.

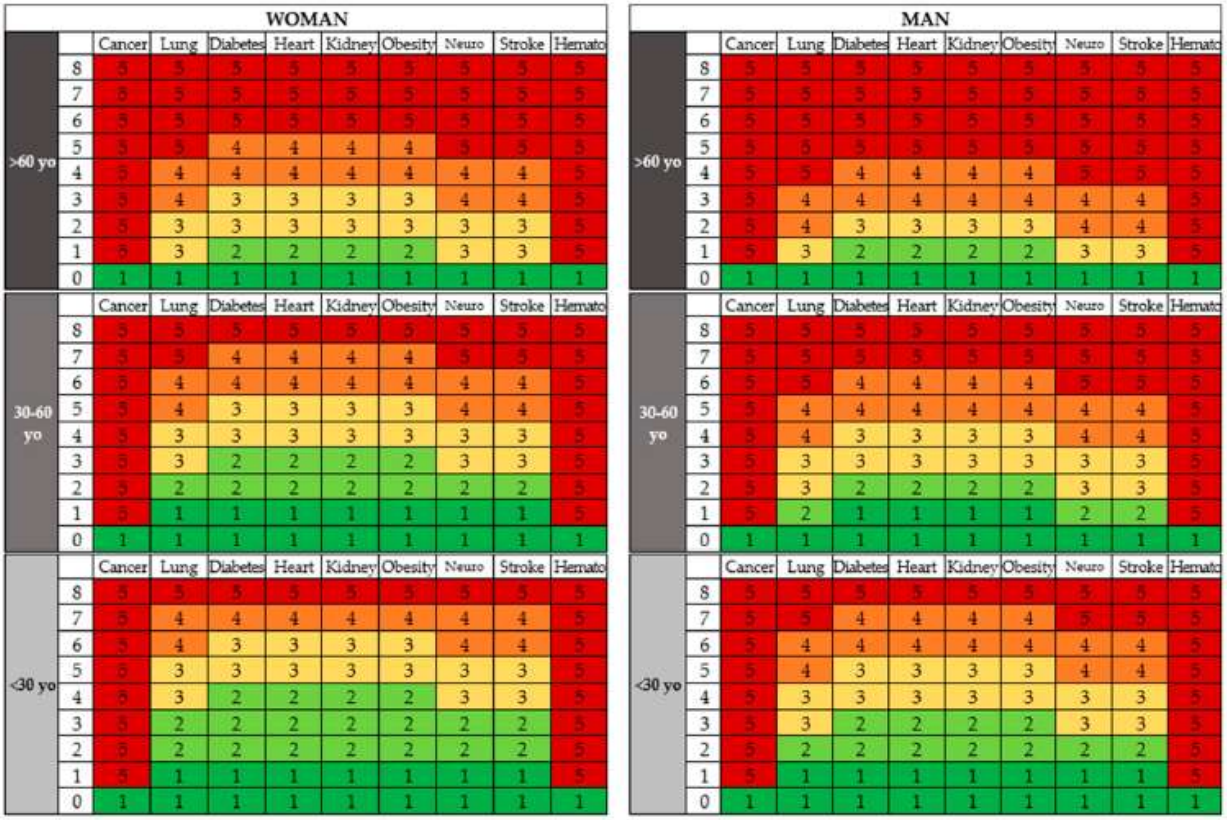
In the validation sample, 299 people survived, while 242 died. A total of 37 people were estimated to have a 10% mortality risk, of whom three (8%) died. It was predicted that 85 people would die in the 10-30% mortality risk category, while 21 (24%) died. Thirty-one to fifty percent of 113 patients were projected to die, and 35 (31%) died. A total of 187 individuals had a mortality risk of between 51% and 70%, with 97 (52%) dying. Finally, 119 were projected to have a more than 70% chance of dying from COVID-19, while 86 (72%) did not survive.

Cancer, all-cause lung damage, diabetes, coronary heart disease, chronic kidney disease, chronic hepatitis, obesity, neurological disease, stroke, and hematologic pathology have been identified as significant comorbidities as predictors of mortality in patients with VOCID. -19, as can be seen in Tables 1 and 2.

Table1–Significant comorbidities in COVID-19 patients

Comorbid condition	p-value	OR (99% CI)
Malignancy	.003	7.6 (1.1-19.6)
Lung disease	.0003	5.1 (1.5-16.5)
Diabetes mellitus	.001	2.3 (1.1-4.4)
Heart disease	< .0001	5.6 (2.6-11.8)
Kidney disease	.0004	5.5 (1.5-19.4)
Liver disease	.03	3.6 (0.7-16.9)
Obesity	.003	3.1 (1.1-8.0)
Neurological disorders	.0001	7.4 (1.9-27.7)
Stroke	.002	7.9 (1.4-43.3)
Hematology disturbances	.0001	8.4 (1.4-3.3)

Table 2 – Risk matrix



When reading the matrix, the patient's gender is the first criterion to consider, followed by the patient's age group. After establishing one of the six major blocks of the matrix, the doctor/nurse will check the comorbidity associated with the highest risk of not surviving the infection and will count the number of associated comorbid conditions out of a possible total of eight, thus determining the risk score, respectively.

Conclusions:

1. A statistically difference was found between survival rates in men and women with COVID-19 with significant associated comorbidities.
2. Chronic hepatitis B or C virus did not show significant predictive power for assessing the risk of mortality in patients with COVID-19. Still, hematological diseases of all causes showed an 8-fold higher risk of death.
3. Our predictive mortality risk matrix is considered to have an acceptable accuracy (AUROC = 0.773). Thus, there is a 77% chance that the doctor who reads the risk matrix while performing the patient assessment will correctly predict a result.
4. Our prediction model helps to identify patients at risk after testing positive for SARS-CoV-2 and suggests early initiation of treatment if the prediction suggests before the clinical presentation of symptoms.

THIRD STUDY:LABORATORY PROFILE OF COVID-19 PATIENTS WITH HEPATITIS C-RELATED LIVER CIRRHOSIS

It was noted that patients in the COVID groups had significantly lower average BMI values (p-value = 0.035), while the proportion of underweight patients was higher in the same COVID group (p-value = 0.041). More than half of our patients with cirrhosis had a history of portal hypertension, among other complications specific to their chronic liver disease, whereas hepatic encephalopathy was observed in a statistically significant higher proportion in patients infected with SARS-CoV-2 (54.3% vs. 36.2%, p-value = 0.023). The study participants were also grouped by the Child–Pugh score, having no significant difference in proportions of Child–Pugh A, B, or C. However, we noticed significant higher number of cirrhosis patients that developed acute-on-chronic liver failure while being infected with SARS-CoV-2 (19.6% vs. 5.1% p-value < 0.001). Similar findings were observed in the rate of ICU admissions in patients with COVID-19 (26.0% vs. 8.2%, p-value < 0.001), and in the mortality rate (15.2% vs. 6.1%, p-value = 0.039).

The assessment of a complete blood count identified significant differences by median values and a departure from the normal range of the WBC (12,200 vs. 4600, p-value < 0.001), lymphocytes (6900 vs. 2500, p-value < 0.001), and ferritin (479 µg/L vs. 355 µg/L, p-value < 0.001). Liver function tests identified significant deviation from normality in fasting glucose levels (146 mmol/L vs. 128 mmol/L, p-value = 0.024), ALT (57 U/L vs. 44 U/L, p-value = 0.049), and PT (13.9 s vs. 11.2 s, p-value = 0.008). Major nutritional deficiency between groups was observed only in thiamine levels, where patients with COVID had significantly lower levels (2.4 µg/dL vs. 2.6 µg/dL, p-value = 0.041). The lipid profile did not differ between groups. Kidney function tests were significantly different in cirrhosis patients infected with SARS-CoV-2, having higher creatinine levels (1.54 µmol/L vs. 1.31 µmol/L, p-value < 0.001) and BUN levels (14 mmol/L vs. 11 mmol/L, p-value = 0.002). Finally, the HCV viral load was significantly higher in the COVID-19 group (p-value < 0.001).

The inflammatory profile was analyzed only in patients positive for SARS-CoV-2 infection. Therefore, data during the acute phase of COVID-19 were obtained from 46 patients, while the second evaluation was performed in 39 patients, since seven of them did not survive. The majority of inflammatory markers were on average higher than the upper limits of the normal range both at the initial evaluation when patients were confirmed with COVID-19, as well as at 4 weeks after being tested negative for SARS-CoV-2. An important finding is the higher mean value of procalcitonin in patients at the second evaluation (0.5ug/L vs. 1.2ug/L, p-value < 0.001), signifying the risk of bacterial infection after the acute viral infection. The other inflammatory markers were elevated in patients during the acute phase of COVID-19, including CRP levels (56mg/L vs. 12mg/L, p-value < 0.001), IL-6 (49pg/mL vs.

17pg/mL, p-value < 0.001), fibrinogen (5.1g/L vs. 3.7g/L, p-value < 0.001), and D-dimers (331ng/mL vs. 262ng/mL, p-value < 0.001).

The odds ratio and 95% confidence intervals are developed based on BMI values that initially showed significant differences between study groups (COVID and non-COVID). The multivariate analysis described significant interactions between WBC and ferritin in all subgroups as predictors for the risk of developing ACLF. Other significant associations with ACLF were observed only in patients with COVID-19 with elevated lymphocytes and ALT levels, respectively, and low fasting glucose levels. Elevated PT, creatinine, BUN, and HCV viral load were associated with an increased risk for ICU admission in cirrhosis patients, regardless of their SARS-CoV-2 status, although the risk was higher once COVID-19 was diagnosed, and stratification by BMI indicated that underweight patients had a higher chance of being admitted to the ICU. Lastly, underweight patients with cirrhosis and COVID-19 were in the group with the highest risk factor for developing ACLF and ICU admission, where procalcitonin levels along with CRP, IL-6, and fibrinogen were all significant independent risk factors. Additionally, d-dimers, pCO2, and SaO2 were all independent risk factors for ICU admission, but not for ACLF.

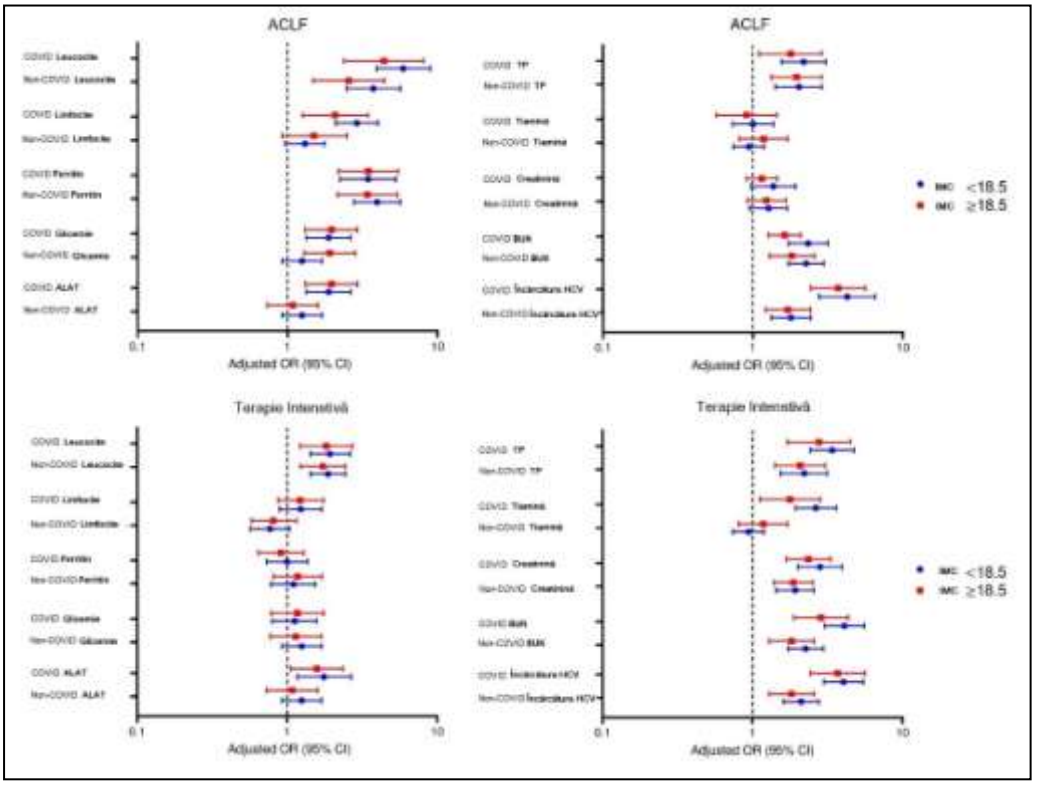


Figure2 – The association of laboratory parameters with ACLF and ICU admission in underweight and normal weighted patients with cirrhosis from the two study groups

ORIGINAL CONTRIBUTIONS

The original contributions made by this paper can be summarized as follows:

1. The present study is the only one known to create a risk matrix for predicting mortality in patients with COVID-19, consisting of clusters of comorbidities frequently encountered in the clinic.
2. Changes in blood parameters usually determined in an emergency have been identified that have been shown to be risk factors for the severity of COVID-19 in patients with HCV-associated infection.
3. Chronic HCV hepatitis has been shown to cause hematological and immunological changes that cause more severe forms of COVID-19 pneumonia but is not an independent risk factor for the risk of mortality in the same patients.

This research did not receive funding.