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VALIDATION OF A NEW INDEX BASED ON LDH, AST AND ALT AS A PROGNOSTIC MARKER IN PATIENTS WITH MAFLD AND COVID-19

TESIS

QUE PARA OBTENER EL TÍTULO DE ESPECIALISTA EN GASTROENTEROLOGÍA

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DEDICATORIA

A mi hijo, Matias hoy que tienes 10 años quiero que sepas que me siento muy orgulloso de ser tu padre, tambien me siento agradecido de poder disfrutar momentos contigo a pesar de la distancia. La conexión y el amor que sentimos es algo único e irrepetible, se que como yo tu tambien sientes que no ha pasado ni un solo día cuando nos volvemos a ver. Quiero que sepas que ya estoy cerca de la meta más importante de mi vida que es compartirla contigo más cerca de ti. No he dejado de esforzarme ni un solo día pensando en el ejemplo que quiero que tomes de mí, ya se acerca el día en que podamos recuperar el tiempo perdido. Gracias por ser paciente conmigo y no perder la fe ni un solo día, te prometo que valdra la pena. Por último quisiera recordarte que existen muchos caminos a la felicidad que todos implican esfuerzo y sacrificio, hoy se que estoy en el correcto al verte sonreir y sentir que me amas, te tocara a ti descubrir cual es tu camino hacia la felicidad.

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ABSTRACT

Background & Aims:

Metabolic associated fatty liver disease (MALFD) is associated with complications and mortality in patients with COVID-19. However, there are no prognostic scores aimed to evaluate the risk of severe disease specifically in patients with MAFLD, despite its high prevalence. LDH, AST and ALT have been used as markers of liver damage. Therefore, we propose an index based on LDH, AST and ALT for the prediction of complications and mortality in patients with MAFLD and COVID-19.

The Aim of this study was to evaluate the prognostic performance of an index based on LDH and transaminases (AST / ALT) in patients with COVID-19 and MAFLD (LFN-COVID-19 index).

Methods: Retrospective cohort study, two cohorts from two different tertiary centers were included, the first was the derivation cohort to obtain the score cutoffs and the second was the validation cohort. We included hospitalized patients with severe COVID-19 and MAFLD. Liver steatosis was evaluated by CT scan. ROC curve analysis and survival analysis were used.

Results: In the derivation cohort 44.6% had MAFLD; ROC analysis yielded a LFN-COVID-19 index >1.67 as the best cutoff, with a sensitivity of 78%, specificity of 63%, NPP of 91% and an AUROC 0.77. In the multivariate analysis, the LFN-COVID 19 index >1.67 was independently associated with the development acute kidney injury (OR; 1.8, CI 95%: 1.3-2.5, p< 0.001), orotracheal intubation (OR: 1.9, CI 95%: 1.4-2.4, p< 0.001), and death (OR: 2.86, CI 95%: 1.6-4.5, p< 0.001) in both cohorts.

Conclusions: LFN-COVID-19 index has a good performance to predict prognosis in patients with MAFLD and COVID-19 which could be useful for the MAFLD population.

Resumen

Antecedentes y objetivos:

La enfermedad por hígado graso asociada a disfunción metabólica (MAFLD) se asocia con complicaciones y mortalidad en pacientes con COVID-19. Sin embargo, no existen puntuaciones de pronóstico dirigidas a evaluar el riesgo de enfermedad grave específicamente en pacientes con MAFLD, a pesar de su alta prevalencia. La DHL, AST y ALT se han utilizado como marcadores de daño hepático. Por ello proponemos un índice basado en estos marcadores para la predicción de complicaciones y mortalidad en pacientes con MAFLD y COVID-19.

El objetivo de este studio fue evaluar el rendimiento pronóstico de un índice basado en LDH y transaminasas (AST/ALT) en pacientes con COVID-19 y MAFLD (índice LFN-COVID-19)

Métodos: Estudio de cohorts retrospective, se incluyeron dos cohorts de dos centros terciarios diferentes, la primera fue la cohort de derivación para obtener los puntos de corte y la segunda fue la cohorte de validación. Incluimos pacientes hospitalizados con COVID-19 grave y MAFLD. La esteatosis hepática se evaluó mediante tomografía computarizada. Se utilizaron análisis de curvas ROC y análisis de supervivencia.

Resultados: En la cohorte de derivación el 44,6% tenía MAFLD; El análisis de curva ROC arrojó un índice LFN-COVID-19 >1.67 como el major punto de corte con una sensibilidad de 78%, una especificidad del 63%, VPN 91% y un AUROC de 0.77. En el análisis multivariado el índice LFN-COVID-19 >1.67 se asoció de forma independiente con el desarrollo de insuficiencia renal aguda (OR: 1,8, IC 95% 1,3-2,5, p < 0.001), intubación orotraqueal (OR: 1.9, IC 95%: 1,4-2,4, p < 0.001) en ambas cohortes.

Conclusiones: El índice LFN-COVID-19 tiene un buen desempeño para predecir el pronóstico en pacientes con MAFLD y COVID-19, lo que podría ser útil para la población con MAFLD.

INTRODUCTION

The SARS-CoV-2 pandemic (COVID-19) still affects the entire world, having as of June 15, 2022, 536,720,870 people infected, of whom 6,312,601 have died(1).

Different risk factors associated with the development of complications and mortality in patients with COVID-19 have been identified, including age >60 years, the presence of cirrhosis, diabetes, immunodeficiencies, obesity, cardiovascular disease, chronic kidney disease, chronic obstructive pulmonary disease, among others(2–4). Metabolic dysfunction associated fatty liver disease (MAFLD), regarded as the hepatic manifestation of metabolic syndrome, has a controversial role in the prognosis of patients with COVID-19. Some studies have reported a poor prognosis in patients with MAFLD, while others have only showed this finding when fibrosis is present. This could be explained by a more pronounced baseline systemic inflammatory profile and activation of the immune response in patients with liver fibrosis, which contributes to increased inflammation when SARS-CoV-2 infection is added(5,6).

Acute respiratory distress syndrome (ARDS) is the major complication in patients with severe COVID-19; other complications such as cardiac or cardiovascular, renal, and secondary infections, among others, may occur.(7) These patients, mainly those admitted to the intensive care unit, may present with laboratory abnormalities, such as leukopenia, lymphopenia (<800 mm3 at admission), elevated prothrombin time, elevated serum levels of D-dimer (> 1000 ng/mL), elevated inflammatory markers (ferritin >300 ug/L), elevated lactate dehydrogenase (LDH), elevated liver enzymes, elevated creatine phosphokinase (CPK twice the upper limit of normal), and elevated troponin I (hs-TpI) (7–9).

In patients with pneumonia associated with SARS-CoV-2 infection, high LDH levels correlate with lung damage, severe disease and mortality at day 30. (10–13) In the study by Yan L et al. LDH (>365 U/l), lymphocyte count (<14.7%) and CRP (>41.2 mg/L) were identified as the three laboratory abnormalities that predict mortality risk with 90% accuracy, which represent a simple way to promptly recognize severe illness.(14)

Likewise, in patients with acute liver injury (non-COVID-19 related), an increase in LDH levels has been reported, secondary to endothelial damage induced by macrophages during acute inflammation, conditioning microcirculation alterations and hypoxia. Thus, it has been suggested that LDH may have a discriminatory role in identifying the etiology of liver damage. As a marker of damage due to liver ischemia, it must be taken into account that LDH has a shorter half-life, therefore a faster fall when the damage disappears, so it has been suggested as a parameter to monitor the evolution of patients with acute liver injury. The ubiquitous nature of LHD in the human body makes it a nonspecific but sensitive biomarker, which in the context of organ damage can provide information with diagnostic and prognostic potential. In the same way, increase in ALT, AST and the AST/ALT ratio has been associated to adverse clinical outcomes including mortality in patients with COVID-19. (10–13,15)

Identifying factors associated with poor prognosis that may be related to a pathophysiological mechanism is ideal in patients with COVID-19, since those patients at risk of progressing to a severe illness could be identified promptly, so measures can be taken to influence the outcomes of those

patients. In this sense, having a prognostic index specific for patients with MAFLD who develop COVID-19, may be useful to identify individuals at risk of developing adverse clinical outcomes.

Therefore, the aim of this study was to evaluate the performance of a prognostic index based on LDH, AST and ALT in patients with MAFLD and COVID-19 and its association with the development of adverse clinical outcomes and mortality.

METHODS

This was a retrospective cohort study performed at two third-level hospitals in Mexico, (INCMNSZ and ISSEMyM) from march to July 2020, during the first phase of the COVID-19 pandemic, and before steroids became a standard of care for severe COVID-19. The study was carried out according to Declaration of Helsinki and was approved by the institutional Ethics Committee (Ref. No. 3777).

Validation process

This study consisted of two phases:

- ***Phase 1 Derivation or training cohort***: The methods described below were used to create and evaluate the newly proposed prognostic index. This cohort was derived from a tertiary care center hospital in Mexico City (INCMNSZ).
- ***Phase 2 Validation cohort:*** This aimed to evaluate the diagnostic performance of the proposed index also in patients with COVID-19 and liver steatosis in a different center. This cohort was derived from a tertiary care center hospital in Toluca, in the center of Mexico (ISSEMYM).

Patients

All patients admitted during the period of study, >18 years of age, any gender, and with a confirmed diagnosis of SARS-CoV-2 infection by RT-PCR and with severe disease (pneumonia + respiratory rate >30/respiratory distress/ SaO2 < 90%) , were included in the study(19). Patients without an adequate follow up were excluded from the analysis (v.gr. those requiring referral to other hospital, with insufficient information in the clinical records, etc.). Follow up and evaluation of the clinical outcomes was conducted through revision of electronic clinical records.

Biochemical tests

Upon admission, a blood sample was drawn for determination of the following tests: complete blood count, glucose, creatinine, electrolytes, ferritin, C reactive protein, LDH, liver chemistry, CPK, arterial blood gases, D dimer, hs-TpI and fibrinogen. HIV and viral hepatitis panel (HCV and HBV) was performed in all the participants. All the tests met the quality standards from our central laboratory, accredited by the College of American Pathologists (CAP).

CT scan

In order to evaluate the severity of pulmonary involvement, all patients underwent a pulmonary CT scan, where a portion of the liver was also evaluated for the presence of steatosis. The methodology was previously described from our group (16). Briefly, an expert radiologist blinded to patient´s clinical status evaluated CT scans to detect liver steatosis, according to the following criteria: (a) attenuation coefficient ≤40 Hounsfield units (HU) in the liver (segments VII and VIII); and b) attenuation coefficient ≥10 HU between the splenic and liver parenchyma.

Estimation of the LFN-COVID-19 Index

The Liver Fibrosis and Nutrition lab (LFN) COVID-19 index was calculated according to the following formula:

$$\text{LFN-COVID-19 index} = \left(\frac{\text{AST}}{\text{ALT}}\right) \times \left(\frac{\text{LDH}}{\text{LDH}_{\text{ULN}}}\right)$$

Where AST/ALT ratio included transaminase levels expressed in U/L and was multiplied by the times above the upper limit of normal value for LDH (U/L). Final value was included in the statistical analysis for characterization of clinical outcomes.

Statistical analysis

Sample size estimation considered a hypothetical area under receiver operating characteristic curve (AUROC) of 0.8 for LFN-COVID-19 index, and 0.7 as null hypothesis. Considering an alpha error of

0.05 and beta 0.20, and a negative/positive ratio of 1/1, estimation yielded 81 negative/positive cases (162 patients in total).

Normality of the data was evaluated with Kolmogorov-Smirnov test. Data is presented as mean ± SD, median (P25-P75) or absolute frequencies. Comparisons between the groups were made through Mann-Whitney U or Student´s t-test. ROC curve analysis was performed to obtain the best cutoff from LFN-COVID-19 index for mortality, through Youden index, as well as sensitivity, specificity, positive and negative predictive values, and likelihood ratio.

Clinical outcomes were evaluated by logistic regression, and a time-dependent survival analysis, including Kaplan-Meier and Cox-regression (Cox proportional-hazards model) for 28-day mortality and general mortality. Statistical analysis was carried out with the statistics software SPSS version 20.0 (IBM, Armonk, NY, EE. UU.) and ROC analysis with MedCalc Statistical Software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium).

RESULTS

In validation cohort a total of 457 patients were included in the final analysis (figure 1), after excluding those without an adequate follow up, CT scan (artifacts, unable to evaluate liver or spleen tissue, post-surgical changes) or those with known autoimmune liver diseases, HIV, hepatitis C or B chronic infection or cancer.

Participant's characteristics:

General characteristics of study population, with and without MAFLD are presented in Table 1. Mean age in total population was 50.4 ± 13.3 years, most of the patients were male (65.2%) and the mean BMI was 30.1 ± 5.6 kg/m2. In general, in the group of patients with MAFLD there was higher prevalence of overweight and obesity, were younger than those without MAFLD, and had higher prevalence of diabetes and metabolic syndrome.

Biochemical tests:

Biochemical tests related to proinflammatory status, such as LDH, CPK, lymphocytes and neutrophils/lymphocytes ratio, were higher in the MAFLD group, as well as liver chemistry abnormalities, glucose, triglycerides and prognostic scores (SOFA).

Index diagnostic performance:

In the group of patients with MAFLD, diagnostic yield of the LFN-COVID-19 index ([AST/ALT]*[DHL/DHL_{ULN}]) was investigated, through area under the ROC curve (AUROC) analysis, to determine the prognostic value of the index as a prognostic marker in patients with COVID-19. Characteristics related to diagnostic yield of the LFN-COVID-19 index are shown in table 2. According to Youden´s index, the best cut off value of the LFN-COVID-19 index for mortality, in patients with MAFLD was >1.67. This cut off value showed an AUROC of 0.77 (CI 95% 0.709 - 0.823, p <0.0001), with a sensitivity of 78.7% and specificity 63.8% (Figure 2a). In general, AUROC in this group of patients was better than in patients without MAFLD (AUROC 0.703, CI 95% 0.647 - 0.755, p<0.0001) (Figure 2b).

Table 3 shows the characteristics of patients with MAFLD according to the LFN-COVID-19 index. Similitudes in both groups regarding metabolic syndrome and BMI were observed, while other variables including age, prognostic scores, and biomarkers related to proinflammatory and prothrombotic status, severe COVID-19 (PaO_2/FiO_2 <100mmHg), orotracheal intubation and other clinical outcomes, including mortality, were higher in the >1.67 group.

Prognostic performance:

In order to determine if the LFN-COVID-19 index is independently associated with the presence of acute kidney injury or orotracheal intubation during hospitalization, a logistic regression was performed, observing that a value of >1.67 is associated to adverse clinical outcomes, independently of metabolic factors, severity scores and demographic variables. (Table 4).

As a marker of mortality was studied by a 28-day Kaplan-Meier survival analysis (Figure 3), observing that patients with a value >1.67, have a lower survival than those with a value <1.67 (p<0.001). The influence of other variables on mortality was evaluated through uni- and multivariate Cox proportional-hazards analysis, in table 5 are summarized the variables that were significant in the univariate analysis, with the results when subjected to the multivariate analysis where the variables that were independently associated with mortality were the LFN-COVID-19 index, the Neutrophil/lymphocyte ratio and the BMI.

In this analysis, a LFN-COVID-19 index >1.67 was associated independently to other variables to mortality, including severity markers, prognostic scores and general characteristics (Figure 4).

Validation cohort

From the 697 patients included in the validation cohort, 104 had MAFLD (15.0%). In general, patients with MAFLD were younger, had higher degrees of obesity and mild abnormalities in liver chemistry (Supplementary table 1). The MAFLD group was further analyzed according to the LFN-COVID-19 index, finding higher levels of CRP and D-dimer in the group >1.67, with little changes in the rest of the variables (Supplementary table 2). Interestingly, mechanical intubation and clinical outcomes including mortality, were more frequent in the >1.67 group, as was found in the initial cohort (Supplementary table 3). These same findings, in another cohort and in a different hospital, highlights the validity of the LFN-COVID-19 index.

DISCUSSION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is currently the main etiology of chronic liver disease in the world. The main associated risk factors are obesity, type 2 diabetes, dyslipidemia and metabolic syndrome, factors with a growing incidence. Both risk factors for MAFLD and MAFLD itself, have also been shown to have prognostic value in COVID-19, associating their presence with higher severity and mortality. However, it remains controversial whether all patients within the spectrum of MAFLD have a worse prognosis or only those who, in addition to steatosis, have fibrosis. (16)

Evidence pointing to MAFLD as a prognostic factor emerges from different studies around the world. A retrospective study in patients with COVID-19, found an association of MAFLD with higher ICU admittance (OR 2.3 CI 95% 1.27-4.17), mechanical ventilation (2.08 CI 95% 1.2-3.6) and in patients with cirrhosis with higher mortality (12.5 CI 95% 2.16-72.5). (6)In a cohort study in patients with COVID-19 and chronic liver disease (42% MAFLD) observed a RR of 2.8 (95% CI 1.9-4.0) for death in these group of patients, regardless of age, race, BMI, presence of hypertension or diabetes.(12) Another study conducted in Zhejiang, China found that hospitalized COVID-19 patients who had MAFLD with fibrosis (evaluated through FIB-4 and NFS scores) were at increased risk of severe disease, regardless of other comorbidities. (5) Lastly, a study conducted by Dong Ji et al showed that

patient with COVID-19 and MAFLD had a higher prevalence of alterations in liver biochemistry test as well as a longer viral clearance time compared with patients without MAFLD.(17)

Considering the evidence mentioned above, it is possible that the synergism between the baseline proinflammatory state of patients with MAFLD together with the body´s inflammatory response to COVID-19 could be the pathophysiological support that explains greater severity and worse prognosis in these patients. Another important component in multiorgan damage in COVID-19, is the state of hypoxemia, cell death and hypoperfusion reflected by biomarkers such as LDH, which correlates positively with worse clinical outcomes (including mortality), and although it is not specific for liver damage, it can be a sensitive and dynamic marker of hypoxic tissue damage due to its short half-life, together with other well-known markers of liver damage, as AST, ALT and the AST/ALT ratio. (10)

Due to the link between MAFLD and COVID-19, and the higher risk of mortality and adverse clinical outcomes, we conceived a prognostic index intended to be used in patients with MAFLD, including variables reflecting somehow the pathophysiology of liver damage, mainly hepatocyte cell death induced by the factors previously mentioned, and associating it with hard clinical outcomes, including mortality (18). The LFN-COVID-19 index, includes the AST/ALT ratio as well as LDH levels normalized by laboratory´s upper limit of normal, facilitating the implementation of the index by non-restricting its usefulness to a specific cutoff value (AST, ALT or LDH), and therefore overcoming the problem of regional variations in laboratory values. The use of this index has potential implications in clinical practice establishing a prognosis of patients, on the other hand the simplicity of the index allows to calculate it without complex calculators, and includes widely available, cheap and reliably laboratory tests.

In the present study, we found a good diagnostic performance of the LFN-COVID-19 index in hospitalized patients with MAFLD and COVID-19. In the ROC curve analysis, a cutoff value of >1.67 was associated with adverse clinical outcomes including need for mechanical ventilation, acute kidney injury and higher mortality. This was reproduced in the validation cohort performed at a different center finding this cut-off point as the best for predicting these outcomes.

An interesting finding was that there were no differences in the days of stay in the ICU based on this cut-off point. The same length of stay in the ICU could be explained by the severity of the disease, where those with an index below 1.67 were discharged from the critical care area earlier and those with an index above 1.67, present earlier mortality.

Among the weaknesses of this study is the fact that the diagnosis of hepatic steatosis was made with computed tomography, however, given the high risk of transmission of SARS-CoV-2 to healthcare workers, this safer approach was chosen in order to reduce the exposure involved in carrying out a study such as transient hepatic elastography or MRI requiring more time to perform it. Another aspect to highlight is that patients with COVID-19 usually present elevated transaminases and LDH of multifactorial cause. Nevertheless, both biomarkers have been widely used as markers of hepatocyte cell death, and may reflect liver damage occurring during SARS-CoV-2 infection, and exacerbated in patients with MAFLD.

This study has several strengths too: sample size, the fact that it was carried out in a center fully converted for the care of COVID-19 patients, which included the general population, in a country with one of the highest prevalence of MAFLD and a genetic profile that predisposes to the development of metabolic diseases such as T2DM, obesity and metabolic syndrome. On the other hand, we included an external validation cohort, where the results were replicated, enhancing the validity of the LFN-COVID-19 index.

CONCLUSION

Finally, based on the findings of this study, we propose a new prognostic index based on markers of liver damage and severity in patients with MAFLD and COVID-19, which can be used in clinical practice to stratify the risk of adverse outcomes in MAFLD patients, and timely set actions to reduce the associated morbidity and mortality in this population.

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ANNEXES

TABLES

Table 1. Baseline characteristics of the total population and according to MAFLD presence.

	All (n=457)	*No-MAFLD (n=253)*	*MAFLD (n=204)*	*p value*
Demographic features				
Sex (% Male / Female)	(65.2/34.8)	(63.6/36.4)	(67.2/32.8)	0.432
Age	50.4 ± 13.3	52.4 ± 14	47.8 ± 11.8	<0.0001
BMI	30.1 ± 5.6	28.7 ± 4.9	31.8 ± 5.8	<0.0001
Comorbidities (n / %)				
Malnutrition	10 (2.8)	7 (3.4)	3 (1.9)	<0.0001
Normal Weight	49 (13.6)	43 (21)	6 (3.9)	
Overweight	136 (37.9)	82 (40)	54 (35.1)	
Obesity G1	110 (30.6)	51 (24.9)	59 (38.3)	
Obesity G2	36 (10)	16 (7.8)	20 (13.0)	
Obesity G3	18 (5.0)	6 (2.9)	12 (7.8)	
T2DM	107 (23.5)	47 (18.7)	60 (29.6)	0.006
Hypertension	122 (26.8)	60 (23.8)	62 (30.5)	0.107
Chronic kidney disease	8 (1.8)	6 (2.4)	2 (1.0)	0.225
Pulmonary obstructive disease	4 (0.9)	1 (0.4)	3 (1.5)	0.235
Autoimmune disease	6 (1.3)	3 (1.2)	3 (1.5)	0.551
Immunosuppression	3 (0.7)	3 (1.2)	0 (0)	0.169
Metabolic syndrome	155 (36.0)	61 (25.5)	94 (49)	<0.0001
Prognostic scores				
qSOFA	1 (0 – 1)	1 (0 – 1)	1 (0 – 1)	0.800
SOFA	2 (1 – 2)	2 (1 – 2)	2 (1 – 2)	0.034
NEWS	6.7 ± 2.3	6.6 ± 2.3	6.8 ± 2.2	0.190
PSI/PORT	62 (50 – 80)	62 (50 – 82)	61 (49 – 77)	0.316
SMART COP	3 (2 – 4)	3 (2 – 4)	3 (2 – 4)	0.091
Biochemical values				
CRP (Ref. value: 0 - 1mg/dL)	13.2 (6.6-20.7)	13.08 (6.6-20)	13.7 (6.2-21.5)	0.286
Ferritin (Ref. value: 11 – 306.8ng/mL)	747.8 ± 665	717.2 ± 662	784 ± 668	0.290

D-dimer (Ref. value: 0- 500ng/mL)	707 (426-1146)	699 (413-1138)	721 (451-1182)	0.418
LDH (Ref. value: 120 - 246U/L)	388 ± 160	374 ± 149	406 ± 173	0.032
Troponins (Ref. value: <15pg/mL)	4.7 (3.2- 8.2)	4.7 (3.1-10.4)	4.6 (3.2-7.1)	0.525
CPK (Ref. value: 30 -233U/L)	110 (59-242)	98 (55-210)	133 (66-311)	0.006
Bilirubin (Ref. value: 0/3- 1mg/dL)	0.68 ± 0.49	0.66 ± 0.54	0.69 ± 0.43	0.593
ALT (Ref. value: 7-52U/L)	37.5 (25-56)	33 (23.8-54.7)	41 (28-59)	0.004
AST (Ref. value: 13 - 39U/L)	42 (30-62)	40 (29-58)	43.9 (32.9-64.3)	0.051
Globulins (Ref. value: 1.9 – 3.7g/dL)	3.2 ± 0.4	3.2 ± 0.43	3.2 ± 0.41	0.560
Albumin (Ref. value: 3.5-5.7g/dL)	3.7 ± 0.4	3.6 ± 0.4	3.7 ± 0.4	0.051
ALP (Ref. value: 34-104U/L)	86 (70-111)	86 (70-113)	85 (69-109)	0.505
Creatinine (Ref. value: 0.6 0 1.2mg/dL)	0.9 (0.75-1.1)	0.9 (0.75 – 1.06)	0.9 (0.71-1.1)	0.877
Glucose (Ref. value: 70-99 mg/dL)	116 (102-144)	110 (99-131)	124 (105-184)	<0.0001
Leukocytes (Ref. value: 4-12X 10^3/uL)	7.6 (5.6-10)	7.2 (5.4-9.8)	7.9 (5.7-10.3)	0.191
Lymphocytes (Ref. value: 1 – 3.9X 10^3/uL)	881.6 ± 509	835 ± 352	938 ± 649	0.043
Platelets (Ref. value: 150 - 450K/uL)	239 ± 88	248 ± 95	227 ± 78	0.012
25 (HO) vitamin D (Ref. value: 30 - 100ng/mL)	21.5 ± 8.0	21.6 ± 8.1	21.5 ± 8.0	0.917
Triglycerides (Ref. value: <150mg7dL)	159 ± 85	155 ± 60	165 ± 110	0.264
CT scan results (pulmonary involvement)				
Mild (Ref <20%)	91 (20)	51 (20.3)	40 (19.6)	0.281
Moderate (20 – 50%)	172 (37.8)	102 (40.6)	70 (34.3)	

Severe (>50%)	192 (42.2)	98 (39)	94 (46.1)	
Treatment n(%)				
Antibiotics	402 (88.4)	228 (90.8)	174 (85.3)	0.096
Antimalarials	132 (28.9)	72 (28.5)	60 (29.4)	0.823
Tocilizumab	51 (11.2)	26 (10.3)	25 (12.3)	0.504
Remdesivir	9 (2)	7 (2.8)	2 (1.0)	0.152
PaO2/FiO2 ratio	233.9 ± 109.9	239 ± 91	227 ± 130	0.011
Neutrophil/Lymphocyte ratio	7 (4.4-11.6)	7.2 (4.5-12.0)	6.7 (4.0-10.8)	0.860
Days between the beginning of symptoms and hospitalization	8.2 ± 4.4	8.6 ± 4.6	7.8 ± 4	0.110

BMI, body mass index; T2DM, type 2 diabetes mellitus; q-SOFA, quick-sequential organ failure assessment; SOFA, sequential organ failure assessment; NEWS, national early warning score; PSI/PORT, pneumonia severity index; CRP, c-reactive protein; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CT, computed tomography.

Table 2. Diagnostic yield of the LFN-COVID-19 index in patients with MAFLD.

Diagnostic yield	
Sensitivity	0.787 (0.643 - 0.893)
Specificity	0.638 (0.563 - 0.709)
Positive predictive value (%)	0.36 (0.273 – 0.468)
Negative predictive value (%)	0.91 (0.855 – 0.960)
+ Likelihood ratio	2.18 (1.7 – 2.8)
- Likelihood ratio	0.33 (0.2 - 0.6)
AUROC	0.77 (0.709 - 0.823) $p < 0.0001$
Youden Index	0.4257

AUROC, area under the receiver operating characteristics

Table 3. Characteristics and outcomes in patients with MAFLD according to the LFN-COVID-19 index.

	<1.67 (n=115)	**>1.67 (n=89)**	**p value**
Demographic features			
Sex (Male/Female %)	63.5/36.5	71.9/28.1	0.203
Age (years)	46 ± 10	50 ± 12	0.011
BMI (kg/m^2)	31.1 ± 4.8	32.5 ± 6.9	0.111
qSOFA	1 (0-1)	1 (1-1)	0.007
SOFA	2 (1-2)	2 (2-3)	0.004
NEWS	7 (5-8)	7 (6-9)	0.035
PSI/PORT	56 (47-69)	66 (53-85)	<0.0001
SMART COP	3 (2-4)	4 (3-4)	0.012
Biochemical values			
CRP (ref: 0-1mg/dl)	8.5 (4.2-18.1)	17.2(11.6 – 23.8)	<0.0001
Ferritin (ref: 11- 306.8ng/ml)	503 (266-970)	795 (412-1114)	0.003
D-dimer (ref: 0-500ng/ml)	587 (399 - 962)	967 (606 – 1549)	<0.0001
LDH (ref: 120 - 246u/l)	312 ± 86	529 ± 180	<0.0001
Troponins (ref:<15pg/ml)	3.7 (2.9 – 5.7)	6.1 (3.8 – 10.9)	<0.0001
CPK (ref: 30-223u/l)	107 (58 – 222)	190 (78 – 414)	0.001
Bilirubin (ref: mg/dl)	0.62 ± 0.3	0.78 ± 0.54	0.017
ALT (ref: 7-52u/l)	43.2 (31 – 61.2)	37 (26.3 – 52.8)	0.026
AST (ref:13-39u/l)	38.3 (27.8 – 52.2)	52.4 (42 – 73.7)	<0.0001
Globulins (ref: 1.9-3.7g/dl)	3.22 ± 0.39	3.29 ± 0.43	0.259
Albumin (ref:3.5 -5.7g/dl)	3.9 ± 0.42	3.5 ± 0.40	<0.0001
ALP (ref: 34-104u/l)	85 (70-109)	85 (67 – 110.5)	0.786
Creatinine (ref: 0.6-1.2mg/dl)	0.85 (0.69 – 1.00)	0.95 (0.79 – 1.16)	0.005
Glucose (ref:70-99 mg/dl)	118 (102 – 180)	135 (114 – 187)	0.03
Leukocytes (ref: 4- 12x 10^3/ul)	7.6 (5.6 – 9.9)	8.3 (6.3 – 10.75)	0.089
Lymphocytes (ref: 3.9x 10^3/ul)	937 (693 – 1210)	715 (510 – 967)	<0.0001
Platelets (ref: 150-450k/ul)	228 ± 78	226 ± 79	0.827
25 oh vitamin D (ref: 30-100ng/ml)	21.9 ± 7.8	20.9 ± 8.3	0.488
Triglycerides (ref:<150mg7dl)	151 (118-187)	137 (111 – 184)	0.13

PaO2/FiO2 ratio	240 (161-287)	159 (96 – 245)	<0.0001
Neutrophil/Lymphocyte ratio	5.9 (3.5 – 9.9)	9.6 (6.4 – 13.7)	<0.0001
Other (n / %)			
Metabolic syndrome	49 (46.2)	45 (52.3)	0.401
Severe COVID-19 (PaO_2/FiO_2 <100mmHg)	9 (8.2)	23 (26.7)	<0.0001
Orotracheal intubation	13 (11.3)	36 (40.9)	<0.0001
Acute kidney injury	11 (11)	26 (34.7)	<0.0001
Thrombotic event	1 (1)	2 (2.7)	0.576
Death	6 (5.3)	25 (29.8)	<0.0001
Days between the beginning of symptoms and hospitalization	7.2 ± 3.4	8.6 ± 4.9	0.027
Length of hospital stay (days)	7 (4-10)	8 (6-10)	0.131
Days in ICU	7 (5-12)	12 (6-13)	0.395
Days between ICU requirement and death	7 (6-7)	5 (3-7)	0.203

BMI, body mass index; Q-SOFA, quick-sequential organ failure assessment; SOFA, sequential organ failure assessment; NEWS, national early warning score; PSI/PORT, pneumonia severity index; NFS, NAFLD fibrosis score; APRI, AST to platelet ratio index; CRP, C-reactive protein; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; COVID-19, coronavirus disease 2019; ICU, Intensive care unit.

Table 4. Logistic regression analysis to evaluate the association between LFN-COVID-19 index and clinical outcomes.

Orotracheal Intubation				
	OR	**CI 95%**	**B coefficient**	**p value**
LFN-COVID-19 index	1.900	1.481 – 2.437	0.642	0.000
Sex	0.605	0.288 – 1.271	-0.502	0.185
Age	0.966	0.939 – 0.993	-0.035	0.015
BMI	1.054	0.997 – 1.114	0.053	0.061
Acute kidney injury				
	OR	**CI 95%**	**B coefficient**	**P value**
LFN-COVID-19 index	1.849	1.366 – 2.504	0.615	0.000
Sex	0.280	0.103 – 0.765	-1.272	0.013
Age	1.021	0.988– 1.054	0.021	0.209
BMI	1.085	1.011 – 1.164	0.081	0.023

OR, odds ratio; CI, confidence interval; BMI, Body mass index.

Table 5. Cox proportional-hazards multivariate analysis for mortality in patients with MAFLD according to the LFN-COVID-19 index.

	OR	**B coefficient**	**P value**	**CI 95%**
LFN-COVID-19 index	0.241	-1.422	0.013	.079-0.741
PaO_2/FiO_2 ratio	1.000	0.000	0.877	.996-1.004
Neutrophil/lymphocyte ratio (NLR)	1.043	0.042	0.030	1.004-1.083
Creatine phosphokinase (CPK)	1.001	0.001	0.340	.999-1.002
Body mass index (BMI, kg/m^2)	1.093	0.089	0.002	1.033-1.157

OR, odds ratio; CI, confidence interval.

FIGURES

Figure 1. Cohort derivation and validation flowchart

Figure 2. Area under the ROC curve for the LFN-COVID-19 index to predict mortality in patients with (a) and without (b) MAFLD and COVID-19.

Figure 3. Kaplan-Meier curve for 28-day mortality according to LFN-COVID-19 index.

Figure 4. Adjusted mortality analysis (Cox regression) for 28-day mortality according to LFN-COVID-19 index.

Figure 1. Cohort derivation and validation flowchart

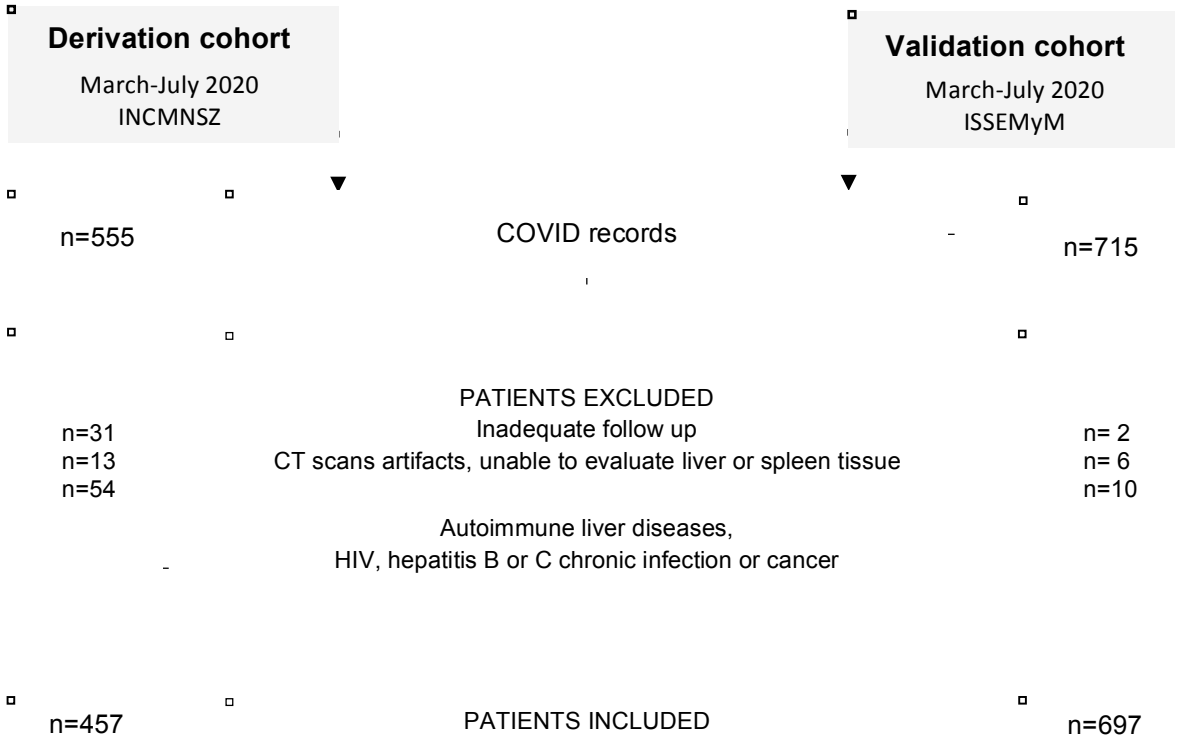

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Figure 3. Kaplan-Meier curve for 28-day mortality according to LFN-COVID-19 index.

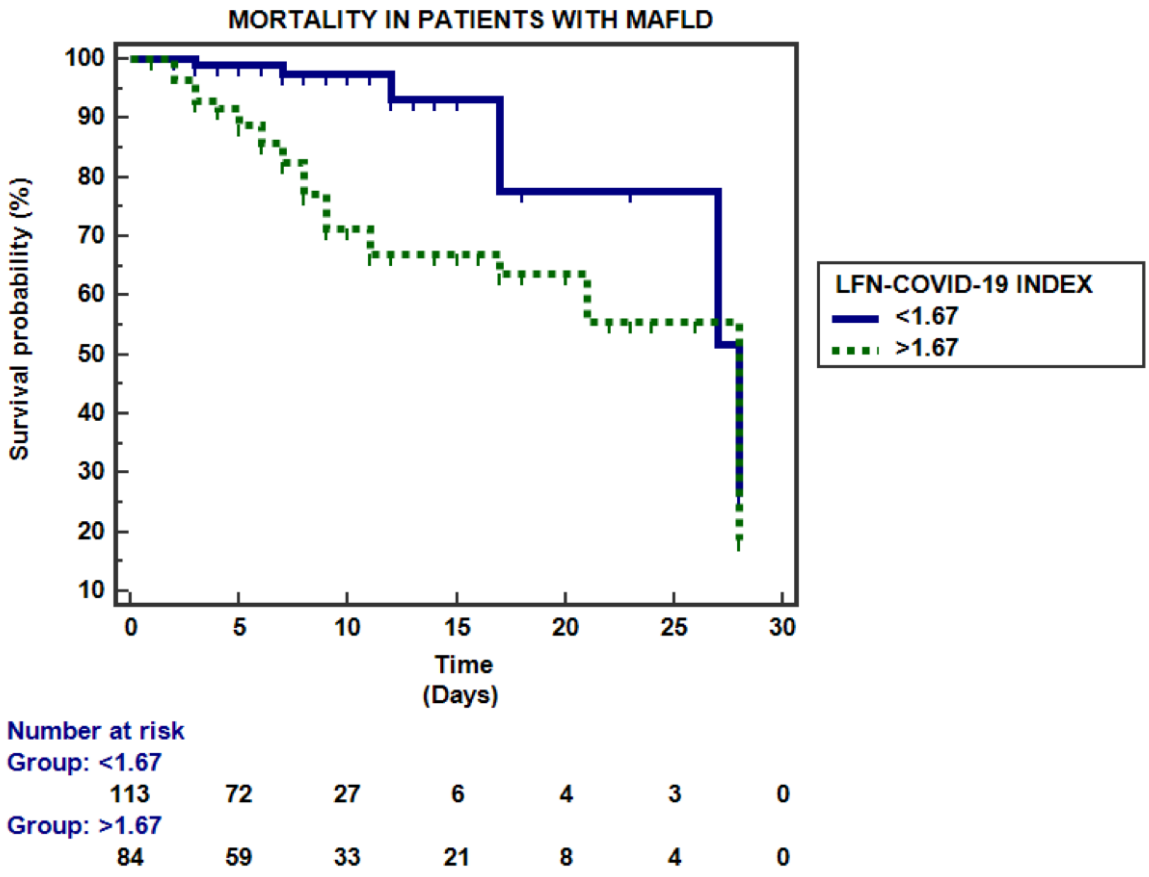

Figure 4. Adjusted mortality analysis (Cox regression) for 28-day mortality according to LFN-COVID-19 index.