Letter to the Editor

Risk of severe illness from COVID-19 in patients with metabolic associated fatty liver disease and increased fibrosis scores

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Abbreviation List

BMI, body mass index

95% CI, 95% confidence interval

COVID-19, coronavirus disease 2019

FIB-4, fibrosis 4 index

MAFLD, metabolic fatty liver disease

NAFLD, nonalcoholic fatty liver disease

NFS, NAFLD fibrosis score

OR, odds ratio

A recent study reported that patients with severe coronavirus disease-2019 (COVID-19) were more likely to have NAFLD compared to those with non-severe COVID-19 illness [1]. However, the prognosis of NAFLD (recently renamed metabolic associated fatty liver disease (MAFLD) [2]) is determined by the severity of liver fibrosis [3,4]. We therefore postulated that MAFLD patients with increased non-invasive liver fibrosis scores are at higher risk for severe illness from COVID-19.

We studied 310 patients with laboratory-confirmed COVID-19, who were consecutively hospitalized at four sites in Zhejiang province, China, between January and February 2020. Some of these patients (n=150) have been included in a prior study examining the association between obesity and COVID-19 severity [5]. Patients with viral hepatitis, excessive alcohol consumption, chronic pulmonary diseases or active cancers were excluded. Clinical and laboratory data were collected at hospital admission. All patients were screened for hepatic steatosis by computed tomography and subsequently diagnosed as MAFLD [6]. The originally validated cut-points for Fibrosis-4 (FIB-4) index and NAFLD fibrosis score (NFS) were used to categorize liver fibrosis probability as low, intermediate, or high [7]. COVID-19 severity was classified as severe and non-severe [8]. The study protocol was approved by the local ethics committees of the four hospitals.

In our cohort, 94 (30.3%) patients had MAFLD. As shown in **Table 1**, MAFLD patients with intermediate or high FIB-4 scores were more likely to be older, obese, have diabetes, and had higher NFS, higher liver enzymes, higher C-reactive protein, as well as lower levels of lymphocyte count, platelet count, triglycerides and high-density lipoprotein-cholesterol compared with their counterparts with low FIB-4 score or those without MAFLD. Notably, the severity of COVID-19 illness markedly increased amongst MAFLD patients with intermediate or high FIB-4 scores.

The severity of COVID-19 illness was associated with intermediate (unadjusted-odds ratio [OR] 4.32, 95%CI 1.94-9.59) or high (unadjusted-OR 5.73, 95%CI 1.84-17.9) FIB4 scores amongst MAFLD patients (**Table 2**, model 1A). This association remained significant after adjusting for sex, obesity and diabetes (model 1B). We did not additionally adjust for age, because this variable was incorporated in the FIB-4 score. Similar to the main analysis, when the

intermediate and high FIB-4 categories were combined, the risk of severe COVID-19 illness was increased with intermediate/high FIB-4 score in unadjusted (model 2A) and multivariate-adjusted analyses (model 2B).

Similarly, the intermediate/high NFS score (unadjusted-OR 5.21, 95%CI 2.39-11.3) was associated with a higher risk of severe COVID-19 illness. This significant association persisted in multivariate-adjusted analyses after controlling for sex, obesity and diabetes (adjusted-OR 2.91, 95%CI 1.20-7.06).

When we included FIB-4 or NFS as continuous measures in multivariable regression models, increasing FIB-4 (adjusted-OR 1.90, 95%CI 1.33-2.72) or NFS scores (adjusted-OR 2.57, 95%CI 1.73-3.82) were associated with greater COVID-19 severity, after adjusting for sex, obesity, diabetes and presence/absence of MAFLD.

Our study has some limitations, including the relatively modest sample size, the Asian ancestry of the cohort, and the use of non-invasive fibrosis scores without a histological diagnosis of liver fibrosis. Despite these limitations, our study is the first to examine the impact of FIB-4 or NFS on COVID-19 severity in patients with imaging-defined MAFLD. These non-invasive fibrosis scores have been shown to predict histological fibrosis stage with reasonable accuracy in cohorts of MAFLD patients [7], and are also associated with increased overall and disease-specific mortality in population-based studies [9,10]. Our data demonstrate that MAFLD patients with increased FIB-4 or NFS scores are at higher likelihood of having severe COVID-19 illness, irrespective of metabolic comorbidities. In the context of COVID-19, the presence of MAFLD with significant/advanced fibrosis might exacerbate the virus-induced cytokine “storm”, possibly through the hepatic release of multiple proinflammatory cytokines, thereby contributing mechanistically to severe COVID-19. Further research is needed to better understand the mechanistic link of advanced MAFLD to the viral disease process.

CONFLICTS OF INTEREST STATEMENT: All authors do not have anything to declare.

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REFERENCES

1. Ji D, Qin E, Xu J, Zhang D, et al. Non-alcoholic fatty liver diseases in patients with COVID-19: a retrospective study. J Hepatol 2020 Apr 8. pii: S0168-8278(20)30206-3. doi: 10.1016/j.jhep.2020.03.044 [Epub ahead of print] No abstract available.
2. Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology 2020 Feb 8:S0016-5085(20)30171-2. doi: 10.1053/j.gastro.2019.11.312. Online ahead of print.
3. Younossi ZM. Non-alcoholic fatty liver disease - a global public health perspective. J Hepatol 2019;70:531-544.
4. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology 2020 Feb 4:S0016-5085(20)30137-2. doi: 10.1053/j.gastro.2020.01.043. Online ahead of print.
5. Gao F, Zheng KI, Wang XB, et al. Obesity as a risk factor for greater severity of COVID-19. eLetter-Observations. Diabetes Care 2020; in press.
6. Eslam M, Newsome PN, Anstee QM, et al. A new definition for metabolic associated fatty liver disease: an international expert consensus statement. J Hepatol 2020 Apr 8. pii: S0168-8278(20)30201-4. doi: 10.1016/j.jhep.2020.03.039 [Epub ahead of print].
7. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: clinical prediction rules and blood-based biomarkers. J Hepatol. 2018;68:305-315.
8. National Health Commission & State Administration of Traditional Chinese Medicine. Diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7). 2020 [EB/OL]. 2020.03.03.
9. Unalp-Arida A, Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. Hepatology 2017;66:84-95.
10. Sung KC, Johnston MP, Lee MY, Byrne CD. Non-invasive liver fibrosis scores are strongly associated with liver cancer mortality in general population without liver disease. Liver Int 2020 Feb 23. doi: 10.1111/liv.14416 [Epub ahead of print].

Table 1. Main clinical and biochemical characteristics of patients with laboratory-confirmed COVID-19, stratified by presence or absence of imaging-defined MAFLD with increasing levels of fibrosis-4 (FIB-4) score.

	No MAFLD	**MAFLD with low FIB-4 (≤1.3)**	**MAFLD with intermediate FIB-4 (1.3-2.67)**	**MAFLD with high FIB-4 (>2.67)**	***P* value**
N	216	44	36	14	
Age (years)	45.9 ± 15.4	41.2 ± 14.2	54.2 ± 10.8	59.9 ± 9.1	**<0.001**
Male sex (%)	43.5	54.6	63.9	57.1	0.086
BMI (kg/m^2)	23.0 ± 3.2	26.5 ± 4.7	26.6 ± 3.2	26.1 ± 2.8	**<0.001**
Obesity (%)	25.9	61.4	75.0	64.3	**<0.001**
Current smokers (%)	8.8	2.3	11.1	7.1	0.768
Systolic blood pressure (mmHg)	130 ± 17	136 ± 15	136 ± 16	136 ± 14	**0.047**
Diastolic blood pressure (mmHg)	80 ± 11	84 ± 11	82 ± 11	79 ± 8	0.117
Prior diabetes (%)	7.4	20.4	13.9	28.6	**0.010**
White blood count (x 10*9/L)	4.90 (3.9-6.2)	5.35 (4.5-6.7)	4.69 (3.8-6.6)	4.90 (3.2-5.8)	0.254
Neutrophil count (x 10*9/L)	3.05 (2.2-4.1)	3.49 (2.6-4.2)	3.0 (2.4-4.7)	3.20 (1.9-4.5)	0.759
Lymphocyte count (x 10*9/L)	1.20 (0.9-1.6)	1.40 (1.2-2.0)	1.10 (0.7-1.3)	0.92 (0.7-1.14)	**<0.001**
Hemoglobin (g/L)	132.4 ± 16	136.6 ± 15	135.5 ±12	135.8 ± 17	0.305
Platelet count (x 100,000/mm^3)	211 ± 72	246 ± 75	183 ± 64	125 ± 24	**<0.001**
Prothrombin time (sec), n=211	12.1 ± 1.3	11.9 ± 2.3	12.2 ± 1.2	12.3 ± 1.1	0.754
APTT (sec), n=211	31.5 ± 3.8	32.7 ± 5.4	32.8 ± 3.7	35.3 ± 7.4	**0.026**
D-dimer (mg/L), n=183	0.18 (0.11-0.29)	0.21 (0.1-0.5)	0.21 (0.1-0.4)	0.27 (0.1-0.4)	0.432
C-reactive protein (mg/L)	7.9 (1.9-24.9)	8.6 (2.9-23.6)	25.4 (8.7-53.3)	25.5 (8.7-53.4)	**<0.001**
Procalcitonin (ng/mL), n=190	0.05 (0.03-0.25)	0.08 (0.04-0.25)	0.06 (0.04-0.10)	0.20 (0.08-0.25)	**0.077**
Albumin (g/L), n=286	41.4 (38.0-44.5)	42.3 (37.3-44.6)	41.0 (36.9-43.1)	38.6 (37.7-42.8)	0.348
Total bilirubin (μmol/L)	10.5 (7.0-15.5)	10.1 (7.5-15.6)	12.0 (9.5-16.6)	15.3 (11.8-17.3)	**0.025**
AST (IU/L)	22 (17-27)	23 (19-32)	32 (26-50)	44 (29-83)	**<0.001**
ALT (IU/L)	18 (13-27)	30 (22-52)	28 (22-48)	27 (16-82)	**<0.001**
GGT (IU/L)	21 (14-33)	31 (22-54)	51 (24-81)	49 (32-102)	**<0.001**
Elevated AST >40 IU/L (%)	7.9	9.1	27.8	57.1	**<0.001**
Elevated ALT >40 IU/L (%)	13.0	29.6	30.6	42.9	**<0.001**
Total cholesterol (mmol/L)	3.98 ± 0.8	4.11 ± 0.9	3.73 ± 0.7	3.82 ± 1.0	0.217
Triglycerides (mmol/L)	1.15 (0.9-1.7)	1.61 (1.0-2.1)	1.48 (1.1-1.8)	1.11 (0.9-1.6)	**<0.005**
HDL cholesterol (mmol/L)	1.18 ± 0.4	0.96 ± 0.2	1.06 ± 0.2	1.09 ± 0.4	**<0.005**
LDL cholesterol (mmol/L)	2.23 ± 0.7	2.54 ± 0.8	2.10 ± 0.7	2.20 ±0.8	0.116
Hospital stay (days)	18 (13-24)	18 (13-22)	22 (16-29)	17 (6-23)	0.122
NAFLD fibrosis score, n=286	-1.82 (-2.8 to -1.0)	-2.61 (-3.1 to -1.9)	-0.68 (-1.4 to -0.2)	+0.26 (-0.03 to 1.6)	**<0.005**
COVID-19 severity					**<0.001**
non-severe (%)	88.4	86.4	63.9	57.1	
severe (%)	11.6	13.6	36.1	42.9	

Sample size, *n*=310, except where indicated. Diabetes was diagnosed as self-reported history of disease and/or specific drug treatment. Obesity was diagnosed as BMI >25 kg/m^2.

Data are expressed as means±SD, medians and interquartile ranges (in parenthesis) or frequencies. Differences among the four groups of patients were tested by the Fisher's exact test for categorical variables, the one-way analysis of variance for normally distributed continuous variables or the Kruskal-Wallis test for not normally distributed continuous variables. For the sake of clarity, significant p-values are highlighted in bold.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Table 2. Association between imaging-defined MAFLD with increasing levels of fibrosis-4 (FIB-4) score and risk of having severe illness associated with COVID-19.

Logistic Regression Analysis	Odds ratio(s)	95% CI	*P* value
Severity of COVID-19 illness (mild/moderate *vs*. severe/critical)			
Unadjusted model 1A			
MAFLD/FIB-4 status			
No MAFLD (n=216)	*Ref.*	*Ref.*	
MAFLD with low FIB-4 (≤1.3; n=44)	1.21	0.46 – 3.14	0.701
MAFLD with intermediate FIB-4 (1.3-2.67; n=36)	4.32	1.94 – 9.59	**<0.001**
MAFLD with high FIB-4 (>2.67; n=14)	5.73	1.84 – 17.9	**<0.005**
Adjusted model 1B			
MAFLD/FIB-4 status			
No MAFLD (n=216)	*Ref.*	*Ref.*	
MAFLD with low FIB-4 (≤1.3; n=44)	0.82	0.30 – 2.24	0.696
MAFLD with intermediate FIB-4 (1.3-2.67; n=36)	2.59	1.09 – 6.13	**0.030**
MAFLD with high FIB-4 (>2.67; n=14)	4.04	1.22 – 13.3	**0.021**
Sex (men vs. women)	1.78	0.93 – 3.44	0.079
Obesity (yes vs. no)	2.62	1.31 – 5.24	**<0.005**
Prior diabetes (yes vs. no)	1.04	0.40 – 2.80	0.928
Unadjusted model 2A			
MAFLD/FIB-4 status			
No MAFLD (n=216)	*Ref.*	*Ref.*	
MAFLD with low FIB-4 (≤1.3; n=44)	1.21	0.46 – 3.14	0.701
MAFLD with intermediate/high FIB-4 (>1.3; n=50)	4.68	2.31 – 9.49	**<0.001**
Adjusted model 2B			
MAFLD/FIB-4 status			
No MAFLD (n=216)	*Ref.*	*Ref.*	
MAFLD with low FIB-4 (≤1.3; n=44)	0.82	0.30 – 2.24	0.696
MAFLD with intermediate/high FIB-4 (>1.3; n=50)	2.95	1.37 – 6.34	**<0.005**
Sex (men vs. women)	1.79	0.94 – 3.45	0.084
Obesity (yes vs. no)	2.60	1.30 – 5.16	**<0.005**
Prior diabetes (yes vs. no)	1.09	0.41 – 2.89	0.862

Sample size*, n*=310. Data are expressed as odds ratios and 95% confidence intervals (CI) as tested by univariable (unadjusted) and multivariable (adjusted) logistic regression analysis. *Ref*., reference category. Diabetes was diagnosed as self-reported history of disease and/or specific drug treatment. Obesity was diagnosed as BMI >25 kg/m^2.

NB: In the adjusted logistic regression models, we did not additionally adjust also for age, because this variable is already incorporated in the FIB-4 score.