Liver Fibrosis Index FIB-4 Is Associated With Mortality in COVID-19

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Coronavirus disease 2019 (COVID-19) is associated with adverse outcomes, including need for invasive mechanical ventilation and death in people with risk factors. Liver enzyme elevation is commonly seen in this group, but its clinical significance remains elusive. In this study, we calculated the Fibrosis-4 (FIB-4) score for a cohort of hospitalized patients with COVID-19 and assessed its association with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA, inflammatory cytokine levels, and clinical outcome. A total of 202 hospitalized participants who tested positive for SARS-CoV-2 by nasopharyngeal sampling were included in this analysis. FIB-4 was calculated for each participant using the alanine aminotransferase, aspartate aminotransferase, age, and platelet count. We evaluated the association between FIB-4 and mortality using both multivariate logistic regression and Cox proportional hazards model. Correlations between FIB-4 and SARS-CoV-2 RNA and cytokine levels were evaluated using the Spearman test. Among the 202 participants, 22 died. The median FIB-4 in participants who survived and died were 1.91 and 3.98 ($P < 0.001$ by Mann-Whitney U test), respectively. Each one-unit increment in FIB-4 was associated with an increased odds of death (odds ratio, 1.79; 95% confidence interval, 1.36, 2.35; $P < 0.001$) after adjusting for baseline characteristics including sex, body mass index, hypertension, diabetes, and history of liver diseases. During hospitalization, FIB-4 peaked and then normalized in the survival group but failed to normalize in the death group. FIB-4 was positively correlated with the level of SARS-CoV-2 viral load and monocyte-associated cytokines, especially interleukin-6 and interferon gamma–induced protein 10. *Conclusion:* FIB-4 is associated with mortality in COVID-19, independent of underlying conditions including liver diseases. FIB-4 may be a simple and inexpensive approach to risk-stratify individuals with COVID-19. (*Hepatology Communications* 2021;5:434-445).

Coronavirus disease 2019 (COVID-19) has led to over 1 million deaths globally.[1] It manifests as a spectrum of clinical syndromes, ranging from asymptomatic infection to severe/critical illness and death.[2] Accumulating evidence suggests that COVID-19 is associated with more than just pulmonary diseases, and its extrapulmonary manifestations, including liver, cardiac, renal and neurologic injury, are receiving increasing attention,[2] as they contribute to significant morbidity and mortality. Liver injury is found to be very common in people with COVID-19 (ranging from ~15% to ~70%[2-4]), and a recent study revealed an elevated aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio, with minimal elevation in bilirubin.[4] This transaminase elevation is thought to originate from hepatocellular injury,

Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase–to-platelet ratio index; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; BWH, Brigham and Women's Hospital; CI, confidence interval; CK, creatine kinase; COVID-19, coronavirus disease 2019; CRP, C reactive protein; FIB-4, Fibrosis-4; IFN, interferon; IL, interleukin; IP-10, IFN-γ-induced protein-10; IQR, interquartile range; IRB, internal review board; LDH, lactate dehydrogenase; MassCPR, Massachusetts Consortium on Pathogen Readiness; OR, odds ratio; PLT, platelet count; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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although muscle and cardiomyocyte injury could also contribute.[4] The mechanisms behind liver injury in COVID-19 remains to be determined, potentially including but not limited to direct virus effects, elevation of certain cytokine levels, hypoxemia, and shock physiology.[5]

A retrospective cohort study with 1,827 participants demonstrated that COVID-19 is associated with mild elevation of ALT and AST, and among those with liver injury, about 60% had ALT and AST at 1-fold to 2-fold the upper limit of normal on admission; it further revealed that AST elevation at admission was associated with severe diseases including intensive care unit admission and mechanical ventilation, but not death.[3]

Fibrosis-4 score (FIB-4), a simple scoring system derived from routine tests including AST, ALT, age, and platelet count (PLT), has been developed to predict advanced fibrosis in hepatitis C infection[6] and was validated in nonalcoholic fatty liver disease (NAFLD), with better performance than other noninvasive markers for liver fibrosis. A recent publication from Europe indicates that an elevation of FIB-4 is associated with poor clinical outcomes in patients with COVID-19,[7] and another study from the United States has shown that a FIB-4 score ≥ 2.67 is associated with both 30-day mortality and the need for ventilation.[8] However, neither study showed temporal trends of FIB-4 changes, and it is difficult to distinguish whether elevations of FIB-4 are related to underlying liver fibrosis versus direct cytopathic effects from COVID-19.

To this end, we used data from two cohorts and aimed to validate the association between FIB-4 and COVID-19 severity. In addition, we evaluated potential mechanisms behind this association by assessing its relationship with levels of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load and inflammatory markers.

Patients and Methods

STUDY PARTICIPANTS

We included hospitalized, SARS-CoV-2-infected participants enrolled in two cohort studies based at two large academic centers in Boston, Massachusetts (Massachusetts Consortium on Pathogen Readiness [MassCPR] cohort and Brigham and Women's Hospital [BWH] Biorepository cohort). Each participant or their health care proxies signed informed consent to participate in the original cohort studies.[9] Participants hospitalized between March 15, 2020, and July 15, 2020, were included in this current study. Inclusion criteria in this analysis included (1) SARS-CoV-2 real-time polymerase chain reaction test positive from nasopharyngeal swab and (2) hospitalized. Exclusion criteria included (1) history of decompensated cirrhosis or cirrhosis with Model for End-Stage Liver Disease–Sodium score above 10 and (2) participants who received chemotherapy within 1 month of hospitalization, as

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both conditions may skew the FIB-4 score. Clinical and laboratory data including age, sex, ethnicity, body mass index (BMI), medical history, liver function test panel, PLT, C reactive protein (CRP), lymphocyte count, D-dimer level, creatine kinase (CK), troponin T level, and lactate dehydrogenase (LDH) were extracted from the medical record. The AST-to-platelet ratio index (APRI) was calculated using 100 × (AST U/L/AST upper limit of normal 40 U/L)/PLT (1,000/μL).[10] FIB-4 was calculated using the following formula[6]:

$$\frac{\text{Age (year)} \times \text{AST (U/L)}}{\text{PLT (1000/}\mu\text{L)} \times \sqrt{\text{ALT (U/L)}}}$$

FIB-4 < 1.45 was considered within the normal range with a negative predictive value of advanced fibrosis of approximately 90%.[6] The original cohort studies were approved by Mass General Brigham institutional review board (IRB).

CLINICAL OUTCOME

The primary outcome of this current analysis was death. Secondary outcomes included severe diseases (requiring invasive mechanical ventilation and death) and time from admission to death (evaluated with Cox proportional model).

SARS-CoV-2 RNA LEVEL QUANTIFICATION

RNA was extracted from clinical samples (plasma respiratory and urine samples) using the TRIzol-based method (Thermo Fisher Scientific, Waltham, MA). Levels of SARS-CoV-2 RNA were then quantified with a modified real-time reverse-transcription quantitative polymerase chain reaction using the US Centers for Disease Control 2019-nCoV_N1 primer and probe set[9] with a limit of detection of 40 SARS-CoV-2 RNA copies/mL.

CYTOKINE ANALYSIS

The following plasma cytokine levels were evaluated using Luminex xMAP assay (Thermo Fisher Scientific): epidermal growth factor, eotaxin, fibroblast growth factor–basic, granulocyte colony–stimulating factor, granulocyte-macrophage colony-stimulating factor, hepatocyte growth factor (HGF), interferon (IFN)-α, IFN-γ, interleukin (IL)-1α, IL-1β, IL-1RA, IL-2, soluble IL-2R, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17A, IL-17F, IL-22, IFN-γ-induced protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1, also known as C-C ligand 2), monokine induced by gamma (MIG, also known as C-X-C ligand 9), macrophage inflammatory protein (MIP-1α), MIP-1β, regulated on activation normal T cell expressed and secreted (RANTES, also known as C-C ligand 5), tumor necrosis factor α, and vascular endothelial growth factor. The cytokine analysis was performed on undiluted plasma, according to the manufacturer's instructions, with duplicates of the standard curve included on the plate. Analysis was performed using GraphPad Prism (San Diego, CA) to extrapolate concentration values based on the standard curve.

ETHICS STATEMENT

Both MassCPR and BWH Biorepository studies were approved by the Mass General Brigham IRB. The MassCPR cohort IRB number was 2020P000804, and the BWH Biorepository cohort IRB number was 2020P000849. Each participant signed an informed consent before enrollment.

STATISTICAL ANALYSES

Continuous variables were summarized using median and interquartile ranges (IQRs), as most of the variables did not fit the normal distribution. We used the Wilcoxon rank-sum test to compare continuous variables from two different categorical groups and Dunn's test for three or more groups. For comparison of longitudinal values within one group, we used the Wilcoxon signed-rank matched-pair test. Categorical variables were evaluated using either the χ^2 test or Fisher's exact test (if the expected value in any category is below 5). Correlation between different continuous variables was evaluated using the Spearman's rank correlation coefficient.

To evaluate the association between FIB-4, other baseline characteristics/ values, and clinical outcomes, we used two different models. In the main analysis, we used logistic regression to calculate odds ratios (ORs) (with 95% confidence interval [CI]) of these variables and their association with the primary outcome

(death). FIB-4 was treated as a continuous variable. In the first multivariate logistic regression model (model 1), we adjusted for baseline characteristics including sex, BMI, ethnicity, hypertension, diabetes, remdesivir use, and history of liver diseases. In the second multivariate logistic regression model (model 2), we added baseline troponin T, CRP, lymphocyte count, LDH, and D-dimer. Age was not included in the multivariate analysis, as it is part of the FIB-4 score. To avoid collinearity and overadjustment, we used the backward stepwise regression method, excluding variables with $P > 0.20$ from the final multivariate model. Collinearity was evaluated using the variance inflation factor and condition number (both indices needed to be <10 to avoid collinearity). In sensitivity analyses, we used the same methods to evaluate the association of baseline characteristics and severe diseases (mechanical ventilation and death). We also evaluated the association between FIB-4 and outcomes by treating FIB-4 as a categorical variable (≤2.67 vs. >2.67, cutoff value based on NAFLD rather than hepatitis C[11]). In another sensitivity analysis, the Cox proportional hazards model was used to calculate the hazard ratio of FIB-4 and other baseline characteristics. Time-to-event was calculated for each participant, counting from admission date to discharge or death. Accuracy of prediction by FIB-4 and other related variables was evaluated by area under receiver operating characteristic curve (AUROC).

We used Stata 13.1 (StataCorp, College Station, TX) to perform all of the statistical analyses and Prism to prepare the figures. *P* value less than 0.05 was considered statistically significant unless stated otherwise.

Results

PARTICIPANT SELECTION AND BASELINE CHARACTERISTICS

A total of 202 participants were selected from the two aforementioned cohorts (n = 87 from the MassCPR cohort and n = 115 from the BWH cohort) (Supporting Fig. S1). The median age was 58 years (IQR = 48, 69), and 93 were female participants (46%). People of color represented 60% of the participants (n = 122). Participants were largely obese or overweight (median BMI = 29 kg/m^2) and hypertensive (n = 123, 61%). Thirty-eight percent had diabetes, and 33% had a diagnosis of liver diseases (mostly steatosis based on imaging findings). Most participants had ALT, AST, and total bilirubin within normal ranges on admission (median = 28 U/L, 38 U/L, and 0.4 mg/dL, respectively). Median FIB-4 was 2.03 with 31% of participants having FIB-4 > 2.67, within the range used to define probable advanced fibrosis. We also observed elevated d-dimer, LDH, CRP levels, as well as decreased lymphocyte count, especially in the death group (Table 1). Compared with the survival group, participants from the death group were older and tended to be male. Otherwise, two groups had comparable BMI, rates of hypertension/diabetes/liver diseases, and rates of enrollment to remdesivir trial (Table 1).

Given this prominent AST > ALT pattern in COVID-19, we suspected that AST could also come from muscle or heart tissue in addition to the liver. While AST and ALT were highly correlated (Spearman rho = 0.82, $P < 0.001$), AST was also moderately correlated to CK (rho = 0.41, $P < 0.001$) and LDH (rho = 0.60, $P < 0.001$); we did not find a significant correlation between AST and troponin T at admission (rho = −0.10, $P = 0.17$).

ELEVATED FIB-4 WAS ASSOCIATED WITH MORTALITY AND SEVERE COVID-19

Most participants (75%) demonstrated an AST/ALT ratio > 1. There were no significant differences in liver enzymes, including ALT, AST, and total bilirubin, between the survival and death groups (Table 1). However, in the death group, we observed significantly elevated FIB-4 compared with those in the survival group ($P < 0.001$; Table 1 and Fig. 1A). APRI, another fibrosis marker that only includes AST and PLT, showed a nonsignificant difference between the survival and death groups (Fig. 1B-D). FIB-4 levels on admission did not differ between participants with and without a pre-existing diagnosis of liver disease (median FIB-4 = 2.03 and 2.03; $P = 0.36$). This finding suggests that a significant number of patients had undiagnosed pre-existing liver disease, most likely NAFLD.

In logistic regression analysis, higher FIB-4 was associated with mortality and an unadjusted OR = 1.75 (95% CI, 1.37, 2.23; $P < 0.001$). We also noted that

TABLE 1. BASELINE CHARACTERISTICS AND LABORATORY VALUES

	Total (n = 202)	Survival (n = 180)	Death (n = 22)	*P* Value
Median age, years (IQR)	58 (49, 69)	57 (45, 66)	73 (65, 79)	<0.001
Age > 60 years, n (%)	91 (45.1)	73 (40.6)	18 (81.8)	<0.001
Sex, n (%)				0.020
Female	93 (46.0)	88 (48.9)	5 (22.7)	
Male	109 (54.0)	92 (51.1)	17 (77.3)	
Ethnicity, n (%)				0.76
African American	48 (23.8)	42 (23.3)	6 (27.3)	
Asian	9 (4.5)	9 (5.0)	0 (0.0)	
Caucasian	80 (39.6)	70 (38.9)	10 (45.4)	
Hispanic	52 (25.7)	48 (26.7)	4 (18.2)	
Other	13 (6.4)	11 (6.1)	2 (9.1)	
Median BMI, kg/m^2 (IQR)	29.0 (25.5, 34.5)	29.3 (25.7, 34.6)	27.1 (25.0, 33.7)	0.22
BMI ≥ 30 kg/m^2, n (%)	92 (45.5)	83 (46.1)	9 (40.9)	0.64
Hypertension, n (%)	123 (60.9)	108 (60.0)	15 (68.2)	0.46
Diabetes, n (%)	77 (38.1)	67 (37.2)	10 (45.5)	0.45
Enrolled in remdesivir trial, n (%)	83 (41.1)	77 (42.8)	6 (27.3)	0.16
Mechanical ventilation, n (%)	76 (37.6)	57 (31.7)	19 (86.4)	<0.001
Median duration of hospitalization, days (IQR)	10 (5, 25)	9 (5, 25)	22 (12, 25)	0.015
History of liver diseases, n (%)	65 (32.2)	57 (31.7)	8 (36.4)	0.66
Classification of liver diseases,* n (%)				0.62
Chronic viral hepatitis without steatosis or cirrhosis	1 (1.6)	1 (1.7)	0 (0.0)	
Steatosis	58 (89.2)	51 (89.5)	7 (87.5)	
Cirrhosis	6 (9.2)	5 (8.8)	1 (12.5)	
Median MELD-Na score† (IQR)	7.5 (6.8, 8.3)	7.0 (6.5, 8.0)	9.0 (9.0, 9.0)	N/A
Median ALT, U/L (IQR)	28 (17, 51)	29 (17, 51)	23 (14, 45)	0.29
Median AST, U/L (IQR)	38 (26, 61)	37 (25, 61)	52 (29, 73)	0.19
Median PLT, ×1,000/uL (IQR)	210 (160, 259)	213 (165, 267)	173 (134, 233)	0.028
Median FIB-4 (IQR)	2.03 (1.34, 3.06)	1.91 (1.24, 2.70)	3.98 (2.51, 6.68)	<0.001
FIB-4 > 2.67, n (%)	63 (31.2)	47 (26.1)	16 (72.7)	<0.001
Median APRI (IQR)	0.5 (0.28, 0.88)	0.50 (0.27, 0.82)	0.70 (0.35, 1.09)	0.054
Median total bilirubin, mg/dL (IQR)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.5 (0.4, 0.7)	0.29
Median troponin T, ng/L (IQR)	10 (5, 24)	9 (5, 20)‡	29 (14, 61)	<0.001
Median CRP, mg/L (IQR)	88.9 (42.0, 168.4)	85.2 (38.9, 162.0)§	119.3 (48.9, 191.5)	0.24
Median lymphocyte count, ×1,000/uL (IQR)	0.94 (0.67, 1.30)	0.96 (0.70, 1.33)	0.60 (0.41, 1.22)	0.005
Median LDH, U/L	349 (260, 506)	346 (254, 478)§	524 (322, 603)	0.003
Median D-dimer, ng/mL (IQR)	1,059 (611, 1,940)	983 (582, 1,669)‖	1713 (1,134, 4,000)	<0.001

*In participants with liver diseases (n = 65).
†In participants with cirrhosis (n = 6).
‡n = 178.
§n = 175.
‖n = 172.
Abbreviations: MELD-Na, Model For End-Stage Liver Disease–Sodium; and N/A, not available.

older age, troponin elevation at admission, higher LDH, and d-dimer levels were risk factors for mortality, whereas female sex and higher lymphocyte count were protective against mortality in the univariate analysis (Table 2). In model 1, after adjusting for sex, BMI, ethnicity, hypertension, diabetes, remdesivir use, and liver diseases, FIB-4 remained to be associated with mortality (adjusted OR [aOR], 1.79; 95% CI, 1.36, 2.35; $P < 0.001$; Table 2). In model 2, the backward stepwise regression model retained FIB-4, sex, remdesivir use, troponin T elevation, and d-dimer level, and FIB-4 remained a significant risk factor for mortality (OR, 1.63; 95% CI, 1.22, 2.17; $P = 0.001$; Table 2). When treated as a categorical variable, FIB-4 > 2.67 was

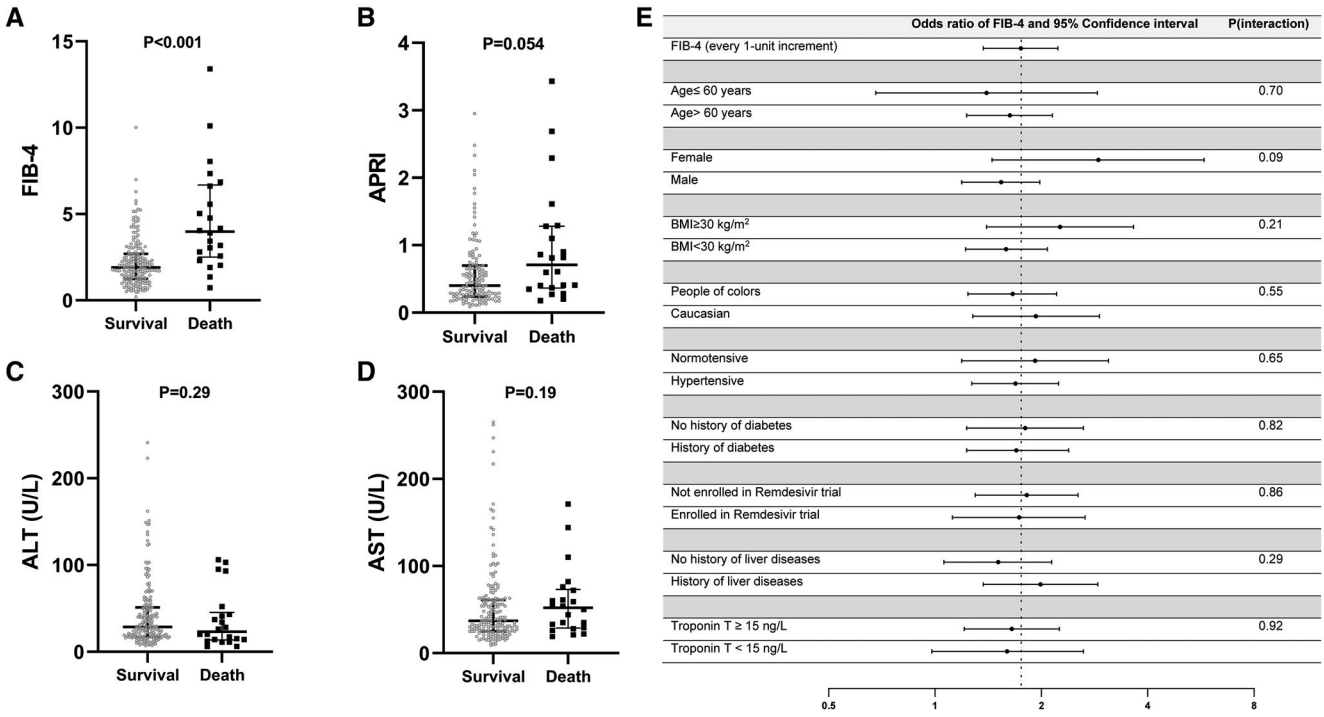

FIG. 1. FIB-4 was associated with death in COVID-19. (A-D) FIB-4 rather than APRI, ALT, or AST was significantly associated with death in COVID-19. (E) The association between FIB-4 and death is consistent across different participant categories.

associated with higher mortality (OR = 7.62 in model 1 [$P < 0.001$] and OR = 6.29 in model 2 [$P = 0.001$]; Supporting Table S1). We also conducted logistic regression analyses stratified by several baseline characteristics and observed no significant interaction between those factors and FIB-4 (Fig. 1E). In another sensitivity analysis assessing the association of FIB-4 and severe diseases (mechanical ventilation and/or death), FIB-4 was significantly associated with severe disease with aOR = 1.37 (95% CI, 1.12, 1.68; $P = 0.002$) in model 1 and 1.26 in model 2 (95% CI, 1.01, 1.58; $P = 0.045$).

We then evaluated the association between FIB-4 and time to events (death). In the group with FIB-4 > 2.67, the median time to events was 40 days, whereas the group with FIB-4 ≤ 2.67 did not reach a median time to events ($P = 0.001$; Fig. 2). In the multivariate Cox proportional hazards model, FIB-4 was again significantly associated with death with an adjusted hazard ratio = 1.46 in model 1 (95% CI, 1.24, 1.70; $P < 0.001$) and 1.33 in model 2 (95% CI, 1.16, 1.52; $P < 0.001$) (Supporting Table S2).

FIB-4 at admission showed good performance in predicting death, with an AUROC of 0.79 (Supporting Fig. S2A). It especially has good performance in participants older than 60 years, with an AUROC of 0.79 (Supporting Fig. S2B,C), better than age, ALT, AST, PLT, age, or troponin levels individually at admission. Its performance is better for those without a diagnosis of liver diseases, with an AUROC of 0.85 (Supporting Fig. S2D).

FIB-4 FAILED TO NORMALIZE IN THE DEATH GROUP

Next, we evaluated longitudinal changes of FIB-4 during hospitalization. In 81 participants from the MassCPR cohort, we observed that FIB-4 peaked during hospitalization ($P < 0.001$ compared with FIB-4 at admission), then fell to a lower level following discharge or death ($P < 0.001$ compared with both FIB-4 admission level and peak level; Fig. 3A), with 71.6% of participants having normalized FIB-4 level (<1.45). When grouped by clinical outcomes, however, FIB-4 in the death group failed to normalize during hospitalization (shaded area indicated by FIB-4 < 1.45), compared with the survival

TABLE 2. ASSOCIATION BETWEEN BASELINE CHARACTERISTICS AND CLINICAL OUTCOME

	Crude OR (95% CI)	*P* Value	Adjusted OR, Model 1* (95% CI)	*P* Value	Adjusted OR, model 2† (95% CI)	*P* Value
FIB-4 (every 1-unit increment)	1.75 (1.37, 2.23)	<0.001	1.79 (1.36, 2.35)	<0.001	1.63 (1.22, 2.17)	0.001
Age (every 10 years increment)	2.12 (1.46, 3.08)	<0.001				
Female sex (male as reference)	0.31 (0.11, 0.87)	0.026	0.37 (0.12, 1.20)	0.10	0.35 (0.11, 1.14)	0.082
BMI ≥ 30 kg/m^2	0.81 (0.33, 1.99)	0.64	1.45 (0.50, 4.20)	0.49		
Ethnicity						
Caucasian	Reference		Reference			
People of color	0.76 (0.31, 1.86)	0.55	0.63 (0.22, 1.81)	0.39		
Hypertension	1.43 (0.56, 3.68)	0.46	0.90 (0.29, 2.82)	0.86		
Diabetes	1.41 (0.58, 3.43)	0.46	1.27 (0.42, 3.88)	0.68		
Enrolled in remdesivir trial	0.50 (0.19, 1.34)	0.17	0.48 (0.16, 1.47)	0.20	0.44 (0.13, 1.41)	0.17
History of liver diseases	1.23 (0.49, 3.11)	0.66	0.75 (0.25, 2.29)	0.61		
Troponin T ≥ 15 ng/L	6.64 (2.46, 17.92)	<0.001			3.78 (1.21, 11.79)	0.022
CRP (every 10-mg/L increment)*	1.02 (0.98, 1.07)	0.36				
Lymphocyte count (every 1,000/uL increment)	0.17 (0.05, 0.58)	0.005				
LDH (every 10-U/L increment)*	1.03 (1.01, 1.05)	0.004				
D-dimer (every 100-ng/mL increment)†	1.05 (1.02, 1.08)	0.004			1.05 (1.00, 1.09)	0.032

*Adjusted for sex, BMI, ethnicity, hypertension, diabetes, remdesivir trial enrollment, and history of liver diseases. Age is part of the FIB-4 score and therefore was not included.
†Backward stepwise regression adjusted for sex, BMI, ethnicity, hypertension, diabetes, remdesivir trial enrollment, history of liver diseases, CRP, lymphocyte count, LDH, and D-dimer. Variable will be eliminated from the model if its *P* value is greater than 0.20 in the backward stepwise regression (n = 188).

group (either with or without mechanical ventilation; Fig. 3B). In the survival group, participants with pre-existing liver diseases (mostly steatosis by imaging findings) were only associated with a nonsignificant increase in FIB-4 during hospitalization, and eventually, most of the participants with or without liver diseases had FIB-4 normalized on discharge (Fig. 3C). Among the 23 participants who did not normalize their FIB-4 to <1.45 on discharge or death, we were able to calculate FIB-4 from 18 participants based on their latest laboratory values before COVID-19. The median FIB-4 was 1.62 (IQR = 1.26, 2.47) before COVID-19 compared with 2.50 (IQR = 1.89, 4.16) on discharge (P = 0.003 using Wilcoxon signed-rank paired sample test). Four of 18 participants (22.2%) had FIB-4 level > 2.67, and 10 of 18 (55.6%) had FIB-4 > 1.45 before COVID-19. Among 8 participants who survived and had follow-up laboratory tests, their median FIB-4 on discharge was 2.01 (IQR = 1.64, 2.62), and during post-hospitalization follow-up, their median FIB-4 further decreased to 1.31 (IQR = 0.99, 1.67; P = 0.093).

FIB-4 WAS ASSOCIATED WITH SARS-CoV-2 RNAemia LEVEL

There are reports that elevated levels of SARS-CoV-2 RNA may be associated with more severe disease and worse outcomes.[9,12,13] In the MassCPR cohort, there were 69 participants with plasma RNA, and 73 had respiratory RNA level (nasopharyngeal swabs, oropharyngeal swabs, and/or sputum) available. FIB-4 level at admission and during sample collection (date when plasma was collected for biorepository purpose) were both correlated with plasma SARS-CoV-2 RNA levels (Spearman rho = 0.41; P < 0.001 in both admission level and sample collection level; Fig. 4A,B). In subgroup analyses, FIB-4 levels at admission and during sample collection were significantly correlated with plasma RNA level in participants with severe disease and participants who survived (Fig. 3A,B). Furthermore, we noted that FIB-4 levels were significantly higher in participants with detectable SARS-CoV-2 RNA in plasma (Fig. 4C,D).

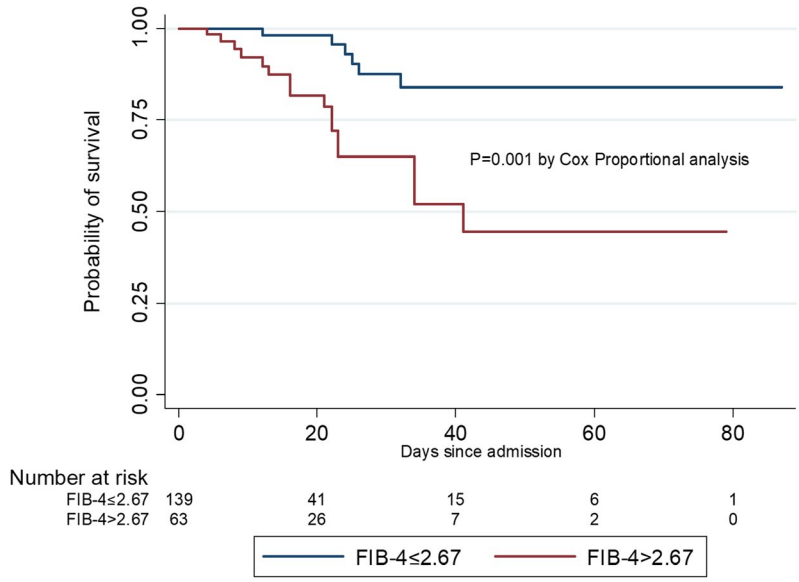

FIG. 2. Probability of survival during hospitalization is based on initial FIB-4 score. *P* value was calculated by Cox proportional model.

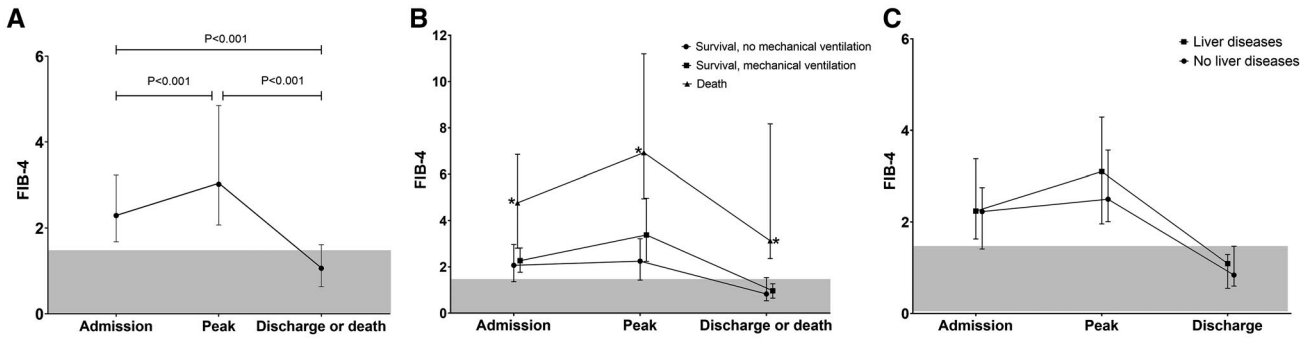

FIG. 3. Longitudinal changes in FIB-4 in MassCPR cohort. (A) FIB-4 peaked during hospitalization and normalized in most participants. (B) In participants with or without mechanical ventilation from the survival group, FIB-4 peaked then normalized during hospitalization. In the death group, FIB-4 was significantly higher than the survival group (*$P < 0.005$) and failed to normalize. (C) Participants with or without liver diseases had similar trajectory in FIB-4 temporal changes. Shaded area indicates FIB-4 < 1.45.

FIB-4 WAS ASSOCIATED WITH LEVELS OF MONOCYTE-ASSOCIATED CYTOKINES

In 66 participants from the MassCPR cohort with available FIB-4 and cytokine levels at the time of enrollment/sample collection, we evaluated the association of FIB-4 and cytokine levels. FIB-4 was positively correlated with the cytokine levels related to monocyte/IFN-I activity, especially IL-6, IP-10, LDH, and MIG (Supporting Fig. S3). There were moderate correlations to anti-inflammatory/tissue repair/fibrogenesis–related cytokines (i.e., HGF and IL-10) (Supporting Fig. S3A). The association between FIB-4 and certain pro-inflammatory cytokines, especially IL-6 and IP-10, were across the board in terms of different disease severity, death/survival, age groups, and presence of RNAemia.

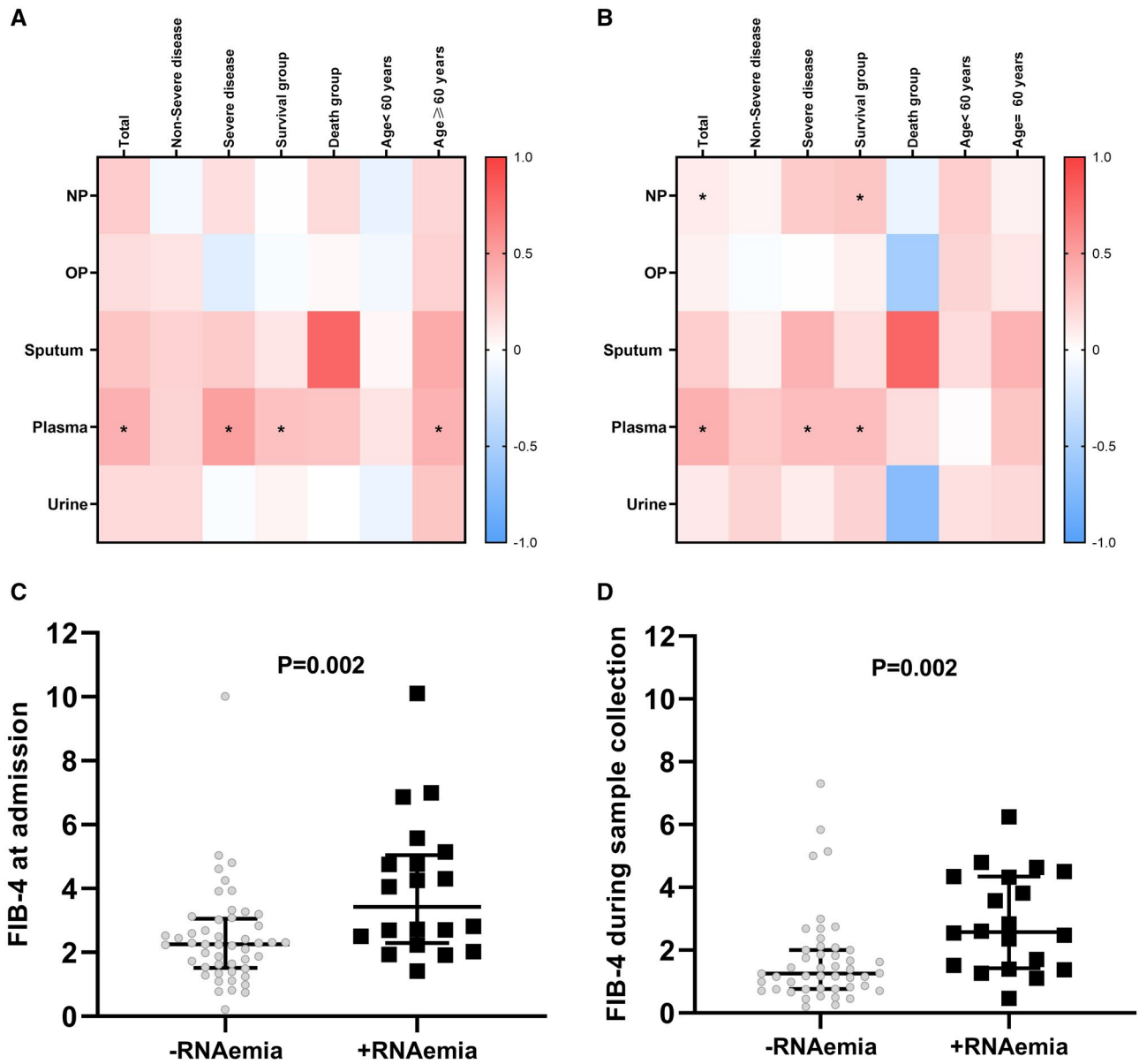

FIG. 4. FIB-4 is associated with SARS-CoV-2 RNAemia. Heatmap summarizing Spearman's rank correlation rho between SARS-CoV-2 RNA level from different body compartments (A) FIB-4 levels at admission. (B) FIB-4 levels during RNA sample collection. Higher FIB-4 levels at admission (C) or RNA sample collection (D) were both associated with SARS-CoV-2 RNAemia. *$P < 0.05$ using Spearman's rank correlation test. NP, nasopharyngeal swab; OP, oropharyngeal swab.

Discussion

In this study, we demonstrated that FIB-4, a simple clinical score derived from routine laboratory values, is significantly associated with COVID-19-related clinical outcomes. This association is likely mediated by COVID-19-related inflammation and potentially direct virological effects, and less so by underlying liver diseases.

In our study, we did not see a statistically significant difference in AST or ALT between the survival and death groups. The nonsignificant difference in median AST between the two groups was 15 U/L (Fig. 1D), which is somewhat consistent with the result from a meta-analysis that shows a 11.7-U/L difference between people who survived and who died.[14] This trivial difference makes AST alone less ideal to predict COVID-19 outcomes. In contrast,

FIB-4 includes AST and age as numerators, which are positively correlated to liver fibrosis[6,10,15]; it also reflects the AST dominant pattern in COVID-19 reported by earlier studies,[4,16] as well as age, which has been shown since early in this pandemic to be one of the strongest indicators of adverse outcomes, including death.[2] These features make the FIB-4 score a better predictor compared with any of its components alone. In contrast, other noninvasive fibrosis scores like APRI fails to incorporate age and reflect the AST > ALT pattern, and therefore did not show a meaningful difference between survival and death groups (Fig. 1B). In terms of its accuracy in predicting COVID-19-related death, FIB-4 has an AUROC of 0.79, which is quite consistent with a recent report from another COVID-19 cohort in North America.[8] Its performance is better than any of its components as well as troponin T level in participants older than 60 years old and those without a diagnosis of liver diseases.

Our results suggest that FIB-4 elevation is likely multifactorial but linked to COVID-19 disease pathogenesis and severity. We speculate that skeletal muscle injury, liver stiffness due to raised pressure in the right heart system, as well as hepatocellular and portal system changes due to SARS-CoV-2 infection and systemic inflammation are all playing a role. First, we noted that AST is moderately correlated to CK levels at admission but not troponin T, indicating that skeletal muscle damage could also be a potential source to fuel this AST elevation and consequently FIB-4 elevation. Second, recent studies have shown that raised right heart pressure is associated with mortality in COVID-19[17,18]; thus, we speculate that this may lead to hepatic congestion, increased liver stiffness, and liver damage. Third, direct virological effects could be an explanation of the persistently elevated FIB-4 in participants who succumbed to COVID-19. We noted that FIB-4 peaked during hospitalization but eventually normalized in most participants who survived, regardless of pre-existing liver diseases. To put this into perspective, even in people with human immunodeficiency virus and viral hepatitis infection (hepatitis B or hepatitis C), the median FIB-4 level is only in the range of 1.00 to 1.80,[19,20] in comparison with the median initial FIB-4 of 1.90 in the survival group from our study. It would be difficult to anticipate that COVID-19 causes this much liver fibrosis in the survival group at the beginning of the disease course, and the fibrosis resolves rapidly following discharge. In fact, even in participants without FIB-4 normalization on discharge, only 22.2% had a pre-existing FIB-4 value above 2.67. In the death group, the failure of FIB-4 normalization can be also be explained by their critical illness (usually associated with liver enzyme elevation, thrombocytopenia) and older age. However, two autopsy studies revealed that early signs of liver fibrosis (i.e., portal fibrosis) are commonly seen in people who succumbed to COVID-19[21,22]; one of them also demonstrated portal dilation, Kupfer cell activation, and detection of SARS-CoV-2 in the portal system from 68% of samples. These pathological findings are consistent with our analyses that the FIB-4 level was correlated to SARS-CoV-2 plasma RNA level as well as monocyte-associated cytokine levels.[23] In summary, it remains unclear whether this represents a pre-existing condition, direct virological effects, response to florid inflammation, or a combination of all of these factors. Further autopsy or pathology studies are warranted to address this issue.

There are several strengths in this study. First, the associations between FIB-4 level on admission and both death and severe diseases offer a timely and inexpensive way to triage patients and predict outcomes. Many of the inflammatory markers and cytokine levels (e.g., IL-6) may take days to a week to come back and are expensive to check in resource-limited settings. We show in this study that FIB-4 scores correlate to many of them, especially cytokine related to monocyte activation and IFN-I, which is cardinal in COVID-19-related inflammation.[24] Second, we demonstrated temporal changes of FIB-4 during hospitalization, especially that FIB-4 levels are elevated in the early phase of admission but normalize in most participants who survive. Third, we were able to demonstrate that the FIB-4 level was associated with plasma levels of SARS-CoV-2 RNA and monocyte/IFN-I-associated cytokine profile, indicating that either virological effects and/or inflammation may play a role in the elevation of FIB-4.

There are some limitations to our study. First, it is unknown how the FIB-4 would predict outcomes in those with pre-existing conditions, which may severely skew AST, ALT, and PLT, as we excluded participants with decompensated cirrhosis and recent chemotherapy in our analyses. Second, we lack elastography and/or

liver biopsy results to confirm that FIB-4 elevation was truly related to liver stiffness or fibrosis; further prospective studies are warranted to evaluate this. Third, we have a limited sample size; thus, it was not practical for us to fully adjust for all of the baseline characteristics that may confound the results. Despite this, we generated two multivariate regression models to account for most of the confounders (e.g., sex, hypertension, BMI, cardiac injury), while balancing the risk of having colinearity. In addition, most participants in our study are overweight or obese, with a diagnosis of hypertension; therefore, it is difficult to single out hypertension or obesity as risk factors for unfavorable outcomes, as reported in other studies.[2]

In conclusion, our study showed that the simple FIB-4 scoring system can predict COVID-19-related mortality, and this association may be mediated by SARS-CoV-2-associated damage and monocyte-associated cytokines. Further studies are warranted to validate this finding in larger prospective cohorts, ideally including elastography evaluation, and to evaluate detailed mechanisms at the tissue level.

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