

## Risk Factors of Viral RNAemia and Its Association With Clinical Prognosis Among Patients With Severe COVID-19



### To the Editor:

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses a great threat to public health worldwide. Approximately 5% of patients are complicated with critical illness, with some of them experiencing multi-organ dysfunction and even death.<sup>1,2</sup>

One of the possible pathogenesis of multi-organ dysfunction in critical COVID-19 is the direct attack of SARS-CoV-2 in multiple organs. Live virus particles have been isolated from multiple sample types of specimens including stool,<sup>3</sup> urine,<sup>4</sup> and respiratory

specimens. However, how the virus further infects kidney and other organs after infecting humans through the respiratory tract remains unknown. One possible explanation is the spread of virus through blood, because viral RNA has been detected in blood of patients with COVID-19,<sup>5</sup> though live virus particle has not been isolated successfully. In theory, patients with viremia are more prone to multiple organ involvement and worse outcome. However, epidemiologic evidence about hypotheses of these associations and of the risk factors of viral RNAemia is lacking.

The aim of this study was to explore the dynamic features of viral RNA in blood and risk factors of viral RNAemia and the association between RNAemia and prognosis among 192 patients with severe COVID-19, with longitudinal blood specimens collected from Lopinavir Trial for Suppression of SARS-Cov-2 (LOTUS) China trial.

### Methods

This study was a post hoc analysis of data prospectively collected from 199 patients aged  $\geq 18$  years old with pneumonia caused by SARS-CoV-2 enrolled in the randomized, controlled trial, LOTUS China.<sup>6</sup> Plasma specimens, oropharyngeal swabs, and anal swabs of patients with COVID-19 were collected on days 1, 5, 10, 14, 21, and 28 after enrollment or until discharge or death. Detection of viral RNA was described previously.<sup>6</sup> E gene, RdRp gene, and N gene of SARS-CoV-2 were tested qualitatively; only E genes were detected quantitatively. Limit of detection was 1000 copies/mL. Viral RNAemia was defined as a positive result by real-time reverse transcriptase-polymerase chain reaction for E gene, RdRp gene, or N gene in the plasma samples at any time point. Disease severity of the patients was evaluated according to the seven-category ordinal scale.<sup>7,8</sup>

The Mann-Whitney *U* tests and  $\chi^2$  tests were used for comparisons of viral load and positive rate of RNAemia among patients who survived and died at different time points after illness onset, respectively. Bonferroni correction was used to determine statistical significance ( $P = .05/7 = .007$ ) for multiple tests at different time points. Spearman correlation analysis was performed to assess the association of viral load in throat and anal swabs with that in blood. Factors associated with viral RNAemia and associations between viral RNAemia with clinical outcomes were analyzed with the use of multivariable adjusted logistic regression models. Multivariable adjusted logistic regression models were constructed according to results from univariable analysis combined with our understanding about potential risk factors biologically associated with RNAemia. The tests were two-sided; a probability value of  $<.05$  was considered statistical significance. All results were analyzed with SAS software (version 9.4; SAS Institute, Inc).

### Results

Of the 199 patients in the LOTUS study, seven cases were excluded due to the lack of viral RNA information in the blood. Of the 192 patients enrolled in this study, 71 (37%) were confirmed with viral RNAemia via reverse transcriptase-polymerase chain reaction. SARS-CoV-2 viral RNA was first detected in plasma of the patients with COVID-19 at a mean time of 12.1 days after disease onset, with no significant difference observed between those who died and those who

survived ( $11.6 \pm 4.0$  d vs  $12.5 \pm 3.8$  d;  $P = .339$ ) (Fig 1A). Viral nucleic acid was detected as early as 5 days after disease onset and remained positive as long as 30 days after disease onset in the plasma of some patients (Fig 1A). Initial viral load in the plasma of the 71 patients ranged from 3.37 to 6.63 log<sub>10</sub> copies/mL. Positive rate of viral RNAemia was higher in deceased group before day 14 after illness onset, compared with the group who survived (Fig 1B). Viral load in plasma of the patients with COVID-19 who died was slightly

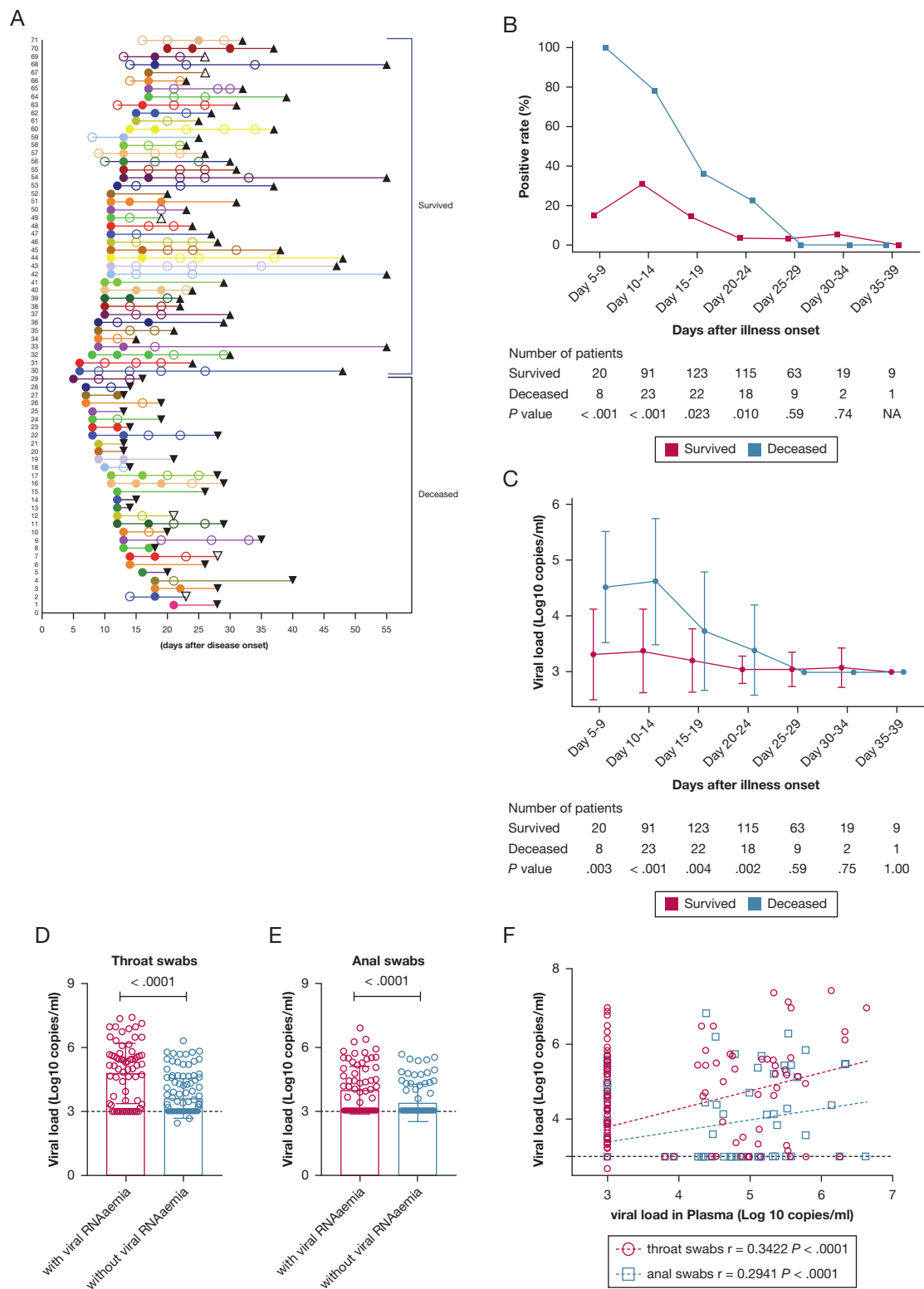


Figure 1 – A-F, Severe acute respiratory syndrome coronavirus 2 viral RNA detected via reverse transcription-polymerase chain reaction in plasma of the patients with coronavirus disease 2019 and viral RNAemia. A, Each line represents a single viral RNAemia positive patient, filled circles refer positive viral nucleic acids in plasma, and open circles represent no detectable viral RNA in plasma of patients with coronavirus disease 2019. Black up-pointing triangle ( $\Delta$ ) represents the time point of discharge. White up-pointing triangle ( $\Delta$ ) represents the time point of discharge and no detectable viral RNA in plasma. Black down-pointing triangle ( $\blacktriangledown$ ) represents the time of death. White down-pointing triangle ( $\blacktriangledown$ ) represents the time of death and no detectable viral RNA in plasma. B, Positive rate and C, dynamic change of viral load in plasma detected via reverse transcriptase-polymerase chain reaction among 192 patients with coronavirus disease 2019. The change of viral load and positive rate of RNAemia were shown with time interval as 5 days (days after illness onset: day 5-9; day 10-14; day 15-19; day 20-24; day 25-29; day 30-34; day 35-39). Difference of viral load in D, throat swabs and E, anal swabs between the patients with viral RNAemia and patients without viral RNAemia. F, Correlation between viral RNAemia and viral load in throat swabs and anal swabs. The dash line represents limit of detection.

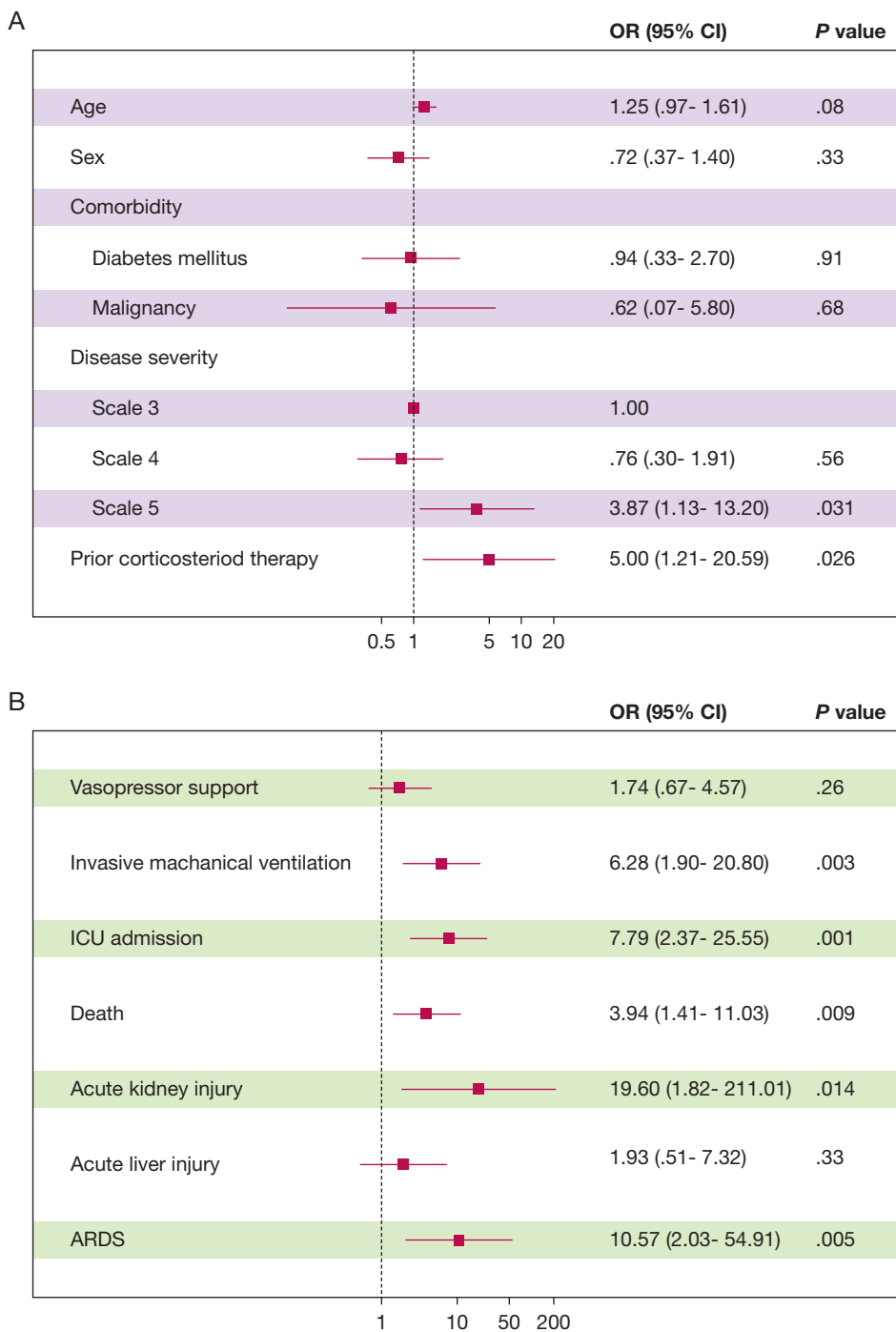


Figure 2 – A, Risk factors associated with viral RNAemia in patients with severe coronavirus disease 2019. Age, sex, comorbidity (diabetes mellitus and malignancy), disease severity (seven-category scale at day 1 after being enrolled into lopinavir-ritonavir trial), and corticosteroids treatment before hospitalization were included in the model. Prior corticosteroid therapy for underlying diseases refers to therapy with corticosteroids dosage  $\geq 15$  mg of prednisone per day for at least 30 continuous days before the onset of illness. B, Association of viral RNAemia with prognosis of patients with severe coronavirus disease 2019. Adjusted for age, sex, seven-category scale at baseline (day 1 after being enrolled into lopinavir-ritonavir trial), comorbidity, duration from illness onset to admission, antiviral treatment during hospitalization, and corticosteroid treatment before and during hospitalization. Acute kidney injury was identified according to Kidney Disease Improving Global Outcomes clinical practice guideline for acute kidney injury. Acute liver injury was considered as the levels of serum alanine aminotransferase above 3-fold of the upper limit of normal. ARDS was defined according to the interim guidance of the World Health Organization for severe acute respiratory syndrome coronavirus 2.

higher than that of the patients who survived before day 25 after illness onset (Fig 1C). Baseline viral load in throat swabs ( $4.8 \pm 1.4$  vs  $3.6 \pm 0.9$  Log<sub>10</sub> copies/mL;

$P < .001$ ) and anal swabs ( $3.9 \pm 1.1$  vs  $3.3 \pm 0.9$  Log<sub>10</sub> copies/mL;  $P < .001$ ) were higher in patients whose condition was complicated with viral RNAemia than in

those without. Further correlation analysis shown that viral load in plasma was associated positively with the baseline viral load in both throat swabs ( $r = 0.34$ ;  $P < .001$ ) and anal swabs ( $r = 0.29$ ;  $P < .001$ ) (Fig 1D-F).

Disease severity at baseline and prior corticosteroid therapy for underlying diseases were independent risk factors for the development of viral RNAemia in patients with COVID-19 (Fig 2A). Compared with patients with a seven-category scale score of 3 at day 1 after being enrolled, multivariable adjusted ORs and 95% CIs for patients with a seven-category scale score of 4 and 5 were 0.76 (95% CI, 0.30-1.91) and 3.87 (95% CI, 1.13-13.20), respectively. Long-term prior corticosteroid therapy before hospitalization was associated with higher risk of viral RNAemia (OR, 5.00; 95% CI, 1.21-20.59).

After multivariable adjustment for age, sex, and seven-category scale at baseline, comorbidity, duration from illness onset to admission, antiviral treatment during hospitalization, and corticosteroid treatment before and during hospitalization, viral RNAemia was found to be associated with increased risk of invasive mechanical ventilation, ICU admission, and in-hospital death among patients with severe COVID-19. RNAemia was also found to be associated with acute kidney injury and ARDS, with the risk of >10 fold among patients with viral RNAemia compared with those without RNAemia (Fig 2B).

## Discussion

In this study with 192 patients with severe COVID-19 prospectively included and longitudinal specimens collected, we found 37% of patients with severe COVID-19 who were hospitalized with pneumonia showed detectable SARS-CoV-2 viral RNA in plasma. Compared with patients COVID-19 without viral RNAemia, those with viral RNAemia have a higher risk for invasive mechanical ventilation support, ICU admission, and in-hospital death. Multiorgan dysfunction was also much more prominent in the patients with viral RNAemia. Possible mechanisms for the worse clinical outcomes in patients with COVID-19 with viral RNAemia include direct attacks by the virus in extrapulmonary organs and immunopathology induced by cytokine storm.<sup>9</sup>

This study elucidated that higher viral loads in respiratory specimens and anal swabs were associated positively with that in plasma, which indicated that, for sites without the ability to test viral RNA in blood, viral

load in respiratory specimens and anal swabs could indicate the possibility of viral RNAemia. However, the indication may be limited because the correlation was not strong. Furthermore, because no plaque assay was performed to confirm the presence of live virus particles, it still needs further investigation to determine whether the virus could be disseminated via blood.

To our knowledge, there are no reports about the risk factors of viral RNAemia in patients with COVID-19. As a universal immune-modulating agent, corticosteroid has been considered for severely ill patients with respiratory virus infections. However, the use of corticosteroids for patients with severe respiratory infection has always been controversial for its adverse effect on delaying viral shedding and increasing the risk of secondary infection.<sup>10</sup> Corticosteroid use was not found to be associated with the increased occurrence of viral RNAemia in patients with COVID-19. However, prior corticosteroids therapy for underlying diseases was found to be an independent risk factor for viral RNAemia in patients with COVID-19.

In conclusion, our study revealed that a high percentage of patients with severe COVID-19 complicated with viral RNAemia is significantly associated with worse outcomes. Patients with more severe baseline disease condition and prior corticosteroid therapy for underlying diseases have higher risk of experiencing the development of viral RNAemia. Our data benefit a better understanding of pathogenesis of COVID-19 and provide a basis for the early identification and treatment of critically ill patients.

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