Virology Journal

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SARS-CoV-2 RNAemia as a reliable predictor of long-term mortality among older adults hospitalized in pulmonary intermediate care units: a prospective cohort study

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Abstract

Background SARS-CoV-2 viremia is associated with disease severity and high risk for in-hospital mortality. However, the impact of SARS-CoV-2 viremia on long-term outcomes in hospitalized patients with COVID-19 is poorly understood.

Methods We conducted a prospective cohort study and recruited a group of older adult patients with COVID-19 admitted to pulmonary intermediate care units of Peking University Third Hospital during December 2022 and January 2023. The plasma level of SARS-CoV-2 RNA was determined by a standardized RT-PCR technique, and SARS-CoV-2 RNAemia was defined as a plasma viral load ≥ 50 copies/ml. In-hospital and follow-up (180-day) outcome data were collected.

Results A total of 101 patients with an average of 80.4 years were recruited, and 63.4% of them were severe or very severe cases. Twenty-eight patients (27.7%) had SARS-CoV-2 RNAemia, with a median viral RNA load of 422.1 [261.3, 1085.6] copies/ml. Patients with SARS-CoV-2 RNAemia were more likely to develop critical cases and had a higher incidence of sepsis. Accordingly, they had a higher 180-day mortality (57.1% vs. 19.7%, $P < 0.001$), as well as in-hospital mortality (50.0% vs. 13.7%, $P < 0.001$), independent of age, disease severity, sepsis, lymphocyte count and C-Reactive protein. In addition, the risk for 180-day mortality increased with the SARS-CoV-2 RNA load in plasma. Plasma cytokines, including IL-6, IL-8 and IL-10, were higher in patients with SARS-CoV-2 RNAemia.

Conclusions Our study indicates that SARS-CoV-2 RNAemia serves as a useful biomarker for predicting mortality, especially long-term mortality, in older adult patients hospitalized in pulmonary intermediate care units.

Trial registration Chinese Clinical Trial Registry website (No. ChiCTR2300067434).

Keywords COVID-19, SARS-CoV-2, Viremia, RNAemia, Mortality

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Background

Coronavirus disease 2019 (COVID-19) can progress to severe and critical illness among older adults infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), especially in those with underlying comorbidities. Studies show that SARS-CoV-2 viremia is an important predictor for disease severity and

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associated with poor outcomes in hospitalized patients with COVID-19. SARS-CoV-2 RNA could be detectable in 19.5–48% of hospitalized patients [1–6], namely SARS-CoV-2 RNAemia, which was even higher in patients requiring ICU hospitalization [7]. Also, SARS-CoV-2 virions could be detectable by electron microscopy in most of patients with SARS-CoV-2 RNAemia, which was at least partly explained by viremia [8]. SARS-CoV-2 RNAemia or viremia was associated with severity of COVID-19 and increased risk of in-hospital mortality [1, 2, 7–9], as well as serving as a good biomarker for rapid deterioration of COVID-19 [10]. Patients with SARS-CoV-2 RNAemia required more frequent oxygen administration, intensive care unit (ICU) admission and invasive mechanical ventilation [6]. Prolonged viral clearance in blood increased the risk of mortality in hospitalized patients [11]. Persistent viremia, defined as two or more consecutive positive SARS-CoV-2 RNA in blood samples, could lead to more ICU admission and death in-hospital [12]. In addition, patients with SARS-CoV-2 RNAemia had higher levels of C-Reactive protein (CRP), Interleukin (IL)-6 and lactic dehydrogenase (LDH), indicating more intensive systemic inflammation [3, 7]. SARS-CoV-2 viremia could also result in other extrapulmonary complications, such as increased prevalence of myocardial injury [4]. SARS-CoV-2 viremia was more likely to occur in patients with hematologic malignancies and resulted in poor prognosis [2, 13]. SARS-CoV-2 viral load could help clinicians in the risk stratification of those with COVID-19 [1].

Nevertheless, previous studies mainly focused on the in-hospital or short-term outcomes among patients with SARS-CoV-2 RNAemia. The association between SARS-CoV-2 RNAemia or viremia and long-term outcomes after discharge from hospitalization was not clear.

Since the strict measures for COVID-19 control were lifted in mainland China in early December 2022, a large number of older adult patients with severe or critical COVID-19 were admitted to the hospital, and some of them required advanced respiratory supports such as high-flow nasal cannula and/or non-invasive positive pressure ventilation in pulmonary intermediate care units set up in accordance with the international specialist consensus and experience [14, 15], during a surge of COVID-19 when intensive care resources were limited. Therefore, we took this opportunity to prospectively evaluate the predictive capacity of SARS-CoV-2 RNAemia, as well as viral RNA load, for in-hospital and long-term mortality among older adults with COVID-19 hospitalized in pulmonary intermediate care units.

Methods

Study design and subject enrollment

We conducted a prospective cohort study from December 2022 to January 2023 and consecutively enrolled the patients who were hospitalized in pulmonary intermediate care units of Peking University Third Hospital. The aim of establishing pulmonary intermediate care unit was to prevent COVID-19 patients with moderate to severe hypoxemia from ICU admission or invasive mechanical ventilation, as well as preserving ICU capacity and beds for patients with more critical illness [14]. All patients in this study met the diagnosis criteria of the 10th guideline for the diagnosis and treatment of COVID-19 from the National Health Commission of the People's Republic of China [16]. Diagnosis of COVID-19 was confirmed by positive RT-PCR for SARS-CoV-2 in pharyngeal swabs or positive antigen detection for SARS-CoV-2 in nasal swabs. Patients' treatment and management was according to the same guideline.

Patients were classified into four severity degrees according to the guideline [16]: mild cases were those with relevant clinical symptoms without lung involvement on chest CT scan; moderate cases were those with relevant clinical symptoms and typical signs of viral pneumonia on CT imaging, but without a respiratory rate $\geq$ 30 breaths/min or oxygen saturation $\leq$ 93% at rest; severe cases were defined as those who met any of the following criteria: (1) respiratory distress with a respiratory rate $\geq$ 30 breaths/min; (2) oxygen saturation $\leq$ 93% at rest on room air; (3) $PaO_2/FiO_2 \leq 300$ mmHg; (4) the clinical symptoms gradually worsened, and CT imaging showed significant progression of the lesion within 24–48 h, with a rate of > 50%. Very severe cases were defined as those who met any of the following criteria: (1) respiratory failure requiring mechanical ventilation; (2) shock.

The study protocol had been registered on the Chinese Clinical Trial Registry website (ChiCTR2300067434). This study was approved by the Ethics Committee of Peking University Third Hospital (M2023006) and consent was obtained from the patients or their close relatives. Data were analyzed anonymously.

Clinical data collection

Demographic data and major comorbidities (including hypertension, coronary heart disease, chronic liver disease and chronic obstructive pulmonary disease) were collected. Information of COVID-19-related treatments including antiviral agents, anti-inflammatory therapies, and anticoagulation were also collected.

Clinical laboratory measurements on admission were recorded, including blood white blood cells (WBC), lymphocytes, neutrophils, platelets, hemoglobin, C-Reactive protein (CRP), D-Dimer, fibrinogen, albumin, alanine

aminotransferase (ALT), aspartic acid transferase (AST), bilirubin, creatine kinase (CK) and its isozyme CKMB, creatinine, and N-terminal pro-brain natriuretic peptide (NT-proBNP).

Plasma cytokines on admission including interleukin (IL)-1β, IL-6, IL-8, IL-10, interferon (IFN)-α, IFN-γ and tumor necrosis factor (TNF)-α were determined based on immunofluorescence assay, using the multi-cytokine detection kit (Cellgene Biotech, Hangzhou, CN).

Follow-up

Patients discharged from hospital were interviewed in our outpatient department or via telephone call at approximately 30-day, 90-day and 180-day after they were recruited in our study.

Outcomes

The primary outcome of our study was 180-day mortality. The secondary outcomes included sepsis during hospitalization, length of stay in hospital, in-hospital mortality, 30-day and 90-day mortalities. Diagnosis of sepsis was made if a patient had organ dysfunction caused by a dysregulated host response to COVID-19, manifested as an increase in the Sequential Organ Failure Assessment (SOFA) of 2 points or more [17].

Blood sample collection and RT-qPCR for SARS-CoV-2 RNA quantification

Peripheral blood samples were collected on admission or the first day of hospitalization. Within 2 h, the samples were centrifuged at 2000 g for 10 min and then the plasma on the top layer of the EDTA Vacutainer tubes (BD, NJ, USA) was aliquoted and stored at −80 °C for further analysis.

RNA copy number quantification standards were constructed from SARS-CoV-2 pseudoviruses subjected to absolute copy number quantification by digital PCR (Bio-Rad). RNA was isolated from 0.5ml plasma from COVID-19 patients and from quantitative standard pseudovirus samples, respectively, using the QIAamp ViralRNA Mini kit (Qiagen, Valencia, CA). RNA concentration was measured using a Qubit fluorometer (Thermo Fisher Scientific, Carlsbad, CA). Negative control samples, RNA from clinical samples and quantitative standards were performed simultaneously using an ABI-7500 Real-Time PCR system (Thermofisher Scientific, Carlsbad, CA) and a clinically validated SARS-CoV-2 Nucleic Acid Detection Kit (Daan Co., Ltd, Guangzhou, CN), and a Ct value lower than 40 was regarded as positive. Based on the gradient dilution of the quantitative standards, the lowest detection limit of the SARS-CoV-2 reagent used in this study was 50 copies/ml. The RNA load of SARS-CoV-2 in clinical samples was calibrated using the RNA load standard curve established by the quantitative standards.

The plasma level of SARS-CoV-2 RNA load were stratified as negative (< 50 copies/ml), RNA load 50–1000 copies/ml and RNA load ≥ 1000 copies/ml.

Statistical analysis

Statistical analyses were performed with SPSS software, version 23.0. Continuous variables following a normal distribution were recorded as mean ± standard deviation and unpaired *t*-test was used to assess the differences between groups. Continuous variables not following a normal distribution were presented as median [interquartile ranges (IQR)] and Mann–Whitney *U* test were used for difference evaluation. Categorical variables were presented as numbers (%) and Chi-square or Fisher exact test was used for difference comparation.

Binary logistic regression model was performed to determine the independent association between SARS-CoV-2 RNAemia and in-hospital mortality and variables included in the model had a $P < 0.1$ in univariate analysis.

Kaplan–Meier curve was used to evaluate the differences of cumulative survival rate between patients with and without SARS-CoV-2 RNAemia and among different viral RNA load stratification. For further assessing the independent predictive capacity of SARS-CoV-2 RNAemia for in-hospital mortality and 180-d mortality, Cox regression model were performed, in which the variables with $P < 0.1$ in univariate analysis were included.

P-value < 0.05 was considered statistically significant. All data were analyzed anonymously. Clinical Epidemiological Research Center of Peking University Third Hospital were responsible for data quality control.

Results

Baseline characteristics of the patients

A total of 101 patients with confirmed COVID-19 were recruited in this study during the study period, all of whom were tested for SARS-CoV-2 RNA in plasma. There were 57 (56.4%) male and 44 (43.6%) female patients, with an average age of 80.4 years, ranging from 55 to 102 years. Thirty-seven (36.6%) cases were defined as moderate, while 50 (49.5%) and 14 (13.9%) as severe and very severe in disease severity, respectively. Twenty-eight (27.7%) patients had a positive SARS-CoV-2 RNA in plasma and therefore were regarded as having SARS-CoV-2 RNAemia, with a median viral RNA load of 422.1 [261.3, 1085.6] copies/ml. Among the patients with SARS-CoV-2 RNAemia, 7 had a viral RNA load in plasma ≥ 1000 copies/ml, while 21 patients had a viral RNA load of 50–1000 copies/ml.

Table 1 showed the demographic and clinical characteristics of our patients. The age, sex, BMI, smoking

Table 1 Demographic and clinical characteristics between patients with and without SARS-CoV-2 RNAemia

	Overall (n = 101)	SARS-CoV-2 RNAemia (−) (n = 73)	SARS-CoV-2 RNAemia (+) (n = 28)	*P* value
Age (years)	80.4 (11.3)	79.9 (11.9)	81.6 (9.4)	0.421
Male (%)	57 (56.4%)	38 (52.1%)	19 (67.9%)	0.152
BMI (kg/m^2)	23.8 (4.1)	23.9 (4.2)	23.6 (3.8)	0.730
Smoking status				0.495
Never-smoker	67 (66.3%)	46 (63.0%)	21 (75.0%)	
Ever-smoker	18 (17.8%)	14 (19.2%)	4 (14.3%)	
Current-smoker	16 (15.8%)	13 (17.8%)	3 (10.7%)	
Hypertension	53 (52.5%)	39 (53.4%)	14 (50.0%)	0.758
Coronary heart disease	23 (22.8%)	15 (20.5%)	8 (28.6%)	0.389
Chronic kidney disease	6 (5.9%)	5 (6.8%)	1 (3.6%)	1.000
COPD	11 (10.9%)	10 (13.1%)	1 (3.6%)	0.144
COVID-19 vaccination				0.221
0-dose	75 (74.3%)	52 (71.2%)	23 (82.1%)	
1-dose	0 (0.0%)	0 (0.0%)	0 (0.0%)	
2-dose	12 (11.9%)	9 (12.3%)	3 (10.7%)	
3-dose	14 (13.9%)	12 (16.4%)	2 (7.1%)	
WBC ($\times 10^9$/L)	7.80 (3.56)	7.86 (3.80)	7.62 (2.88)	0.762
Lymphocyte ($\times 10^9$/L)	0.80 (0.55)	0.88 (0.60)	0.60 (0.30)	0.003
Neutrophil ($\times 10^9$/L)	6.54 (3.28)	6.49 (3.47)	6.67 (2.74)	0.800
Platelet ($\times 10^9$/L)	214.4 (82.9)	227.9 (89.0)	179.1 (57.1)	0.001
C-Reactive protein (mg/L)	62.0 (53.0)	53.3 (49.0)	84.5 (51.3)	0.007
D-Dimer (μg/ml)	4.30 (2.36)	1.98 (3.39)	3.36 (6.03)	0.261
Fibrinogen (g/L)	4.03 (1.26)	4.01 (1.35)	4.10 (0.99)	0.695
ALT (U/L)	25.0 [16.5, 41.5]	24.0 [15.5, 44.5]	25.5 [20.0, 40.0]	0.587
AST (U/L)	30.0 [22.5, 48.5]	28 [21.5, 41.5]	47.0 [27.8, 61.0]	0.006
Bilirubin (μmol/L)	11.2 [8.3, 14.8]	11.2 [8.5, 14.0]	11.3 [7.1, 16.9]	0.826
Albumin (g/L)	31.7 [29.6, 34.4]	32.5 (4.6)	30.8 (3.4)	0.089
CK (U/L)	52.0 [32.5, 105.0]	47.0 [30.0, 99.0]	79.0 [50.8, 148.5]	0.015
CKMB (U/L)	9.0 [7.0, 12.8]	8.5 [6.3, 12.0]	10.0 [7.0, 14.0]	0.288
Creatinine (μmol/L)	74.0 [60.0, 95.0]	70.0 [59.0, 92.0]	79.5 [65, 111.5]	0.137
NT-proBNP (pg/ml)	975.0 [279.5, 2384.0]	867.0 [230.5, 2113.5]	1333.0 [433.3, 2643.3]	0.088
Drug Treatments				
Systemic glucocorticoids	84 (83.2%)	58 (79.5%)	26 (92.9%)	0.142
Tocilizumab	11 (16.8%)	4 (5.5%)	7 (25.0%)	0.009
Baricitinib	20 (19.8%)	14 (19.2%)	6 (21.4%)	0.799
Nirmatrelvir/Ritonavir	17 (16.8%)	10 (13.7%)	7 (25.0%)	0.234
Azvudine	32 (31.7%)	19 (26.0%)	13 (46.4%)	0.049

Data were presented as Mean (SD), Median [IQR] or n (%)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BMI, body mass index; COPD, chronic obstructive pulmonary disease; ALT, alanine aminotransferase; AST, aspartate transaminase; CK, creatine kinase; CKMB, creatine kinase isoenzyme MB; NT-proBNP, N-terminal pro-brain natriuretic peptide

status, major comorbidities, and COVID-19 vaccination were not different between patients with and without SARS-CoV-2 RNAemia. Those with SARS-CoV-2 RNAemia had lower lymphocyte and platelet counts and a higher level of CRP. Blood biochemical measurements were comparable between the two groups, except for AST and CK.

Use of tocilizumab and azvudine was more frequent in patients with SARS-CoV-2 RNAemia. Other medications, including systemic glucocorticoids, baricitinib, and nirmatrelvir/ritonavir, were comparable between groups.

SARS-CoV-2 RNAemia and disease severity, in-hospital and follow-up outcomes

Plasma SARS-CoV-2 RNAemia was associated with increased disease severity among our patients, as 82.1% of patients with SARS-CoV-2 RNAemia were severe or very severe cases compared to 56.2% of those without SARS-CoV-2 RNAemia. Sepsis occurred more often in patients with SARS-CoV-2 RNAemia. In the patients who were discharged from hospital, SARS-CoV-2 RNAemia was associated with a prolonged length of stay in the hospital. Furthermore, patients with SARS-CoV-2 RNAemia had a higher 180-d mortality, as well as in-hospital mortality, 30-d and 90-d mortality, compared to those without SARS-CoV-2 RNAemia (Table 2). Kaplan–Meier curve analysis showed that cumulative survival rate of 180-day in patients with SARS-CoV-2 RNAemia was significantly lower, with Log-Rank $P<0.001$ (Fig. 1).

The following measurements were associated with in-hospital mortality when comparing the survivors and non-survivors: age (77.8 ± 11.5 years vs. 85.8 ± 8.7 years, $P=0.007$), lymphocyte count ($0.87\pm0.60\times10^9$/L vs. $0.58\pm0.25\times10^9$/L, $P=0.001$), platelet count ($224.4\pm85\times10^9$/L vs. $182.2\pm67.9\times10^9$/L, $P=0.029$), CRP (51.7 ± 47.3 mg/L vs. 90.1 ± 57.4 mg/L, $P<0.001$), sepsis (62.3% vs. 83.3%, $P=0.056$), severe and very severe illness (55.8% vs. 87.5%, $P=0.005$) and SARS-CoV-2 RNAemia (18.2% vs. 58.3%, $P<0.001$). These variables were included in the multivariate analysis. The gender proportion, smoking exposure, comorbidities and COVID-19 vaccination status were not different between survivors and non-survivors.

In the binary logistics regression model, SARS-CoV-2 RNAemia was independently associated with a higher in-hospital mortality, with odds ratio (OR) 4.909 (95% confidence interval (CI) 1.485–16.221), adjusted for age, severity of COVID-19, sepsis, lymphocyte and platelet count, and CRP (Table 3). In addition, we analyzed the association between SARS-CoV-2 RNAemia and 180-d mortality, using Cox regression model. After adjustment by age, severity of COVID-19, sepsis, lymphocyte

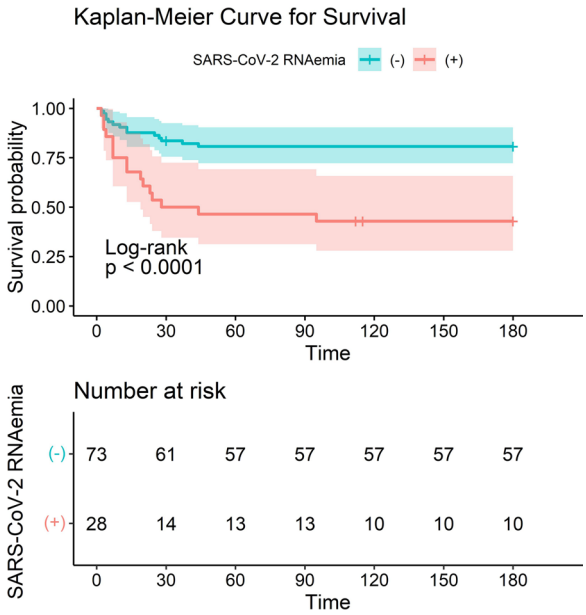

Fig. 1 Comparison of 180-d mortality between SARS-CoV-2 RNAemia (+) and (–). Kaplan-Meier survival curve analysis was performed and the Log Rank $P < 0.001$. Upper Panel: Displays the Kaplan-Meier survival curve, illustrating the survival probabilities over time for patients with SARS-CoV-2 RNAemia and those without. Lower Panel: Presents the number of patients alive at each specified time point during the follow-up period for both the RNAemia (+) and RNAemia (–) groups

Table 2 SARS-CoV-2 RNAemia and disease severity and prognosis

	SARS-CoV-2 RNAemia (–) (n = 73)	SARS-CoV-2 RNAemia (+) (n = 28)	*P* value
Severity			0.015
Moderate	32 (43.8%)	5 (17.9%)	
Severe + Very severe	41 (56.2%)	23 (82.1%)	
Primary outcome			
180-day mortality [a]	14 (19.7%)	16 (57.1%)	< 0.001
Secondary outcomes			
Sepsis	44 (60.3%)	24 (85.7%)	0.015
Length of hospitalization (d)[b]	8.4 (4.3)	16.4 (10.0)	0.008
In-hospital mortality	10 (13.7%)	14 (50.0%)	< 0.001
30-day mortality	12 (16.4%)	14 (50.0%)	0.001
90-day mortality[a]	14 (19.7%)	15 (53.6%)	0.001

Data were presented as n (%)

[a] Two patients were missing during the follow-up period

[b] Analyzed in the discharged alive patients (n = 77)

Table 3 Univariate and multivariate analyses for the association between in-hospital mortality and SARS-CoV-2 RNAemia and other factors

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*	aOR	95% CI	*P*
SARS-CoV-2 RNAemia	6.300	2.325–17.073	< 0.001	4.909	1.485–16.221	0.009
Age	1.082	1.021–1.147	0.008	1.123	1.042–1.211	0.002
Severity of COVID-19	5.535	1.523–20.120	0.009	3.947	0.913–17.060	0.066
Sepsis	3.021	0.939–9.717	0.064			
Lymphocyte	0.218	0.056–0.848	0.028			
Platelet	0.993	0.987–0.999	0.033			
C-Reactive protein	1.015	1.006–1.025	0.001	1.018	1.005–1.211	0.006

Binary logistics regression model was performed to evaluate the association between the risk factors and in-hospital mortality. The measurements with $P < 0.1$ in univariate analysis were shown in this table and included in the multivariate model for final analysis

OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio

and platelet count, and CRP, SARS-CoV-2 RNAemia remained an independent factor to predict death in 180 days, with the hazard ratio (HR) 2.749 (95% CI 1.315–5.747) (Table 4).

SARS-CoV-2 RNAemia load and disease severity, in-hospital and follow-up outcomes

The in-hospital mortality increased with the increased stratification of SARS-CoV-2 RNA load, with 13.7%, 47.6% and 57.1% ($P = 0.001$), respectively. Similarly, the same trends could be observed in the 180-d mortality, with 19.7%, 57.1%, 57.1%, respectively (Fig. 2). We also used the binary logistic regression and Cox regression models to assess the associations between SARS-CoV-2 RNAemia load and in-hospital mortality, as well as 180-d mortality. After adjusting for age, severity of illness, sepsis, counts of lymphocyte and platelet, and CRP, SARS-CoV-2 RNA load 50–1000 copies/ml was independently associated with in-hospital mortality, while SARS-CoV-2 RNA load > 1000 copies/ml did not remain this association (Supplementary Table 1). For 180-d mortality, SARS-CoV-2 RNA load > 1000 copies/ml and 50–1000 copies/ml both had the independent predictive capacity (Supplementary Table 2).

Association between SARS-CoV-2 RNAemia and plasma cytokines

Plasma levels of cytokines were performed in 92 patients. Higher plasma levels of IL-6, IL-8, and IL-10 were found in patients with SARS-CoV-2 RNAemia. Other cytokines did not differ between the two groups (Table 5 and Fig. 3). Spearman's correlation analysis showed that plasma SARS-CoV-2 RNA load was associated positively with IL-6 ($r = 0.313$, $P = 0.002$), IL-8 ($r = 0.370$, $P < 0.001$) and IL-10 ($r = 0.437$, $P < 0.001$). The median levels of these three cytokines increased with the increased stratification of virus RNA load in plasma (Supplementary Table 3). Additionally, the patients who died during the 180-d follow-up period exhibited higher levels of IL-6,

Table 4 Univariate and multivariate analyses for the association between 180-day mortality and SARS-CoV-2 RNAemia and other factors

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	aHR	95% CI	*P*
SARS-CoV-2 RNAemia	3.655	1.778–7.511	< 0.001	2.749	1.315–5.747	0.007
Age	1.074	1.025–1.125	0.003	1.071	1.025–1.119	0.002
Severity of COVID-19	4.305	1.501–12.346	0.007	3.241	1.102–9.533	0.033
Sepsis	2.729	1.044–7.134	0.041			
Lymphocyte	0.332	0.124–0.887	0.028			
Platelet	0.994	0.989–0.999	0.014			
C-Reactive protein	1.008	1.002–1.014	0.005			

Cox regression model was performed to evaluate the association between the risk factors and 180-d mortality. The measurements with $P < 0.1$ in univariate analysis were shown in this table and included in the multivariate model for final analysis

HR, hazard ratio; CI, confidence interval; aHR, adjusted hazard ratio

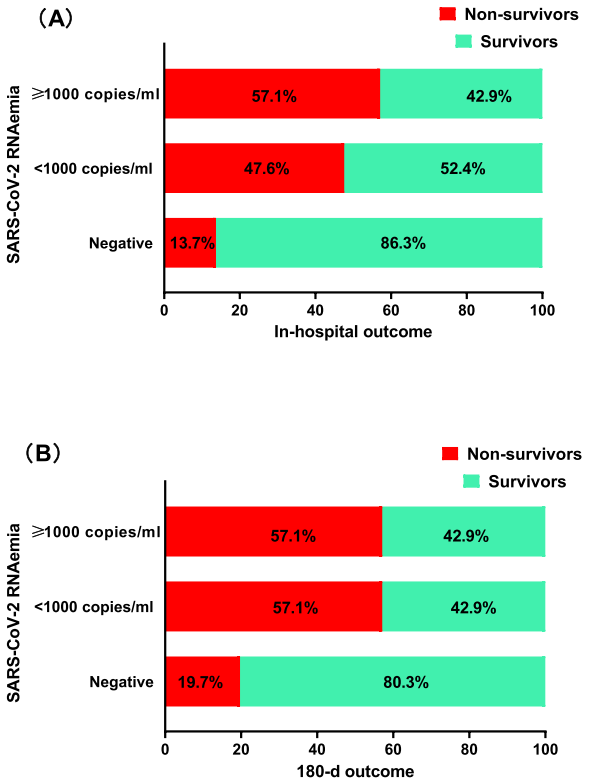

Fig. 2 **A** in-hospital mortalities among patients with non-SARS-CoV-2 RNAemia, SARS-CoV-2 RNA load 50–1000 copies/ml and ≥ 1000 copies/ml. **B** 180-d mortalities among patients with non-SARS-CoV-2 RNAemia, SARS-CoV-2 RNA load 50–1000 copies/ml and ≥ 1000 copies/ml

Table 5 Levels of plasma cytokines in patients with and without SARS-CoV-2 RNAemia

	SARS-CoV-2 RNAemia (−) (n = 65)	SARS-CoV-2 RNAemia (+) (n = 27)	*P* value
IL-1β (pg/ml)	1.95 [1.41, 2.47]	1.80 [1.39, 2.60]	0.928
IL-6 (pg/ml)	10.33 [6.79, 24.30]	36.49 [10.59, 93.19] [a]	0.004
IL-8 (pg/ml)	12.71 [7.47, 21.41]	27.35 [13.00, 45.12] [a]	0.001
IL-10 (pg/ml)	3.32 [2.49, 4.04]	6.53 [4.02, 9.39]	< 0.001
IFN-α (pg/ml)	1.87 [1.46, 3.06]	2.11 [1.52, 3.66]	0.474
IFN-γ (pg/ml)	1.89 [1.47, 2.61]	2.16 [1.58, 2.92]	0.354
TNF-α (pg/ml)	2.01 [1.67, 2.36]	2.22 [1.45, 2.63]	0.631

Data were presented as Median [IQR]. Plasma levels of cytokines were performed in 92 patients, with 65 SARS-CoV-2 RNAemia negative and 27 SARS-CoV-2 RNAemia positive

a: One patient with an extreme value of IL-6 and IL-8 was not included

IL-8, and IL-10 upon admission compared to those who did survive (Supplementary Table 4).

Discussion

The most important finding in our study was that SARS-CoV-2 RNAemia was associated with and served as an independent risk factor for 180-d mortality in a cohort of older adults with COVID-19 requiring medical care in pulmonary intermediate care units. We also found that the risk of death at 180-d increased with the increase of SARS-CoV-2 RNA load in blood. Our results suggest that SARS-CoV-2 RNAemia is an important indicator of poor long-term prognosis in elderly patients after discharge. We also observed a higher in-hospital mortality (> 50%) in patients with a virus RNA load over 1000 copies/ml. However, during the follow-up period of 180 days, the mortality was similar between patients with a virus RNA load of ≥ 1000 copies/ml and those of 50–1000 copies/ml, but both higher than those without RNAemia. We speculated that SARS-CoV-2 RNAemia per se, rather than RNA load in blood, could serve as a predictor for long-term prognosis. Due to the small number of patients with RNAemia in our study, we did not compare the differences of clinical outcomes between these two groups. Previous studies showed that patients who died in the hospital had significantly higher levels of plasma viral RNA load than those discharged alive. In patients who received ventilatory support, those with detectable plasma SARS-CoV-2 RNAemia had higher mortality compared to those without [1]. Another study showed that SARS-CoV-2 RNAemia was associated not only with the severity of illness, but also with the viral loads of nasopharyngeal swabs collected at the same time as the serum sample. Additionally, combining serum and nasopharyngeal swab SARS-CoV-2 RNA testing could improve the predictive capacity for disease severity and in-hospital mortality [6]. Even in patients with mild or moderate severity of disease, SARS-CoV-2 viremia still increased the risk of in-hospital death to more than six folds [9]. Duration of viremia and viral clearance were also associated with mortality in hospital. Prolonged viral clearance in blood increased the risk of death and contributed to severity of illness [11].

Some studies had described the possible mechanisms behind the association between SARS-CoV-2 viremia and the prognosis. Basing on proteomic analysis, some prominent proteomic pathways associated with SARS-CoV-2 viremia were determined, including upregulation of SARS-CoV-2 entry factors (ACE2, CTSL, FURIN), increased expression of proinflammatory factors (IL-6, CCL7, CXCL10/IP-10, CXCL11), heightened tissue damage markers of lungs (SFTPD, SFTPA1/2, AGER) and other organs, and activation of coagulation pathways. In addition, prolonged tissue damage was more likely to occur in patients with SARS-CoV-2 viremia [18]. Whole transcriptome analysis demonstrated that

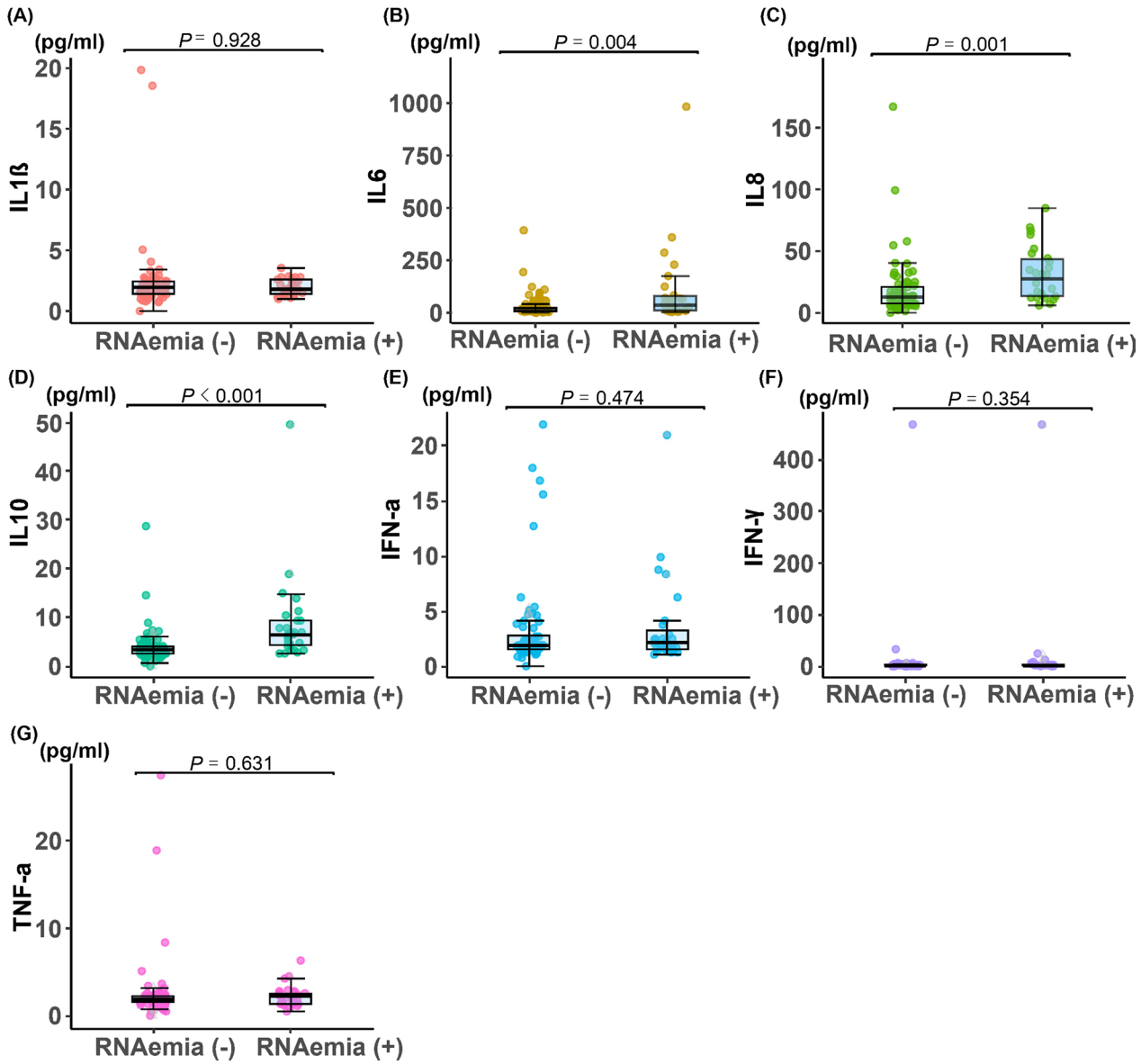

Fig. 3 Plasma cytokines between patients with and without SARS-CoV-2 RNAemia. IL, interleukin; IFN, interferon; TNF, tumor necrosis factor

severe COVID-19 patients with detectable plasma viral RNA had lower frequencies of $CD8^+$ T lymphocytes and nature killer cells but a higher frequency of monocytes in peripheral blood, impaired induction of Type I interferon-driven inflammation, which plays a central role in clearance of viruses, and an excessive systemic inflammation which contributes to multi-organ damages, indicating dysregulation of innate immune response in these patients [19]. Humoral immune response was also impaired in patients with SARS-CoV-2 RNAemia, resulting in worse outcomes. The spike (S)-specific IgG3 level, IgG Fc-receptor spike receptor binding domain (RBD)-specific FcγR3B and S-specific FcγR2B binding were decreased in patients with RNAemia compared to those without, which was linked with the perturbation of viral clearance [20]. A recent study revealed that delayed engagement of host antivirus response in the airways after SARS-CoV-2 infection was an important trigger for viremia, resulting in systemic disseminated infection and damage. Diverse host immune responses to viral replication in the respiratory tract can result in different clinical presentations and outcomes [21].

The prevalence of SARS-CoV-2 viremia varied among different studies, depending on the age of patients, site

of care, and severity of illness. The patients in our study were of advanced age and hospitalized, with mostly severe to very severe disease. The prevalence of SARS-CoV-2 RNAemia in our patients was 27.7%, which was very similar to the prevalence reported by Fajnzylber and colleagues [1], but a much lower prevalence was also shown in their outpatient participants [1]. Kawasuji and colleagues reported that SARS-CoV-2 RNAemia was detected in 19.6% of the patients whose age was much younger than ours [6]. In the patients who required ICU admission, the frequency of detectable SARS-CoV-2 RNAemia was even more than 50% [7].

Sepsis is a life-threatening condition caused by dysregulated host response to various pathogens [17]. Sepsis and septic shock were common in critical cases of COVID-19 according to Sepsis 3.0 criteria [22] and contributed to mortality [22–26]. Innate and adaptive immune response dysfunction and bacterial coinfections contributed to development of sepsis or hyperinflammation status in COVID-19 patients [27]. In our study, patients with SARS-CoV-2 RNAemia had a very high prevalence of sepsis compared to those without detectable RNAemia, indicating a causal relationship between SARS-CoV-2 viremia and sepsis. Another study showed that increasing levels of RNAemia correlated with qSOFA score [7]. Probably due to the relatively small size of our cohort, the relationship between sepsis and mortality was not confirmed by multivariate analysis in our study.

The associations between SARS-CoV-2 RNAemia and mortality, disease severity, sepsis/septic shock, and multiorgan dysfunction/failure could be explained by the excessive systemic inflammatory response and tissue injury. Similar to most of the previous studies [3, 11, 19, 28], our study demonstrated an association between SARS-CoV-2 RNAemia and various inflammatory cytokines, such as CRP, IL-6, IL-8 and IL-10, indicating SARS-CoV-2 viremia could result in systemic hyperinflammation contributing to severe COVID-19. COVID-19 can result in host immune dysfunction, manifesting as down-regulation of Type I interferon-driven inflammation which is crucial for inhibiting virus replication [29, 30] and up-regulation of systemic inflammatory response and inducing cytokine storm, including TNF-α and IL-6 [31]. The consequence of these changes is tissue damage and cell death, resulting in poor clinical outcomes.

There are several limitations to our study. Firstly, we did not observe the dynamics of SARS-CoV-2 RNA load in peripheral blood and the duration of SARS-CoV-2 RNAemia. Previous studies showed that prolonged duration of viremia was strongly associated with mortality in hospital [11]. Secondly, the proportion of patients receiving antiviral agents was relatively low in our study, which was mostly due to the temporary antiviral drug shortage in the very early stage of this wave of epidemic. Therefore, it might delay the clearance of viremia in some patients, affecting the outcome of treatment and long-term prognosis. Thirdly, other long-term outcomes such as quality of life, activity of daily life, re-admission to hospital due to any respiratory diseases were not assessed in our study. Finally, the exact causes of death in patients discharged from hospital were not recorded, because we were unable to collect this information by a telephone interview.

Conclusions

Among the older adults with COVID-19 admitted to pulmonary intermediate care units, SARS-CoV-2 RNAemia was common and shown to be a useful predictor for in-hospital and long-term mortality. The risk of death increased with the viral RNA load in plasma. SARS-CoV-2 RNAemia was also associated with the severity of COVID-19 and linked with systemic hyperinflammation, which played a crucial role in the poor prognosis in these patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-024-02526-4.

Additional file 1.

Acknowledgements
We thanked the Hangzhou Matridx Biotechnology Co.,Ltd. and its staffs Xuefang Cao and Jun Wang. They provided the RT-PCR technique for plasma SARS-CoV-2 RNA load detection and validation.

Author contributions
Ying Liang and Yongchang Sun contributed to study concept and design, preparation of manuscript. Ying Liang, Chun Chang, Yanling Ding, Xiaoyan Gai and Qingtao Zhou contributed to acquisition of subjects and data, analysis and interpretation of data. Hongling Chu and Lin Zeng contributed to analysis and interpretation of data. All of the authors contributed to revision of manuscript.

Funding
This work was supported by the Peking University Third Hospital-MatriDx Biotechnology Joint Research & Development Center of Molecular Diagnostics [H63512-19 to Yongchang Sun, H82499-03 to Ying Liang].

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Peking University Third Hospital (M2023006) and consent was obtained from the patients or their close relatives.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 20 March 2024 Accepted: 2 October 2024
Published online: 07 October 2024

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