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RESEARCH ARTICLE

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SARS-CoV-2 RNAemia with a higher nasopharyngeal viral load is strongly associated with disease severity and mortality in patients with COVID-19

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Abstract

This study aimed to determine the frequency of SARS-CoV-2 RNA in serum and its association with the clinical severity of COVID-19. This retrospective cohort study performed at Toyama University Hospital included consecutive patients with confirmed COVID-19. The prevalence of SARS-CoV-2 RNAemia and the strength of its association with clinical severity variables were examined. Fifty-six patients were included in this study. RNAemia was detected in 19.6% (11/56) patients on admission, and subsequently in 1.0% (1/25), 50.0% (6/12), and 100.0% (4/4) moderate, severe, and critically ill patients, respectively. Patients with RNAemia required more frequent oxygen supplementation (90.0% vs. 13.3%), ICU admission (81.8% vs. 6.7%), and invasive mechanical ventilation (27.3% vs. 0.0%). Among patients with RNAemia, the median viral loads of nasopharyngeal (NP) swabs that were collected around the same time as the serum sample were significantly higher in critically ill (5.4 $\log_{10}$ copies/μl; interquartile range [IQR]: 4.2–6.3) than in moderate-severe cases (2.6 $\log_{10}$ copies/μl; [IQR: 1.1–4.5]; $p = 0.030$) and were significantly higher in nonsurvivors (6.2 $\log_{10}$ copies/μl [IQR: 6.0–6.5]) than in survivors (3.9 $\log_{10}$ copies/μl [IQR: 1.6–4.6]; $p = 0.045$). This study demonstrated a relatively high proportion of SARS-CoV-2 RNAemia and an association between RNAemia and clinical severity. Moreover, among the patients with RNAemia, the viral loads of NP swabs were correlated with disease severity and mortality, suggesting the potential utility of combining serum testing with NP tests as a prognostic indicator for COVID-19, with higher quality than each separate test.

KEYWORDS

COVID-19, mortality, nasopharyngeal viral load, RNAemia, SARS-CoV-2, severity

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1 | INTRODUCTION

Knowledge of the viral load dynamics of SARS-CoV-2 in nasopharyngeal (NP) swabs and other tissues plays an important role in understanding the pathogenesis, transmission, and management of patients with coronavirus disease (COVID-19).[1] Previous studies have found that the viral load in NP swab specimens peaks around the time of symptom onset and generally becomes undetectable about 2 or 3 weeks after symptom onset[2,3]; however, there is evidence of prolonged virus detection in elderly patients,[4] with previous studies showing that the viral load in the respiratory specimens of symptomatic patients was similar to that of asymptomatic patients,[5] thus implying that the viral load in respiratory specimens may not objectively reflect disease severity.[6]

On the other hand, some studies have reported that a high viral load in NP swabs was an independent risk factor for intubation and/or death.[7,8] Thus, the real impact of initial SARS-CoV-2 viral load in NP swabs on clinical outcomes is not been clearly elucidated.

Previous studies have indicated that SARS-CoV-2 RNA may also be detected in plasma and serum,[9,10] and serum SARS-CoV-2 viral RNA (termed RNAemia by the authors in a recent study) was detected in 15% of COVID-19 patients.[11] Recent studies demonstrated that RNAemia is associated with disease severity and unfavorable clinical outcomes.[12,13] This suggests that extra-respiratory spread of SARS-CoV-2 contributes to systemic inflammatory responses, which are an important factor in the systemic pathogenesis of COVID-19, including thrombosis and coagulopathy.[13,14]

However, the clinical implication with SARS-CoV-2 RNAemia detection have not been completely clarified and information regarding the use of NP swabs and blood samples to improve patient management is insufficient.

In this study, we conducted a retrospective, monocentric cohort study of consecutive COVID-19 patients to investigate the frequency of SARS-CoV-2 RNA in serum and its association with the clinical severity of COVID-19.

2 | MATERIALS AND METHODS

2.1 | Study design

This cohort study enrolled consecutive patients who were admitted to and/or from whom viral load measurements and blood tests were conducted at Toyama University Hospital with confirmed COVID-19 from April 13, 2020, to September 28, 2020, were tested for SARS-CoV-2 RNA in serum collected at the initial visit. NP samples that were collected approximately at the same time of serum collection around admission were included in the study as paired NP samples if residual tested serum was collected within seven days before or after NP collection. Given that viral culture was not performed to determine virus viability, the presence of viral RNA in serum is referred to as RNAemia instead of viremia.

2.2 | Data collection

A retrospective chart review was performed for all individuals in the study to identify basic demographic data, medical history, clinical presentation, and laboratory test results on hospital admission. The collected outcome data included the need for oxygen supplementation, admission to an intensive care unit (ICU), invasive mechanical ventilation, and all-cause mortality. Chest computed tomography was carried out in all patients around admission and severity was divided into five categories: asymptomatic, mild (symptomatic patients without pneumonia), moderate (pneumonia patients without required oxygen supplementation), severe (required oxygen supplementation), and critically ill (requiring invasive mechanical ventilation, shock, and/or multiple organ dysfunction). The sample size was based on a convenience set of all available clinical samples, without formal power calculations.

2.3 | Quantitative reverse transcriptase-polymerase transcription assay (RT-qPCR)

Serum samples and NP swabs were collected from all patients and RNA was extracted. NP swab specimens were pretreated with 500 µl of sputazyme (Kyokuto Pharmaceutical). After centrifugation at 20 000 g for 30 min at 4°C, the supernatant was used for RNA extraction. A total of 60 µl RNA solution was obtained from 140 µl of the supernatant or 140 µl of the serum using the QIAamp ViralRNA Mini Kit (QIAGEN) or Nippongene Isospin RNA Virus (Nippongene), according to the manufacturer's instructions. The viral loads of SARS-CoV-2 were quantified on the basis of an N2 gene-specific primer/probe set by RT-qPCR, according to the protocol of the National Institute of Infectious Diseases of Japan.[15] Quantification quality was controlled using AcroMetrix COVID-19 RNA Control (Thermo Fisher Scientific). The detection limit was approximately 0.4 copies/µl (two copies/5 µl). When undetectable, viral load data were hypothetically calculated as −0.5 $\log_{10}$ copies/µl.

2.4 | Statistical analysis

Continuous and categorical variables are presented as median (interquartile range [IQR]) and *n* (%), respectively. We used the Mann–Whitney *U* test, χ^2 test, or Fisher's exact test to compare the differences between patients with and without RNAemia. In all analyses, we preliminarily confirmed the effect of multicollinearity of the covariates used in the statistical analysis. Univariate logistic regression analysis was used to investigate variables potentially associated with clinical severity. Multivariate logistic regression analyses were performed with all the independent variables ($p \le 0.20$) on univariate analysis as well as with the main variable of RNAemia and variables deemed either clinically relevant or supported in the medical literature. Data were analyzed using JMP Pro version 14.2.0 software (SAS Institute Inc.).

TABLE 1 Demographic and clinical characteristics of 56 patients with paired nasopharyngeal and serum samples tested for SARS-CoV-2

Characteristics	Overall patients, *n* = 56	With RNAemia, *n* = 11	Without RNAemia, *n* = 45	*p*
Age, median, y	54.5	78	50	0.0013
0–19, *n* (%)	4 (7.1)	0 (0.0)	4 (7.14)	0.58
20–64, *n* (%)	32 (57.1)	4 (36.4)	28 (62.2)	0.18
≥65, *n* (%)	20 (35.7)	7 (63.6)	13 (28.9)	0.042
Sex				
Male, *n* (%)	24 (42.9)	5 (45.5)	19 (42.2)	1
Presence of symptoms, *n* (%)				
Asymptomatic	6 (10.7)	0 (0.0)	6 (13.3)	0.33
Symptomatic	50 (89.3)	11 (100.0)	39 (86.7)	0.33
Mild	9 (18.0)	0 (0.0)	9 (23.1)	0.18
Pneumonia	41 (73.2)	11 (100.0)	30 (66.7)	0.026
Moderate	25 (50.0)	1 (9.1)	24 (61.5)	0.0046
Severe	12 (24.0)	6 (54.6)	6 (15.4)	0.014
Critically ill	4 (8.0)	4 (36.4)	0 (0.0)	0.0014
Comorbidities, *n* (%)				
Hypertension	13 (23.2)	6 (54.6)	7 (15.6)	0.013
Dyslipidemia	9 (16.1)	2 (18.2)	7 (15.6)	1
Diabetes	5 (8.9)	1 (9.1)	4 (8.9)	1
Bronchial asthma	4 (7.1)	0 (0.0)	4 (8.9)	0.58
Charlson comorbidity index ≥ 2	8 (14.3)	2 (18.2)	6 (13.3)	0.65
Laboratory findings, median (IQR)				
White blood cell count ($\times 10^3/\mu l$)	5.1 (3.7–5.9)	5.1 (2.9–5.7)	5.1 (3.7–5.9)	0.77
Neutrophil count (/μl)	2780.1 (1991.4–3915.3)	3099.8 (1918.6–4936.4)	2672.1 (1996.2–3783.2)	0.48
Lymphocyte count (/μl)	1234.2 (725.2–1801.0)	1283.3 (861.2–2030.5)	699.4 (430.3–1360.8)	0.010
Hemoglobin (g/dl)	14.0 (13.0–15.5)	13.3 (11.7–14.3)	14.1 (13.1–15.6)	0.11
Platelet count ($\times 10^3/\mu l$)	202.5 (133.8–234.5)	186 (142–219)	207 (128.5–250.5)	0.34
Albumin (g/dl)	4.1 (3.4–4.4)	4.2 (3.9–4.4)	3.1 (2.9–3.3)	<0.0001
AST (U/L)	30 (19.5–45.5)	41 (31–61)	28 (18.5–36)	0.0023
ALT (U/L)	22 (13.3–42.5)	20 (12–42)	26 (16–60)	0.23
Lactate dehydrogenase (U/L)	224 (182–320)	425 (279–559)	213.5 (177.5–276.3)	<0.0001
Total bilirubin (mg/dl)	0.5 (0.3–0.6)	7.06 (2.76–16.7)	0.44 (0.06–1.77)	0.0019
	13.4 (10.5–18.6)	23.3 (13.4–27.6)	12.7 (10–15.7)	0.0063

(Continues)

TABLE 1 (Continued)

Characteristics	Overall patients, *n* = 56	With RNAemia, *n* = 11	Without RNAemia, *n* = 45	*p*
Urea nitrogen (mg/dl)				
Creatinine (mg/dl)	0.80 (0.57–0.92)	1.01 (0.56–1.19)	0.78 (0.57–0.89)	0.076
CRP (mg/dl)	0.7 (0.083–3.1)	7.1 (2.8–16.7)	0.4 (0.06–1.8)	0.0019
D-dimer (μg/ml)	0.7 (0.5–1.3)	1.5 (1.4–3.2)	0.7 (0.5–0.9)	<0.0001
Treatment, *n* (%)				
Antiviral therapy	19 (33.9)	9 (81.8)	10 (22.2)	0.0004
Favipiravir	17 (30.4)	8 (72.7)	9 (20.0)	0.0016
Remdesivir	4 (7.1)	4 (36.4)	0 (0.0)	0.0009
Antibiotic therapy	15 (26.8)	9 (81.8)	6 (13.3)	<0.0001
Clinical outcomes, *n* (%)				
Required oxygen supplementation	16 (28.6)	10 (90.9)	6 (13.3)	<0.0001
ICU admission	12 (21.4)	9 (81.8)	3 (6.7)	<0.0001
Invasive mechanical ventilation	3 (5.4)	3 (27.3)	0 (0.0)	0.006
In-hospital mortality	3 (5.4)	2 (18.2)	1 (2.2)	0.095

2.5 | Ethics approval

This study was performed in accordance with the Declaration of Helsinki and was approved by the ethical review board of the University of Toyama (approval no.: R2019167). Written informed consent was obtained from all patients.

3 | RESULTS

Fifty-six patients were included, with a median age of 54.5 years. A total of 24 (42.9%) patients were men, and eight (14.3%) patients had Charlson comorbidity index ≥2 (Table 1). Hypertension was the most common comorbidity, followed by dyslipidemia, diabetes, and bronchial asthma. Hypertension was significantly more frequent in patients with detectable RNAemia than in those without.

The serum tested for the detection of RNA was collected at the initial visit in all patients except for two (collected 2 or 5 days after hospital admission). Paired NP swabs were collected within one day before and after the day of serum collection in 51 (91.1%) patients. SARS-CoV-2 RNAemia was detected in 11 (19.6%) patients, and patients with detectable RNAemia were significantly older than those without RNAemia (78 vs. 50 years; $p = 0.0013$). Lymphocyte count and serum albumin level were significantly lower in patients with RNAemia than in those without RNAemia, and serum levels of aspartate aminotransferase (AST), lactate dehydrogenase, total bilirubin urea nitrogen, CRP, and D-dimer were significantly higher in the patients with than in those without RNAemia.

The numbers of asymptomatic, mild, moderate, severe, and critically ill cases were 6, 9, 25, 12, and 4, respectively. Figure 1 shows the proportion of patients with detectable RNAemia, stratified by severity. The proportions of patients with RNAemia in moderate, severe, and critically ill cases were 1.0% (1/25), 50.0% (6/12), and 100.0% (4/4), respectively, but there were no asymptomatic and mild cases.

In terms of clinical outcomes, patients with RNAemia required more frequent oxygen supplementation (90.0% vs. 13.3%; $p < 0.0001$) and ICU admission (81.8% vs. 6.7%; $p < 0.0001$), and also required invasive mechanical ventilation (27.3% vs. 0.0%; $p < 0.0001$). In addition, RNAemia was independently associated with an increased risk of requiring more frequent oxygen supplementation and ICU admission in univariate and multivariate conditional logistic regression analyses (Tables 1 and 2). RNAemia was also associated with required invasive mechanical ventilation in univariate analysis, but multivariate conditional logistic regression analysis could not be conducted due to complete separation because of strong relationships. In-hospital mortality was more frequent in patients with SARS-CoV-2 RNAemia than in those without SARS-CoV-2 RNAemia; however, this difference was not significant (18.2% vs. 2.2%; $p = 0.095$), possibly because of the small sample size (Table 1).

There was no significant difference in viral loads in the NP swabs of patients with RNAemia (4.0 $\log_{10}$ copies/μl [IQR: 2.1–4.9]) and without RNAemia (4.2 $\log_{10}$ copies/μl [2.0–5.7]; $p = 0.85$) (Table 3).

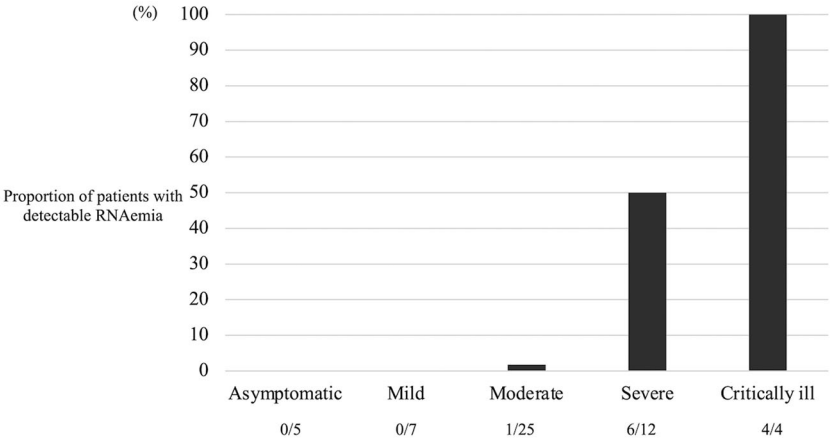

FIGURE 1 Proportion of patients with detectable RNAemia stratified by severity

TABLE 2 Univariate and multivariate conditional logistic regression analyses of required oxygen supplementation and ICU admission

Variables (required oxygen supplementation)	Univariate OR (95% CI)	*p*	Multivariate OR (95% CI)	*p*
RNAemia	65.0 (7.0–603.3)	<0.0001	232.3 (17.2–12468.5)	0.0007
Male	0.73 (0.22–2.41)	0.77	0.040 (0.00089–0.78)	0.052
Charlson comorbidity index (per 1-point increment)	1.85 (0.87–3.94)	0.11	2.35 (0.67–10.57)	0.20
Hemoglobin (per 1-g/dl increment)	0.68 (0.47–0.98)	0.026	1.19 (0.59–2.63)	0.65
Platelet count (per 1.0×10^3/μl increment)	0.9918 (0.9850–0.9986)	0.0067	0.9898 (0.9756–1.001)	0.11
Creatinine (per 1-mg/dl increment)	39.98 (2.274–702.6)	0.0013	995.6 (1.286–4858026)	0.063
Variables (ICU admission)	**Univariate OR (95% CI)**	***p***	**Multivariate OR (95% CI)**	***p***
RNAemia	63.0 (9.2–433.4)	<0.0001	440.1 (22.3–91446.6)	0.0022
Male	1.44 (0.39–5.33)	0.74	0.81 (0.041–15.62)	0.88
Charlson comorbidity index (per 1-point increment)	2.19 (0.99–4.83)	0.068	3.79 (0.91–30.01)	0.11
Platelet count (per 1.0×10^3/μl increment)	0.9922 (0.9850–0.9995)	0.013	0.9857 (0.9647–0.9998)	0.092
ALT (per 1-U/L increment)	1.014 (0.9908–1.037)	0.053	1.025 (0.9541–1.082)	0.37
Creatinine (per 1-mg/dl increment)	48.13 (2.106–1099.6)	0.0023	3.054 (0.001935–4174.8)	0.75

The overall median number of days of symptoms at the time of NP testing was six (IQR: 3–9), which was similar in moderately to critically ill patients with and without RNAemia.

In an in-depth study of RNAemia patients, although the days after symptom onset at the time of NP testing were not significantly different, the median viral loads of NP swabs were significantly higher in critically ill (5.4 $\log_{10}$ copies/μl [IQR: 4.2–6.3]) than in moderate-severe cases (2.6 $\log_{10}$ copies/μl [1.1–4.5]; $p = 0.030$) and in nonsurvivors (6.2 $\log_{10}$ copies/μl [IQR: 6.0–6.5]) than in survivors (3.9 $\log_{10}$ copies/μl [1.6–4.6]; $p = 0.045$).

4 | DISCUSSION

In this cohort study, 56 consecutive patients were assessed for RNAemia detection in serum samples. These findings show that RNAemia was present in almost 20% of the samples tested. Previous

TABLE 3 Nasopharyngeal viral load in 56 patients with and without RNAemia

Characteristics	Overall patients, *n* = 56	With RNAemia, *n* = 11	Without RNAemia, *n* = 45	*p*
Viral load (log_{10} copies/μl) of nasopharyngeal swab collected around admission, median (IQR)	4.2 (2.1–5.5)	4.0 (2.1–4.9)	4.2 (2.0–5.7)	0.85
Severity				
Moderate	4.1 (2.0–5.3)	0.7	4.2 (2.4–5.4)	0.24
Severe	3.5 (2.1–4.9)	3.2 (1.9–4.5)	4.0 (2.1–5.5)	0.47
Critically ill	5.4 (4.2–6.3)	5.4 (4.2–6.3)	–	–
In-hospital mortality				
Survivor	4.2 (2.0–5.4)	3.9 (1.6–4.6)	4.3 (2.0–5.8)	0.30
Nonsurvivor	6.0 (2.1–6.5)	6.2 (6.0–6.5)	2.1	0.22

studies have reported different rates of SARS-CoV-2 detection in serum (ranging from 10.4% to 74.1%),[1,8,9,16–18] whereas other studies did not find any patients or reported only 1% of RNAemia.[16,17] However, a direct comparison of these results is difficult because of the use of different testing protocols, including differences in specimen extraction volumes, sample types (plasma, serum, and whole blood), viral genomic targets (N, S, E genes, or ORF1ab region), variable timing of blood collection, and different patient populations.[9]

Regarding the clinical importance of the presence of SARS-CoV-2 RNAemia, our results are consistent with previous reports, suggesting an association with ICU admission, invasive mechanical ventilation, and mortality.[19,20]

However, the association between viral load in respiratory specimens and severity remains unclear and controversial in the literature. Multiple case series have reported that the viral load in asymptomatic patients is as high as that in asymptomatic patients,[5,18] while recent studies have found that NP viral load at admission is independently correlated with the risk of intubation and in-hospital mortality.[8] This discrepancy may be attributed to the different patient populations, including the levels of severity.

In this study, there was no significant difference in the viral load in the NP swabs of patients with and without RNAemia. These results confirm those of Berastegui-Cabrera et al.[1] and Prebensen et al.,[21] who did not find any association between the viral load in NP samples and the presence of SARS-CoV-2 RNAemia. However, a recent study in ICU patients reported that the nasopharyngeal viral load was associated with RNAemia as well as 28-day mortality.[12] Therefore, in some severe and critical cases, both NP viral load and RNAemia may be useful as prognostic markers of condition development and mortality.

To date, information regarding the combined use of NP swabs and blood samples to improve patient management is insufficient. To the best of our knowledge, no previous study investigates the prognostic potential of RNAemia combined with the viral load of NP swabs in hospitalized patients with COVID-19. Among the patients with RNAemia in the present study, the higher viral load of NP swabs was correlated with severity and mortality. This result suggested that NP viral load may also be useful as an aid to provide a more accurate prediction of the severity and mortality of patients with RNAemia than RNAemia alone.

Although this study was not performed in a controlled setting, most serum and NP samples were collected on the same day as their admission, thus supporting a potential role for the early detection of individuals at risk of severe clinical illness. Thus, in addition to assessing the viral load of NP swabs, which is the technique most frequently used for COVID-19 diagnosis, serum tests may be considered a complementary modality for the early identification of individuals who are likely to develop severe COVID-19.[9]

This study has several limitations inherent to its small sample size, retrospective design, and potential for confounding the detection of SARS-CoV-2 RNA from the serum, NP viral load, and clinical conditions that cannot be excluded. In addition, we did not conduct multivariate or age-adjusted analyses owing to the strong multicollinearity between variables and the small sample size. Longitudinal analyses were also not performed because viral load dynamics could be modified by the treatment, including combinations of antivirals and antibiotics during hospitalization.

5 | CONCLUSIONS

In conclusion, this study demonstrated a relatively high overall proportion of detectable SARS-CoV-2 RNAemia as well as a strong association between serum viral detection and severe clinical disease, including the need for oxygen supplementation and ICU admission, invasive mechanical ventilation, and the likelihood of in-hospital mortality. In addition, among the patients with RNAemia, the viral loads of NP swabs were significantly higher in critically ill and nonsurvivor cases than in moderate-severe and survivor cases. Further studies are required to investigate the prognostic potential of RNAemia and those combined with the viral load of NP

swabs to facilitate early therapeutic and supportive interventions before severe clinical deterioration of COVID-19.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Hitoshi Kawasuji, Yoshitomo Morinaga, and Yoshihiro Yamamoto planned the study design, contributed to data analysis, and wrote and revised the manuscript. Yoshitomo Morinaga, Hideki Tani, Yoshihiro Yoshida, Miyuki Kimura, and Hiroshi Yamada contributed to conduct and perform RT-qPCR. Hitoshi Kawasuji, Yusuke Takegoshi, Makito Kaneda, Yushi Murai, Kou Kimoto, Akitoshi Ueno, Yuki Miyajima, Yasutaka Fukui, Ippei Sakamaki, and Yoshihiro Yamamoto contributed to obtaining informed consent, collecting specimens, and patient management. All the authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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