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RESEARCH ARTICLE

RNAemia and Clinical Outcome in COVID-19 Patients

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ABSTRACT

Objectives: Objectives: SARS-CoV-2 virus dynamics in the human body and its correlation with disease severity and biomarkers have not been elucidated. This study aims to evaluate the viral load in the blood sample of COVID-19 patients and viral load association with disease severity and progression.

Methods: Blood samples from COVID-19 positive inpatients were collected sequentially, at admission, and during hospitalization. Depending on the clinical presentation, the patients were classified into mild, moderate, and severe categories. The blood samples were subjected to SARS-CoV-2 Real-time polymerase chain reaction (RT-PCR); positive samples were then tested for viral load using digital drop RT-PCR. Patients were followed up until they were discharged.

Results: Of 83 enrolled patients, 15 (18.1%) were mild, 45 (54%) were moderate, and 23 (27.7%) had severe symptoms. The blood PCR in 21 patients was positive (rate was 25.3); one was mild, four moderate, and 16 severe on the day of admission. The mean cycle threshold (CT) of RNAemia in these 21 patients was 24.7+3.1. The viral load was significantly higher in patients with severe symptoms than others. Serum ferritin, D-dimer, and plasma fibrinogen were found raised along with lymphopenia in all patients. Viremia was found persistently detectable (or even rising) in patients in the severe category and associated with a fatal outcome.

Conclusion: A prolonged elevation of D-dimer, serum ferritin, and plasma fibrinogen together with lymphopenia lead to a greater risk of mortality and morbidity. Quantitative estimation of virus in blood has a clinical significance which may help in prognostication and management of patients of COVID-19. *J Microbiol Infect Dis 2021; 11(3):116-123.*

Keywords: *SARS-CoV-2, Viremia, Digital drop PCR, Serum ferritin, D-Dimer, Lymphopenia, Plasma fibrinogen, Procalcitonin*

INTRODUCTION

COVID-19 caused by the SARS-CoV-2 virus is a challenging disease. It appeared to be just an upper respiratory tract infection at first. Eventually turned out to be a complicated disease with broad clinical manifestations ranging from asymptomatic and mild to life-threatening systemic infection, inflammation, and thrombosis. It is still not fully understood why some patients exhibit mild or no symptoms while others suffer severe complications. Neither the length of time for

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which SARS-CoV-2 RNA survives in the upper respiratory tract nor the viral load in the throat correlates with prognosis [1]. Many other factors are known to affect the course of COVID-19, such as age, gender, comorbid diseases, and genetic makeup.

At present, there are many unclear issues regarding the relationship between the course of the disease and virus dynamics in patients, especially in hosts with associated comorbidities. The optimal method to detect COVID-19 is real-time reverse transcription-polymerase chain reaction (RT-PCR) in respiratory samples. Although respiratory samples have the most significant yield, other samples, including stool and blood, could also be used to detect the SARS-CoV-2 virus, same as for other coronaviruses like Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) [2,3]. Currently, the literature available on RNAemia in COVID-19 patients is scanty, with limited sample size, reporting varying PCR positivity rates in blood. [4,5]. The primary objective of this study is to evaluate the correlation of viral load in blood with disease severity in COVID-19 patients. The secondary aim is to determine the correlation between viremia levels and clinic, demographic, hematological parameters, and outcomes in these patients.

METHODS

A hospital-based prospective observational study was conducted in Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, in North India, from August 2020 to November 2020. This tertiary care hospital has a dedicated 200 COVID-19 beds and COVID diagnostic laboratory. The BSL-II laboratory has been approved by the Indian Council of Medical Research (ICMR), New Delhi. The COVID-19 patients that were confirmed with a positive RT-PCR test of the nasopharyngeal swab were included in the study. They had been admitted to the COVID-19 hospital and were followed at isolation ward, high dependency unit (HDU), or intensive care unit (ICU). The patients were classified as mild, moderate, and severe using clinical management protocol COVID-19 (https://www.mohfw.gov.in/pdf/ClinicalManagementProtocolforCOVID19.pdf) [6]. Written informed consent was obtained before enrollment from all patients. The following information was retrieved from the patient records in the hospital information system: i) Demographic profile; ii) Clinical characteristics (Mild/Moderate/Severe) and outcome (Survived/Died); iii) Hematological investigations: D-dimer, serum ferritin, neutrophil to lymphocyte ratio, serum procalcitonin level, plasma fibrinogen level, and other additional tests as necessary.

A blood sample (2-3 ml) for viral load detection was collected at the time of admission in a citrated vial, transported to COVID lab immediately, maintaining the appropriate cold chain. The enrolled patients showed COVID virus RNA on RT-PCR in the 1st sample; the second blood sample was collected on day four. Attempts were made to collect a third blood specimen too on day 8 of admission from all patients with RNAemia in the first blood sample. The institutional ethics committee approved the study (ref. IEC No. 64/20; RMLIMS, Lucknow).

RNA extraction

Standard Protocol for RNA Extraction from blood sample was performed following Graham et al. [5], using QIAamp DSP Virus Kit (Cat# 60704, Qiagen, Hilden, Germany). For extraction; 75 µL of QP (protease) buffer, 500 µL of the AL (lysis) buffer, and 11.2 µg/mL of RNA Carrier were added to 500 µL of plasma in a 2 ml tube. The tube was incubated at 56°C for 15 minutes then centrifuged at 13,000 g for 1 minute. Next, 600 µL of 97% ethanol was added, mixed by pulse-vortex, incubated at room temperature for 5 minutes, and centrifuged at 13,000 g for 1 minute. Then 600 µL of AW1 (wash buffer 1) was added before centrifuging the tubes at 13,000 g for 1 minute, and the same process was repeated using 750 µL of AW2 (wash buffer 2). After a final step of washing using 750 µL of 97% ethanol, the tube was centrifuged at 16,000 g for 1 minute, and the tube cap was left open to dry any residual ethanol at 56°C for 3 minutes. Viral nucleic acid was eventually eluted by adding 50 µL of AVE buffer and centrifuging the tubes at 16,000 g for 3 minutes.

In Vitro Transcription of SARS-COV-2 control RNA

According to the manufacturer's instruction, in vitro transcription (IVT) of SARS-COV-2 control-RNA was performed using MEGA script® Kits (Life Technologies Corporation,

Carlsbad, California, USA). For Poly A tailing, three µl of RNA (5ng) and two µl of diluted poly-A controls were added. For first-strand cDNA synthesis, the reaction mixture contained 10µl of the poly-A tailed RNA, 2.0 µl 10× RT Buffer, 0.8 µl 25XdNTP Mix (100 mM), 1.0 µl 10×T7 oligo (dT)24V anchored primers (Applied Biosystems), 1.0µl MultiScribe™ Reverse Transcriptase, and 5.2 µl Nuclease-free water (High-Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, CA, USA). 2 µl DNase recombinant Turbo DNase I was added to cRNA and incubated at 37°C for 30 minutes. RNA was then purified by MEGAclearKit (Thermo Fisher Scientific, Carlsbad, California, USA).

Real-time quantitative PCR

Real-time quantitative RT-PCR in 20µl was carried out using 8µl of extracted RNA 10µl of TB Green Premix Ex Taq (Takara Bio. Inc, Shiga, Japan) and RdRP primers (RdRP_SARSr-F2 5'-GTGARATGGTCATGTGTGGCGG-3', RdRP_SARSr-R1 5'-CARATGTTAAASACACTATTAGCATA-3'; Cat No 4304970, Thermo Fisher Scientific, Carlsbad, CA, USA). Master Mix containing ROX was used as a passive reference. Amplification and detection were performed in ABI PRISM 7900 Fast sequence detection system (Applied Biosystems, Foster City, CA, USA) under the following conditions: an initial incubation at 50°C for 2 minutes, followed by PCR activation at 95°C for 10 minutes and 40 cycles of amplification (15 seconds at 95°C and 1 minute at 60°C). Serial dilutions of SARS-CoV RNA standards (Taqman™ 2019nCoV Control Kit v1, Thermo Fisher Scientific, Carlsbad, California, USA) were used to determine the viral RNA load in patient samples and the construction of the standard curve (Figure 1).

Statistical Analysis

All data were statistically analyzed using the SPSS software package (version 18.0; IBM Corp, Armonk, NY, USA). Frequency rates and percentages were used to describe categorical data and were analyzed using χ 2 test and Fisher exact test. Quantitative variables were presented as mean and analyzed utilizing a t-test. Statistical significance was set at $p < 0.05$.

RESULTS

A total of 83 confirmed COVID-19 patients (M: F ratio was 4:1) were enrolled in the study. The mean age was 56.3 (range: 16 to 76) years. Characteristics of the patients are given in Table 1. RNAemia was detected in 21 (25.3%) of these cases in blood samples collected at admission. There was no significant difference in age and sex distribution among RNAemia and non RNAemia patients. However, the SARS CoV-2 RNA detection rate in blood samples was higher in patients with severe and moderate disease than in patients with mild disease (16 vs. 4 vs. 1, respectively), and this difference was statistically significant.

At admission, all patients with detectable RNAemia needed ventilatory support. On the other hand, only ten of the patients (16.4%) without detectable RNAemia required ventilatory support.

At least one comorbidity was present in all RNAemia positive patients. The most common comorbidity associated was hypertension, followed by chronic obstructive pulmonary disease (COPD). Cough and dyspnea were the most commonly observed symptoms on admission, 71.2% and 60.2%, respectively.

A significant difference was found between viremia and non-viremia patients for elevated d-dimer/ serum ferritin/ plasma fibrinogen levels (p=0.0001). A reverse correlation was seen between lymphocyte count and RNAemia detection.

A second blood sample was collected from 21 patients who showed RNAemia, on the 4th day of admission. Table 3 shows results of RNAemia and viral load (2nd sample is designated as suffix '2' after patient's code). Since some were discharged or expired, the third blood sample could be obtained from only 15 patients (on day eight of their hospital stay). All these 15 samples tested negative for viral RNA. In four patients (18.2%) (with codes D, I, O, and S as mentioned in Table 3), viral load in blood was found to be on the rise on the 4th day of illness; unfortunately, all these four patients expired between days 5 to 7, and hence third blood sample could not be obtained. Along with the presence of RNAemia and increased viral load, a constant increase in d-dimer, serum ferritin, and plasma fibrinogen, along with lymphopenia, was also noted in these patients' samples.

Table 1. Demographic and clinical Characteristics of study patients on the admission.

Characteristics	RNAemia Detectable (21 samples) n (%)	RNAemia Not-detectable (62 samples) n (%)	p Value
Age, median (range) years	54.24 (16-90)	57.06 (35-76)	0.07
Sex (Male) n, %	17, 80.95%	42, 67.7%	0.25
Hypertension	6 (27.3)	22 (36.1)	0.45
Diabetes Mellitus	5 (22.7)	18 (29.5)	0.54
Hypothyroidism	2 (9.1)	0 (0)	0.1
COPD*	9 (40.9)	10 (16.3)	0.02
TBM*	1 (4.8)	0 (0)	0.2
CKD*	1 (4.8)	0 (0)	0.2
Hospital Stay, median, (range) days	6.9 (3-15)	4.5 (3-7)	0.04
Cough	15, 71.2	34, 54.8	0.2
Dyspnea	15, 71.2	41, 66.1	0.65
Fever	10, 47.6	18, 29.0	0.12
Rhinorrhea	8, 38.1	40, 64.5	0.03
Anosmia & Hypogeusia	8, 38.1	37, 59.7	0.09
Kidney problems	5, 23.8	9, 14.5	0.33
Conjunctival congestion	3, 14.3	27, 43.5	0.02
Diarrhea	0, 0	6, 9.6	0.3
Average days to PCR negative NP sample, median (range)	9.63 (7-15)	4.84 (3-6days)	0.03
Death n, %	4 (19.1)	0 (0)	0.02

*COPD- Chronic Obstructive pulmonary disease; TBM – Tuberculous meningitis; CKD – Chronic kidney disease

Table 2: Laboratory Parameters of study patients at the time of admission

Laboratory parameter	RNAemia Patients (n=21), %	Non RNAemia Patients (n=61), %	P Value
Elevated D-Dimer (>0.5 µg/ml)	20, 91%	15, 24.6%	<0.0001
Raised Serum Ferritin>250 ng/ml	18, 81.8%	13, 21.3%	<0.0001
Plasma fibrinogen>400 mg/dl	19, 86.3%	17, 27.9%	<0.0001
Lymphopenia (< 1000cells/mm^3)	10, 45.5%	15, 24.6%	0.05
Total WBCs x 10^3/cu.mm	4.6 x 10^3 (4.0-5.2)	6.5 x 10^3 (5.5-8.0)	0.26
Neutrophil to lymphocyte ratio (Mean + SD)	20.7 ± 24.1	4.8 ± 3.5	<0.001
Hemoglobin g/dl	12.5 (11.0-13.6)	12.8 (11.2 – 14.3)	0.35
Platelet count (cells/cu.mm)	1.8 x 10^5 (1.5-2.1 x 10^5 cells/cu.mm)	2.2 x 10^5 (1.6-2.8 x 10^5 cells/cu.mm)	0.44
APTT (sec)	28.4 (25.1 – 31.2 sec)	30.2 (25.3 – 35.2 sec)	0.52
Elevated C reactive protein (>8mg/dl)	21, 100%	60, 96.7%	0.7
Serum Procalcitonin >0.5µg/l	1, 4.8%	10, 16.1%	0.14

Table 3: Absolute quantification of RNA isolated from the blood sample of cases.

Patient Code (First sample on Day 0)	Result	CT value	Viral genome copy number	Patient Code (Second sample) on day 4)	Result	CT value	Viral genome copy number	Outcome
A1	Positive	27.5	$4.73X10^{7}$	A2	Negative			Survived
B1	Positive	27.6	$4.73X10^{7}$	B2	Negative			Survived
C1	Positive	22.6	$2.3X10^{11}$	C2	Positive	25.4	$4.73X10^{10}$	Survived
D1	Positive	26.8	$4.73X10^{10}$	D2	Positive	17.4	$1.88X10^{12}$	Died
E1	Positive	27.1	$4.73X10^{10}$	E2	Negative			Survived
F1	Positive	22.3	$2.3X10^{11}$	F2	Negative			Survived
G1	Positive	24.2	$4.73X10^{10}$	G2	Positive	23.9	$4.73X10^{10}$	Survived
H1	Positive	21.7	$4.73X10^{11}$	H2	Negative			Survived
I1	Positive	25.2	$4.73X10^{10}$	I2	Positive	21.7	$4.73X10^{11}$	Survived
J1	Positive	22.3	$2.3X10^{11}$	J2	Negative			Survived
K1	Positive	22.1	$2.3X10^{11}$	K2	Negative			Survived
L1	Positive	22.7	$2.3X10^{11}$	L2	Negative			Survived
M1	Positive	26.9	$4.73X10^{10}$	M2	Negative			Survived
O1	Positive	27.8	$4.73X10^{7}$	O2	Positive	16.2	$3.76X10^{12}$	Died
P1	Positive	27.3	$1.5X10^{7}$	P2	Positive	23.1	$4.73X10^{10}$	Died
Q1	Positive	26.5	$4.73X10^{10}$	Q2	Positive	21.8	$4.73X10^{11}$	Survived
R1	Positive	18.5	$3.76X10^{12}$	R2	Positive	18.5	$1.88X10^{12}$	Survived
S1	Positive	24.8	$4.73X10^{10}$	S2	Positive	17.8	$1.88X10^{12}$	Died
T1	Positive	24.9	$4.73X10^{10}$	T2	Positive	24.0	$4.73X10^{10}$	Survived
U1	Positive	27.0	$4.73X10^{9}$	U2	Negative			Survived
V1	Positive	22.8	$2.3X10^{11}$	V2	Positive	18.8	$3.76X10^{12}$	Survived

DISCUSSION

It is well documented that COVID-19 primarily manifests as a respiratory tract infection. However, emerging data indicate that it should be regarded as a multi-systemic disease involving cardiovascular, respiratory, gastrointestinal, neurological, hematopoietic, and immune systems [7, 8]. Although it has been more than a year and millions of patients down the line, yet many facets of SARS- CoV-2 virus dynamics in different hosts' settings and their relationship with disease severity are not yet clearly known.

The present work is a hospital-based prospective study, reporting the viral load of SARS-CoV-2 in blood from COVID-19 patients admitted to our COVID hospital. Among 83 enrolled patients, the PCR positivity rate in blood was 25.3% (21/83) on the day of admission. Varying percentages of RNAemia have been reported from different centers across the globe from 0% to 37% (9, 10, 11, and 12). this difference can be attributed to varying sample population characteristics and sample size studied. For example, the high prevalence of viremia (37%), as reported by Hagman et al., has a large sample size, and all their viremia cases are from ICU (61/167) [13].

The present study's relatively high yield of RNAemia may be attributed to immediate blood transport for viral load determination under appropriate cold chain conditions and enrolment of more moderately and severely ill patients with comorbid conditions.

In the present study, viral loads in blood samples varied significantly among patients with mild, moderate, and severe COVID-19 disease. Among 15 mild patients in the Covid

isolation ward, only 1 showed the presence of viral RNA. In contrast, four among 45 and 16 among 23 patients admitted in HDU and ICU respectively had RNAemia at admission. The mean CT of RNAemia in 21 patients at the time of admission was 24.7+ 3.1, and viral load ranged from 1.5 x 107 to 3.76 x 1012, as per the clinical severity (Table 3).

Zheng et al. [14] reported that, from the first week, the load of viral RNA in serum samples gradually increased, followed by a decline in the third week of the disease. According to the authors, serial sampling of blood samples demonstrated that the presence of virus in blood extends when there is elevated D-dimer, serum ferritin, and decreased lymphocyte count, which together contribute to cytokine storm. In our study, on the other hand, from amongst 21 patients who had RNAemia on admission, only 15 showed continuous viral RNA in blood on day 4 of admission; by the 8th day, in eleven of these patients, RNAemia was cleared. The remaining four patients expired (on or before day seven) before their third sample could be obtained. RNAemia didn't extend beyond one week of illness in the present study, demonstrating a decline in viral dynamics by the second week. However, 11 patients showing no RNAemia in the third sample continued to show slightly elevated D-dimer and serum ferritin in the second-week blood sample. Hereby suggesting that once the cytokine storm is activated by viral RNA in the blood, it may continue to persist for some days, even after the absence of RNAemia. It was also noticed that detectable RNAemia was not significantly related to the presence of chronic comorbidities like hypertension, diabetes mellitus, hypothyroidism, tuberculosis. However, it was significantly associated with chronic obstructive pulmonary disease [15, 16].

The mean age of the study population was 56.3 (16–76) years, and it was towards the higher side in patients admitted in ICU. Like others, we also observed higher rates of severe illness in patients older than 60 years [17,18]. This is partly because of immunosenescence. Another reason is that older people have higher in their alveoli, which is thought to be an Angiotensin-converting enzyme-2 receptor for novel coronaviruses [19,20]. The sex of the patient does not seem to play any role in RNAemia, as observed in our study.

We found a significant association between lymphopenia and RNAemia at the time of hospital admission. In patients with severe disease and fatal outcomes, there was a rise in viral load in the second sample. In addition, these patients during illness had increased neutrophils to lymphocyte ratio ($p< 0.001$) compared to 62 non-RNAemia cases [10,20]. It is evident from the result in Table-2 that raised d-dimer and serum ferritin and decreasing lymphocyte counts have a significant role in predicting the prognosis of COVID-19. In Singapore, Fan et al. also found that patients requiring ICU support had significantly lower lymphocyte levels ($p<0.001$) at baseline [21]. In another retrospective analysis including 52 critically ill patients from Wuhan, China, lymphopenia was reported in 85% of patients [22].

Other hematological parameters like total leukocyte/ red cell/ platelet counts were normal in all study participants. No significant differences in these parameters were observed between patients with RNAemia and non-RNAemia.

We found that CRP was elevated in 100% of patients, and severe cases with RNAemia showed a more marked increase than severe but non-viremic ones (78.6% vs. 58.4%). Furthermore, higher CRP has been linked to unfavorable outcomes of COVID-19 disease [23]. In addition, elevated Procalcitonin was observed in 4.8% of our patients, although none had any secondary bacterial infection reported by earlier workers [22].

In our prospective study, the elevated D-dimer (>2 µg/L) was detected in 56% of viremia patients. In the study by Wang et al., patients requiring ICU treatment had significantly higher D-dimers ($p<0.001$). D-dimer >2 µg/ml at the time of admission may lead to a poor prognosis as it is associated with a higher risk of developing acute respiratory distress syndrome [20,24].

Another significant finding was the presence of elevated serum ferritin in viremia patients compared to non-viremic ones. Active ferritin production during the course of inflammatory diseases can occur from macrophages, which also produce cytokines. Moreover, ferritin synthesis can be induced by cytokines, such as IL-6. Interestingly, high IL-6 concentrations in COVID-19 patients have been correlated to disease severity. Thus, it is possible that

ferritin, apart from its classic role as an iron storage protein, can assume other functions like induce the expression of pro-and anti-inflammatory cytokines, contributing to cytokine storm [24].

The strengths of our study were evaluating serial samples of blood from COVID-19 patients of varying clinical severity revealing the relationship between disease course and SARS-CoV-2 viral load in blood, along with other blood parameters. Our study has some limitations; a more extensive patient number would have strengthened the present study. The blood from some COVID-19 patients may contain viral RNA but in low copy numbers or below the detection limit, often outside the threshold of a clinical diagnostic laboratory. Also, we tested blood samples after they had been subjected to a freeze/thaw cycle, which can potentially influence the retrieval of viral RNA.

Conclusion

This study demonstrates that the rising level of SARS-CoV-2 RNAemia is associated with poorer outcomes. Thus continuous monitoring during illness via measuring viral load and other biomarkers in the blood may have a role in decision making regarding the management of the disease. COVID-19 is like a complex chess game where, with every move, the virus seems to be adapting/ evolving. Further large-scale studies on viral and immunological parameters are urgently needed to checkmate this virus and reveal the big picture.

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Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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REFERENCES

1. Peng L, Liu J, XuW, et al. SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. J Med Virol 2020; 92 (9): 1676-1680. https://doi.org/10.1002/jmv.25936.
2. Chen JH-K, Yip CC-Y, Poon RW-S, et al. Evaluating the use of posterior oropharyngeal saliva in a point-of-care assay for the detection of SARS-CoV-2. Emerg Microbes Infect 2020;9(1):1–14. https://doi.org/10.1080/22221751.2020.1775133.
3. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis.2020;20(4):411–2. https://doi.org/10.1016/S1473-3099(20)30113-4
4. Wang W, XuY, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA.2020; 323(18) :1843–4. https://doi.org/10.1001/jama.2020.3786
5. Graham TGW, Dugast-Darzacq C, Dailey GM, et al. Open-source RNA extraction and RTqPCR methods for SARS-CoV-2 detection. PLOS ONE. 2021 16(2): e0246647. https://doi.org/10.1371/journal.pone.0246647
6. Clinical management protocol for COVID-19. https://www.mohfw.gov.in/pdf/Updated ClinicalManagementProtocolforCOVID19dated0307 2020.pdf (accessed on December 29, 2020).
7. Bangash MN, Patel J, Parekh D. COVID-19 and the liver: little cause for concern. Lancet Gastroenterol Hepatol 2020; 5(6):529-530. https://doi.org/10.1016/S2468-1253(20)30084-4.
8. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020; 395(10229):1033-1034. https://doi.org/10.1016/S0140-6736(20)30628-0
9. Chen W, LanY, Yuan X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect.2020;9:469–73. https://doi.org/10.1080/22221751.2020.1732837.
10. Huang C,Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
11. Young, BE, Ong SWX, Kalimudiin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA 323, 1488–1494 (2020). https://doi.org/10.1001/jama.2020.3204
12. Chang L, Yan Y, Wang L. Coronavirus disease 2019: corona viruses and blood safety. Transfus Med Rev. 2020; 34(2):75–80. https://doi.org/10.1016/j.tmrv.2020.02.003.
13. Hagman K, Hedenstierna M, Gille-Johnson P, et al. SARS-CoV-2 RNA in serum as predictor of severe outcome in COVID-19: a retrospective

cohort study. Clin Infect Dis. 2020; 2020:1285. https://doi.org/10.1093/cid/ciaa1285
14. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study. BMJ. 2020; 369:m1443. https://doi.org/10.1136/bmj.m1443
15. Dodd RY, Stramer SL. COVID-19 and blood safety: help with a dilemma.Transfus Med Rev. 2020; 34(2):73–4. https://doi.org/10.1016/j.tmrv.2020.02.004
16. Chen X, Zhao B, Qu Y, et al. Detectable serum SARS-CoV-2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. Clin Infect Dis. 2020; ciaa449. https://doi.org/10.1093/cid/ciaa449
17. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020; 581:465–9. https://doi.org/10.1038/s41586-020-2196-x
18. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020; 12(1):8. https://doi.org/10.1038/s41368-020-0074-x
19. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020; 323(11):1061-1069. https://doi.org/10.1001/jama.2020.1585.
20. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol. 2020; 95(6):E131-E134. https:// doi.org/10.1002/ajh.25774.
21. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020; 8 (5):475-481. https://doi.org/10.1016/S2213-2600(20)30079-5.
22. Deng Y, Liu W, Liu K, et al. Clinical characteristics of fatal and recovered cases of coronavirus disease 2019 (COVID-19) in Wuhan, China: a retrospective study. Chin Med J (Engl). 2020; 133 (11):1261-1267. https://doi.org/10.1097/CM9.0000000000000824
23. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost JTH. 2020; 18(4):844–847. https://doi.org/10.1111/jth.14768
24. T. Liu, J. Zhang, Y. Yang, et al. The potential role of IL-6 in monitoring severe case of coronavirus disease 2019. Available: https://www.medrxiv.org/content/ 10.1101/2020.03.01.20029769v2 (accessed on December 29, 2020).