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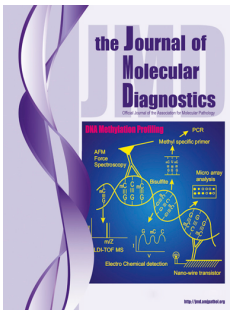

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Dynamics of Blood Viral Load is Strongly Associated with Clinical Outcomes in COVID-19 Patients: A Prospective Cohort Study

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ABSTRACT

The prevalence and clinical relevance of viremia in patients with coronavirus disease 2019 (COVID-19) are not well-studied. A prospective cohort study was designed to investigate blood viral load and clearance kinetics in 52 patients (median age, 62 years; 31 [59.6%] male) and explore their association with clinical features and outcomes based on a novel one-step reverse-transcription droplet digital PCR (RT-ddPCR). By using one-step RT-ddPCR, 92.3% (48/52) of this cohort was quantitatively detected with viremia. The concordance between the blood and oropharyngeal swab tests was 60.92% (53/87). One-step RT-ddPCR was tested with a 3.03% of false positive rate and lower 50% confidence interval of detection (LOD_{50}) at 54.026 copies/ml plasma. In all critical patients, the blood viral load was not eliminated, while the general and severe patients showed similar ability to clear the viral load. The viral loads in critical patients were significantly higher than those in general and severe counterparts. Among the 52 patients, 30 (58%) were discharged from hospital. Among half of 30 discharged patients, blood viral load remained positive, of which 76.9% (10/13) completely cleared their blood viral load at follow-up. Meanwhile, none of their close contacts had the evidence of infection. Quantitative determination of blood viral test is of great clinical significance to the management of COVID-19 patients.

Introduction

The end of 2019 witnessed an outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and its associated coronavirus disease 2019 (COVID-19) in Wuhan, China (1). By April 3, 2020, more than 1,000,000 cases have been confirmed, and among them over 50,000 died worldwide. Its rapid global spread has led to subsequent declaration of a pandemic by the World Health Organization (2). Currently, a great many clinical challenges concerning COVID-19 need to be addressed urgently. Mounting evidence has shown that, the SARS-CoV-2 test of respiratory specimens turned positive for the virus after discharge (3) which rises the concerns about the possible transmission via these patients.

To improve SARS-CoV-2 testing sensitivity, researchers tried different sampling locations. Peripheral blood should be theoretically an ideal sampling input for viral test since it contains a variety of biomarkers shed from the whole body, including cell-free DNA (cfDNA) and RNA (cfRNA), *et al* (4). Despite the critical clinical importance of viremia in pathogenesis and progression of COVID-19 (5), so far, only two studies examined the viral load in peripheral blood but the result was unsatisfactory due to limited sensitivity of real-time PCR (6). The prevalence and clinical significance of viremia in patients with COVID-19 are unknown. Recently, two studies had shown that droplet digital PCR (ddPCR), possesses much higher sensitivity and specificity than conventional traditional real-time PCR in SARS-CoV-2 test of respiratory specimens (7), which provides the possibility for studying the prevalence and clinical significance of viremia in COVID-19 patients. Furthermore, many evidences had proposed that one-step RT-droplet digital PCR (RT-ddPCR) proved to be more precise and fully amenable to the viral RNA absolute quantification, especially when RNA

concentration was low (8).

In this prospective cohort study, we developed a novel test for quantitative and dynamic assessment of the blood viral load of SARS-CoV-2 and validated it in 52 cases of confirmed COVID-19. By using this method, we attempted to study the prevalence and clinical significance of viremia in COVID-19 patients.

Materials and Methods

Study approval: This study was approved by the Medical ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Written informed consent was obtained from all patients or the patients' legal representative if the patient was too unwell to provide the consent.

Patients

Between January 30 to February 26, 2020, 52 patients with COVID-19 were enrolled into this prospective study after screening (Supplemental Figure S1). The diagnosis and the illness severity of COVID-19 were defined according to the Chinese management guideline for COVID-19 (version 7.0) (https://www.who.int/docs/default-source/wpro---documents/countries/china/covid-19-briefing-nhc/1-clinical-protocols-for-the-diagnosis-and-treatment-of-covid-19-v7.pdf **March 2020**) and the World Health Organization interim guidance (9). Methods for laboratory confirmation of SARS-CoV-2 infection have been described elsewhere (10). Exclusion criteria included: (1) no clinical and chest radiographic findings were available; (2) no written informed consent could be obtained; (3) patients had received remdesivir or were involved in other clinical

trials.

Study Design, Specimens and Data Collection

Specimens and data of the enrolled participants were collected prospectively according to study design (Supplemental Figure S1). The first and the third day after the enrollment were designated D_1 and D_3, respectively. D_x referred to the day when disease progressed according to the Chinese management guideline for COVID-19 (version 7.0). Dend was the day before discharge or the death. A patient had to meet the following criteria to be deemed clinically cured and eligible for discharge: (1) body temperature remained normal for more than 3 days; (2) respiratory symptoms resolved; (3) chest CT showed substantial improvement of acute exudative lesions; (4) two consecutive real-time PCR assays, at least 24 h apart, yielded negative results.

Peripheral blood was taken from patients on D_1, D_3, D_x and/or D_{end} for the determination of viral load by RT-ddPCR. Blood samples might be collected at other time points whenever necessary. Chest CT scan was performed before the enrollment and on D_{end}. Additional chest CT might be performed if the condition deteriorated. The real-time PCR for detecting SARS-CoV-2 RNA in oropharyngeal swab samples was conducted as described previously (10) and at an interval routine clinical practice requires. Anti-SARS-CoV-2-IgG/IgM was serologically detected on D_1, D_3, D_x and D_{end} by using chemiluminescence immunoassay kit (YHLO BIOTECH, Shenzhen, China). All the other treatments, laboratory examinations and clinical evaluation followed the routine clinical practice.

Patients' data were entered into an electronic data capture system (EDCS), including demographics, medical history, daily clinical findings, oximetric measurements and

laboratory data, involving complete blood count, serum biochemical parameters, high sensitivity C-reactive protein (hsCRP), serum interleukin-6 (IL-6), IL-8 and ferritin and treatments they received.

Follow-up

The patients were followed up by post-discharge clinic visit. The close contacts of the patients, who were eligible for discharge but were positive for plasma SARS-CoV-2, were also included for clinical and laboratory examination to rule out possible transmission by the patients.

Determination of SARS-CoV-2 copies number by One-step RT-ddPCR

For quantitative detection of SARS-CoV-2 copies number, Viral RNA purification kit (QIAamp Viral RNA Mini Kit, Qiagen，Germany), one-step RT-ddPCR advanced kit, QX200 droplet generator (BioRad，USA) and QX200 droplet reader (BioRad，USA) were used following the manufacturer's instructions. To increase sensitivity, 4-wells were used for each sample. The SARS-CoV-2 specific minor groove binder (MGB) probe-primer set was designed for targeting the *ORF1ab* region and the sequences were as follows: forward primer 5′-TGACCCTGTGGGTTTTACACTTAA-3′; reverse primer 5′-CAGCCATAACCTTTCCACATACC-3′; probe 5′FAM-AACACAGTCTGTACCGTCT-3′MGB. Primer blast results showed that 58,667 of 58,799 (99.78%) SARS-CoV-2 genomic sequences collected in GISAID database (https://db.cngb.org/gisaid/ **September 2020**) were on target templates (Supplemental Table **S1**). To test the quantitative capacity, SARS-CoV-2 plasmid standards (Sangon Biotech, Shanghai，China) were linearized by restriction enzyme BamHI , five-fold serially diluted at the concentrations ranging from approximately 50000

copies/μl to 16 copies/μl and subjected to ddPCR amplification. To evaluate the lower limit of detection, which were defined as the lower 50% confidence interval of detection (LOD_{50}), plasma samples from patient DF were used as standards (11). Plasma samples from 33 healthy donors served as negative control to estimate the false positivity in healthy samples of the test.

SARS-CoV-2-specific IgM and IgG detection

The SARS-CoV-2-specific IgM and IgG were detected by paramagnetic particle chemiluminescent immunoassay (CLIA) using iFlash-SARS-CoV-2 IgM/IgG assay kit (SHENZHEN YHLO BIOTECH CO., LTD., Shenzhen, China) and iFlash Immunoassay Analyzer (SHENZHEN YHLO BIOTECH CO., LTD.，Shenzhen， China).

Statistical Analysis

Categorical variables were presented as frequency and proportions. Mean ± standard deviation is given for continuous variables, median and ranges are given for variables that were not normally distributed. Means were compared by using *t* tests for normally distributed continuous variable. Otherwise, the Mann-Whitney U test was used. Multiple comparisons were made by utilizing Turkey's test. Coefficient of determination (R^2) was computed by linear regression analysis. Probit analysis for LOD_{50} was conducted with SPSS Statistics 25 (IBM Corp., USA).

Results

Patient Characteristics

The study included 52 patients with COVID-19 and their data are summarized in Table 1. The median age was 62 years (range, 26~83) with a sex ratio of 31/21(male/female). The general, severe and critical status of this cohort made up 40%, 33%, and 27%, respectively. Of note, the proportion of cases of various severities in our study did not reflect the actual case distribution since Tongji hospital is a designated medical center taking care of severe and critical patients. Comorbidities were present in 54% of the patients, with hypertension and diabetes being the most common ones. Of these patients, 4 (7.7%) and 2 (3.8%) had cancers or immunodeficiency diseases before. The median time from illness onset (always before admission) to enrollment was 17 days (range, 3-29). 49 of 52 (94%) patients were enrolled into our observational investigation over 7 days after the onset. About 80% of the patients received corticosteroid treatment. Finally, 30 (58%) patients who met the criteria were discharged with two consecutive real-time PCR assays, 15 (29%) patients died and 7 (13%) patients were still hospitalized for further observation or managements.

Detection and Validation of Plasma SARS-CoV-2 by One-step RT-ddPCR

We developed a SARS-CoV-2 detection system based on RT-ddPCR. Linearized plasmids with *ORF1ab* inserted were serially diluted and used as standards to test the quantitative capacity of the system. Each concentration, measured in triplicate, was well in line with expected concentration ($R^2 = 0.998$, $P < 0.001$), indicating that ddPCR using the probe-primer set for SARS-CoV-2 could accurately quantify SARS-CoV-2 copies (Figure 1A). Next, a COVID-19 patient DF with severe defect in humoral immunity, was continuously measured for SARS-CoV-2 load in blood and oropharyngeal swabs by using one-step RT-ddPCR. The

patient DF had a completed depletion of plasma cells due to anti-B cell mature antigen (BCMA) chimeric antigen receptor (CAR) T treatment for his relapsed multiple myeloma 3 months ago. BCMA CAR T cells could be persistently detected in the patient and caused the humoral immune deficiency to persist in the patient. Although his viral load in oropharyngeal swabs stayed at a constant level, his plasma SARS-CoV-2 load showed a continuous rise with his disease progressing to death (Figure 1B).

We then determined the LOD50 of one-step RT-ddPCR by using serially diluted blood samples of patient DF. Eight replicates of each concentration were tested. As shown in figure 1C, the LOD50 of plasma SARS-CoV-2 load was 54.026 copies/ml plasma. On the other hand, the false-positive rate was estimated from 33 healthy plasma samples, only one of them exhibited one positive droplet. Thus, the false positive rate was 3.03% (1/33).

To investigate the correlation in the SARS-CoV-2 load between oropharyngeal swabs and the corresponding plasma samples, eleven time-matched SARS-CoV-2-positive oropharyngeal swab samples were re-detected by one step RT-ddPCR. While the SARS-CoV-2 load in oropharyngeal swab were highly variable, the plasma viral load was relatively low, ranging from 80.4 to 187.5 copies/ml (excluding samples of patient DF) and bore no correlation with the load of their paired swab samples (P = 0.488) (Figure 1D).

Clinical Concordance between Blood Test and Oropharyngeal Swab Test

We compared 87 pairs of time-matched samples: i.e., plasma samples tested by one-step RT-ddPCR and oropharyngeal swab samples by real-time PCR. 16 out of 87 (18.39%) pairs of plasma and oropharyngeal swab samples were positive with both tests; 37 out of 87 (42.53%) were negative with the two tests. Thus, the concordance between these two testing

methods was 60.92% (53/87). Among 17 positive oropharyngeal swab samples, 16（94.12%） were also positive for plasma SARS-CoV-2. On the other hand, there were 33 paired tests with plasma viral positivity but negative oropharyngeal swabs.

Clearance of Blood SARS-CoV-2 in the Course of Disease

By applying one-step RT-ddPCR, 92.3% (48/52) of our cohort could be quantitatively detected with viremia. Clearance of blood SARS-CoV-2 in the course of disease was tracked according to the classification of clinical severity (Figure 2). Continuous increase of viremia was only observed in patient DF who had no appreciable level of SARS-CoV-2 antibody. At the cut-off day of the study, clearance of blood viral load was observed in 42.86% (9/21) of the general patients, with a median clearance time of 24 days (13~38 days). 43.75% (7/16) of the severe patients cleared the virus in their blood, with a median clearance time of 21 days (9~39 days). There was no statistical difference in the median clearance time between mild and severe patients (P = 0.484). Remarkably, all critical patients were unable to clear blood SARS-CoV-2. The critical patients C4 and C10 were followed up on day 45 and day 56, respectively. Their plasma viral loads remained positive.

Correlation of Blood Viral Load, Antibodies and Inflammatory Factors to Illness Severity of COVID-19

The average plasma viral load in general, severe and critical patients was 81.68 (0~312.5) copies/ml, 73.62 (0~317) copies/ml and 176 (0~392) copies/ml, respectively (Figure 3A). While the plasma viral load was significantly higher in critical patients than in general and severe patients (P < 0.001), no significant difference was found between the general and severe patients (P = 0.885). In our cohort, disease progression occurred in 2 general (G19 and

G9) and 3 severe patients (S13, S15 and S16) and 4 of the 5 patients concomitantly experienced a substantial increase in the blood viral load (Figure 3B). The peak level of anti-IgG of SARS-CoV-2 in critical patients was lower than in general (P = 0.044) and severe (P = 0.011) patients (Figure 3D), while no significant difference was found in peak IgM among the three groups (Figure 3C). All inflammatory factors including hsCRP, ferritin, sIL6 and sIL8 were profoundly higher in critically ill patients in comparison to all the other patients (Figure 3E-H). Interestingly, while hsCRP was significantly higher in severe patients than in general patients, no significant difference was revealed in the levels of ferritin, sIL6 and sIL8, suggesting that other cytokines might be responsible for the increased inflammatory reaction in severe patients.

Outcomes of Discharged Patients with Positive Blood Test and Their Close Contacts

Among 30 discharged patients, 18 were general patients and 12 were severe patients (Figure 2). At the time when they satisfied the discharge criteria, the blood viral test yielded positive results in 50% (15/30) of the patients. Important indicators, including disease severity, serum antibody level, viral cfRNA copies, time from illness onset to discharge, absolute lymphocyte count and corticosteroid exposure were compared between the positive and negative patients (Supplemental Table S**2**). There were no significant differences in these indicators between the two subgroups (Supplemental Table S**2**). To address the concerns of the final clinical outcome as well as contagion in these blood-positive patients, we followed up 13 of 15 patients and their close contacts. Physical examination, oropharyngeal and rectal swabs and blood viral tests of SARS-CoV-2 were performed (Table 2). The median days from the illness onset to the follow-up lasted 49 (45~61) days. The median day from close family contact to

follow-up was 7 (4~14) days. Interestingly, 10 of 13 patients eventually cleared their blood viral load without any further treatment. All 7 close contacts showed no evidence of SARS-CoV-2 infection.

Discussion

In this prospective cohort study, we developed and validated a novel detection method to quantitatively measure the viral load of SARS-CoV-2 in the blood of COVID-19 patients. Quantitative capacity was evaluated by linearized DNA, within which the reverse-transcriptase step was not included, hence the results were not completely applicable to RNA.

By using this technique, we were able to address the prevalence and clinical significance of viremia in COVID-19 patients. To our knowledge, this is the first report to depict the clearance kinetics of blood SARS-CoV-2 over the entire course of COVID-19. Remarkably, our study, including a protracted follow-up of survivors, suggested that all critical patients failed to eliminate blood viral load. In contrast, the general and severe patients were equally capable of completely clearing the blood viral load. Second, blood viral load was found to be associated with the severity of COVID-19. The viral load was significantly higher in the critical patients than in their general/severe counterparts, which could be, at least partially, ascribed to the impaired capability to generate SARS-CoV-2-specific antibodies on the part of critical patients. Among the patients whose disease deteriorated, a corresponding rise in blood viral load was detected. Interestingly, although there was no significant difference in the level of blood viral load between the general and severe patients, the severe patients showed stronger inflammatory response as defined by hsCRP level. This finding provides a rationale

for prospective clinical trial to reduce the over-activated host inflammatory response in severe patients. Moreover, no significant difference was found in the levels of cytokines between the general and severe patients, suggesting that other cytokines or chemokines might be more crucial for the immunopathological changes. Finally, 30 patients who met the current discharge criteria, of which 15 patients' blood viral load remained positive. Consistent with our data, other independent studies had reported that nucleic acid of SARS-CoV-2 can be frequently detected in urine, stool *etc* (12). All these findings raised serious concerns over the long-term outcome and infectivity of these patients, which seems to present public health issues in future. Fortunately, 10 out 13 the discharged patients completely cleared their blood viral load as revealed by our follow-up (spanning 45~61 days from the illness onset to the follow-up). Moreover, ddPCR detection showed that none of their close contacts exhibited the evidence of infection. Our results suggested that the clearance of blood SARS-CoV-2 might take longer time than expected and occurred in a gradual manner. Nevertheless, no evidence suggested that the patients in the course of viral clearance were contagious. The most recent online publication from German group has confirmed our finding (13). Although further investigation in a large cohort is warranted to draw definitive conclusions, the present findings were very encouraging.

This study has important clinical implications. By using this method, 92.3% of our cohort could be quantitatively detected with viremia, which serves as a useful tool for diagnosis, monitoring therapeutic response in terms of viral load and confirming the viral clearance and following patients up after discharge *etc*. Our study further supports the notion that COVID-19 is a self-limited disease with most of the general and severe cases who can

completely clear their viral load. On the other hand, critical patients appears to be a distinct subgroup characteristically with defective viral clearance (14), lower level of SARS-CoV-2-specific antibodies and strong inflammatory response. Therefore, the management of COVID-19 should be tailored since these patients are heterogeneous. For the general/severe cases, supportive therapies should be essential, and, with some severe cases, immunomodulatory therapies might be explored to suppress aberrant host inflammatory response. For the critical patients, anti-viral therapy, in combination with anti-inflammatory and supportive treatments should be considered and tested in clinical trial setting to improve their clinical outcomes. In this regard, dynamic monitoring of blood viral load by RT-ddPCR will assist physicians in identifying and monitoring the early signs of aggravation in critical cases. Finally, our study showed that the viremia in patient DF and other critical patients didn't suffice to produce SARS-CoV-2-specific antibodies, suggesting that the host humoral immunity plays a critical part in the removal of SARS-CoV-2 and the vaccines and therapeutic antibodies promise to be effective for the prevention and cure of COVID-19.

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References

1) Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung K, Lau E, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam T, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020, 382:1199-1207.

2) Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020, 382:727-733.

3) Mahase E. Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. BMJ. 2020, 368:m1036. doi: 10.1136/bmj.m1036

4) Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D, Tan S, Yin L, Xu J, Zhou C, Jiang C, Liu L. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020, 63:364-374.

5) Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, Su X, Cao B. SARS-CoV-2 and viral sepsis: observations and hypotheses. Lancet. 2020, 395:1517-1520.

6) Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, Li L, He R, Tan Y, Deng X, Gao M, Tang G, Zhao L, Wang J, Fan Q, Wen C, Tong Y, Tang Y, Hu F, Li F, Tang X. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect. 2020, 9:469-473.

7) Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, Gao G, Wang S, Ma C, Xie R, Wang F, Tan C, Zhu L, Guo Y, Zhang F. Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients. Clin Infect Dis. 2020, 71:793-798.

8) Racki N, Morisset D, Gutierrez-Aguirre I, Ravnikar M. One-step RT-droplet digital PCR: a breakthrough in the quantification of waterborne RNA viruses. Anal Bioanal Chem. 2014, 406:661-7.

9) World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance. WHO/2019-nCoV/clinical/2020.5

10) Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, Tao Q, Sun Z, Xia L. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology. 2020, 296:E32-E40.

11) Stone M, Lanteri MC, Bakkour S, Deng X, Galel SA, Linnen JM, Munoz-Jordan JL, Lanciotti RS, Rios M, Gallian P, Musso D, Levi JE, Sabino EC, Coffey LL, Busch MP. Relative analytical sensitivity of donor nucleic acid amplification technology screening and diagnostic real-time polymerase chain reaction assays for detection of Zika virus RNA. Transfusion. 2017, 57:734-747.

12) Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y, Guo Q, Sun X, Zhao D, Shen J, Zhang H, Liu H, Xia H, Tang J, Zhang K, Gong S. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat Med. 2020, 26:502-505.

13) Wolfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Muller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brunink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020, 581:465-469.

14) Chen J, Qi T, Liu L, Ling Y, Qian Z, Li T, Li F, Xu Q, Zhang Y, Xu S, Song Z, Zeng Y, Shen Y, Shi Y, Zhu

T, Lu H. Clinical progression of patients with COVID-19 in Shanghai, China. J Infect. 2020, 80:e1-e6.

Figure legends

Figure 1. The sensitivity and accuracy of plasma SARS-CoV-2 cfRNA quantification.

(A) Dilution curve of plasmid standards quantification by ddPCR. Correlation between expected and observed copy number were showed. Each black square represents a single replicate well of the dilution experiment, whereas the regression line is based on the average concentration at each dilution.

(B) SARS-CoV-2 concentration-time curve of plasma (blue) and oropharyngeal swab (red) samples from patient DF.

(C) Probit analysis sigmoid curve reporting the LOD_{50} of one-step RT-ddPCR. The red-dashed line represents the plasma SARS-CoV-2 concentration when the detection probability is 50%.

(D) Comparison of plasma and oropharyngeal swab SARS-CoV-2 concentration of time-matched samples.

Figure 2. SARS-CoV-2 dynamics of 52 patients.

One humoral immuno-deficient, 21 general, 16 severe and 14 critical patients are presented. Squares in different colors represent different virus loads. Star: patients met discharge standards at the specific days; circle: patients were dead at the specific days; triangle: patients were getting worse at the specific days.

Figure 3. Analysis of severity-associate clinical factors.

(A) Plasma SARS-CoV-2 loads in general, severe and critical patients were compared.

(B) Levels of plasma SARS-CoV-2 cfRNA before and after disease progression were analyzed. Paired t-test showed significance within these two groups.

(C-H) The levels of peak SARS-CoV-2 specific IgM (C), IgG (D), as well as hsCRP (E), peak serum ferritin (F), peak sIL6 (G) and peak sIL8 (H) were compared among general, severe and critical patients. Error bars, mean ± SD. Results were test for significance with Tukey's multiple comparisons test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. A *U* test was used.

Table 1. Patients characteristics

Characteristics	Data (N = 52)
Age, median (range) y	62 (26-83)
Sex (M/F)	31/21
Disease Severity status, No. (%)	
Mild	0 (0)
General	21 (40)
Severe	17 (33)
Critical	14 (27)
Comorbidities, No. (%)	28 (54)
Hypertension	17 (33)
Diabetes	9 (17)
Coronary heart disease	6 (12)
Chronic obstructive Pulmonary disease	1 (2)
Cancer*	4 (8)
Immunodeficiency†	2 (4)
Smoke history, No. (%)	7 (14)
Time from illness onset to enrollment, median (range) d	17 (3-40)
> 7 days, No. (%)	49 (94)
≤ 7 days, No. (%)	3 (6)
Treatments, No. (%)	
Antibiotics	35 (67)
Antiviral treatment	38 (73)
Intravenous Immunoglobin	13 (25)
Corticosteroids	42 (81)
High-flow nasal cannula oxygen therapy	5 (12)
Non-invasive mechanical ventilation	2 (4)
Invasive mechanical ventilation	11 (21)
ECMO	4 (8)
Renal replacement therapy	9 (17)
Clinical outcomes at data cutoff, No (%)	
Discharge from Hospital	30 (58)
Death	15 (29)
Hospitalization	7 (13)

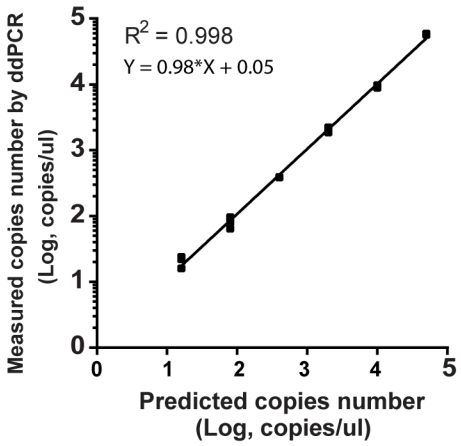
Abbreviations: y, years; M, male; F, female; d, days; ECMO, Extracorporeal Membrane Oxygenation; * They include: G8: acute B-cell lymphoblastic leukemia (no remission, NR), G21: endometrial cancer (NR), S8: endometrial cancer (no assessment) , D1: multiple myeloma , MM (complete remission ,CR); † They include: D1: MM post anti-BCMA CAR-T therapy, C9: pulmonary sarcoidosis with long-term glucocorticoid administration.

Table 2. Follow-up outcome of discharged patients with positive blood test and their close contacts

Participants		Relationship to patients	COVID-19 associated clinical manifestations	Follow-up to illness onset(d)	Follow-up to discharge(d)	Close contact time(d)	Viral load (copies/ml plasma)			Swabs on follow-up		
							Discharge point	Follow-up point				
Patients	Contacts							Patients	Contacts	nasal	throat	rectal
G10			No	55	22		71.4	0		N	N	N
G11			No	49	21		71.4	98		N	N	N
	C1 of G11	wife	No			7			0	N	N	N
G13			No	52	17		89.3	0		N	N	N
G14			No	61	17		89.3	0		N	N	N
G15			No	47	22		142.9	0		N	N	N
G18			No	49	22		258.9	0		N	N	N
	C1 of G18	husband	No			8			0	N	N	N
	C2 of G18	father in-law	No			8			0	N	N	N
G16			No	46	16		169.6	107		N	N	N
G17			No	46	28		250.0	0		N	N	N
	C1 of G17	husband	No			14			0	N	N	N
S8			No	45	20		62.5	0		N	N	N
	C1 of S8	daughter	No			6			0	N	N	N
	C2 of S8	son in-law	No			6			0	N	N	N
S9			No	47	21		71.4	0		N	N	N
S10			No	58	30		80.4	0		N	N	N
S11			No	54	18		98.2	0		N	N	NA
	C1 of S11	wife	No			4			0	N	N	N
S12			No	46	20		232.1	250		N	N	N
Total			0/20	49 (45-61)	21 (16-30)	7 (4-14)	89.3(62.5-258.9)	10/13	7/7	20/20	20/20	19/19

Abbreviations：N, Negative; NA, not available; d, days

A



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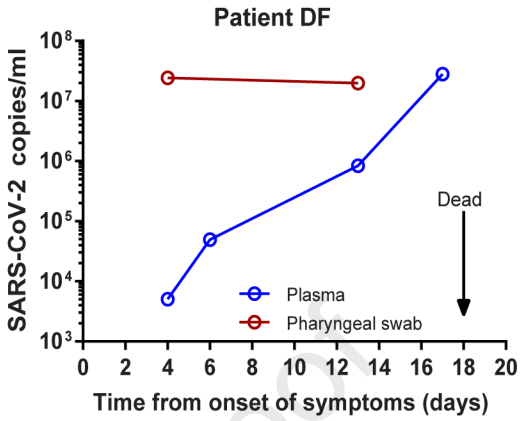

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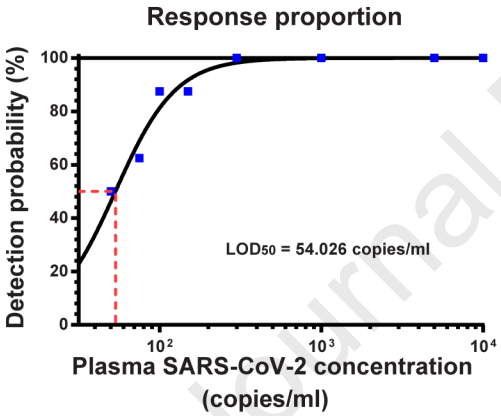

D