BRIEF REPORT

Decreased B Cells on Admission Associated With Prolonged Viral RNA Shedding From the Respiratory Tract in Coronavirus Disease 2019: A Case-Control Study

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The viral RNA shedding time (VST) for severe acute respiratory syndrome coronavirus 2 has not been well characterized. Clinical data were collected and compared between patients with short and long VSTs (in the lower and upper quartiles, respectively). The probability of recurrent positive reverse-transcription polymerase chain reaction results decreased sharply to 4.8% after 3 consecutive negative results. A series of ≥3 consecutive negative results was suitable as a criterion for the end of viral RNA shedding. The VST for shedding from the respiratory tract was significantly shorter in patients with normal B-cell counts on admission than in those with decreased B-cell counts (median [interquartile range], 11 [9–13] vs 16 [12–20] days, respectively; P = .001).

Keywords. B cells; COVID-19; viral RNA shedding; IL-10; T cells.

The outbreak of coronavirus disease 2019 (COVID-19) in Wuhan, China, beginning in late December 2019, has caused millions of infections around the world. Person-to-person transmission was proved shortly after the outbreak began [1]. The traditional public health measures used to contain severe acute respiratory syndrome (SARS), including syndrome surveillance, isolation, and quarantine, could be effective for containing the global outbreak of COVID-19 [2]. Unlike with SARS, the viral loads detected in asymptomatic patients with COVID-19 were as high as those in symptomatic patients [3], which explained why SARS coronavirus 2 (SARS-CoV-2) could be transmitted by either symptomatic or asymptomatic infected persons [4].

More alarmingly, some patients with COVID-19 who met criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative reverse-transcription polymerase chain reaction [RT-PCR] test results) had positive RT-PCR test results 5–13 days later [5]. Not identifying asymptomatic or mild symptomatic patients with long viral RNA shedding times (VSTs) will undoubtedly increase the risk of unrecognized infections and weaken the herd effort of community quarantine. Because SARS-CoV-2 has higher transmissibility than SARS, more rigorous criteria should be used for determining disease control measures. However, it should be noted that viral RNA may persist in respiratory specimens long after the disappearance of infectious virus [6].

The VST is closely related to the host immune status. In immunocompromised patients, influenza A can shed for as long as 18 months and cause problems with drug resistance [7]. In the setting of human coronavirus infection, immunosuppressive status was also associated with prolonged viral RNA shedding. Recovery from Middle East respiratory syndrome (MERS) and SARS have been associated with adaptive immunity [8]. In the current article, we report the duration of SARS-CoV-2 shedding from the respiratory tract, analyze risk factors associated with longer VSTs, and discuss the association of B-cell counts on admission with prolonged viral RNA shedding from the respiratory tract.

METHODS

Study Design and Participants

A case-control single-center study was performed in patients with COVID-19 hospitalized from 19 January to 19 February 2020 at the First Affiliated Hospital, College of Medicine, Zhejiang University, in Hangzhou, China. SARS-CoV-2 RT-PCR tests of respiratory specimens were conducted daily in these patients until discharge, and only qualitative data were available. The criteria for discharge were absence of fever for ≥3 days, improvement in chest computed tomographic and clinical symptoms, and 2 consecutive negative results for SARS-CoV-2 RNA in respiratory tract samples (nasal/throat swab or sputum) obtained ≥24 hours apart. The clinical characteristics and treatment outcomes were compared between patients with
short and those with long VSTs (in the lower and upper quartiles for VST, respectively).

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (no. IIT20200005C). The requirement for written informed consent was waived by the ethics commission for this retrospective study.

Definition of Variables

All cases were laboratory confirmed, with the presence of SARS-CoV-2 in respiratory specimens demonstrated by RT-PCR. The end of viral RNA shedding was determined by ≥2 consecutive negative RT-PCR results. For patients still shedding viral RNA, the time between the date of confirmed diagnosis to the final follow-up date of 3 March 2020 was used to calculate. Acute respiratory distress syndrome was defined according to the World Health Organization interim guidance [9], and acute kidney injury was defined according to the Kidney Disease: Improving Global Outcomes definition [10]. Disease severity was determined as described elsewhere [11].

Statistical Analysis

Logistic regression and Cox proportional hazards regression analysis were used to determine risk factors for prolonged VST. The Kaplan-Meier method was used to estimate VST in the groups with normal or decreased B-cell counts, and the log-rank test was applied for comparison. Cases with missing lymphocyte counts were excluded for the corresponding calculations. Statistical analyses were performed using SPSS (version 19.0) and R (version 3.6.2) software.

RESULTS

VST Findings

In all, 104 patients with confirmed COVID-19 were included in this study; their median age was 55 years, and 42 (40.4%) were female. Of the 104 patients, 94 (90.4%) were identified as negative for viral RNA shedding and 82 (78.8%) patients were discharged by the final follow-up. No patient had died by the final follow-up. The median number of consecutive RT-PCR-negative respiratory specimens before discharge was 5 (interquartile range [IQR], 4–8).

Of the 104 patients, the median VST (IQR) was 11 (7–18) days, with a range from 1 to 39 days (see Supplementary Figure 1). Seven patients (6.7%) had extremely long VSTs (>30 days). The proportions of patients with prolonged VSTs (≥7 days) from the respiratory tract were comparable between our study and that of Young et al [12] in Singapore (76.9% vs 83.3%; P = .76). The median VST (IQR) for discharged patients was 10 (6–10) days for non-intensive care unit (ICU) and 13 (3–23) days for ICU patients (P = .62).

Recurrent Positive RT-PCR Results

Recurrent positive RT-PCR results in respiratory specimens after ≥2 consecutive negative results were observed in 24 patients (23%). The probabilities of positive RT-PCR results in the next test after 1 or 2 consecutive negative results were as high as 30.5% and 16.4%, respectively. As shown in Table 1, with the increase from 2 to 3 consecutive negative results, the rate of recurrent positive RT-PCR results decreased sharply to 4.8%. There were no recurrent positive results after ≥7 consecutive negative results (Supplementary Table 1).

Patient Characteristics in Short-VST and Long-VST Groups

Among the 104 enrolled patients with COVID-19, 24 patients with VSTs in the lower quartile (1–6 days) and 25 with VSTs in the upper quartile (19–39 days) were identified as the short-VST and long-VST groups, respectively. As shown in Supplementary Table 2, patients in the in the short-VST group were more likely to be female and had lower temperatures on admission than those in the long-VST group. The time from onset of illness to RT-PCR confirmation was longer in the short-VST group (median [IQR], 8 [4–10] vs 5 [3–8] days; P = .041). On admission, more patients in the long-VST group had lymphocytopenia (48.0% vs 20.8% in the short-VST group; P = .046), and the platelet counts were significantly lower in the long-VST group (median [IQR], 145 × 10^9/L [121–178 × 10^9/L] vs 193/μL [144–243 × 10^9/L] in the short-VST group; P = .02). There were no differences in chest computed tomographic manifestations between the 2 groups (Supplementary Table 3).

Although disease severity was comparable in the groups on admission, the long-VST group included more patients admitted to the ICU and treated with mechanical ventilation, and extracorporeal membrane oxygenation, through the final follow-up. At the end of follow-up, all patients in the short-ST group had been discharged, compared with 44% in the long-VST group (P < .001), and the hospitalization time for discharged patients was significantly shorter in

| No. of Consecutive Negative RT-PCR Results | Positive Results in Next RT-PCR, No. | Negative Results in Next RT-PCR, No. | Probability of Positive Results in Next RT-PCR, % |
|------------------------------------------|-------------------------------------|-------------------------------------|-----------------------------------------------|
| 1                                        | 54                                  | 123                                | 30.5                                          |
| 2                                        | 19                                  | 97                                 | 16.4                                          |
| 3                                        | 4                                   | 80                                 | 4.8                                           |
| 4                                        | 2                                   | 65                                 | 3.0                                           |
| 5                                        | 1                                   | 49                                 | 2.0                                           |
| 6                                        | 2                                   | 36                                 | 5.3                                           |

Table 1. Recurrent Positive Reverse-Transcription Polymerase Chain Reaction Results in Respiratory Specimens

Abbreviation: RT-PCR, reverse-transcription polymerase chain reaction.
the short-VST than in the long-VST group (11 vs 28 days; \( P < .001 \)) (Supplementary Table 4).

**Cytokines and Lymphocyte Subtype Counts**

Cytokines, including interleukin 2, 4, and 6, interleukin 10 (IL-10), tumor necrosis factor α, and interferon γ were measured. IL-10 levels were elevated in the long-VST group (median, 5.53 pg/mL) and much higher than in the short-VST group (3.09 pg/mL; \( P = .02 \)). Lymphocyte subtype counts were available in 13 (54.2%) and 24 (96%) of the patients in the short- and long-VST groups, respectively. T-, B-, and natural killer cell counts were decreased in the long-VST group compared with the normal reference range. Cell counts were higher in the short-VST than in the long-VST group for T cells (median, 829/μL vs 220/μL, respectively; \( P = .01 \)), CD4+ T cells (441/μL vs 97/μL; \( P = .01 \)), CD8+ T cells (346/μL vs 95/μL; \( P = .001 \)), and B cells (170/μL vs 73/μL; \( P = .001 \)) (Table 2).

**Factors Related to Prolonged VST**

Within the subcohort of patients in the short- and long-VST groups (\( n = 49 \)), all differential variables, except treatments and outcomes, were tested in univariable logistic regression to evaluate their association with prolonged VST. As shown in Supplementary Table 5, decreased B-cell counts, IL-10 levels elevated to twice the upper reference limit, temperature >37.5°C on admission, and decreased T-cell counts were risk factors for prolonged VST, whereas female sex, time from onset to positive RT-PCR results, and interferon treatment were protective factors. These factors were further analyzed in a multivariable logistic regression model (backward selection), and B-cell count decreases and IL-10 increases were independently significant in predicting prolonged VST.

Cox regression analysis was further used to verify these results in the full cohort (\( n = 104 \)). Decreased B-cell count, time from onset to positive RT-PCR result, female sex, and temperature >37.5°C on admission were associated with the probability of ending viral RNA shedding. With multivariable Cox regression analysis (backward selection), only decreased B-cell count and time from onset to first positive RT-PCR result were independently significant in predicting the probability of ending viral RNA shedding (Supplementary Table 6). Comparing the results of the above applied sensitive analysis, B-cell counts were the only factor associated with prolonged VST. Supplementary Figure 2 displays Kaplan-Meier analysis showing that the median VST in the group with decreased B-cell counts was significantly longer than that in the median VST in the group without decreased counts (16 \([95\% \text{ confidence interval, } 12–20]\) vs 11 \([9–13]\) days; \( P = .001 \)).

**DISCUSSION**

In the current study, we determined the VSTs and recurrent positive RT-PCR results in respiratory specimens based on daily virus monitoring in 104 patients with confirmed COVID-19. Patients in the long-VST group had lymphocytopenia and more severe illness than those in the short-VST group. The IL-10 levels increased and the B-cell counts decreased in the long-VST group.

A recent study by Zhou et al [13] found that the median VST (IQR) after illness onset among adult inpatients with COVID-19 was 20 (17–24) days in survivors, and the longest observed VST among survivors in Wuhan was 37 days. Our study was conducted outside Wuhan, and the median VST (11 days) was shorter than that reported by Zhou et al. The longest VST in our study was 39 days, comparable to 37 days in Wuhan.

**Table 2. Immune Factors in 104 Patients With Coronavirus Disease 2019 by Viral RNA Shedding Time**

| Variable                 | Median Value (IQR) | \( P \) Value |
|--------------------------|-------------------|---------------|
| **Cytokine levels, pg/mL (reference range)** |                   |               |
| IL-2 (0–4.13)            | 0.43 (0.00–1.49)  | .61           |
| IL-4 (0–8.37)            | 0.00 (0.00–0.63)  | .88           |
| IL-6 (0–6.61)            | 20.85 (8.65–56.31)| .050          |
| IL-10 (0–2.31)           | 4.46 (2.77–7.74)  | .02*          |
| TNF-α (0–33.27)           | 16.04 (0.00–53.29)| .42           |
| IFN-γ (0–20.06)           | 0.12 (4.96–27.99) | .70           |
| **Lymphocyte counts, cells/μL (reference range)** |                   |               |
| T cells (955–2860)       | 319 (201–840)     | .01*          |
| CD4+ T cells (550–1440)  | 152 (89–482)     | .01*          |
| CD8+ T cells (320–1250)  | 131 (77–286)     | .02*          |
| B cells (90–560)         | 115 (67–193)     | .001*         |
| NK cells (150–1100)      | 99 (52–177)      | .31           |

Abbreviations: IFN, interferon; IL-2, IL-4, IL-6, and IL-10, interleukin 2, 4, 6, and 10; IQR, interquartile range; NK, natural killer; TNF, tumor necrosis factor; VST, viral shedding time.

*Significant at \( P < .05 \).
Interestingly, our median VST (12 days) and our proportion of patients with prolonged VST (≥7 days) from the respiratory tract (76.9% vs 83.3%) were similar to the findings reported by Young et al [12] in Singapore, indicating that VSTs were comparable outside Wuhan.

Although 2 consecutive negative RT-PCR results for SARS-CoV-2 separated by ≥24 hours was a widely accepted criterion for the end of viral RNA shedding, we found that the probabilities of positive RT-PCR results in the next test after 1 or 2 consecutive negative results were as high as 30.5% and 16.4% respectively. The probability of recurrent positive RT-PCR results decreased sharply to 4.8% after 3 consecutive negative results. Therefore, ≥3 consecutive negative results were suitable as the criterion to determine the end of viral RNA shedding. To our knowledge, ours is the first study to characterize the probability of recurrent positive RT-PCR results.

Decreased T-cell counts have been observed in patients SARS and MERS. MERS-CoV can infect T cells and induce T-cell apoptosis [14]. Peripheral CD4+ and CD8+ T-cell counts were substantially reduced in a single-case COVID-19 pathological report [15]. In the current study, decreased T-cell and T-cell subtype counts were observed. Further evidence is needed to determine whether SARS-CoV-2 infects T cells directly and to clarify the mechanism of T cells in COVID-19. It is well acknowledged that virus can suppress B-cell proliferation with viral proteins by means of various strategies. We found decreased B cells in the long-VST group, which indicated compromised B-cell activation with the infection of SARS-CoV-2. Nevertheless, whether convalescent plasma or B-cell targeting therapy has therapeutic potential in COVID-19 needs further study.

A series of ≥3 consecutive negative results was suitable as a criterion for the end of viral RNA shedding. Increased IL-10 levels and decreased of T-cell and B-cell counts were associated with prolonged viral RNA shedding. Because patients with decreased B-cell counts had significant longer VSTs than patients with normal B-cell counts, more attention should be paid in clinical practice to patients in this subgroup.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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