Research Article

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Significance of nucleic acid positive anal swab in COVID-19 patients

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Abstract

Aim – We compared the clinical characteristics of patients with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) positive and negative anal swabs during coronavirus disease 2019 (COVID-19) recovery and investigated the clinical significance and influence factors of anal swab detection.

Methods – This study retrospectively analyzed 23 moderate COVID-19 patients in the recovery phase. They were divided into anal swab positive group (n = 13) (negative for pharyngeal swabs but positive for anal swabs) and anal swab negative group (n = 10) (negative for pharyngeal and anal swabs). The epidemiology, clinical symptoms, time of pharyngeal swabs turning negative, and laboratory results were compared.

Results – The time of pharyngeal swabs turning negative in the anal swab positive group was 6 (5–8.5) days, significantly longer than that in the anal swab negative group (1 (1–4.25) days), P = 0.0002. The platelet count of the anal swab positive group was significantly lower than that of the anal swab negative group (198 (135–235) × 10^9/L vs 240.5 (227–264.75) × 10^9/L, P = 0.0248). No significant difference was observed between the two groups in other variables.

Conclusions – The time of pharyngeal swab turning negative in anal swab positive patients is longer than that in anal swab negative patients. The platelet count can be used as an indicator for viral infection evaluation. For patients with a longer time of pharyngeal swabs turning negative, the combined testing of the anal swab and platelet counts may help to avoid pharyngeal swab false negatives, premature discharge, and the possibility of fecal-oral transmission.

Keywords: coronavirus disease 2019, anal swab, pharyngeal swab, platelet count

1 Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), was first found in Wuhan, China, in late December 2019 [1]. It is highly contagious and currently circulating worldwide. As of June 28, 2020, SARS-CoV-2 has caused over 1 billion infections and a cumulative death toll of more than 5,00,000. Its main transmission route is respiratory droplet transmission and contact transmission [2]. It has been confirmed that the RNA fragments of SARS-CoV-2 can be detected in the fecal specimens and anal swabs of COVID-19 patients [3–7]. Additionally, the gastrointestinal tissue samples of COVID-19 infected patients are also tested positive for SARS-CoV-2 [7]. It is also observed that after the patient’s pharyngeal swab became negative, the nucleic acid test of the fecal samples was still positive and that the median time for viral shedding from feces after the pharyngeal swab turned negative was 7 days [7]. The SARS-CoV-2 RNA can be detected in feces only from the 5th day of infection, and the positive rate gradually increases over time, reaching a peak on the 11th day [8]. Interestingly, SARS-CoV-2 RNA fragments can still be detected in the feces of a small number of people after 30 days of infection [8]. These studies suggest the possibility of fecal-oral transmission of COVID-19, and that detection of SARS-CoV-2 RNA can also be performed in anal swabs.
Clinically, the negative pharyngeal swab is used as the standard to rule out SARS-CoV-2 infection, and the pharyngeal swab negative for two consecutive times of more than 24 h apart is used as the standard for discharge [9]. However, the results of pharyngeal swabs can be falsely negative due to the non-standard sampling method or sampling site and low viral load. The results of the pharyngeal swab nucleic acid test may not truly reflect the viral load in the body, especially in patients with convalescence and asymptomatic infections [10]. Thus, supplementary methods for SARS-CoV-2 RNA detection are necessary. As mentioned above, SARS-CoV-2 RNA fragments can be detected in fecal samples. Although the fecal samples of COVID-19 patients are not available at any time, the anal swab is feasible for nucleic acid detection. The anal swab is used as a supplementary method to pharyngeal swab in detecting SARS-CoV-2 [11].

In this study, we analyzed the clinical characteristics of COVID-19 patients with positive and negative SARS-CoV-2 anal swabs and explored the clinical significance and possible influence factors of anal swab detection of SARS-CoV-2 in COVID-19 patients during recovery.

### 2 Material and methods

#### 2.1 Study design and patients

This study is a retrospective study. We enrolled 23 moderate COVID-19 patients who were in the recovery phase and were hospitalized in the Hezheng ward of Shenzhen Hospital of Southern Medical University from February 11, 2020, to March 5, 2020. They were transferred from Shenzhen Third People’s hospital when two consecutive times of pharyngeal swab more than 24 h were negative and vital signs were stable. Moderate COVID-19 was defined when there were symptoms of fever and respiratory symptoms such as dry cough, running nose, and features of pneumonia on imaging. Diagnostic criteria and therapy for COVID-19 were according National Health Commission of the People’s Republic of China Diagnostic and Treatment Protocol for COVID-19 (trial fifth Edition) [9]. During the observation, pharyngeal swabs and anal swabs were reviewed at the same time every 3–5 days, and relevant laboratory tests were reviewed regularly. Exclusion criteria were as follows: patients with obvious dysfunction of heart, liver, kidney, and brain organs were excluded. According to the anal swab test results, patients were divided into anal swab positive group \((n = 13)\) and anal swab negative group \((n = 10)\).

#### 2.2 Data collection

Patients’ basic information, epidemiological history, clinical symptoms (including medical history, comorbidities, physical signs, gastrointestinal symptoms (such as diarrhea), etc.), time of pharyngeal swab nucleic acid turning negative, as well as results of laboratory tests, including white blood cell (WBC), neutrophils percentage (NEUT%), lymphocyte percentage (LYMPH%), lymphocyte absolute value (LYMPH #), platelet count (PLT), D-dimer, alanine aminotransferase (ALT), aspartate aminotransferase (AST), activation partial thrombin time (APTT), C-reactive protein (CRP), and days of chest CT absorption greater than 50%, were obtained from the electronic medical records.

#### 2.3 Laboratory examination

Pharyngeal swabs and anal swabs of patients were tested for SARS-CoV-2 RNA by RT-PCR. Laboratory confirmation of SARS-CoV-2 RNA was performed by the clinical laboratory in Shenzhen Hospital of Southern Medical University. The RT-PCR assay was conducted in accordance with the protocol established by the WHO [12].

#### 2.4 Statistical analysis

Statistical analysis was conducted using SPSS 16.0 statistical software. Data were expressed as the median and interquartile range or \(n\) (%). The comparison of measurement data was conducted using a non-parametric test of two independent sample \(t\)-tests. The count data were analyzed by the chi-square test or Fisher exact tests. The continuous variables of non-normal distribution were compared with the Mann–Whitney \(U\) test. \(P < 0.05\) was considered statistically significant.

**Ethics statement:** This study was approved by the Ethics Committee of Shenzhen Hospital of Southern Medical University (NYSZYYEC202000017). The data were anonymous and thus the requirement for informed consent was therefore waived.
3 Results

3.1 Demographics and baseline characteristics

The demographic, epidemiological, and clinical data of the COVID-19 patients are shown in Table 1. Among the 23 patients, 13 were in the anal swab positive group, including 6 males and 7 females, with a median age of 25.0 (6.34-43.5) years old. Among these 13 patients, there were 4 pediatric patients. Their ages were 5.58 years (5 years and 7 months), 6.43 years (6 years and 5 months), 2.83 years (2 years and 10 months), and 6.25 years (6 years and 3 months). There were 6 males and 4 females in the anal swab negative group, and their median age was 34.0 (24.50-56.75) years old. Of the 13 patients with positive anal swab results, 10 (76.92%) had a history of travel or residence in Wuhan and 6 (46.15%) had a history of contact with COVID patients. In the anal swab negative group (n = 10), 5 patients (50.0%) had a history of travel or residence in Wuhan and 2 (20.0%) had a history of contact with COVID patients. The difference between the two groups in epidemiology was not statistically significant (P = 0.398) (Table 1).

3.2 Symptoms and time of pharyngeal swab turning negative

As shown in Table 1, of the 13 anal swab positive patients, 10 (76.92%) had fever, 7 (53.85%) had cough, 10 (76.92%) had fatigue, 2 (15.38%) had throat discomfort, and 2 (15.38%) had diarrhea. Of the 10 anal swab negative patients, 6 (60%) had fever, 3 (30%) had cough, 7 (70%) had fatigue, 1 (10%) had throat discomfort, and 1 (10%) had diarrhea. The clinical symptoms of fever, fatigue, cough, throat discomfort, and diarrhea between the two groups were not statistically significant (P > 0.05). None of the included patients had the gastrointestinal symptoms of nausea, vomiting, or abdominal pain. One anal swab negative patient had decreased appetite. The time of pharyngeal swab turning negative was 6 (5-8.5) days in the anal swab positive group, significantly longer than that in the anal swab negative group (1 (1-4.25) days) (P = 0.0002) (Table 1 and Figure 1a).

3.3 Laboratory test results

The contents of WBC, NEUT, LYMPH, PLT, D-dimer, ALT, AST, APTT, and CRP and the time of chest CT absorption greater than 50% were also analyzed (Table 2). We found that the results of WBC, NEUT, LYMPH, D-dimer, ALT, AST, APTT, CRP, and time of chest CT absorption greater than 50% showed no statistically significant difference between the two groups (P > 0.05). The PLT in the anal swab positive group was 198 (135-235) × 10^9/L, significantly lower than that in the anal swab negative group 240.5 (227-264.75) (P = 0.0248) (Table 2 and Figure 1b).

| Items                                      | Anal swab positive (n = 13) | Anal swab negative (n = 10) | χ^2     | P value |
|--------------------------------------------|----------------------------|-----------------------------|---------|---------|
| Male                                       | 6                          | 6                           | 4.261   | 0.119   |
| Female                                     | 7                          | 4                           |         |         |
| Age (years)                                | 25.0 (6.34-43.5)           | 34.0 (24.50-56.75)          | 0.101   |         |
| Exposure history                           | 12 (92.3%)                 | 7 (70.0%)                   | 0.713   | 0.398   |
| History of Travel or residence in Wuhan, China | 10 (76.92%)                | 5 (50.0%)                   |         |         |
| History of contact with COVID-19 patients | 6 (46.15%)                 | 2 (20.0%)                   |         |         |
| Combination                                | 0                          | 2                           | 7.913   | 0.178   |
| Hypertension                               | 0                          | 1                           |         |         |
| Diabetes                                   | 0                          | 2                           |         |         |
| Symptoms                                   |                            |                             |         |         |
| Fever                                      | 10 (76.92%)                | 6 (60.0%)                   | 0.174   | 0.676   |
| Cough                                      | 7 (53.85%)                 | 3 (30.0%)                   | 0.518   | 0.472   |
| Throat discomfort                           | 2 (15.38%)                 | 1 (10.0%)                   |         | 1.000   |
| Diarrhea                                   | 2 (15.38%)                 | 1 (10.0%)                   |         | 0.000   |
| Fatigue                                    | 10 (76.92%)                | 7 (70.0%)                   |         | 1.000   |
| Time of pharyngeal swab turning negative (days) | 6 (5-8.5)                  | 1 (1-4.25)                  |         | 0.0002  |

Note: Data were expressed as the median and interquartile range or n (%). Comparison of measurement data was conducted using a non-parametric test of two independent sample t-tests. Count data were analyzed by the chi-square test or Fisher exact tests. Continuous variables of non-normal distribution were compared with the Mann–Whitney U test. P < 0.05 was considered statistically significant.
4 Discussion

Common clinical symptoms of patients with COVID-19 include fever, cough, fatigue, chest tightness, and difficulty breathing. Compared with mild patients, about 10% of severe patients have diarrhea and nausea 1–2 days before the onset of fever and respiratory symptoms [13,14]. It is suggested that SARS-CoV-2 enters human cells through binding with angiotensin-converting enzyme 2 (ACE2) [15]. ACE2 is highly expressed not only in type II alveolar epithelial cells and esophageal epithelial cells but also in absorptive intestinal epithelial cells of the ileum and colon [16]. This study observed that both groups of patients had fever, cough, throat discomfort, and diarrhea, but there was no significant statistical difference in fever, fatigue, cough, throat discomfort, and diarrhea between the two groups. However, the small sample size was relatively small, and further studies are needed to verify this result.

The data of this study showed that 84.6% of anal swab positive patients mainly had fever or respiratory symptoms, while only 15.4% of them had diarrhea, suggesting that SARS-CoV-2 can be detected in anal swabs or fecal samples of COVID-19 patients without gastrointestinal symptoms. A study performed dynamic monitoring of the COVID-19 nucleic acid in the blood, pharyngeal swab, and anal swab in 39 COVID-19 hospitalized patients [3]. It was found that after several days of

| Laboratory test results | Anal swab positive (n = 13) | Anal swab negative (n = 10) | P value |
|-------------------------|-----------------------------|-----------------------------|---------|
| WBC (×10^9/L)           | 5.06 (4.26–5.54)            | 6.07 (4.34–6.83)            | 0.239   |
| NEUT%                   | 55.3 (47.35–65.9)           | 52.5 (47.54–61.13)          | 0.901   |
| LYMPH%                  | 33.4 (22.6–41)              | 31.4 (25.6–41.05)           | 0.852   |
| LYMPH# (×10^9/L)        | 1.28 (1.10–3.77)            | 1.72 (1.37–2.54)            | 0.410   |
| PLT (×10^9/L)           | 198 (135–235)               | 240.5 (227–264.75)          | 0.0248  |
| ALT (U/L)               | 15.1 (12.5–28.25)           | 28.5 (15.5–49.25)           | 0.186   |
| AST (U/L)               | 32.3 (22.5–40)              | 25 (20–28)                  | 0.148   |
| APTT (s)                | 34.7 (33.35–38.6)           | 36.25 (27.58–42.68)         | 0.784   |
| CRP (mg/L)              | 3.83 (0.92–19.55)           | 1.74 (0.56–3.99)            | 0.193   |
| D-dimers (µg/ml)        | 0.38 (0.29–0.71)            | 0.35 (0.22–0.68)            | 0.247   |
| Chest CT absorption >50% (days) | 10 (6–12)                  | 9.5 (3.5–12)                | 0.428   |

Note: WBC, white blood cell; NEUT%, neutrophils percentage; LYMPH%, lymphocyte percentage; LYMPH#, lymphocyte absolute value; PLT, platelet count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; APTT, activation partial thrombin time; CRP, C-reactive protein.

Data were expressed as the median and interquartile range. Comparison of measurement data was conducted using a non-parametric test of two independent sample t-tests. Count data were analyzed by the chi-square test or Fisher exact tests. Continuous variables of non-normal distribution were compared with the Mann–Whitney U test. P < 0.05 was considered statistically significant.
treatment, there were still 8 cases positive for a pharyngeal swab and 4 cases positive for an anal swab. The study also found that the pharyngeal swab nucleic acid test was negative, whereas the anal swab or blood sample nucleic acid test was positive. On the first day, 50% of patients had a positive pharyngeal swab, and 25% of patients had a positive anal swab. However, by day 5, the positive rate of pharyngeal swab decreased to 25%, while the positive rate of anal swab rose to 37.5%. These results imply that the positive rate of nucleic acid in pharyngeal swabs in the early stage of infection is higher, while in the late stage of infection, the positive rate of anal swabs is higher than that in pharyngeal swabs. Our study found that the absorption of the ground glass opacity of the lungs on chest CT was significantly improved by more than 50% after treatment. Nucleic acid tests of pharyngeal swabs were negative for many consecutive times, and thus, the patients met the discharge standards. After discharge, the patients were further observed for recovery at our hospital. Re-examination (every 3–5 days) during the recovery period showed that the pharyngeal swab was negative, but the anal swab was positive in some patients, suggesting that the virus may still be actively replicated in the gastrointestinal tract after the respiratory tract is cleared. Therefore, anal swab detection has important epidemiological significance for the management of COVID-19 patients in the recovery phase.

We found that the time of pharyngeal swabs turning negative in the anal swab positive group was 6 (5–8.5) days, significantly longer than that in the anal swab negative group (1 (1–4.5) days) \((P = 0.0002)\). However, the comparison of epidemiology of anal swab positive and anal swab negative groups (including patients with close contact with COVID-19) was not statistically significant. This suggests that epidemiology history is not an influencing factor of a positive anal swab. It has been reported that SARS-CoV-2 could be isolated from the feces of patients infected with COVID-19. However, Wu’s research team [8] and Roman’s research team [17] did not isolate the virus from the fecal samples of patients. This inconsistency may be related to the type of the included patients. The viral load of SARS-CoV-2 in critically ill or severe patients may be much higher than that in mild patients [18]. A systematic literature review, which included 26 articles on positive stool SARS-CoV-2 in COVID-19 patients, was conducted [19]. The results showed that the presence of SARS-CoV-2 in stool samples of COVID-19 patients was high, with 53.9% of fecal RNA was positive. The duration of fecal virus shedding was 1–33 days after a negative nasopharyngeal swab, and one of the results even remained positive 47 days after the onset of symptoms. Thus, further isolation is needed to observe whether the viral shedding is still in progress.

This study found that the PLT of patients in the anal swab positive group was lower than that in the anal swab negative group. It is suggested that during viral infections, platelet activity is increased [20]. Platelets can be activated by viral antigen-antibody complexes [21], and B lymphocyes also produce anti-platelet antibodies to certain viruses [22]. These processes that promote platelet activation will cause increased platelet consumption and clearance, leading to a reduction in PLT. However, the fastest way to destroy platelets is through the direct interaction between platelets and viruses [23]. It has also been found that decreased PLT is inversely proportional to the risk of death and acute respiratory failure [24]. Thus, PLT may be used as an indicator for evaluating viral infection.

The findings of this study have to be seen in the light of some limitations. First, the sample size was relatively small, which may lead to bias in the results. Larger sample size would help to better understand SARS-CoV-2 infection and COVID-19. Second, since RT-PCR detects only nucleic acid fragments of SARS-CoV-2, whether there is still a live virus in the body still needs further investigation. Third, the nucleic acid of pharyngeal swabs and anal swabs were not tested quantitatively. If they were, the relationship between them and PLT would be clearer. Further study is warranted.

5 Conclusion

In conclusion, for patients with a longer time of pharyngeal swab turning negative, it is highly recommended to be careful to use negative pharyngeal swabs as a discharge standard. The decrease of PLT in COVID-19 patients may be related to the viral load and the destruction of platelets by the virus. Therefore, PLT may be used as a reference indicator to evaluate the viral infection. For patients with a longer time of pharyngeal swabs turning negative, positive anal swabs is still possible. Combining PLT with anal swab testing may reduce the risk of premature discharge due to false-negative pharyngeal swab and the possible fecal-oral transmission.

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