Original Article

DOI: 10.5582/bst.2021.01090

Rapid SARS-CoV-2 antigen detection potentiates early diagnosis of COVID-19 disease

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SUMMARY As the COVID-19 epidemic is still ongoing, a more rapid detection of SARS-CoV-2 infection such as viral antigen-detection needs to be evaluated for early diagnosis of COVID-19 disease. Here, we report the dynamic changes of SARS-CoV-2 viral antigens in nasopharyngeal swabs of COVID-19 patients and its association with the viral nucleic acid clearance and clinical outcomes. Eighty-five COVID-19 patients were enrolled for detection of SARS-CoV-2 viral antigens, including 57 anti-SARS-CoV-2 antibody negative cases and 28 antibody positive cases. The viral antigen could be detected in 52.63% (30/57) patients with SARS-CoV-2 antibody negative at the early stage of SARS-CoV-2 infection, especially in the first 5 days after disease onset ($p = 0.0018$) and disappeared in about 8 days after disease onset. Viral antigens were highly detectable in patients with low Ct value (less than 30) of SARS-CoV-2 nucleic acid RT-PCT assay, suggesting the expression of viral antigen was associated with high viral load. Furthermore, positive antigen detection indicated disease progression, nine cases with positive antigen (9/30, 30.0%), in contrast to two cases (2/27, 7.40%) ($p = 0.0444$) with negative antigen, which progressed into severe disease. Thus, the viral antigens were persistent in early stages of infection when virus was in highly replicating status, and viral antigen detection promises to rapidly screen positive patients in the early stage of SARS-CoV-2 infection.

Keywords COVID-19, nasopharyngeal swab virus nucleic acid, antigen-detection, RT-qPCR Ct value, radiographic progression

1. Introduction

The pandemic of SARS-CoV-2 virus infections has caused 113,315,218 confirmed cases of coronavirus disease COVID-19, including 2,517,964 deaths up to 28 February 2021 in the world by World Health Organization (WHO) (*1*). Despite tremendous efforts to prevent the spread of SARS-CoV-2 worldwide, the high rate of person-to-person transmission with a large number of deaths poses a significant threat to global public health (*2*). The mortality in several countries exceeded 10% in the early stage of COVID-19 pandemic, which brought substantial economic losses and life threats. According to Chinese Center for Disease Control and Prevention, the overall mortality of COVID-19 patients is approximately 2.3% (*3*), which is obviously lower than Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) (*4*), however, the number of COVID-19 deaths is still high because of the lager quantity of COVID-19 patients (*5*).

At present, the diagnosis of COVID-19 mainly depends on RT-qPCR-based nucleic acid testing of SARS-CoV-2 virus (*6*). Specific testing for SARS-CoV-2 has significantly contributed to controlling this public health emergency and clinical practice. Further studies have demonstrated that the combined RT-qPCR detection with serological testing enhances diagnostic sensitivity and specificity (*7*). Recently new methods have developed to detect SARS-CoV-2 antigen(s) for diagnosis of acute or early infections, because SARS-CoV-2 antigen(s) are highly expressed in the respiratory

tract when the virus is actively replicating. For instance, monoclonal antibodies (mAbs) against the nucleocapsid protein of SARS-CoV-2 have promised a rapid antigen detection test (*2*).

To validate the diagnostic significance of SARS-CoV-2 antigen detection, here, we focused on the dynamic changes of virus antigen in COVID-19 patients during the course of SARS-CoV-2 nucleic acid clearance and the correlation of SARS-CoV-2 antigen existence with clinical outcome.

2. Materials and Methods

2.1. Subjects

This study included 85 patients (≥ 18 years old) with COVID-19 from December 9, 2020 to January 26, 2021, in the Shanghai Public Health Clinical Center. All patients were imported cases and diagnosed with COVID-19 according to the Eighth Edition of the Guidance for COVID-19 of China (5) and confirmed by nasopharyngeal swab nucleic acid test at the airport or quarantine hotels. This study was approved by the Ethics Committee of Shanghai Public Health Clinical Center (No. 2020-E142-01) and all participants consented.

All patients were classified into anti-SARS-CoV-2 antibody positive and negative groups based on the serological test of anti-SARS-CoV-2 IgG and IgM antibodies on admission day. Clinical and lab data were collected at admission, including sex, age, blood cell counts of $CD4^+$ and $CD8^+$ T lymphocytes, $CD19^+$ B lymphocyte and $CD14^+$ monocytes, erythrocyte sedimentation rate (ESR), and chest Computed Tomography (chest CT). The participant's histories of clinical and lab exams, together with the SARS-CoV-2 viral RNA detection data, were prospectively collected.

2. 2. Detection of SARS-CoV-2 viral RNA

Total RNA was extracted from a 200-mL sample of nasopharyngeal swabs using a magnetic bead-based nucleic acid extraction kit in a fully automated nucleic acid extraction instrument (Master Biotechnology, China). Dual fluorescence RT-PCR (Applied Biosystems 7500 Real-Time PCR Systems, Foster City, CA, USA) was performed according to the manufacturer's instructions. Gene ORF-1ab and gene N of SARS-CoV-2 virus were used as target sequences of PCR primers, respectively. A Ct value of greater than 40 was considered as negative detection.

2.3. Detection of SARS-CoV-2 viral antigen in nasopharyngeal swab specimens

As the low antigen expression in antibody positive cases, a rapid chromatographic immunoassay for the qualitative detection of specific antigens of SARS-CoV-2 virus in human nasopharynx was performed in antibody negative patients on different days of hospitalization using Diagnostic Kit for COVID-19 Antigen Test (Colloidal Gold) (Kehua Bio-engineering, China). This test device contains two antibody pre-coated lines, the "C" (control) and "T" (test) lines on the surface of the nitrocellulose membrane. The C line was pre-coated with anti-Chicken IgY antibody and the T line with anti-SARS-CoV-2 antibody. Color particle-conjugated anti-SARS-CoV-2 antibody was used as detector for SARS-CoV-2 antigen. During the test, SARS-CoV-2 antigens in the specimen interact with color particle-conjugated monoclonal anti-SARS-CoV-2 antibody, forming a color antigen-antibody complex. This complex migrates on the membrane *via* capillary action until the test line, where it will be captured by pre-coated anti-SARS-CoV-2 antibody. A colored test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen. The intensity of colored test line varies with the amount of SARS-CoV-2 antigen in the specimen. Color particle-conjugated Chicken IgY was used as detector for the control line.

2.4. Definitions

Based on the fact that some patients have no clinical symptoms at the time of COVID-19 disease confirmation, disease onset time was defined as first appearance of symptoms or first positive viral nucleic acid screening. The severity of COVID-19 was categorized into 4 groups according to the Chinese management guidelines for COVID-19 (version 8.0) (*8*): mild cases presented with mild symptoms without manifestation of pneumonia on imaging; moderate cases have fever, cough, sputum production, and other respiratory tract or non-specific symptoms along with manifestation of pneumonia on imaging; severe cases suffer from respiratory distress with respiratory frequency ≥ 30/min, SaO_2/SpO_2 below 94% on room air or a PaO_2 to FiO_2 ratio of 300 or lower; and critical cases show respiratory failure and need for mechanical ventilation, or shock or combination with other organ failure and need ICU care. Disease progression indicates that (*9*) mild or moderate disease on admission progressed to moderate or severe/critical disease; or (*10*) severe disease on admission progressed to critical disease.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 25.0 (International Business Machines Corporation, IBM, Armonk, New York, USA). Non-normally distributed data were presented as median and interquartile range (IQR) as appropriate. Categorical variables were

expressed as counts and percentages for each category. The Wilcoxon rank-sum tests and Kruskal-Wallis tests were applied to test differences between two groups, Fisher exact tests or Chi-square tests were used for categorical variables. Multiple linear regression was applied to determine the relationship between outcomes and the exploratory factor. $p < 0.05$ was considered significant. Figures were constructed using GraphPad Prism 8.0.

3. Results

3.1. Comparison of antibody positive and negative groups

This study enrolled 85 COVID-19 confirmed patients who are Chinese citizens returning from Italy, Russia, US, UK, Nigeria and France and their age ranged from 18 to 67 years old. Twenty-eight patients (32.94%) were positive for serum anti-SARS-Cov-2 IgG/IgM and 57 patients (67.06%) were negative. The age in antibody positive group was older than antibody negative group (Z = -2.256, p = 0.0241). There was no difference in sex (p = 0.3434) and body mass index (BMI) (Z = -0.683, p = 0.4949) between the two groups. Twenty-seven (31.76%) patients had radiographic progression during the first week of hospitalization, 24 of them (88.9%) were from antibody negative group, and 3 of them (11.1%) from antibody positive group. As a consequence, 8 cases (8/24, 33.3%) progressed from mild to moderate type, and 3 cases (3/24, 12.5%) from mild to severe type in the antibody negative group, in contrast to 3 of 3 cases (100%) from mild to moderate type with no severe progression in antibody positive group. The cell counts of $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ B cells in antibody negative group were significantly lower than those in antibody positive group (Z = -3.469, p = 0.0005; Z = -4.119, $p < 0.0001$; Z = -3.932, $p < 0.0001$, respectively), whereas, the thyroid stimulating hormone (TSH) level in antibody negative group was higher than antibody positive group (Z = -2.525, p = 0.0116). There was no significant difference between the two groups in ESR (Z = -0.595, p = 0.5519) and $CD14^+$ monocyte (Z = -0.463, p = 0.6434) level (Table 1).

3.2. Comparison of SARS-CoV-2 antigen positive and negative groups

Among the 57 antibody negative patients, 30 patients (52.63%) were SARS-CoV-2 antigen positive and 27 patients (47.36%) were negative. There was no difference in sex (χ^2 = 0.4838, p = 0.6285), age (Z = -0.392, p = 0.6951), BMI (Z = -0.655, p = 0.5123) and disease severity on admission (χ^2 = 1.233, p = 0.2174) between SARS-CoV-2 antigen positive and negative groups. Positive SARS-CoV-2 antigen detection likely indicates disease progression, as nine cases with positive antigen (9/30, 30.0%) progressed, in contrast to disease progression in 2 cases (2/27, 7.40%) with negative antigen detection (p = 0.0444). In the antigen positive group, there were 18 cases (18/30, 60.00%) with radiographic progression during the first week of hospitalization, in contrast to 6 of 27 cases (22.22%, p = 0.0068) in antigen negative group. There was no significant difference in levels of $CD4^+$ T cells, $CD8^+$ T

Table 1. The comparison of the groups with positive antibody and negative antibody

Items	Antibody (-) n = 57	Antibody (+) n = 28	Statistics	P
Gender (n,%)			-	0.3434
Female	23 (43.33%)	8 (28.57%)		
Male	34 (56.67%)	20 (71.43%)		
Age (Median)	38.0 (24.0-52.0)	29.5 (23.0-34.8)	Z = -2.256	0.0241
BMI (kg/m^2)	22.60 (20.35-25.14)	23.12 (21.37-26.02)	Z = -0.683	0.4949
Nasopharyngeal swab virus nucleic acid negative time*				
days of hospitalization	16.0 (8.0-24.0)	12.0 (3.0-22.0)	Z = -3.173	0.0015
days after onset	20.0 (10.0-25.0)	ND	ND	ND
Severity on admission (n, %)			-	0.0163
Mild	31 (46.67%)	23 (82.14%)		
Moderate	26 (53.33%)	5 (17.86%)		
Severe	0 (0)	0 (0)		
Disease progression (n, %)	11 (19.29%)	3 (10.71%)	-	0.3707
Mild to moderate	8 (14.03%)	3 (10.71%)	-	1.0000
Mild to severe	3 (5.26%)	0 (0)	-	-
Radiographic progression (n, %)	24 (42.11%)	3 (10.7%)	-	< 0.0001
$CD4^+$ cells (/μL)	480.0 (350.5-664.5)	647.5 (539.5-808.8)	Z = -3.469	0.0005
$CD8^+$ cells (/μL)	344.0 (216.5-426.5)	527 (362.3-639.0)	Z = -4.119	< 0.0001
$CD19^+$ cells (/μL)	171.0 (115.0-210.0)	277.0 (190.3-385.0)	Z = -3.932	< 0.0001
ESR (mm/H)	27.0 (8.5-41.5)	18.5 (9.3-33.0)	Z = -0.595	0.5519
Peripheral blood monocyte (10^9/L)	0.570 (0.365-0.720)	0.50 (0.423-0.593)	Z = -0.463	0.6434
TSH (μIU/mL)	2.38 (1.29-3.09)	1.64 (1.15-2.15)	Z = -2.525	0.0116

*55 cases with negative antibody were followed up until nasopharyngeal swab virus clearance.

Table 2. Comparison of groups with positive and negative antigen

Items	Antigen (+) n = 30	Antigen (-) n = 27	Statistics	P
Gender (n,%)			χ^2 = 0.4838	0.6285
Female	13 (43.33%)	10 (37.04%)		
Male	17 (56.67%)	17 (62.96%)		
Age (Median)	39.0 (24.75-56.25)	33.0 (24.00-50.00)	Z = -0.392	0.6951
BMI (kg/m^2)	23.09 (21.03-25.98)	22.04 (19.69-24.22)	Z = -0.655	0.5123
Nasopharyngeal swab virus nucleic acid negative time				
days of hospitalization	19.5 (13.3-25.0)	12.0 (3.0-22.0)	Z = -2.521	0.0117
days after onset	30.5 (15.3-27.0)	14.0 (5.0-23.0)	Z = -1.432	0.1520
Antigen negative time				
days of hospitalization	6.5 (5-9)	-	-	-
days after onset	8 (6-11)	-	-	-
Antibody appearance time				
days of hospitalization	12.0 (9.0-16.0)	10.0 (7.8-16.3)	Z = -0.813	0.4160
days after onset	13.0 (10.0-17.0)	12.5 (9.0-17.5)	Z = 0.000	1.0000
Severity on admission (n,%)			χ^2 = 1.233	0.2174
Mild	14 (46.67%)	17 (62.96%)		
Moderate	16 (53.33%)	10 (37.04%)		
Severe	0 (0)	0 (0)		
Disease progression (n,%)	9 (30.00%)	2 (7.41%)	-	0.0444
Mild to moderate	7 (23.34%)	1 (3.70%)	-	0.0543
Mild to severe	2 (6.67%)	1 (3.70%)	-	1.0000
Radiographic progression (n,%)	18 (60.0%)	6 (22.2%)	-	0.0068
CD4$^+$ cells (/μL)	465.5 (342.5-552.0)	513.0 (401.5-677.3)	Z = -1.135	0.2565
CD8$^+$ cells (/μL)	286 (162.8-446)	369 (228-420)	Z = -0.967	0.3336
CD19$^+$ cells (/μL)	143 (113.8-207)	183 (116-219)	Z = -1.031	0.3026
ESR (mm/H)	27.0 (10.0-38.8)	27.0 (8.0-47.0)	Z = -0.160	0.8728
Peripheral blood monocyte (10^9/L)	0.67 (0.40-0.76)	0.45 (0.35-0.66)	Z = -1.823	0.0684
TSH (μIU/mL)	2.33 (1.13-2.95)	2.47 (1.33-3.26)	Z = -0.751	0.4526

cells, CD19$^+$ B cells, CD14$^+$ monocyte, ESR, and TSH between the two groups (p > 0.05) (Table 2).

3.3. Dynamic changes of serum antibody and antigen and virus nucleic acid in nasopharyngeal swabs

All of the 30 cases positive for SARS-CoV-2 antigen detection were followed up until negative for antigen detection. The mean time of virus antigen disappearance was about 6.5(5-9) days of hospitalization or 8 (6-11) days after disease onset (Table 2). The median nasopharyngeal swab SARS-CoV-2 virus RNA clearance time in the antibody negative group was 20 (10.0-25.0) days after onset and 16.0 (8.0-24.0) days of hospitalization, which was significantly longer than that in antibody positive group {12.0 (3.0-22.0), Z = -3.173, p = 0.0015} (Table 1). The median hospitalization time for virus nucleic acid disappearance in nasopharyngeal swabs was significantly different between antigen positive group {19.5 days (13.3-25.0)} and antigen negative group {12.0 days (3.0-22.0)}(Z = -2.521, p = 0.0117), however, there was no difference on virus clearance time after disease onset (Z = -1.432, p = 1.1520). There was also no difference in antibody positive time between the two groups (p > 0.05) (Table 2). The positive rate of antigen was 57.78% (26/45) in 3 days, 60% (3/5) in 5 days and 0% (0/1) in 8 days of hospitalization (χ^2 = 4.0474, p = 0.0436). After 14 days of hospitalization, the antigen positive rate dropped to 20% (1/5). Taking the disease course into consideration, the antigen positive rate was 72% (18/25) in 3 days, 52.94% (9/17) in 5 days, 40% (2/5) in 7 days and 12.5%(1/8) after onset (p < 0.05) (Figure 1A, B). The viral antigen can be detected from nasopharyngeal swabs in 52.63% (30/57) patients with COVID-19 antibody negative at the early stage of SARS-CoV-2 infection, especially in the first 5 days after admission (p = 0.0007) (Table 4). According to follow-up data in 30 patients with positive antigen, the positive rate of antigen began to decrease gradually on the 3rd day, while the antibody began to appear gradually. The time for virus nucleic acid disappearance in nasopharyngeal swabs was longer than that for viral antibody and antigen disappearance (Figure 1C, D).

3.4. Factors related to antigen detection

A total of 35 samples, including 10 antigen negative samples and 25 antigen positive samples, were classified into three grades based on CT values of SARS-CoV-2 nucleic acid RT-PCR assay: less than 30, 30-35, 35-40, and two grades by the days after disease onset: less than 5 days, more than 5 days. The antigen was highly detected in cases with less than 30 CT value of the gene ORF-1ab (86.96%, 20/23, p = 0.0048) or gene N (84.62%, 22/26, p = 0.0074) (Table 3). In multi-analysis, the CT value of gene N (less than 30) and days after disease onset (less than 5 days) were positively correlated with the positive rate of antigen detection (p = 0.0018, p = 0.0018) (Table 4).

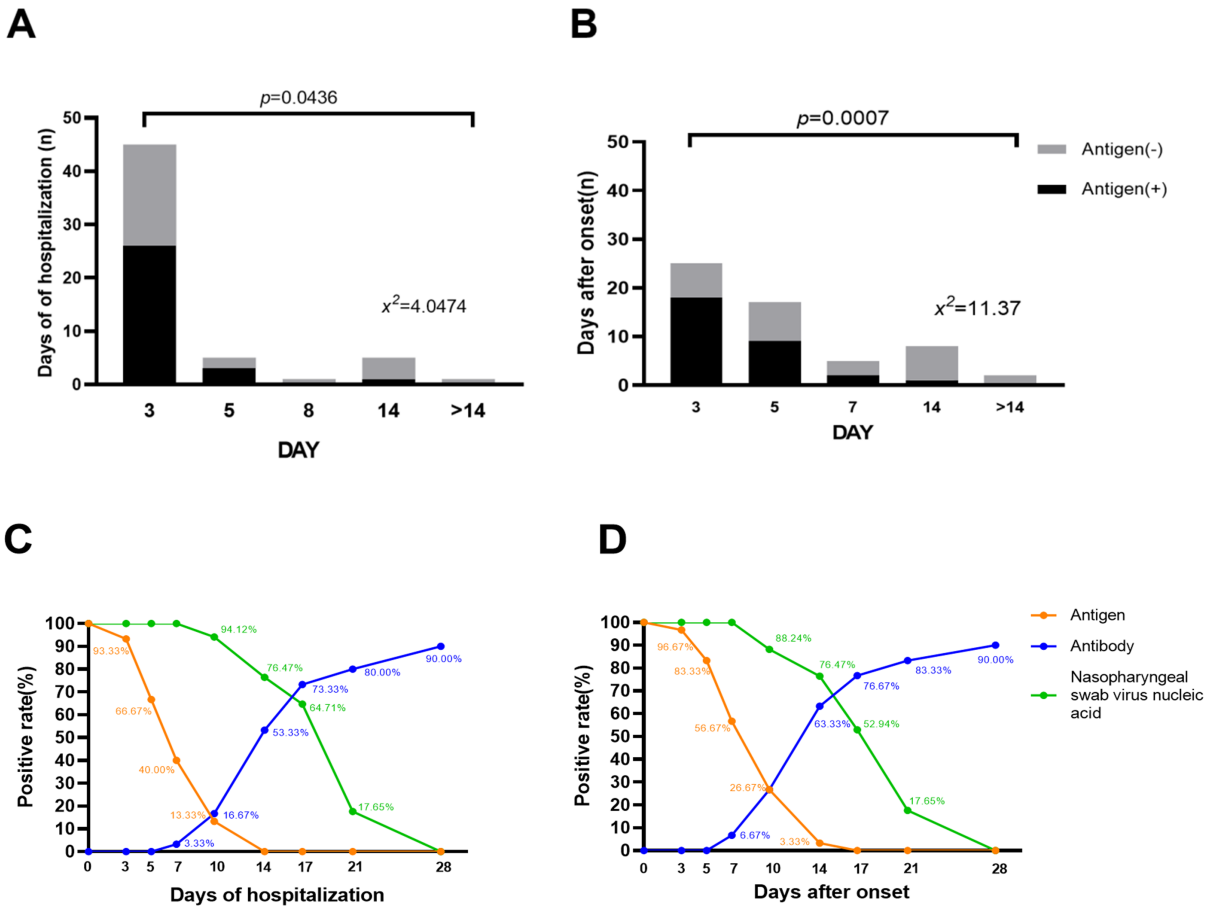

Figure 1. Positive rate of antigen in different days of hospitalization or after onset. The rate in different hospitalizations is shown in **(A)**. The positive rate of antigen was 57.78% (26/45) in 3 days, 60% (3/5) in 5 days, 0% (0/1) in 8 days and 20% (1/5) of hospitalization ($\chi^2 = 4.0474$, $p = 0.0436$). The rate in different days after onset is shown in **(B)**. Taking the disease course into consideration, the antigen positive rate was 72% (18/25) in 3 days, 52.94% (9/17) in 5 days, 40% (2/5) in 7 days and 12.5% (1/8) after onset ($p < 0.05$). Dynamic changes of antigen, antibody and viral RNA in nasopharyngeal swab of 17 antigen positive patients on the days after hospitalization **(C)** and disease onset **(D)**.

Table 3. The correlation of RT-qPCR Ct value of viral RNA and antigen detection

RT-qPCR (Ct value)	Gene ORF-1ab			Gene N		
	Antigen (+)	Antigen (-)	$P^{\ddagger}$	Antigen (+)	Antigen (-)	$P^{\ddagger}$
- 30.0	20	3	0.0048	20	3	0.0074
30.0 - 35.0	2	2		2	2	
35.0 - 40.0	1	3		1	3	
40.0 -	1	2		1	2	

‡The p value was calculated by comparison between the patients with Ct value less than 30 and those with more than 30.

Table 4. Multiple linear regression analysis of parameters with respect to virus antigen detection

Independent variables	Coefficient	St. Error	Beta	*t*	*P*
CT value < 30 (Gene N)	0.496	0.137	0.450	3.403	0.0018
Disease course (≤ 5 days)	0.496	0.137	0.450	3.403	0.0018

4. Discussion

The dynamic changes of SARS-CoV-2 antigens, serum anti-SARS-CoV-2 antibody, SARS-CoV-2 viral RNA clearance, and disease progression were evaluated in the early stages of infection in this study. The viral antigens were persistent in cases after less than 5 days infection with high viral load.

Molecular-based approaches are the first-line methods for diagnosis of SARS-CoV-2 acute infection. RT-qPCR assay of respiratory samples is the currently recommended method to confirm suspected cases

(*11*). However, this method is not efficient in rapidly screening a large number of individuals in places where thousands of people transit per hour. In addition, the accuracy of RT-qPCR depends on many factors, such as the sample type, stage of infection, skill of sample collection, and quality and consistency of the PCR assay (*12,13*). A new type of rapid diagnostic test (RDT) has been recently developed. It detects the presence of SARS-CoV-2 viral antigens in a respiratory tract sample, it is simple and can be completed typically within 30 minutes. However, recent research in Belgium showed that the poor sensitivity of the SARS-CoV-2 Ag Respi-Strip leads to false negative results, and suggested that SARS-CoV-2 Ag Respi-Strip should not be used alone for COVID-19 diagnosis (*14*). Viral antigen(s) are expressed only when the virus is in an actively replicating stage; thus, such tests are best for identification of acute or early infection (*15*), especially in the first 5 days after disease onset. In comparison with molecular techniques, antigen detection has several advantages such as ease, speed, low cost and non-requirement of special equipment or skills (*24*), and meets the need to rapidly screen positive patients at early infection.

Viral antigen clearance is earlier than viral RNA clearance after SARS-CoV-2 infection. In this study, we found that hospitalized patients of confirmed COVID-19 in Shanghai were at different stages of disease, mostly because all of them were diagnosed by viral nucleic acid screening after disembarking. Some of them had viral antibodies in their serum, while others did not. We found that the median negative turning time for virus nucleic acid in nasopharyngeal swab was about 16 days of hospitalization and 20 days after onset in the viral antibody negative group who showed longer virus clearance than those in the antibody positive group (12 days of hospitalization). Viral antigen can be detected in 52.63% (30/57) patients with anti-SARS-CoV-2 antibody negative from nasopharyngeal swabs in the first 5 days of hospitalization. When the CT value of viral nucleic acid RT-qPCR was less than 30, the positive rate of viral antigen was high either for gene ORF-1ab or for gene N. When the CT value was more than 30, the positive rate of antigen was significantly decreased. The grades of disease course and CT value in gene ORF-1ab may help to predict viral antigen detection. The continuous detection of viral antigen in nasopharyngeal swabs suggests that the antigen may disappear in about 6.5 days of hospitalization and 8 days after onset. According to the follow-up of 17 cases with antigen positive patients, the virus antigen disappeared earlier than both the nucleic acid and antibody disappearance. All of these tests may be helpful to estimate the stage of the disease.

Positive viral antigen detection suggests disease in progression. We found that nine cases with antigen positive (9/30, 30.0%) progressed, in contrast with only two cases with negative antigen (2/27, 7.40%) (p = 0.0444), which probably related to the strong immune response of B cells in the early stage of the disease, which is consistent with previous reports (*16,17*). An analysis of the correlation among factors such as CT value, disease course and detection of virus antigen in the two target genes showed that the disease course (less than 5 days) and CT value(less than 30) may help predict viral antigen detection, respectively, and the disease course was strongly correlated with antigen detection, that suggests the importance of early viral antigens detection. However, there was no significant difference in these immune indexes between antigen positive and negative groups, suggesting no significant change in cellular immune status during the disappearance of virus antigen. During the outbreak of SARS-CoV-2 infection, serum levels of TSH, T3 and T4 in COVID-19 patients were significantly lower than those in controls, and levels of T3 were a positive correlation with the severity of the disease (*22*). In our study, we found a negative correlation between TSH and virus antigen clearance, however, positive antibody detection indicates recovery from illness. The levels of $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ B cells in the antibody negative group were also lower than those in antibody positive group. The age and the thyroid stimulating hormone (TSH) level in antibody negative group were higher than antibody negative group. This suggests that the existence of antibody indicates the recovery of immune function.

In summary, the viral antigens were persistent in the nasopharyngeal place less than 5 days in early stages of infection and is related to high viral load, Viral antigen detection may be helpful to screen the positive patients early and rapidly. However, the small amount of samples and loss of quantitative detection of viral antigen and virus nucleic acid in this study limited its value in clinic application, further multi-center studies are needed in the future to validate its clinical significance.

Acknowledgements

We would like to thank all the participants whose data were used in this study. We are most grateful for the assistance and support of Shanghai Public Health Clinical Center for providing clinical data.

Funding: The work was supported by Guangdong Province Science and Technology Project (2018B020241002) Shanghai Science and Technology Innovation Action Plan, Medical Innovation Research Special Project (20Z11900900), National Natural Science Foundation of China (82072260) and Shanghai "Medical Garden Rising Star" Youth Medical Talent Training Funding Program in 2021.

Conflict of Interest: The authors have no conflicts of interest to disclose.

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Received March 7, 2021; Revised March 19, 2021; Accepted March 22, 2021.

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Released online in J-STAGE as advance publication March 26, 2021.