Characteristics and Prognosis of Antibody Non-Responders with COVID-19

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Research Article

Keywords: antibody, SARS-COV-2, COVID-19

Posted Date: November 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1015563/v1

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Abstract

Background: The coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been spreading globally. The information regarding the characteristics and prognosis of antibody non-responders with COVID-19 is scarce.

Method: In this retrospective, single-center study, we included all the patients with confirmed COVID-19 using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) admitted to the Fire God Mountain hospital from February 3, 2020, to April 14, 2020. A total of 1921 patients were divided into the antibody-negative group (n=94) and antibody-positive group (n=1827), and the 1:1 propensity score matching (PSM) was used to match two groups.

Results: In the antibody negative group, 40 patients (42.6%) were male, 54 patients (57.4%) were female, and 49 patients (52.1%) were older than 65 years old. Cough was the most common symptoms in the antibody negative group. White blood cell counts (WBC) 6.6×10⁹/L [5.0, 9.1], Neutrophils 4.3×10⁹/L [3.1, 6.6], C-reactive protein 7.3 mg/L [1.3, 49.0], Procalcitonin (PCT) 0.1 ng/mL [0.0, 0.2], Interleukin-6 (IL-6) 64.2 [1.5, 28.7], Lactate dehydrogenase (LDH) 193.8 U/L [154.9,260.6], Creatine kinase 60.5 U/L [40.5, 103.7], Creatine kinase isoenzyme 10.3 ng/mL [8.2, 14.5], Urea nitrogen 5.3 mmol/L [4.0, 8.7] and Creatinine 77.7 μmol/L [60.6, 98.7] were significantly higher in antibody negative patients than in antibody positive group (P<0.005). The days of nucleic acid negative conversion in the antibody negative group was shorter than that in the antibody positive group (P < 0.001). Meanwhile, the hospitalization time of antibody negative patients was shorter than that of antibody positive patients (8.0 [6.0, 10.0] VS 13.0 [8.2, 23.0], P < 0.001).

Conclusion: Some COVID-19 patients without specific antibodies had mild symptoms, but the inflammatory reaction caused by innate clinical immunity was more intense than those with antibodies, and the virus was cleared faster. The production of specific antibodies was unnecessary for SARS-CoV-2 clearance, and non-specific immune responses played an essential role in virus clearance.

Introduction

Since December 2019, a series of unexplained pneumonia cases have occurred in Wuhan, Hubei province, China. The pathogen has been identified as a novel enveloped RNA beta-coronavirus by gene sequencing and has subsequently been named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. The World Health Organization (WHO) has declared that the disease caused by SARS-CoV-2 was officially named: Coronavirus Disease 2019 (COVID-19)[2]. With the rapid spread of Covid-19, As of July 11th, 2021, a total of 186,232,998 laboratory-confirmed cases had been documented globally, with 4027,858 deceased[3].

At present, information regarding the characteristics and prognosis of antibody non-responders with COVID-19 is scarce. In this study, we did a retrospective review of electronic medical records from the patients with confirmed COVID-19 admitted to the Fire God Mountain hospital to determine that some patients do not produce specific antibody against SARS-CoV-2 and explore the immune system mechanism of clearing SARS-CoV-2.
Study design and participants

We did a retrospective review of electronic medical records from COVID-19 patients admitted to the Fire God Mountain hospital in Wuhan, from February 3, 2020, to April 14, 2020.

All the patients with confirmed COVID-19 using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) admitted to the Fire God Mountain hospital from February 3, 2020, to April 14, 2020, were enrolled with selectivity. The exclusion criteria of the patients included age less than 18 years old, more than three months from the initial diagnosis to admission, and no detection of serum IgM/IgG antibody during hospitalization. 1921 patients were divided into the antibody-negative group (n=94) and the antibody-positive group (n=1827). Each patient had at least one antibody test during the course of the disease. When all the antibody tests are negative, the patient is considered as the antibody-negative group; when one antibody test is positive, the patient is considered as the antibody-positive group.

Meanwhile, According to Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (trial version 7), COVID-19 patients are divided into mild cases, moderate cases, severe cases, critical cases. Moderate cases showing fever and respiratory symptoms with radiological findings of pneumonia. Severe cases were defined when one of the following criteria is met: (1) shortness of breath, RR $\geq$ 30 times/min; (2) Oxygen saturation is less than 93% in resting-state; (3) partial pressure of arterial oxygen (PaO2)/oxygen concentration (FiO2) $\leq$ 300mmHg; Critical cases were defined when one of the following criteria is met: (1) respiratory failure; (2) septic shock; (3) other organ failures.

Data Collection

Data of all patients were obtained using the standardized data collection form from the electronic health records by the research team of the department of respiratory, Chinese People’s liberation army general hospital. The data collection form included demographic data, the date of disease onset, comorbidities, symptoms, signs, laboratory findings, and serum antibody test and prognosis results.

Detection Of The Antibodies

Serum IgM and IgG antibodies against SARS-COV-2 were detected using a CFDA approved chemiluminescence kit by Bioscience Biotechnology Co., Ltd (Chongqing, China, REF of IgM: C86095M, IgG: C86095G), according to the manufacturer’s instructions. A threshold of 10 AU/ml was used for both IgM and IgG as the manufacturer recommended. When the sample threshold $\leq$ 10.00AU/mL, it is regarded as non-reactive, and when the sample threshold $\geq$ 10.00AU/mL, it is regarded as reactive.

Statistical analysis

The 1:1 propensity score matching (PSM) was used to match two groups, and the scoring factors were age, sex, hypertension, diabetes, CLD, malignancy, CKD, COPD. Statistical analysis was completed with Python (version 3.7). We use the mean (SD) to express continuous variables that follow a normal distribution, the
median (IQR) to express non-normally distributed values, and the frequency and percentage (%) were used to indicate categorical variables. Continuous variables were compared with a t-test when the variables were normally distributed; otherwise, the Mann-Whitney U test was used. Proportions for categorical variables were compared using the χ² test and prognostic correlation analysis. Furthermore, logistic regression, survival curves were also used. We judged that the difference was statistically significant when P <0.05.

Results

Demographic characteristics

From February 3, 2020, to April 14, 2020. A total of 1921 COVID-19 patients with laboratory-confirmed in Fire God Mountain hospital were included in this study; during hospitalization, 1921 patients were divided into the antibody-negative group (n=94) and the antibody-positive group (n=1827). Their data were analyzed in this study.

The demographic characteristics of the COVID-19 patient are shown in Table 1. In the antibody-negative group, 40 patients (42.6%) were male, 54 patients (57.4%) were female, and 49 patients (52.1%) were older than 65 years old. There was no significant difference in gender and age between the antibody-negative and antibody-positive groups after matching two groups with 1:1 propensity score matching (PSM).
|                               | Antibody-negative | Antibody-positive | P       | Antibody-positive (after matching) | P   |
|-------------------------------|-------------------|-------------------|---------|------------------------------------|-----|
|                               | n                 |                   |         |                                    |     |
| Gender                        |                   |                   |         |                                    |     |
| female                        | 54 (57.4)         | 898 (49.2)        | 0.143   | 49 (52.1)                          | 0.558 |
| male                          | 40 (42.6)         | 929 (50.8)        |         | 45 (47.9)                          |      |
| Age                           |                   |                   |         |                                    |     |
| <65                           | 45 (47.9)         | 1152 (63.1)       | 0.004   | 56 (59.6)                          | 0.144 |
| ≥65                           | 49 (52.1)         | 675 (36.9)        |         | 38 (40.4)                          |      |
| Fever                         |                   |                   |         |                                    |     |
| 0                             | 55 (58.5)         | 535 (29.3)        | <0.001  | 26 (27.7)                          | <0.001 |
| 1                             | 39 (41.5)         | 1292 (70.7)       |         | 68 (72.3)                          |      |
| Cough                         |                   |                   |         |                                    |     |
| 0                             | 43 (45.7)         | 582 (31.9)        | 0.007   | 38 (40.4)                          | 0.556 |
| 1                             | 51 (54.3)         | 1245 (68.1)       |         | 56 (59.6)                          |      |
| Sputum production             |                   |                   |         |                                    |     |
| 0                             | 73 (77.7)         | 1543 (84.5)       | 0.107   | 79 (84.0)                          | 0.354 |
| 1                             | 21 (22.3)         | 284 (15.5)        |         | 15 (16.0)                          |      |
| Myalgia                       |                   |                   |         |                                    |     |
| 0                             | 85 (90.4)         | 1349 (73.8)       | <0.001  | 67 (71.3)                          | 0.002 |
| 1                             | 9 (9.6)           | 478 (26.2)        |         | 27 (28.7)                          |      |
| Fatigue                       |                   |                   |         |                                    |     |
| 0                             | 67 (71.3)         | 912 (49.9)        | <0.001  | 45 (47.9)                          | 0.002 |
| 1                             | 27 (28.7)         | 915 (50.1)        |         | 49 (52.1)                          |      |
| Dyspnea                       |                   |                   |         |                                    |     |
| 0                             | 63 (67.0)         | 894 (48.9)        | 0.001   | 45 (47.9)                          | 0.012 |
| 1                             | 31 (33.0)         | 933 (51.1)        |         | 49 (52.1)                          |      |
| Diarrhoea                     |                   |                   |         |                                    |     |
| 0                             | 94 (100.0)        | 1720 (94.1)       | 0.029   | 90 (95.7)                          | 0.121 |
| 1                             |                   | 107 (5.9)         |         | 4 (4.3)                            |      |
| Family history of tumor       |                   |                   |         |                                    |     |
| 0                             | 93 (98.9)         | 1773 (97.0)       | 0.519   | 89 (94.7)                          | 0.211 |
| 1                             | 1 (1.1)           | 54 (3.0)          |         | 5 (5.3)                            |      |
| Hypertension                  |                   |                   |         |                                    |     |
| 0                             | 68 (72.3)         | 1376 (75.3)       | 0.597   | 65 (69.1)                          | 0.748 |
| 1                             | 26 (27.7)         | 451 (24.7)        |         | 29 (30.9)                          |      |
| Diabetes                      |                   |                   |         |                                    |     |
| 0                             | 83 (88.3)         | 1607 (88.0)       | 0.949   | 83 (88.3)                          | 1.000 |
| 1                             | 11 (11.7)         | 220 (12.0)        |         | 11 (11.7)                          |      |
| Chronic liver disease         |                   |                   |         |                                    |     |
| 0                             | 87 (92.6)         | 1747 (95.6)       | 0.194   | 89 (94.7)                          | 0.765 |

(0: There is no such symptom or coexisting illness; 1: patients have this symptom or coexisting illness; CKD: Chronic Kidney Disease; COPD: chronic obstructive pulmonary diseases)
Clinical Features

The Clinical features of the COVID-19 patient are shown in Table 1. Hypertension (27.7%) and diabetes (11.7%) were the most common coexisting illness in COVID-19 patients. Cough was the most common symptom in the antibody-negative group. Other prevalent symptoms at the onset of illness of COVID-19 patients in the antibody-negative group included fever (41.5%), dyspnea (33.0%), fatigue (28.7%), expectoration (22.3%), and myalgia (9.6%), which were different between the antibody-negative group and the antibody-positive group. The proportions of clinical classification between the antibody-negative and antibody-positive groups (common cases 66.0% VS 68.1%, severe cases 30.9% VS 30.9%, critical cases 3.2% VS 1.1%) were no significant difference.

The laboratory findings were observed to have substantial differences between antibody-negative patients and antibody-positive patients (Table 2). Among patients with available data, white blood cell counts (WBC) 6.6 [5.0, 9.1], Neutrophils 4.3[3.1, 6.6], C-reactive protein 7.3 [1.3, 49.0], Procalcitonin (PCT) 0.1 [0.0, 0.2], Interleukin-6 (IL-6) 64.2 [1.5, 28.7], Lactate dehydrogenase (LDH) 193.8 [154.9,260.6], Creatine kinase 60.5 [40.5, 103.7], Creatine kinase isoenzyme 10.3 [8.2, 14.5], Urea nitrogen 5.3 [4.0, 8.7] and Creatinine 77.7 [60.6, 98.7] were significantly higher in antibody-negative patients than in antibody-positive group (P<0.005), with 143 (81.2%), 20 (18.3%), 34 (44.2%) antibody-negative blood samples having increased C-reactive protein, Procalcitonin, Interleukin-6 and 37 (19.9%) antibody-negative blood samples having Lymphocytopenia, respectively. In addition, those of antibody-negative group were all higher than antibody-positive group (P<0.05).
Table 2
Laboratory findings on admission of patients with COVID-19.

|                  | Antibody-negative | Antibody-positive |            | Antibody-positive (after matching) |            |
|------------------|-------------------|-------------------|-----------|-------------------------------------|-----------|
|                  | n                 |                   | P         |                                     | P         |
| White-cell       | 94                | 1827              | 0.003     | 6.0 [4.8,7.6]                      | 0.004     |
|                  | 6.6 [5.0,9.1]     | 6.0 [4.8,7.7]     | <0.001    | 6.0 [4.8,7.6]                      | <0.001    |
|                  | 14 (8.5)          | 428 (10.2)        | 0.552     | 15 (6.8)                           | 0.676     |
|                  | 151 (91.5)        | 3757 (89.8)       |           | 205 (93.2)                         |           |
| Neutrophils      | 4.3 [3.1,6.6]     | 3.7 [2.8,5.2]     | <0.001    | 3.6 [2.8,5.0]                      | <0.001    |
|                  | 4 (2.2)           | 102 (2.1)         | 1.000     | 1 (0.4)                            | 0.173     |
|                  | 182 (97.8)        | 4657 (97.9)       |           | 238 (99.6)                         |           |
| Lymphocyte       | 1.4 [0.9,1.8]     | 1.4 [1.0,1.8]     | 0.528     | 1.5 [1.0,2.0]                      | 0.110     |
|                  | 37 (19.9)         | 734 (15.4)        | 0.122     | 28 (11.7)                          | 0.029     |
|                  | 149 (80.1)        | 4025 (84.6)       |           | 211 (88.3)                         |           |
| Platelets        | 209.0 [158.2,262.8] | 213.0 [169.0,261.0] | 0.138 | 215.5 [171.8,265.2] | 0.140 |
| C-reactive protein | 7.3 [1.3,49.0] | 2.4 [0.8,9.2] | <0.001 | 1.7 [0.7,6.8] | <0.001 |
|                  | 33 (18.8)         | 1084 (24.1)       | 0.124     | 65 (29.1)                          | 0.023     |
|                  | 143 (81.2)        | 3417 (75.9)       |           | 158 (70.9)                         |           |
| PCT              | 0.1 [0.0,0.2]     | 0.1 [0.0,0.1]     | <0.001    | 0.0 [0.0,0.1]                      | <0.001    |
|                  | 89 (81.7)         | 2374 (94.1)       | <0.001    | 98 (100.0)                         | <0.001    |
|                  | 20 (18.3)         | 148 (5.9)         |           |                                   |           |
| IL-6             | 4.2 [1.5,28.7]    | 2.2 [1.5,7.6]     | 0.001     | 2.1 [1.5,5.1]                      | 0.001     |
|                  | 43 (55.8)         | 1436 (71.9)       | 0.003     | 72 (81.8)                          | 0.001     |
|                  | 34 (44.2)         | 560 (28.1)        |           | 16 (18.2)                          |           |
| ALT              | 17.4 [10.8,30.4]  | 23.7 [15.1,39.3]  | <0.001    | 24.6 [13.8,39.5]                   | <0.001    |
| AST              | 19.7 [15.9,29.4]  | 20.3 [15.9,28.4]  | 0.898     | 19.9 [15.5,28.1]                   | 0.587     |
| LDH              | 193.8 [154.9,260.6] | 187.3 [158.2,232.1] | 0.258 | 180.3 [157.5,209.8] | 0.006 |
| CK               | 60.5 [40.5,103.7] | 42.2 [28.7,62.4]  | <0.001    | 37.3 [28.4,61.5]                   | <0.001    |
| CK-MB            | 10.3 [8.2,14.5]   | 8.8 [7.0,11.5]    | <0.001    | 8.1 [6.4,10.8]                     | <0.001    |

(PCT: Procalcitonin; IL-6: Interleukin-6; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase; CK-MB: Creatine Kinase-MB; BUN: Blood Urea Nitrogen.)
|                      | Antibody-negative | Antibody-positive | P    | Antibody-positive (after matching) | P    |
|----------------------|-------------------|-------------------|------|-----------------------------------|------|
| Albumin              | 36.7 [32.9,40.6]  | 37.3 [34.0,40.1]  | 0.308| 37.5 [35.1,40.0]                 | 0.183|
| BUN                  | 5.3 [4.0,8.7]     | 4.9 [3.9,6.2]     | <0.001| 5.1 [4.3,6.4]                  | 0.042|
| Creatinine           | 77.7 [60.6,98.7]  | 63.6 [53.5,75.9]  | <0.001| 69.8 [58.6,81.7]                | <0.001|

(PCT: Procalcitonin; IL-6: Interleukin-6; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase; CK-MB: Creatine Kinase-MB; BUN: Blood Urea Nitrogen.)

Table 3
Dynamic changes of antibodies in patients with COVID-19.

|                              | Antibody-negative | Antibody-positive (after matching) | P    |
|------------------------------|-------------------|-----------------------------------|------|
| n                            | 156               | 142                               |      |
| IgM titer (AU/ml)            | 1.0 [0.6,2.0]     | 33.5 [11.5,65.1]                  | <0.001|
| IgM emergence after onset (d)| 20.0 [13.0,31.2]  | 40.0 [31.0,48.8]                  | <0.001|
| IgG titer (AU/ml)            | 1.7 [0.9,2.6]     | 155.5 [101.6,185.8]               | <0.001|
| IgG emergence after onset(d) | 20.0 [13.0,31.2]  | 40.0 [31.0,48.8]                  | <0.001|
| The Time of nucleic acid turning negative(d) | 19.0 [10.0,30.0] | 37.0 [26.0,46.8] | <0.001 |
| The time of hospitalization(d) | 8.0 [6.0,10.0]   | 13.0 [8.2,23.0]                  | <0.001|
| outcome                     |                   |                                   |      |
| survivors                   | 91 (96.8)         | 94 (100.0)                       | 0.246|
| Non-survivors               | 3 (3.2)           |                                   |      |

Clinical Outcome

There were 142 IgG detection results (155.51 [101.64,185.83] AU/ml) and 141 IgM detection results (33.48 [11.51, 65.1] AU/ml) of 94 patients in the antibody-positive group (figure 1). There was a significant correlation between antibody production and nucleic acid negative conversion. The days of nucleic acid negative conversion in the antibody-negative group was shorter than that in the antibody-positive group (P < 0.001) (figure 2). Meanwhile, the hospitalization time of antibody-negative patients was shorter than that of antibody-positive patients (8.0 [6.0, 10.0] VS 13.0 [8.2, 23.0], P < 0.001). Three patients died during hospitalization in the antibody-negative group (3.2%), and there was no death in the positive group. There was no significant difference between the two groups.

Discussion
As we all know, when bacteria, viruses, and other microorganisms invade the human body, the body removes harmful microorganisms through Innate immunity and adaptive immunity. It produces immunoglobulins that can bind to target antigens through adaptive immunity, which can accurately and efficiently remove harmful substances. However, some COVID-19 patients can still clear the virus without producing specific antibodies. What are the characteristics of these patients, how to rely on Innate immunity to remove the virus, what is the relationship between novel coronavirus and human immune response? In this study, we analyzed the clinical characteristics and clinical outcomes of COVID-19 patients without serum conversion using electronic medical records. We reported there was no significant difference in gender, age, non-survivors and the clinical classification between the antibody-negative and antibody-positive groups. Fever, dyspnea, fatigue, expectoration, and myalgia, which were observed more in the antibody-positive group. In terms of laboratory findings, white blood cell counts (WBC), Neutrophils, C-reactive protein, Procalcitonin, Interleukin-6 (IL-6), Lactate dehydrogenase, Creatine kinase, etc, were significantly higher in antibody-negative patients than in antibody-positive group. Specially, 143 (81.2%), 20 (18.3%), 34 (44.2%) antibody-negative blood samples having increased C-reactive protein, Procalcitonin, Interleukin-6 and 37 (19.9%) antibody-negative blood samples having Lymphocytopenia. Our results showed that innate immunity played an essential role in clearing the virus.

The common symptoms of the antibody-negative patients included in this study were cough, fever, dyspnea, fatigue, expectoration, and myalgia. The incidence rate was lower than that of the antibody-positive group and other related studies[4, 5]. This might be related to the inhibition of virus activity and weakening of virus pathogenicity by an inflammatory reaction in vivo. The types of coexisting illness and the proportion of severe/critical cases in the antibody-negative group were similar to those in the antibody-positive group and other studies[6, 7], suggesting that the antibody non-responders accorded with the overall distribution characteristics of COVID-19 patients. Wang, B., et al. studied the relationship between antibody and virus clearance time in 26 patients with COVID-19; they found the early production of antibodies does not mean early elimination of this virus, not observe a correlation between early adaptive immune responses and better clinical outcomes. One of the cases did not produce specific antibodies to SARS-CoV2 within 66 days of observation. However, eventually, the nucleic acid test of SARS-CoV2 turned negative, revealing that some individuals may not produce antibodies after being infected with SARS-CoV2[8]. In this study, it was found that 94 of the 1921 patients did not produce specific antibodies. However, the virus nucleic acid was still cleared during hospitalization, which confirmed that the production of specific antibodies by adaptive immunity was not necessary for the clearance of SARS-CoV-2.

C-reactive protein (CRP) is a non-specific inflammatory marker and a kind of acute-phase reaction protein, which can activate complements, strengthen phagocytes' function, and remove pathogenic microorganisms invading the body and tissue cells that are damaged, necrotic and apoptotic. Interleukin-6 is also a non-specific indicator of inflammation. Inflammatory cytokines produced by various cells after inflammatory stimulation are the critical components of the inflammatory response and can induce the increase of CRP and PCT at 2h and 6h after infection, respectively. PCT reflects the activity of systemic inflammation, which increases slightly when the virus is infected. When the pathogen invaded the body, the human immune system entered the immediate innate immune response stage, neutrophils were the central effector cells, and the total number of leukocytes and neutrophils increased significantly. In the early stage of the innate immune response, activated
Neutrophils produce pro-inflammatory cytokines such as interleukin-6, while hepatocytes produce a series of acute-phase proteins after being stimulated by pro-inflammatory cytokines such as interleukin-1, of which C-reactive protein is the most significant. Zhu, L, et al found that interferon-stimulated genes (such as ISG15, IFI44L, and MX1) in peripheral blood immune cells of patients with COVID-19 were significantly up-regulated, which confirmed that the innate immune response was significantly activated in patients with COVID-19.

Meanwhile, it was found that the concentration of interleukin-6 (IL-6) in patients with COVID-19 was significantly higher than that in ordinary people\(^9\). We did not find that the total number of white blood cells, neutrophils, C-reactive protein, IL-6, PCT, and other inflammatory cells and factors were higher in the antibody-negative group in this study. It was confirmed that after SARS-COV-2 entered the body, the innate immune response of this population was rapid, intense, and cleared the virus quickly. Other studies found that pathogenic T cells were activated rapidly to produce GM-CSF and IL-6, and then GM-CSF further activated CD14+, CD16+ inflammatory monocytes to produce more IL-6 and other inflammatory cytokines, resulting in an inflammatory storm\(^10\). The number of lymphocytes-decreased patients in the antibody-negative group was more than that in the antibody-positive group, suggesting that the inhibition of the virus on lymphocytes\(^11\) weakened the adaptive immune response and delayed the production of specific neutralizing antibodies by B lymphocytes. Strong inflammatory reactions spread throughout the body, involving many target organs such as the liver and kidney, resulting in a significant increase in lactate dehydrogenase, creatine kinase, creatine kinase isoenzyme, urea nitrogen, and creatinine.

The virus clearance time of antibody-negative patients was significantly shorter than that of antibody-positive groups. The shedding time of viruses in other related studies varied greatly from 11 to 20 days\(^{12, 13}\). After eliminating the confounding factors such as age and coexisting illness, the hospitalization time of the antibody-negative group was significantly shorter than that of the antibody-positive group, which confirmed that the rapid clearance of the virus was related to the short hospitalization time. 3/94 (3.2%) deaths were all critically ill patients, and the mortality rate was lower than the current results of related studies\(^5, 14\). The activation of innate immune response is necessary to eliminate the invading virus effectively, but its abnormal activation and excessive production of pro-inflammatory cytokines may cause damage to the host tissue. Galloway, J. B., et al. scored COVID-19 patients on 12 items, and the higher the score, the greater the risk of CCU or death, including neutrophil count > 8.0 x10⁹/L, CRP > 40 mg/L. Another study listed leukocyte > 10x10⁹/L and neutrophil > 7.5x10⁹/L as risk factors for adverse outcomes\(^15\). In another paper\(^16\), it was found that SARS-CoV-2 infected human macrophages could induce the release of intracellular ISG15 to extracellular through papain-like protease PLpro encoded by SARS-CoV-2, and extracellular free ISG15 continued to amplify the expression of a variety of pro-inflammatory cytokines and chemokines in a manner similar to cytokines, which is one of the possible causes of excessive inflammatory response in patients with COVID-19. Whether these studies suggest that excessive inflammation is related to the death of critically ill patients is worthy of further study.

Our study has some notable limitations. First, because of the retrospective study, there was a lack of more detailed laboratory testing of immune cell inflammatory factors, such as all kinds of T cell count, tumor necrosis factor, and various types of interleukins and so on, to more accurately judge the degree of the inflammatory response of COVID-19 patients. Second, there was a significant gap between the antibody-
negative group and the positive group. Although we used the 1:1 propensity score matching, the bias has inevitably affected our assessment.

**Conclusion**

Some COVID-19 patients without specific antibodies had mild symptoms, but the inflammatory reaction caused by innate clinical immunity was more intense than those with antibodies, and the virus was cleared faster. The production of specific antibodies was unnecessary for SARS-CoV-2 clearance, and non-specific immune responses played an essential role in virus clearance.

**Declarations**

**Ethics approval and consent to participate**

All methods of this study were carried out in accordance with relevant guidelines and regulations. This study was approved by Ethical Review of Scientific Research Projects of the Medical Ethics Committee of the Chinese PLA General Hospital, and obtained a waiver of one or more elements of informed consent form. The need for informed consent was waived by Medical Ethics Committee of the Chinese PLA General Hospital. The name of the ethics committee: The Medical Ethics Committee of the Chinese PLA General Hospital. The approval committee’s reference number: No. S2020-161-01.

**Consent for publication**

Not applicable

**Availability of data and materials**

The data that support the findings of this study are available from the Huoshenshan hospital and Chinese PLA General Hospital, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the Huoshenshan hospital and Chinese PLA General Hospital.

**Competing interests**

All authors declared there were no conflicts of interests.

**Funding**

This article had no funding source.

**Authors’ contribution**

All authors reviewed and edited all sections of the article. JunYu Ding Changxin Liu wrote the first draft of the manuscript. Hua Guo and Zhao Wang handled laboratory logistics and generated data. Zhao Wang analyzed and summarized the data. Kan Zhang and Lin Ma provided input on assay design and interpretation of results.
Bo Wang, Huijun Zhao and Manya Song provided input on data collection and interpretation of results. XiZhou Guan developed project concept and 60 guided the laboratory work.

Acknowledgements

We thank all medical staff, researchers and patients who participated in this study.

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Figures

Figure 1

Antibody expression after onset of patients with COVID-19
Figure 2

The Time of nucleic acid turning negative in patients with COVID-19