Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Associations of medications used during hospitalization and immunological changes in patients with COVID-19 during 3-month follow-up

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ABSTRACT

Background: Understanding the immunological responses in COVID-19 patients during their recovery period is essential to the development of a vaccine and herd immunity.
Methods: This retrospective cohort study screened 233 patients admitted to the First Hospital of Changsha, China with COVID-19 from January 17th to February 29th, 2020. After completion of SARS-CoV2-specific immunoglobulins, and T cells tests at 2-week and 3-month follow-up points after discharge, 87 were enrolled. Wilcoxon signed-rank test was performed to assess changes in the values of IgG and IgM, the number of CD3+, CD4+ and CD8+ T cells, and CD4+/CD8+ ratio during the 3-month follow-up. Linear regressions were used to evaluate the associations of immunological changes and medications during hospitalization.
Results: The positive rate of IgG decreased from 98.6% (40/41) to 85.4% (35/41) in men and 100% (43/43) to 76.7% (33/43) in women, whereas IgM declined from 34.1% (14/41) to 12.2% (5/41) in men and 37.2% (16/43) to 27.9% (12/43) in women during the follow-up. CD4+ T cells increased from (median (IQR), 484 (384–635)) cells/ul to 543 (414–657) cells/ul ($P = 0.01$). Antibiotic use was negatively associated with IgG change (mean change [95%CI], 8.08 [0.80–15.37] U, $P = 0.03$), and glucocorticoid use was positively related to increased CD4+ T cells (100.85 [16.56–185.15] cells/ul, $P = 0.02$).
Conclusion: This study demonstrated that the positive rates and values of IgG and IgM decreased in COVID-19 patients over a 3-month follow-up, while CD4+ T cells significantly increased. Moreover, we found that antibiotic use during hospitalization was associated with IgG decrease, and glucocorticoid use was associated with increases in CD4+ T cells.

1. Introduction

A cluster of pneumonia cases of unknown origins was reported in Wuhan on December 31, 2019[1]. The infectious agent was determined to be severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease has since been named coronavirus disease 2019 (COVID-19) by the World Health Organization[2]. As of Aug 16th, 2020, the COVID-19 pandemic has affected more than 17 million people around the world[3].

The SARS-CoV-2 infection activates the innate and adaptive immune response. While a well-coordinated immune response can eliminate the virus, an excessive inflammatory innate response and dysregulated adaptive host immune defense may cause harmful tissue damage. This excessive pro-inflammatory host response might be the main cause of acute lung injury (ALI) and ARDS in SARS-CoV-2 infected patients[4].

An effective immune response is also the physiological basis behind a vaccine and herd immunity. However, the waning of antibody response could be a potential difficulty[5]. In patients that survived SARS-CoV-1 or Middle East Respiratory Syndrome (MERS)-CoV infections, the antibody titers often waned after 2 years[6,7]. Notably, re-infections of SARS-CoV-2 after days of recovery have been recently reported[8]. It will be a challenge for an effective SARS-CoV-2 vaccine if the virus

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causes recurrent seasonal epidemics. Therefore, it is important to explore immunological changes in patients with COVID-19 and to explain the potential reason. In the hospital, patients are usually treated with multiple medications including antiviral agents, antibiotics, immunoglobulin, CVD agents, statin, and hypoglycemic agents. However, the relationship between immunological changes and these medications, and the predictive effect of them remains unclear in patients with COVID-19.

Here we describe the clinical characteristics, including medication and immunological changes, in patients with COVID-19 over a 3-month follow-up, which may provide insight for the clinical management in consideration for further acquired immunity.

2. Methods

2.1. Study design and participants

This retrospective cohort study screened 233 consecutive patients with COVID-19 who were admitted to the First Hospital of Changsha, China from January 17th to February 29th, 2020. Of the 233 patients, two died, 144 are living well but had not completed all follow-up tests, a total of 87 patients who completed both the T cells and SARS-CoV2-specific immunoglobulins tests at 2-week and the 3-month follow-up after discharge were enrolled (Fig. 1). The present study was approved by the Ethics Committee of the First Hospital of Changsha (approval No. KX-2020047).

2.2. Data collection

The characteristics of all participants (sex, age, body weight, body mass index, smoking status), time of admission, length of hospital stay, clinical classification of COVID-19, comorbidity, the number of medications used during hospitalization, the nucleic acid test results of SARS-CoV2 when the patients were discharged, the positive rate and the values of virus specific-IgG and IgM, the numbers of CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD4+/CD8+ ratio at 2-week and the 3-month follow-up points were extracted from the electronic medical system. All patients were codified and anonymized to protect the confidentiality of individual participants.

2.3. Definitions

Criteria for COVID-19 diagnosis and clinical classification referred to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)[9].

2.4. Discharge criteria

In clinical practice, the discharge time for all patients was 1–2 days after their SARS-CoV2 nucleic acid test returned negative. SARS-CoV2 nucleic acid test was performed via RT-PCR in a nasopharyngeal specimen collected by a healthcare provider. As for the image criteria, 1) for critical and severe cases without underlying disease, the discharge time was after a CT scan showed that the pneumonia lesion was almost absorbed; 2) for mild and moderate cases, the lung lesion should be fully absorbed before discharge.

2.5. Blood specimen collection and processing

About 7 ml of blood was aseptically collected through venipuncture into two sterile BD vacutainers respectively. Out of them, 5 ml of blood in a general vacutainer (non-anticoagulated) was centrifuged, collecting serum for antibody detection, while 2 ml of blood in an anticoagulated vacutainer (ethylenediaminetetraacetic acid, EDTA) was prepared for T cells detection. Processed serum and whole blood samples were stored at room temperature (20 °C – 25 °C). A minimum of 100 μL of serum or whole blood tests were extracted for antibody or T cell detection respectively within six hours after the blood specimen collection.

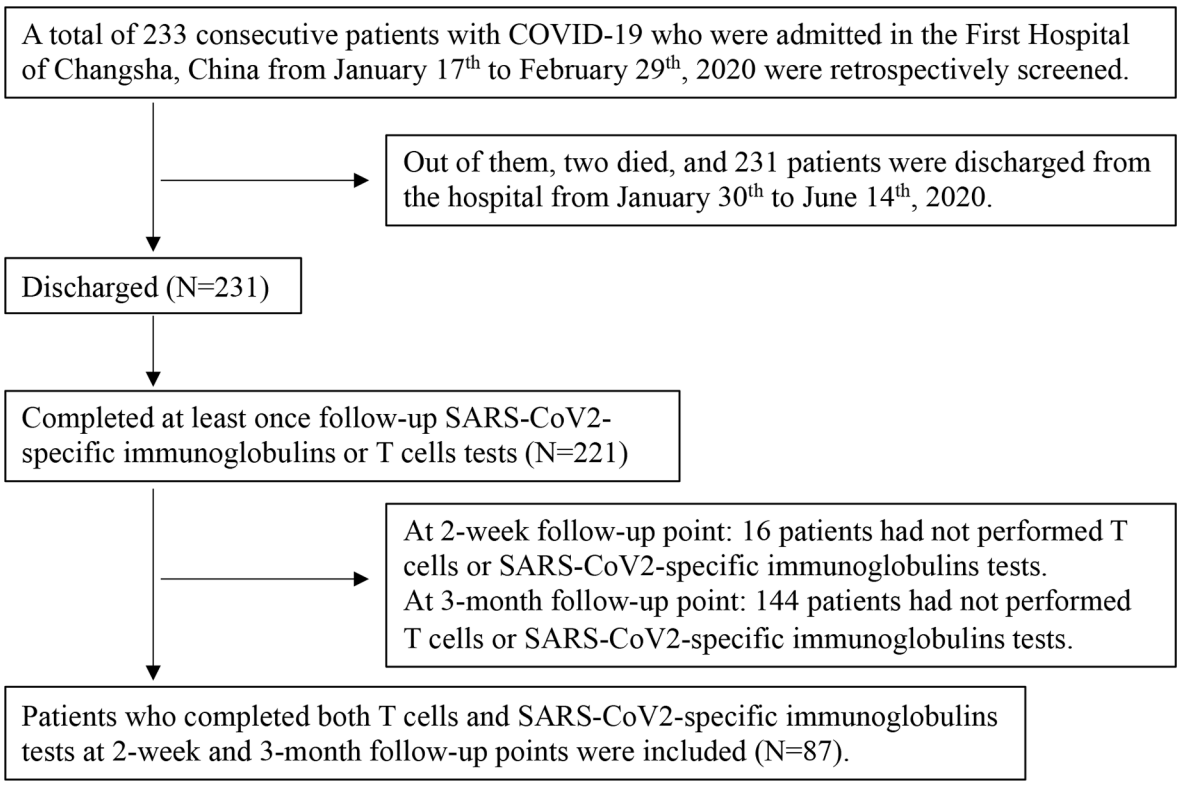

Fig. 1. Study enrollment. COVID-19, coronavirus disease-19; SARS-CoV2, severe acute respiratory syndrome coronavirus 2.

2.6. Detection of IgG and IgM against SARS-CoV-2

IgG and IgM against SARS-CoV-2 were detected in serum samples using 2019-nCoV Ab test kits (Innovita, China), according to the manufacturer's instructions.

2.7. Detection of the numbers of CD3+, CD4+, CD8+ T cells

Mature human T lymphocytes (CD3+), helper/inducer (CD3+ CD4+) T cell, suppressor/cytotoxic (CD3+ CD8+) T cell were detected in blood samples stored in an anticoagulated vacutainer at room temperature using BD FACSCalibur Cell Sorting System (Biosciences, US), with BD Tirtest CD4/CD8/CD3 reagent according to the manufacturer's instructions.

2.8. Statistical analysis

Continuous and categorical variables are described as median (IQR) and number (percent) accordingly. The primary outcome of the present study is the changes in SARS-CoV-2-specific IgG, IgM, CD3+ T cell, CD4+ T cell, CD8+ T cell, CD4+/CD8+ ratio in patients with COVID-19 over the 3-month follow-up. The secondary outcomes are the associations of the aforementioned changes and the medications used during hospitalization. Wilcoxon signed-rank test was used to assess the mean difference between 2-week and 3-month follow-up of the value of SARS-CoV-2-specific IgG and IgM, and the numbers of CD3+ T cells, CD4+ T cells and CD8+ T cells, CD4+/CD8+. The relationships of the changes above and the medications used during hospitalization were assessed using both univariate and multivariate linear regressions, and sex, age, BMI were adjusted. No data was missed. Analyses were carried out with the use of SAS software, version 9.4 (SAS Institute), a two-tailed alpha level of 0.05 was considered significant.

3. Results

3.1. Demographic characteristics

The demographics of 87 participants are presented in Table 1. The median (IQR) of participant's age was 37 (17–59) years. The majority of participants (78/87, 78.1%) suffered from moderate and severe COVID-19. Twenty-seven (31%) participants were administrated glucocorticoid, and almost all participants used at least one kind of antiviral agent. All participants' nucleic acid test results of SARS-CoV2 were negative when they were discharged.

3.2. Changes in IgG and IgM against SARS-CoV-2 over the 3-month follow-up

To investigate the change in antibodies to SARS-CoV-2 during the convalescent phase, virus-specific IgG and IgM were measured in serum samples at both the 2-week and 3-month follow-up points. At the 2-week follow-up point, 98.3% (83/84) tested positive for IgG, and 35.7% (30/84) for IgM. At the 3-month follow-up point, the positive rates of IgG and IgM decreased to 81.0% (68/84) and 20.2% (17/84) respectively. The nasopharyngeal swab tests for all participants were negative at both follow-up points. Both men and women had similar decremental trends in the positive rate of IgG and IgM. The positive rate of IgG reduced from 98.6% (40/41) to 85.4% (35/41) in men and from 100% (43/43) to 76.7 (33/43) in women, whereas IgM declined from 34.1% (14/41) to 12.2% (5/41) in men and from 37.2% (16/43) to 27.9% (12/43) in women. The test values of IgG and IgM are shown in Fig. 2 A-C.

3.3. Changes in CD3+, CD4+, CD8+ T cells, and CD4+/CD8+ ratio over 3-month follow-up

To investigate the changes in T cell response to SARS-CoV-2 during the convalescent phase, CD3+, CD4+, CD8+ T cells, CD4+/CD8+ ratio were also measured in whole blood samples at both the 2-week and 3-month follow-up points. The CD4+ T cell increased from 484 (384–635) [median (IQR)] cells/ul to 543 (414–657) cells/ul from the beginning to end of the 3-month follow-up, the mean change (95% CI) was 49.20 (10.86–87.78) cells/ul, $P = 0.01$ (Fig. 2E). There were no significant changes in CD3+ T cells, CD8+ T cells, CD4+/CD8+ over the 3-month follow-up. More details are shown in Fig. 2D, 2F, and 2G.

Table 1
Characteristics of Patients.

	Men (N = 42)	Women (N = 45)	Total (N = 87)
Age, yrs	48 (37–59)	49 (38–60)	37 (17–59)
Body weight, kg	72.6 (61.5–78.5)	59.6 (53.5–62.0)	65.9 (57.75–72.25)
BMI, kg/m^2	25.1 (22.9–26.5)	23.6 (21.5–24.9)	24.4 (21.8–25.8)
Smoking status			
Smoking	7 (16.7)	0 (0)	7 (8.0)
Previously smoked	1 (2.3)	1 (2.2)	2 (2.3)
Never	34 (81.0)	44 (97.8)	78 (89.7)
Clinical classification of COVID-19			
Mild cases	3 (7.1)	3 (6.7)	6 (7.0)
Moderate cases	1 (2.4)	35 (77.8)	36 (41.3)
Severe cases	27 (64.3)	5 (11.1)	32 (36.8)
Critical cases	11 (26.2)	2 (4.4)	13 (14.9)
Length of stay	18 (12–25)	20 (12–28)	19 (12–26)
Comorbidity			
Hypertension	6 (14.3)	9 (20.0)	15 (17.2)
Diabetes mellitus	4 (9.5)	3 (6.7)	7 (8.0)
Dyslipidemia	4 (9.5)	3 (6.7)	7 (8.0)
Coronary artery disease	2 (4.8)	1 (2.2)	3 (3.4)
Cerebrovascular disease	2 (4.8)	2 (4.4)	4 (4.6)
Chronic kidney disease	1 (2.4)	0 (0)	1 (1.1)
Peptic ulcer	1 (2.4)	1 (2.2)	2 (2.3)
Cancer	1 (2.4)	1 (2.2)	2 (2.3)
Medications used during hospitalization			
Glucocorticoid	14 (33.3)	13 (28.9)	27 (31.0)
Lopinavir/Ritonavir	29 (69.0)	37 (82.2)	66 (75.9)
Arbidol	20 (47.6)	23 (51.1)	43 (49.4)
Interferon	28 (66.7)	33 (73.3)	61 (70.1)
Chloroquine phosphate	8 (19.0)	11 (24.4)	19 (21.8)
Antibiotics	22 (52.4)	17 (38.6)	39 (44.8)
Immunoglobulin	16 (38.1)	14 (31.1)	30 (33.5)
CVD agents[†]	13 (31.7)	9 (20.0)	22 (25.3)
Statin	4 (9.5)	3 (6.7)	7 (8.0)
Hypoglycemic agents[‡]	4 (9.5)	2 (4.4)	6 (7.0)

COVID-19, coronavirus disease-19; CVD, cardiovascular disease. Continuous and categorical variables are expressed as median (interquartile range, IQR) or number (percent) accordingly.

[†] CVD agents include anti-platelets, anti-coagulants, Beta-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors/ angiotensin II receptor blockers, diuretics, nitrate, Digoxin.

[‡] Hypoglycemic agents include oral hypoglycemic drugs and insulin.

3.4. The relationships of medications used during hospitalization to the changes in IgG, CD4+ T cell.

The main medications used during hospitalization included antiviral agents (Lopinavir/Ritonavir, Arbidol, Interferon, Chloroquine phosphate), antibiotics, immunoglobulin, glucocorticoid, CVD agents (anti-platelets, anti-coagulants, Beta-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors/ angiotensin II receptor blockers, diuretics, nitrate, Digoxin), Statin, hypoglycemic agents (oral

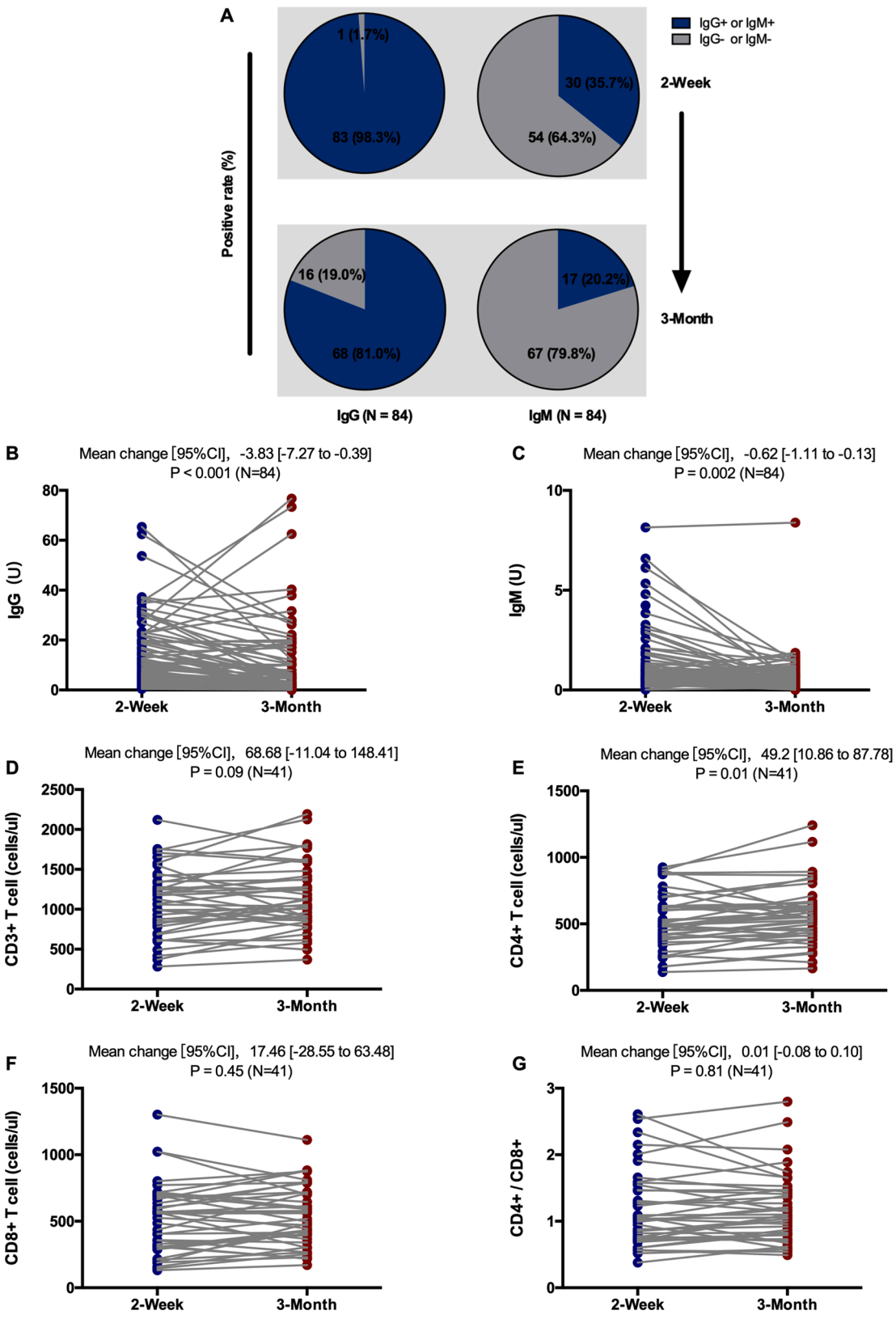

Fig. 2. Changes in the positive rate of IgG and IgM (A), the values of IgG (B) and IgM (C), the numbers of CD3+ (D), CD4+ (E), and CD8+ T cells (F), and the ratio of CD4+/CD8+ (G) in patients with COVID-19 over 3-Month Follow-up.

hypoglycemic drugs and insulin). After adjustment for sex, age and, BMI, the use of antibiotics was negatively associated with the change in IgG [mean change (95%CI), (8.08 (0.80–15.37) U, $P = 0.03$]. Moreover, the use of glucocorticoid was positively related to the change in the number of total CD4+ T cells (100.85 [16.56–185.15] cells/ul, $P = 0.02$). More details are shown in Table 2 and 3. No relationships between medication used during hospitalization and change in IgM were found (data has not been shown).

Table 2
Univariate and Multivariate Linear Regression Analyses for the Association of Medication History and Change in IgG in Patients with COVID-19 over 3-Month Follow-up.

Variables	Univariate analysis (N = 84)		Multivariate analysis (N = 84)	
	Mean change in IgG (95% CI) (U)	*P* Value	Mean change in IgG (95% CI) (U)	*P* Value
Change in IgG (U)	−3.83 (−7.27 to −0.39)	<0.001	–	–
Sex*	−1.65 (−8.56 to 5.27)	0.64	1.80 (−5.58 to 9.19)	0.63
Age, yrs	0.02 (−0.21 to 0.25)	0.85	0.04 (−0.20 to 0.27)	0.59
Body weight, kg	0.15 (−0.08 to 0.38)	0.19	0.43 (0.01–0.85)	0.04
BMI, kg/m^2	0.63 (−0.20 to 1.47)	0.13	0.41 (−1.11 to 1.93)	0.59
Medication used during hospitalization§				
Glucocorticoid	2.24 (−5.23 to 9.71)	0.55	3.31 (−4.66 to 11.28)	0.41
Lopinavir/ Ritonavir	3.70 (−4.26 to 11.65)	0.36	2.78 (−5.72 to 11.28)	0.52
Arbidol	0.56 (−6.36 to 7.49)	0.87	2.16 (−5.09 to 9.41)	0.55
Interferon	3.05 (−4.58 to 10.69)	0.43	4.88 (−3.32 to 13.08)	0.24
Chloroquine phosphate	−3.25 (−11.66 to 5.16)	0.44	−2.49 (−11.19 to 6.22)	0.57
Antibiotics	6.01 (−0.89 to 12.91)	0.09	8.08 (0.80–15.37)	0.03
Immunoglobulin	0.39 (−6.89 to 7.67)	0.92	1.27 (−6.49 to 9.04)	0.74
CVD agents	6.67 (−1.24 to 14.37)	0.10	8.31 (−0.58 to 17.20)	0.07
Statin	6.79 (−5.65 to 19.23)	0.28	6.97 (−6.22 to 20.16)	0.30
Hypoglycemic agents	1.35 (−12.18 to 14.88)	0.84	1.34 (−12.76 to 15.45)	0.85

IgG, immunoglobulin G; COVID-19, coronavirus disease-19; CVD, cardiovascular disease. Multivariate linear regression adjusted for sex, age, BMI.
* For sex, female was the reference group.
§ For medications, no use was the reference group for each medication analysis.

Table 3
Univariate and Multivariate Linear Regressions for Association of Medication History and Change in CD4+ of Patients with COVID-19 over 3-Month Follow-up.

Variables	Univariate analysis (N = 41)		Multivariate analysis (N = 41)	
	Change in CD4+ (95% CI) (cells/ul)	*P* Value	Change in CD4+ (95% CI) (cells/ul)	*P* Value
Change in CD4+, cells/ul	49.20 (10.86, 87.78)	0.01	–	–
Sex*	−42.99 (−119.72 to 33.75)	0.26	−37.19 (−118.75 to 44.37)	0.36
Age, yrs	0.09 (−2.49 to 2.66)	0.95	0.50 (−2.35 to 3.35)	0.72
Body weight, kg	0.62 (−2.58 to 3.82)	0.70	2.65 (−8.22 to 13.51)	0.62
BMI, kg/m^2	4.53 (−7.77 to 16.84)	0.46	5.37 (−7.92 to 18.66)	0.42
Medication used during hospitalization§				
Glucocorticoid	73.82 (−4.83 to 152.47)	0.07	100.85 (16.56–185.15)	0.02
Lopinavir/ Ritonavir	−27.69 (−146.48 to 91.09)	0.64	11.42 (−129.98 to 152.82)	0.87
Arbidol	−4.90 (−83.06 to 73.25)	0.9	−12.4 (−100.28 to 75.47)	0.78
Interferon	15.50 (−78.54 to 109.54)	0.74	5.96 (−95.62 to 107.55)	0.91
Chloroquine phosphate	−31.89 (−115.02 to 51.24)	0.44	−28.58 (−121.53 to 64.37)	0.54
Antibiotics	52.68 (−23.97 to 129.32)	0.17	52.56 (−33.78 to 138.89)	0.22
Immunoglobulin	44.28 (−33.54 to 122.09)	0.26	60.89 (−21.98 to 142.56)	0.15
CVD agents	55.72 (−30.38 to 141.83)	0.2	62.06 (−28.65 to 152.77)	0.17
Statin	55.61 (−74.52 to 185.74)	0.39	54.25 (−82.72 to 191.22)	0.43
Hypoglycemic agents	−45.01 (−225.39 to 135.36)	0.62	−52.30 (−240.8 to 136.00)	0.58

COVID-19, coronavirus disease-19; CVD, cardiovascular disease. Multivariate linear regression adjusted for sex, age, BMI.
* For sex, women was the reference group.
§ For medications, no use was the reference group for each medication analysis.

4. Discussion

The present study demonstrates, for the first time, the virus-specific immunoglobulin value and T cells number in patients with COVID-19 over a 3-month follow-up. It was found that at two specific time points: 2-weeks and three months after leaving hospital, there was considerable immunoglobin value in patients, 98% IgG and 35.7% for IgM, decreasing to 81.0% and 20.2% respectively after 3 months. Furthermore, the CD4+ T cells number significantly increased over the same period. We also found that use of antibiotics was associated with a decrease in IgG while the use of glucocorticoid was related to the increase in the number of CD4+ T cells.

At present, studies concerning immunological changes in COVID-19 have primarily concentrated on the acute immunological response to the virus. In this study, we found at the 2-week follow-up point, 98.3% (83/84) tested positive for IgG, and 35.7% (30/84) for IgM, at the 3-month follow-up point, the positive rates of IgG and IgM decreased to 81.0% (68/84) and 20.2% (17/84) respectively. The opposite result was observed in CD4+ T cells of COVID-19 patient, the cell concentration increased 49.2[10.86–87.78] cells/ul ($P = 0.01$) from 2-week to the 3-month follow-up point. Based on data from SARS patients in 2003 to 2004 and the fact that most acute viral infections result in the development of protective immunity[10], researchers proposed that substantial CD4+ T cell, CD8+ T cell, and neutralizing antibody responses contribute to clearance of the acute infection of SARS-CoV-2, and some of the T and B cells are retained as immunological memory against SARS-CoV-2 infection. A previous study demonstrated that patients who recovered from SARS had SARS-CoV-specific T cells that persist for 11 years after infection[11]. For the management of the current pandemic and for the vaccine development against SARS-CoV-2, it is important to investigate the duration of acquired immunity and how medication or any other factors may influence it.

Participants in the present study accepted multiple medications during their hospitalization. We performed univariate and multivariate linear regression analyses to investigate the relationship between medication history and immunological changes, including IgG, IgM, and CD4+ T cell. Each drug, including antiviral agents, antibiotics, immunoglobulin, CVD agents, statin, and hypoglycemic agents, were analyzed, and finally, we found the use of antibiotics might decrease the value of IgG and the use of glucocorticoid might increase the number of CD4+ T cells. A recent study has shown that within 17–19 days after symptoms onset, the virus specific-IgG and/or IgM could be 100% positive[12] which could be considered a reliable diagnostic method like nucleic acid detection assay for patients with suspected COVID-19 [13]. In fact, specific IgG is known to last long in the body and to provide persisting protective immunity. Moreover, it could be the main mechanism of convalescent plasma treatment for COVID-19[14]. But

research about the association between medication and SARS-CoV-2-specific IgG is rarely reported. Although the underlying mechanisms remain unclear, we found the use of antibiotics might decrease the value of IgG in this study. This finding may change the priority of such drugs in COVID-19 treatment.

Usage of glucocorticoid could help improve fever in the early stage, absorption of pneumonia, and obtain better oxygenation as immunomodulatory therapy in COVID-19. However, some studies during the SARS epidemic in 2003 showed no beneficial effects to glucocorticoid ingestion, or that it even delayed the virus clearance, leading to the progression of the disease[15,16]. In this study, glucocorticoid usage history increased 138.06 (10.18–265.93) cells/ul CD4+ T cells, which may provide further protective immunity. However, glucocorticoids were used for participants with progressive deterioration of oxygenation indicators, rapid progress in imaging, and excessive activation of the body's inflammatory response, according to the protocol. In order to eliminate the influence of different clinical features, multivariate linear regression was adjusted for comorbidity and clinical classification of COVID-19 when analyzing for an association, and the results didn't show any difference (data not shown).

4.1. Limitations

Just as with any medical intervention or organic finding, these results represent the average public effect, and variations within individuals exist. The present retrospective cohort study only enrolled eighty-seven COVID-19 patients and followed up 3-months after they had discharged, which potentially limits the generalizability of the findings.

5. Conclusion

We found the positive rate and the values of IgG and IgM decreased in patients with COVID-19 over a 3-month follow-up, while the CD4+ T cells number increased significantly. These results were caused by multiple factors, but our analysis indicated that usage of antibiotics in hospitalization was associated with the decrease in IgG while the use of glucocorticoid was associated with the increase in the number of CD4+ T cells, which may influence the duration of post-discharge acquired immunity. These findings may provide data for prospective vaccine application and herd immunity model establishment, and provides insight for clinical management.

CRediT authorship contribution statement

Chao Liu: Conceptualization, Methodology, Writing - original draft, Investigation. **Yaoshan Dun:** Conceptualization, Methodology, Writing - original draft, Formal analysis. **Ping Liu:** Conceptualization, Methodology, Project administration. **Baiyang You:** Writing - original draft, Visualization. **Kongliang Shu:** Software, Data curation. **Huijun Luo:** Software, Data curation. **Jeffrey W. Ripley-Gonzalez:** Writing - review & editing, Visualization, Writing - review & editing, Visualization. **Suixin Liu:** Conceptualization, Methodology, Resources, Supervision. **Jiyang Liu:** Conceptualization, Methodology, Funding acquisition, Resources. **:** . **Bo Li:** Conceptualization, Methodology, Resources, Supervision.

Declaration of Competing Interest

The authors declare no competing interests.

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Data availability

The data that supports the findings of this study are available upon request from the corresponding author, BL. The data have not been made publicly available due to their containing information that could compromise the privacy of research participants.

Ethics

The study was approved by the Ethics Committee of the First Hospital of Changsha (approval No. KX-2020047) and written informed consent was waived due to the retrospective nature of the study. All patients were codified and anonymized to protect the confidentiality of individuals.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.107121.

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