Am J Transl Res 2022;14(4):2655-2667
www.ajtr.org /ISSN:1943-8141/AJTR0141219

*Original Article*

# Research on antibody changes and nucleic acid clearance in COVID-19 patients treated with convalescent plasma

Li Duan[1*], Yudi Xie[2*], Qiang Wang[3*], Xianli Sun[1], Wenlong Guan[1], Jun Xu[1], Zhiqiang Lin[1], Quan Wang[1], Ying Qin[1], Chi Zhang[1], Yuanda Li[1], Xiaojuan Qu[1], Ling Li[2], Jianping Li[4], Guoyue Lin[1]

*[1]The Eighth Affiliated Hospital of Xinjiang Medical University, Urumqi 830013, Xinjiang, China; [2]Institute of Blood Transfusion, Chinese Academy of Medical Sciences and Peking Union Medical College, Chengdu 610052, Sichuan, China; [3]Department of Dermatology, Seventh Peoples Hospital of Shenyang, Shenyang 110003, Liaoning, China; [4]Liaoning Blood Center, Shenyang 110044, Liaoning, China. [*]Equal contributors.*

Received December 20, 2021; Accepted March 17, 2022; Epub April 15, 2022; Published April 30, 2022

**Abstract:** Purpose: To investigate changes in the production of IgM and IgG antibodies and the negative transformation of viral nucleic acids in COVID-19 patients after convalescent plasma therapy, and also to discuss the clinical therapeutic effect, so as to provide a basis for the treatment of COVID-19 using specific antibodies. Methods: The convalescent plasma of recovered patients from COVID-19 was used to treat other patients, and the levels of antibodies IgM and IgG and the nucleic acid genes ORF1ab and N in the patients were tested regularly for statistical comparison and analysis. Results: In general, the Ct value and concentration of IgM and IgG antibodies in the plasma infusion group were significantly higher (1-3 times higher) than those in the non-plasma infusion group, respectively, but these differences were not significant (P>0.05). However, the content of antibodies in severe patients in the plasma transfusion group was significantly higher than those in the non-plasma transfusion group at discharge, the results being statistically significant (P<0.05). Conclusions: The application of convalescent plasma significantly increases the antibody content in severe and critical inpatients, effectively enhances immune function, accelerates the clearance of virus and the nucleic acid negative conversion rate, and significantly promotes early improvement in COVID-19 patients.

**Keywords:** COVID-19, SARS-CoV-2, convalescent plasma, antibody, nucleic acid

## Introduction

There is currently no specific antiviral drug available to treat COVID-19, and the best preventive measure is quarantine [1]. For mild- and moderate-type patients, treatment strategies with a combination of Chinese and Western medicine antiviral treatment are given priority [2]. For patients who have immune system disorders with severe or life-threatening disease, commonly used antiviral treatment, supplemented with oxygen therapy and respiratory support, is primarily enhanced to improve the patient's immunity [3]. Early immune therapy to regulate the patient's immune homeostasis and enhance the body's antiviral ability, such as IFN-γ aerosol inhalation therapy, thymus peptide immunomodulatory substances, antibodies and vaccines and other immunotherapies, can prevent mild- and ordinary-type patients from becoming severely ill or from the disease being life-threatening. It has been reported that collecting serum from patients with COVID-19 in convalescence can neutralize the entry of SARS-CoV-2 [4, 5]. "Convalescent plasma therapy" may be applicable in patients with rapid disease progression and also severe- and life-threatened-type patients [5]. On July 15, 2020, the COVID-19 outbreak occurred in Urumqi, and the situation was very grim. Under the guidance of the domestic expert group, we adopted plasma antibody therapy for recovered patients in the new version of the *COVID-19 Diagnosis and Treatment Protocol (Trial Sixth Edition)* [6]. The mechanism is that patients recovering from COVID-19 infec-

tion will produce polyclonal antibodies against SARS-CoV-2, including neutralizing antibodies and non-neutralizing antibodies. Neutralizing antibodies can bind to viral surface antigens or viral receptor antigens and inhibit the proliferation and amplification of viruses by preventing the virus from invading cells [7]. After binding to the virus, non-neutralizing antibodies mediate the phagocytosis and killing of infected cells by immune cells, such as macrophages and NK cells, through the conditioning effect and antibody-dependent cytotoxicity [8]. This method of treating diseases using convalescence plasma has achieved good clinical effects in SARS-CoV [9], Ebola virus infection [10] and MERS-CoV. However, the technique of treating COVID-19 patients with plasma antibodies from recovered COVID-19 patients and performing clinical observation and evaluation on a large sample has never been done in designated hospitals, and this is the first attempt to do so in Xinjiang. In addition, this kind of plasma is more complex than ordinary frozen plasma, with more allergenic substances, low amounts of viruses and more cytokines, and the residue of pretreatment drugs, which is not clear, may convey some therapeutic risks [11-18]. Therefore, it is of great significance for COVID-19 patients to be treated with frozen convalescent plasma from recovered COVID-19 patients to investigate the changes in antibody content and nucleic acid clearance in their bodies as well as the clinical treatment effect to provide a basis and reference for possible blood transfusion treatment in the outbreak of epidemics.

## Material and methods

### *Participants*

A total of 299 COVID-19 patients admitted to our hospital were all in line with the diagnostic criteria and clinical classification of the COVID-19 diagnosis and treatment plan (trial for the seventh revision) [19]. Oral and pharyngeal swabs were collected twice and analyzed by reverse transcription real-time fluorescence quantitative polymerase chain reaction (RT-PCR) to detect SARS-CoV-2-positive nucleic acids. All cases were confirmed by domestic and Xinjiang provincial expert groups. There were 99 patients in the plasma transfusion group, including 24 patients who did not have a complete antibody detection record after frozen plasma infusion and were not included in the study and 75 patients in the actual statistical study. In addition, the non-plasma transfusion group contained 200 cases. Principles of transfusion were performed in accordance with the "Notice of General Office of National Health Commission and Health Bureau of Logistic Support Department of Central Military Commission on the issuance of clinical plasma treatment plan for COVID-19 convalescent patients (Trial Second Edition)" [20]. All hospitalized patients were approved for inclusion by the medical ethics committee of the hospital.

### *Plasma transfusion group (research group)*

In our study, a total of 99 patients with COVID-19 were treated with convalescent plasma transfusion, among whom the clinical classification composition ratio was 59.6% rapidly developing type, 25.3% severe type, and 15.2% life-threatening type. Among the 99 cases, there were 34 males (45%) and 41 females (55%). Patient ages ranged from 24 to 81 years old. Twenty-six people were between 20 and 50 years old (accounting for 35%), and 49 people were over 50 years old (accounting for 65%). There were 59 Uyghurs (accounting for 79%) and 16 Han (accounting for 21%). Fifty-one patients (68%) had underlying diseases, including diabetes, hypertension and lung disease. Average patient weight was 72.80± 16.81 kg. The average IgG and IgM antibody contents of 229 bags (200 mL per bag) of recovered patients (donors) were 31.61± 23.28 (concentration equivalent to 1:64) and 7.19±11.94 (concentration equivalent to 1:8), respectively. The average hospital stay was 30.80±20.05 days.

### *Non-plasma transfusion group (control group)*

Complete statistical data were available for 200 patients who had not received convalescent plasma transfusion for COVID-19, including 175 patients with mild disease, 25 patients with severe disease, and 14 patients (7%) with basic diseases, such as diabetes mellitus, hypertension and pulmonary disease. Among the 200 patients, there were 88 males (44%) and 112 females (56%). Ages ranged from 4 to 88 years old. A total of 121 people were between 20 and 50 years old (accounting for

61%), 39 people were over 50 years old (accounting for 19%), and 40 people were under 20 years old (accounting for 20%). There were 157 Uyghurs (accounting for 78.5%), 1 Hui (accounting for 0.5%), and 4 Han (accounting for 21%). Average patient weight was 67.30±26.49 kg. The average hospital stay was 32.45±7.15 days.

*Laboratory examination*

The antibodies and nucleic acids were tested several times after admission, after plasma injection and when discharged from the hospital. Detection methods: 2019-nCoV nucleic acid detection using 2019-nCoV ORF1Ab/N gene dual real-time PCR technology and reagents provided by Jiangsu Master Company were performed in accordance with the instructions. Antibody IgM and IgG levels were detected using the magnetic particle chemiluminescence method. The equipment was an automatic chemiluminescence analyzer (model: AutoLumo A2000 Plus). 2019-nCoV IgG/IgM antibody detection kit (batch numbers IgM 20200918 and IgG 20201023) was provided by Zhengzhou Anto Bio-Engineering Co., Ltd. Ct value represents the number of cycles that the fluorescence signal in each PCR reaction tube goes through when it reaches the set threshold value and was shortened from the Cutoff value. The specification of 2019-nCoV IgM/IgG Antibody Test Kit stipulates that Cutoff value = average luminescence value of positive control well × Cutoff coefficient. The Cutoff value of the kit is equal to the average luminescence value of the positive control well × 0.1 (the Cutoff coefficient is 0.1 when the highest sensitivity of 90% and specificity of 100% is taken by ROC curve method for statistical analysis). There is no unit, which is different from the traditional expression method of concentration dilution multiple. There is a linear relationship between the Ct value of each template and the logarithm of the initial copy number of the template. The more the initial copy number, the smaller the Ct value, and vice versa. ABO blood group positive and negative stereotyping reagents were provided by Shanghai Blood Biomedical Co., Ltd. The cross-matching test and antibody screening test were conducted using coagulant amine medium reagent provided by Zhuhai BASO Biotechnology Co., Ltd.

*Statistical methods*

SPSS 21.0 software was used for statistical processing. The measurement data are described as ($\bar{x} \pm s$), and the number of enumeration data points is expressed as a percentage (%). Then, a t-test, $\chi^2$ test or ANOVA test was used for statistical analysis. $P<0.05$ was considered statistically significant.

## Results

*Convalescent plasma antibody therapy in COVID-19 patients*

Ninety-nine patients received convalescent plasma therapy. Among them, 59 patients with rapidly developing disease received 50,450 mL of convalescent plasma, the highest proportion (47.0%) of the total plasma amount, with an average of 855.1 mL per person. Twenty-five severe type patients received 27,800 mL of convalescent plasma, with an average of 1,112 mL per patient. In 15 life-threatening type patients, 29,000 mL of convalescent plasma was used, with an average of 1,933.3 mL. Usage in patients with severe and life-threatening disease was almost the same (25.9% and 27.0%). The effective rate of clinical transfusion was 100%, and the total transfusion rate was 9.31% (99/1064).

*Changes in IgG and IgM antibody levels before and after therapy between the transfusion and non-transfusion groups*

Ninety-nine patients with COVID-19 received clinical convalescent plasma transfusion, 75 of whom had clear antibody statistical data and 24 of whom did not have complete antibody data and were not included in the study. A total of 229 bags (45,800 mL) of frozen convalescent plasma were infused, with an average of 610.67 mL per person. The average IgG Ct value was 31.61±23.28, and IgM was 7.19±11.94 per bag. The total antibody of these plasma donors was equivalent to a 1:8-1:64 concentration. The IgG and IgM levels of the patients were detected at 2-3 days and 7-14 days after transfusion.

In the transfusion group, the Ct concentrations of IgG and IgM antibodies (4.46±13.99 and 4.46±13.99, respectively) in patients before transfusion were significantly lower than

**Table 1.** Comparison of IgG and IgM antibody levels before and after convalescent plasma transfusion in the transfusion and non-transfusion groups

| Groups | n | IgG ($\bar{x} \pm s$) | IgM ($\bar{x} \pm s$) |
|---|---|---|---|
| Convalescent plasma transfusion group | | | |
| Before plasma transfusion | 75 | 4.46±13.99 | 4.55±11.02 |
| Frozen convalescent plasma | 229 | 31.61±23.28 | 7.19±11.94 |
| After plasma transfusion at discharge | 75 | 68.70±69.14 | 47.89±64.63 |
| Comparison before and after plasma transfusion, (t, P) | | 7.8867, p<0.05 | 5.7248, p<0.05 |
| Non-convalescent plasma transfusion group | | | |
| After admission | 200 | 21.49±18.54 | 0.04±0 |
| Before discharge | 200 | 43.41±35.61 | 17.33±16.26 |
| Comparison at admission and discharge, (t, P) | | 4.6259, p<0.05 | 9.2088, p<0.05 |
| Comparison of two groups at admission, (t, P) | | 8.1857, p<0.05 | 3.5443, P>0.05 |
| Comparison of two groups at discharge, (t, P) | | 3.0210, P>0.05 | 4.0472, P>0.05 |

those after plasma infusion (68.70±69.14 and 47.89±64.63, respectively), with significant differences between the two groups (P<0.05).

In the non-transfusion group, the Ct values of IgM and IgG antibodies in patients after admission were also significantly lower than the Ct values of IgM and IgG antibodies in patients after discharge (P<0.05), but the content of IgM and IgG antibodies was significantly lower than the Ct values and concentrations of IgM and IgG antibodies in patients after infusion of frozen convalescent plasma.

In general, the IgM and IgG antibodies in the transfusion group were significantly higher (1-3 folds) than those in the non-transfusion group after clinical treatment, but there was no significant difference (P>0.05).

In addition, the data showed that the increase in IgM antibody production occurred more rapidly in severe and life-threatening type patients (transfusion group) after admission (4.55±11.02) with only 0.04±0 in the mild type group (4.46±13.99), but the increase in IgG antibody was slower (4.46±13.99). However, the IgG antibody produced faster and higher concentrations (21.49±18.54) than the clinical mild group without transfusion (**Table 1**).

*Comparison of rapidly developing, severe and life-threatening type patient antibody changes and hospital stay before and after plasma therapy*

There were 75 cases divided into 3 types of COVID-19 patients. The results showed that in the plasma transfusion group, the severe and life-threatening subgroups had significantly higher antibody IgG content than the rapidly developing subgroup at admission (P<0.05). All 3 types exhibited an increase in antibody IgG content when discharged from the hospital but were less statistically significant (P>0.05). Among the 3 clinical types, antibody IgM content at the time of admission was not different. However, there was a significant increase in IgM antibodies in the 3 types at the time of discharge, the difference between the severe and life-threatening groups was statistically significant (P<0.05), and the antibody IgM content of the severe type group was higher. The contents of IgM and IgG antibodies in all 3 types increased at discharge and were significantly higher than those when admitted to the hospital (P<0.05).

In the non-transfusion group, the antibody IgG content of the severe type was significantly lower than that of the rapidly developing type when admitted to the hospital or when discharged from the hospital (P<0.05). The IgG antibody content of the rapidly developing type was clearly increased when discharged from the hospital, which was statistically significant (P<0.05), while severe type patient IgM antibody content decreased but not statistically significantly (P>0.05). The antibody IgM content of the severe type was significantly higher than that of the rapidly developing type when admitted to the hospital (P<0.05) but was equal to that of the rapidly developing type when discharged from the hospital (P>0.05). When discharged from the hospital, only the

rapidly developing type group had a significant increase in the content of IgM and IgG antibodies, which was significantly higher than that at admission (P<0.05), while the severe type group did not exhibit a significant change (P>0.05).

Rapidly developing type patients in the non-transfusion group had a higher content of IgG antibody than that of transfusion group's rapid-developing type at admission, which was statistically significant (P<0.05). In addition, rapidly developing type patients in the transfusion group had a significantly higher content of IgM antibody than that of the non-transfusion group's rapidly developing type at admission (P<0.05). Importantly, the IgG and IgM antibody content of severe type patients were higher in the transfusion group than in the non-transfusion group at discharge (P<0.05).

For the 3 types of patients in both the transfusion and non-transfusion groups, the days of hospitalization increased as the degree of COVID-19 development increased, and these differences were significant (P<0.05). Among them, the number of days of hospitalization in the rapidly developing type in the transfusion group was significantly less than that of the non-transfusion group (P<0.05), as was the severe type (P<0.05) (**Table 2**).

*Viral nucleic acid removal before and after convalescent plasma therapy*

The results showed the total negative rate of the nucleic acid gene in 75 patients who experienced transfusion of convalescent plasma. That of the transfusion group at 7-14 days (63.89%) was significantly higher than the non-transfusion group during the same period (26.50%) and was statistically different (P<0.005). Moreover, the number of ORF1ab genes and N negative conversion and proportion was also increased significantly, and these differences were statistically significant (P<0.01), as shown in **Table 3**.

*Comparison of key diagnostic indicators of heart, lung, kidney and coagulation in transfusion group*

Statistical analysis results of the data showed that except for cTnI and Myo; the levels of AST, ALT, LDH, CK and CKMB in patients with severe syndrome and critical condition were significantly higher than those in patients with rapid development, with statistically significant differences (P<0.001). Lung function except PH, $PCO_2$ (mmHg), $PO_2$ (mmHg) and LAC (mmol/L) were significantly higher in severe syndrome and critical group than in the rapid development group, with statistical significance (P<0.001). The UREA (mmol/L) and CRE (umol/L) of renal function in the severe syndrome and critical type group were significantly higher than those in the rapid development type group, with statistical significance (P<0.001). Besides PT, APTT and TT, D-Dimer (DD), PLT, FIB, TT, PT-INR, DD, PT-% and PT-R were significantly higher in severe syndrome and critical group than in the rapid development group (P<0.001). The AT3 rapidly developing group was significantly higher than the severe syndrome and critical group (P<0.001). The results are shown in **Table 4**.

*Comparison of peripheral blood main inflammatory cell detection results in transfusion group*

The changes of major cytokines in the peripheral blood of 77 COVID-19 patients were observed according to clinical classification. ANOVA comparison showed that, except RBC ($\times 10^{12}$/L), WBC ($\times 10^9$/L) and neutrophil (%) had statistical significance in the rapidly developing, severe and life-threatening types (P< 0.001). There was no significant difference in the percentage of lymphocyte (%), which was lower only in the severe type and the life-threatening type. In addition, WBC ($\times 10^9$/L) was significantly higher in the severe and life-threatening types than in the rapidly developing type, while neutrophil (%) was significantly higher in the rapidly developing group than in the severe and critical groups (**Table 5**).

*Chest imaging examination*

Chest radiographs and CT examinations were performed in both the transfusion group and the non-transfusion group, and all 275 patients showed pneumonia (**Figure 1**). Chest X-ray: In the transfusion group, 75 patients showed increased and thickening of double lung texture and patchy increased density, and some severe syndromes showed diffuse consolidation like "white lung". CT images showed that in the transfusion group, 39 cases (52.0%)

**Table 2.** Comparison of antibody content and hospital stays in patients in the transfusion and non-transfusion groups

| | Rapid developing type ($\bar{x} \pm s$) n=38 | Severe type ($\bar{x} \pm s$) n=21 | Life-threatening type ($\bar{x} \pm s$) n=16 | t-value | *P*-value |
|---|---|---|---|---|---|
| Convalescent plasma transfusion group (n=75) | | | | | |
| Days in hospital, (d) | 24±8.68* | 28±11.46 | 45±39.11* | 4.5396 | <0.05 |
| IgG at admission | 2.98±12.23* | 6.05±18.24 | 5.88±10.07* | 13.0760 | <0.05 |
| IgG at discharge | 55.15±59.09 | 99.6±79.49 | 57.21±52.50 | | >0.05 |
| IgM at admission | 3.78±9.85 | 3.88±10.07 | 7.49±14.22 | | >0.05 |
| IgM at discharge | 45.63±54.19 | 63.69±85.75* | 28.80±35.54* | 10.7183 | <0.05 |
| Comparison of IgG between hospital admission and discharge, (t, P) | 8.7645, <0.05 | 9.9339, <0.05 | 8.3157, <0.05 | | |
| Comparison of IgM between hospital admission and discharge, (t, P) | 0.7626, >0.05 | 5.9992, <0.05 | 4.8212, <0.05 | | |
| Non-convalescent plasma transfusion group (n=200) | 175 | 25 | 0 | | |
| Days in hospital, (d) | 31±6.33 | 39±8.69* | - | 9.9709 | <0.05 |
| IgG at admission | 30.85±15.89* | 2.70±0 | - | 25.0536 | <0.05 |
| IgG at discharge | 43.40±35.61* | 11.83±65.55 | - | 5.9850 | <0.05 |
| IgM at admission | 0.04±0 | 20.90±15.40* | - | 19.1562 | <0.05 |
| IgM at discharge | 17.33±16.26 | 16.37±37.90 | - | 0.3359 | >0.05 |
| Comparison of IgG between hospital admission and discharge, (t, P) | 4.5515, <0.05 | 1.9697, >0.05 | | | |
| Comparison of IgM between hospital admission and discharge, (t, P) | 15.0379, <0.05 | 1.5659, >0.05 | | | |
| Comparison of IgG in two groups at admission, (t, P) | 15.4434, <0.05 | 1.5906, >0.05 | | | |
| Comparison of IgG in two groups at discharge, (t, P) | 1.6158, >0.05 | 8.5357, <0.05 | | | |
| Comparison of IgM in two groups at admission, (t, P) | 3.2883, >0.05 | 10.6838, <0.05 | | | |
| Comparison of IgM in two groups at discharge, (t, P) | 6.2541, <0.05 | 4.6131, <0.05 | | | |
| Comparison of hospital stays in two groups, (t, P) | 6.3771, P<0.05 | 7.5394, P<0.05 | | | |

*indicates whether the two groups of data are statistically compared by t-test.

**Table 3.** Comparison of the removal of nucleic acids before and after convalescent plasma therapy in patients in the transfusion and non-transfusion groups

| | The number of positive cases | Number of gene ORF1ab and N (-) cases, (%) | Number of gene ORF1ab (-) cases, (%) | Number of gene N (-) cases, (%) | Total nucleic acid to negative rate, (%) |
|---|---|---|---|---|---|
| Convalescent plasma transfusion group (n=75) | | | | | |
| Before plasma therapy | 75 | 0 | 0 | 0 | 0, 0 |
| 7-14 days After plasma treatment | 29 | 17, 22.67 | 13, 17.33 | 16, 21.33 | 46, 61.33 |
| Non-convalescent plasma transfusion group (n=200) | | | | | |
| 7-14 days in the same period | 147 | 21, 10.50 | 15, 7.50 | 17, 8.50 | 53, 26.65 |
| Comparison between transfusion group and non-transfusion group in the same period, ($\chi^2$, P) | | 6.7801 | 5.7673 | 8.5070 | 28.7254 |
| | | <0.01 | <0.025 | <0.005 | <0.005 |

**Table 4.** Comparison of clinical cardiac, lung, kidney and other key functional indicators in the transfusion group ($\bar{x} \pm s$)

| Indicator | Rapid developing type ($\bar{x} \pm s$) n=38 | Severe type ($\bar{x} \pm s$) n=21 | Life-threatening type ($\bar{x} \pm s$) n=16 | t-value | *P*-value |
|---|---|---|---|---|---|
| Cardiac function | | | | | |
| AST (u/L) | 23.48±3.82 | 35.73±4.85 | 48.74±6.72 | 71.633 | <0.001 |
| ALT (u/L) | 18.64±3.27 | 38.49±5.28 | 57.73±6.93 | 56.857 | <0.001 |
| LDH (u/L) | 132.74±21.64 | 253.74±37.68 | 356.28±43.73 | 36.271 | <0.001 |
| CK (u/L) | 54.82±6.39 | 102.37±17.83 | 184.21±32.79 | 36.957 | <0.001 |
| CKMB (u/L) | 3.15±0.48 | 3.54±0.33 | 4.49±0.42 | 31.748 | <0.001 |
| cTnI | 0.32±0.10 | 0.36±0.12 | 0.41±0.11 | 1.765 | 0.183 |
| Myo | 37.54±5.81 | 42.54±6.83 | 39.29±5.37 | 2.928 | 0.064 |
| Lung function | | | | | |
| $PCO_2$ (mmHg) | 33.26±4.93 | 38.54±3.85 | 48.73±4.14 | 36.366 | <0.001 |
| $PO_2$ (mmHg) | 61.25±5.82 | 98.38±8.92 | 142.31±15.48 | 43.271 | <0.001 |
| PH | 7.31±0.23 | 7.33±0.17 | 7.46±0.22 | 1.766 | 0.183 |
| LAC (mmol/L) | 1.94±0.21 | 2.46±0.22 | 3.34±0.24 | 110.079 | <0.001 |
| Renal function | | | | | |
| UREA (mmol/L) | 3.86±0.14 | 5.37±0.53 | 8.65±0.87 | 218.998 | <0.001 |
| CRE (umol/L) | 43.72±5.44 | 76.28±8.75 | 112.36±17.54 | 146.746 | <0.001 |
| Coagulation function | | | | | |
| PLT | 146.38±16.48 | 268.35±28.65 | 421.29±43.91 | 65.761 | <0.001 |
| PT | 11.29±1.43 | 11.43±1.38 | 11.73±1.72 | 0.250 | 0.780 |
| APTT | 29.84±3.93 | 31.28±4.83 | 30.47±3.55 | 0.490 | 0.616 |
| AT3 | 120.48±23.29 | 112.43±19.48 | 93.27±16.35 | 5.067 | <0.011 |
| FIB | 1.43±0.28 | 2.48±0.30 | 3.89±0.32 | 187.163 | <0.001 |
| TT | 18.49±3.29 | 16.94±4.83 | 17.48±3.79 | 0.585 | 0.561 |
| PT-INR | 0.93±0.10 | 0.96±0.14 | 1.18±0.13 | 12.204 | <0.001 |
| D-Dimer | 0.25±0.05 | 0.76±0.08 | 7.13±0.08 | 368.954 | <0.001 |
| PT-% | 85.38±8.93 | 98.37±11.28 | 106.85±14.36 | 10.846 | <0.001 |
| PT-R | 0.84±0.05 | 0.93±0.09 | 1.17±0.11 | 42.943 | <0.001 |

showed small patch shadow, 44 cases (58.7%) showed ground glass shadow, 16 cases (21.3%) showed large consolidation shadow. There were 6 cases complicated with small pleural effusion, 2 cases unilateral and 4 cases bilateral. Pleural thickening and adhesion were complicated in 34 cases (45.3%). Statistical analysis showed that the incidence of mass consolidation shadow and ground glass shadow in severe type (21 cases) and life-threatening type (16 cases) was significantly higher than that in rapidly developing type (38 cases) [37.8% (14/37) vs 10.8% (4/37), P<0.05]. There were 12 cases (16.0%) of chest lesions involving single lung and focal distribution, and 61 cases (81.3%) of chest lesions involving double lung and diffuse distribution. The incidence of double lung diffuse distribution in patients with severe and life-threatening type was significantly higher than that in the rapidly developing type [88.0% (66/75) vs 78.7% (59/75), P<0.05]. After 7 days of plasma infusion, 75 COVID-19 patients were examined, and chest CT showed significant improvement in the above symptoms, with most of them improved in absorption.

*Clinical outcome*

100% (75 cases) of all COVID-19 patients in this study were cured by convalescent plasma transfusion and discharged from hospital, the longest time being 83 days, with no death. The average length of hospital stay in the transfusion group was 24±9 days for the rapid development type, 28±12 days for the severe type,

**Table 5.** Comparison of detection results of inflammatory factors in the transfusion group ($\bar{x} \pm s$)

| | Rapid developing type ($\bar{x} \pm s$) n=38 | Severe type ($\bar{x} \pm s$) n=21 | Life-threatening type ($\bar{x} \pm s$) n=16 | F-value | *P*-value |
|---|---|---|---|---|---|
| WBC ($\times 10^9$/L) | 5.08±1.23 | 7.83±1.98 | 9.62±2.26 | 18.136 | <0.001 |
| RBC ($\times 10^{12}$/L) | 4.54±1.38 | 4.73±1.36 | 4.62±1.22 | 0.0902 | 0.901 |
| NEU (%) | 0.62±0.15 | 0.43±0.11 | 0.33±0.05 | 19.619 | <0.001 |
| LYM (%) | 0.45±0.17 | 0.41±0.12 | 0.37±0.11 | 0.979 | 0.384 |

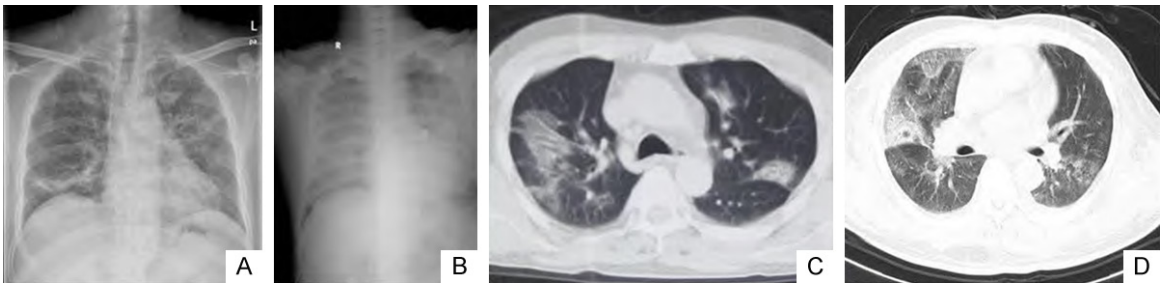


**Figure 1.** Chest imaging results of COVID-19 patients. A. In patients with rapidly development disease, chest X-ray showed increased and thickened lung textures, scattered in small patches and strips. B. In patients with severe disease, x-rays revealed diffuse ground glass shadows in both lungs. C. The CT scan of the rapidly developing patient showed scattered patchy consolidation of both lungs and bronchial air sign. D. In patients with severe disease, CT showed diffuse ground glass shadows and partial consolidation in both lungs.

and 45±39 days for the life-threatening type. In the non-transfusion group, the mean time was 31±6 days for patients with rapidly developing and 39±9 days for patients with severe disease. Statistical analysis showed that between the transfusion group and the non-transfusion group, the length of hospital stays of patients with rapidly-developing COVID-19, severe COVID-19 and critical COVID-19 type became longer and longer with the aggravation of disease progression, and the differences were significant (P<0.05). In addition, the hospitalization days of patients with rapidly developing and severe type in the transfusion group were significantly shorter than those in the non-transfusion group (P<0.05), indicating obvious therapeutic effect. Due to the influence of basic diseases on critically ill patients and the absence of critically ill patients in the non-transfusion group in this study, no specific conclusions can be drawn, but the actual effect of convalescent plasma transfusion therapy is clear.

## Discussion

This round of SARS-CoV-2 spread quickly and widely, and some patients became seriously ill. To quickly control the outbreak and offer useful treatment, in accordance with the advice of the domestic expert group, we used convalescent plasma therapy on patients with "rapid developing, severe and life-threatening types of the disease" [2-6]. Plasma therapy was used during the Spanish influenza pandemic of 1918-1920 and proved to be effective [19, 21, 22], with the identification of a potential treatment for multiple viral infections for the first time [23]. It has also been used in major international outbreaks in recent years, such as SARS, MERS, and Ebola [24]. Convalescent Plasma therapy or plasma replacement therapy can remove excess cytokines from the patient's body and relieve the "cytokine storm" [25], thereby reducing damage of the immune response to the body. This may be the first application of 99 cases of clinical patients with COVID-19 being treated with convalescent plasma and 5 cases with plasma replacement in Xinjiang, China. Active treatment of 99 clinical "rapidly developing, severe and life-threatening types" patients in accordance with the principle of infusion [20], of which 59 patients with rapid developing were given an average of 855.1 mL per person, 25 patients with severe disease had an average infusion of 1,112 mL per person, and 15 cases of life-

threatening type patients infusion rehabilitation of 29,000 mL of convalescent plasma, an average of 1,933.3 mL, were higher than the literature reports for typical convalescent plasma use of 200-500 mL. The results showed that the treatment was 100% effective, and the cure rate was 100%. The total convalescent plasma transfusion rate was 9.31% (99/1064), and the non-transfusion treatment rate was 90.69%. Six adverse events were observed, and no serious adverse reactions to transfusions occurred. In conclusion, the clinical results were satisfactory.

The strength of the immune system determines a patient's disease status and prognosis [26]. The body's immune system plays a vital role in removing virus [27]. It takes a while for the immune system to produce specific antibodies after the virus enters the body, where IgM antibody occurs during the early period after infection and IgG antibody occurs with the progression of the disease. Neutral antibodies produced by the human body in the natural antiviral immune process bind to viral receptor antigens, which can prevent the virus from invading cells to inhibit the proliferation and amplification of the virus, and the combination of non-neutralizing antibodies and viruses mediates the phagocytosis and killing of virus-infected cells by macrophages and NK cells through antibody-dependent cytotoxicity regulation [7, 8]. The content of antibodies and the detection of antibodies are of great significance for viral diagnosis, infection time and infection stage, and the evaluation of therapeutic effect and the outcome of disease. The National COVID-19 Treatment Program (Trial 7th Edition) clearly states that SARS-CoV-2-specific IgM and IgG antibody positivity is one of the diagnostic criteria for COVID-19 [20]. Therefore, the use of convalescent plasma antibody treatment achieves antiviral effects and alleviates the disease. In SARS-CoV, Ebola virus and MERS-CoV infection, plasma treatment has achieved good clinical results [9, 10]. When the virus first invades the human body, the early immune system produces temporary IgM antibody, which peaks after approximately 1 month, neutralizes the invading virus and plays an immune role. IgM antibody gradually decreases with the improvement of the disease, while the body's immune system produces persistent IgG antibody, which is the primary driving force of the body's immunity during the middle and late stages of infection, with high concentrations that can be detected [28]. Patients can be diagnosed with recurrent infection if the recovery period IgG antibody increases fourfold or more than that of the acute stage [29, 30], which is an important basis for diagnosis. In our study, in each bag of convalescent plasma, the average IgG Ct value was 31.61±23.28, IgM was 7.19±11.94, equivalent to the concentration of antibody dilution of 1:8-1:64, and the donor plasma antibody concentration was not high.

For the convalescent plasma transfusion group, the IgM and IgG antibody Ct values, concentration and content were distinctly higher than those before plasma transfusion, and these differences were significant ($P<0.05$). The IgM and IgG antibodies in the non-transfusion group (mild type) Ct values were also significantly lower than those when patients were discharged from the hospital. However, the Ct values and concentrations of IgM and IgG antibodies were obviously less than those of patients after plasma transfusion. Although the plasma transfusion groups were 1-3-fold higher than the non-transfusion patient group after clinical treatment with IgM and IgG antibodies, there was no significant difference ($P>0.05$). This suggests that apart from the antibodies produced by the patients themselves, the convalescent plasma of the infusion increased the concentration of antibodies in the patient's body, demonstrating that convalescent plasma has a role in promoting the increase of the patient's antibody levels. In addition, it was shown that IgM antibodies increased rapidly (4.55±11.02) after admission to the hospital in severe and life-threatening type patients (transfusion groups), while mild type patients were slower (0.04±0), but IgG antibodies produce a slower rise (4.46± 13.99), visibly less rapid and concentrated than clinically mild type and non-transfusion group IgG (21.49±18.54). These results fully demonstrate that convalescent plasma transfusion therapy significantly improves the immunity of patients with COVID-19 and promotes the production of antibodies in the body, facilitating more rapid recovery from the disease.

In the plasma transfusion group, the severe and life-threatening type subgroups displayed a

significantly higher antibody IgG content than the rapidly developing type subgroup at admission (P<0.05). All 3 types exhibited an increase in antibody IgG content when discharged from the hospital but were less statistically significant (P>0.05). This indicates that the immunity of severe and life-threatening patients with early IgG content is enhanced, while the immunity of patients with rapid development is insufficient, and antibodies can only be gradually recovered by adjuvant therapy of convalescent plasma infusion. Among the 3 clinical types, antibody IgM content at the time of admission was not different. However, there was a significant increase in IgM antibodies among the 3 types at the time of discharge, the difference between the severe and life-threatening groups was statistically significant (P<0.05), and the antibody IgM content of the severe type group was higher. The contents of IgM and IgG antibodies in all 3 types increased at discharge and were significantly higher than those when admitted to the hospital (P<0.05). These results indicate that the content of IgM had risen to a higher concentration in patients at different stages during early disease. The more severe the disease was, the greater the amount of IgM antibody consumed by the body, and the antibody concentration even decreased significantly due to immune exhaustion. Therefore, antibody concentration serves the function of indicating the severity of the disease and the strength of immunity, with a high antibody concentration indicating severity of disease. If the disease is severe and the antibody concentration is low, it indicates a risk of poor prognosis. The IgG and IgM antibody contents of severe type patients were higher in the transfusion group than in the non-transfusion group at discharge (P<0.05). This clearly indicates that clinical transfusion promotes an increase in antibody levels in severe patients, and the antibody levels remain high at clinical discharge.

In the non-transfusion group, the antibody IgG content of the severe type was significantly lower than that of the rapidly developing type when admitted to the hospital or when discharged from the hospital (P<0.05). The IgG antibody content of rapidly developing type was clearly increased when discharged from the hospital, which was statistically significant (P<0.05), while severe type patients' IgM antibody content decreased but not statistically significantly (P>0.05). These results suggest that the low levels of IgG and IgM in severe patients at discharge or admission indicate that the patient's immune status is weak, and clinical observation and treatment should be continued. In the non-transfusion group, the antibody IgM content of the severe type was significantly higher than that of the rapid-developing type when admitted to the hospital (P<0.05) but was equal to that of the rapid-developing type when discharged from the hospital (P>0.05). When discharged from the hospital, only the rapidly developing type group exhibited a significant increase in the content of IgM and IgG antibodies, which was significantly higher than that at admission (P<0.05), and the severe type group was not significantly changed (P>0.05). These results also further indicate that patients with severe disease have a stronger ability to produce antibodies to resist the attack of the virus when their immunity is normal until the body gradually recovers and maintains the corresponding antibody concentration. Rapidly developing type patients in the non-transfusion group had a higher content of IgG antibody than that of transfusion group's rapid-developing type at admission, which was statistically significant (P<0.05), suggesting that IgG antibody content is high and exerts strong immunity, which does not require clinical treatment or plasma treatment.

The length of the SARS-CoV-2 genome is 29 KB, two-thirds of which are open reading frames (ORFs), and 3'-end genes encode structural proteins, including the E gene encoding the envelope protein, the M gene encoding the membrane protein, the N gene encoding the nucleocapsid protein, and the S gene encoding the spike-like glycoprotein (which mediates viral entry into the host) [31]. After the human body is infected with the virus, it generally takes 2 weeks to detect viral antibodies in the peripheral blood, which is called the "window period". The virus replicates continuously during the "window period", and the nucleic acid load increases exponentially and becomes positive. Compared to serum antibody detection, it has the advantage of early detection of infected persons, and it is an important indicator for whether the virus is removed and how much exists. However, the IgM or IgG produced during the "window period" is present at very

low levels and is and difficult to quickly detect, coming up as negative [32]. Therefore, the detection and diagnosis of viral nucleic acids is particularly important. Our study compared the nucleic acid negative conversion results of COVID-19 in severe and life-threatening type patients treated with convalescent plasma during the convalescent period and concluded that the viral nucleic acid gene clearance rate of 75 COVID-19 patients was 61.33%. That of the transfusion group was 7-14 d (63.89%), which was significantly higher than the non-transfusion group during the same period (26.50%; P<0.005). Moreover, during the same period, the negative conversion rate of the 7-14d genes ORF1Ab and N (22.67%), the negative conversion rate of the gene ORF1Ab (17.33%), and the negative conversion rate of the gene N (21.33%) were also significantly increased and were significantly higher than those of the non-transfusion group (10.50%, 7.50%, and 8.50%, respectively). These differences were statistically significant (P<0.01). This fully indicates that the infusion of convalescent plasma from convalescent patients has a very important therapeutic effect in severe and life-threatening COVID-19 patients, and plasma antibody therapy has a clear role in clearing viral nucleic acids in patients and promoting the improvement and recovery of COVID-19 patients.

In addition, for the 3 types of patients in both the transfusion and the non-transfusion groups, the days of hospitalization were longer as the degree of development of COVID-19 increased, and these differences were significant (P<0.05). Among them, the number of days of hospitalization in the rapidly developing type in the transfusion group was significantly less than that of the non-transfusion group (P<0.05). These results suggest that blood transfusion therapy significantly shortens the duration of disease, reduces the length of hospital stay, and promotes early recovery and discharge.

In summary, timely and reasonable convalescent plasma treatment for COVID-19 patients with “rapidly developing, severe and life-threatening disease” [2] promotes the increased production of immune antibodies in patients, effectively improves the immunity of patients, accelerates the clearance of SARS-CoV-2 and the negative conversion rate of viral nucleic acid, and significantly promotes the early improvement of COVID-19 patients.

## Conclusions

This study confirms a positive relationship between the content of IgM and IgG antibodies and the clearance of viral nucleic acids in patients in response to infusion of convalescent plasma. The application of COVID-19 convalescence plasma in COVID-19 patients significantly increases the antibody content in the body of severe and critical inpatients, effectively enhances immune function, accelerates the clearance of virus and the nucleic acid negative conversion rate, and significantly promotes the early improvement of COVID-19 patients. The research has some clinical guidance and reference significance for the treatment of COVID-19 in the next step.

## Acknowledgements

This work was supported by a study on the combined detection and diagnosis of COVID-19 virus nucleic acids and antibodies in first-line close contact/asymptomatic infection (QLY-KY-2020001), as well as the CAMS Innovation Fund for Medical Sciences (CIFMS) (Grant Nos. 2020-I2 M-CoV19-006) and the Science & Technology Department of Sichuan Province (2020YFS0583).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Guoyue Lin, The Eighth Affiliated Hospital of Xinjiang Medical University, No. 100, North No.1 Lane, Kashi East Road, Xincheng District, Urumqi 830013, Xinjiang, China. Tel: +86-13565423808; E-mail: lgy474@163.com; Jianping Li, Liaoning Blood Center, No. 13, Beihai Street, Dadong District, Shenyang 110003, Liaoning, China. Tel: +86-24-88334530; E-mail: ljp_63@163.com

## References

[1] Wang XY, Liao J and Chen J. Research progress on immunological characteristics and immunotherapy strategies of novel coronavirus pneumonia. J Trop Med. http://kns.cnki.net/kcms/detail/44.1503.R.20200304.1632.002.html.

[2] General Office of the National Health Commission. Notice on Issuing the New Coronavirus Pneumonia Diagnosis and Treatment Plan (Trial Version 6). Medical Administration and Hospital Administration 2020-02-19/2020-02-26.
[3] General Office of the National Health Commission. Transcript of the press conference on February 17, 2020. Publicity Department 2020-02-17/2020-02-26.
[4] Hoffmann M, Kleine-Weber H, Krueger H, Mueller MA and Poehlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. bioRxiv 2020.
[5] National Health Commission. New coronavirus pneumonia diagnosis and treatment plan (trial version 6). (2020-02-19). [2020-0302]. http://www.nhc.gov.cn/yzygj/s7653p/2020-02/8334a8326dd94d329df351d7da8aefc2.html.
[6] Yang XM and Hou JF. The current situation of the application of plasma in the recovery period to acute viral infectious diseases and the prospects for the treatment of new coronavirus pneumonia. Chinese Journal of Biological Products 2020.
[7] Liu Y, Cao W, Sun M and Li T. Broadly neutralizing antibodies for HIV-1: efficacies, challenges and opportunities. Emerg Microbes Infect 2020; 9: 194-206.
[8] Mellor JD, Brown MP, Irving HR, Zalcberg JR and Dobrovic A. A critical review of the role of Fc gamma receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol 2013; 6: 1.
[9] Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, Chan P, Wong KC, Leung CB and Cheng G. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis 2005; 24: 44-46.
[10] Mupapa K, Massamba M, Kibadi K, Kuvula K, Bwaka A, Kipasa M and Muyembe-Tamfum JJ. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. J Infect Dis 1999; 179 Suppl 1: S18-23.
[11] Arribas JR, Luczkowiak J and Delgado R. Convalescent plasma for Ebola virus disease. N Engl J Med 2016; 374: 2498-2500.
[12] Lu XJ and Ma YL. Investigation and analysis of adverse effects of clinical blood transfusion. Journal of Clinical Hematology (Blood Transfusion and Testing) 2015; 28: 724-725.
[13] Jongerius I, Porcelijn L, van Beek AE, Semple JW, van der Schoot CE, Vlaar APJ and Kapur R. The role of complement in transfusion-related acute lung injury. Transfus Med Rev 2019; 33: 236-242.
[14] Yuan Q, Sun FX, Jiang HM and Wang YG. Application progress of convalescent plasma therapy in the epidemic of respiratory coronavirus infection. Chinese Journal of Rational Drug Use 2020; 7: 1-4.
[15] Qiu X, Hong C, Li Y, Bao W and Gao XM. Calreticulin as a hydrophilic chi-meric molecular adjuvant enhances IgG responses to the spike protein of severe acute respiratory syndrome coronavirus. Microbiol Immunol 2012; 56: 554-61.
[16] Zhu Z, Dimitrov AS, Chakraborti S, Dimitrova D, Xiao X, Broder CC and Dimitrov DS. Development of human monoclonal antibodies against diseases caused by emerging and biodefense-related viruses. Expert Rev Anti Infect Ther 2006; 4: 57-66.
[17] Liu L, Wei Q, Lin QQ, Fang J, Wang HB, Kwok H, Tang HY, Nishiura K, Peng J, Tan ZW, Wu TJ, Cheung KW, Chan KH, Alvarez X, Qin C, Lackner A, Perlman S, Yuen KY and Chen ZW. Anti-spike IgG causes severe a-cute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight 2019; 4: 123-158.
[18] Bogardus FB. Influenza pneumonia treated by blood transfusions. NY Med 1919; 109: 765.
[19] Miller OOMW. Report of influenza treated with serum from recovered cases. KyMed 1919; 17: 218-9.
[20] PM.C. Injection of whole blood in influenza. Br Med 1919.
[21] Lesne EBP and Saint-Girons F. Plasma therapy influenza. PresseMed 1919; 27: 181-2.
[22] Niu PH and Tan WJ. Research progress in antiviral therapy for Middle East respiratory syndrome. Acta Virology 2018; 34: 599-605.
[23] Li JP, Li XF, Zhao LZ, Wang HY, Li WW, Lin Y and Liu JH. Reflections on the application of blood transfusion-related therapies in the treatment of new coronary pneumonia. Clinical Transfusion and Laboratory Medicine 2020; 28: 228-231.
[24] The General Office of the National Health Commission, the Health Bureau of the Logistics Support Department of the Central Military Commission. Notice on the issuance of the clinical treatment plan for convalescent plasma in patients with new coronary pneumonia (trialversion2). (2020-03-04). http://www.gov.cn/zhengce/zhengceku/2020-03/05/content5487145.htm.
[25] Zhu J, Kim JW, Xiao X, Wang YG, Luo DN, Chen R, Xu L, Zhang H, Xiao GH, Zhan XW, Wang T and Xie Y. Profiling the immune vulnerability landscape of the 2019 novel coronavirus. Preprint at https://doi.org/10.1101/2020.02.08.939553 (2020).

[26] Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X, Zhang Q and Wu J. Coronavirus infections and immune responses. J Med Virol 2020; 92: 424-432.
[27] National Health Commission of the People's Republic of China. New Coronavirus Pneumonia Diagnosis and Treatment Plan (Trial Version 7). (2020-03-04)[2020-03-04]. http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.html.
[28] Phan T. Novel coronavirus: from discovery to clinical diagnostics. Infect Genet Evol 2020; 79: 104211.
[29] Ning YT, Hou X, Lu MY, Wu X, Li YZ and Xu YC. Discussion on the application of new coronavirus serum specific antibody detection technology. Concord Medical Journal. http://kns.cnki.net/kcms/detail/11.5882.R.20200305.1652.002.html.
[30] He C, Jiang H, Xie W, Chen J and Yin W. Discussion on the laboratory test path for the diagnosis and treatment of novel coronavirus pneumonia. Chinese Journal of Respiratory and Critical Care 2020. http://kns.cnki.net/kcms/detail/51.1631.R.20200221.1322.002.html.
[31] Cui J, Li F and Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019; 17: 181-192.
[32] Fricker-Hidalgo H, Bailly S, Brenier-Pinchart MP, Dard C, Jean D, Coston AL, Garnaud C and Pelloux H. How toestimate time of infection with toxoplasma gondii in pregnant women. Use of specific IgG and IgM kinetics by 7 techniques on 691 sera. Diagn Microbiol Infect Dis 2020; 15: 114987.