Research Paper

Convalescent plasma to treat COVID-19: clinical experience and efficacy

Shiyao Pei[1,2,*], Xi Yuan[3,*], Zhimin Zhang[3,*], Run Yao[3], Yubin Xie[4], Minxue Shen[2], Bijuan Li[3], Xiang Chen[1,2], Mingzhu Yin[1,2]

[1]Hunan Engineering Research Center of Obstetrics and Gynecological Disease, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China
[2]Department of Dermatology, Hunan Engineering Research Center of Skin Heath and Disease, Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China
[3]Department of Blood Transfusion of Xiangya Hospital, Central South University, Changsha 410008, Hunan Province, China
[4]Department of Blood Transfusion Laboratory of Changsha Blood Center, Changsha 410008, Hunan Province, China
*Equal contribution

Correspondence to: MingZhu Yin, Bijuan Li, Xiang Chen; **email:** yinmingzhu@csu.edu.cn; libijuan0528@126.com, https://orcid.org/0000-0001-6186-7992; chenxiangck@126.com, https://orcid.org/0000-0001-8187-636X
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ABSTRACT

The recent outbreak of COVID-19 in the world is currently a big threat to global health and economy. Convalescent plasma has been confirmed effective against the novel corona virus in preliminary studies. In this paper, we first described the therapeutic schedule, antibody detection method, indications, contraindications of the convalescent plasmas and reported the effectiveness of convalescent plasma therapy by a retrospective cohort study.

INTRODUCTION

In December 2019, pneumonia associated with a novel corona virus 2019 (COVID-19) caused an outbreak, which has posed significant threats to global health and economy [1]. As of 6th April 2020, the World Health Organization Situation Reported that this epidemic had spread to more than 180 countries with 1,113,758 confirmed cases, including 62,784 deaths [2]. It was reported that severely/critically ill case ratio was approximately 7-10% [3], while the current treatment strategy mainly rely on the supportive care since specific drugs of COVID-19 are still being researched. On March 4, 2020, in order to improve the therapeutic effect of COVID-19, the National Health Commission of the People's Republic of China organized Chinese experts to make revisions of the "Clinical treatment of COVID-19 Convalescent Plasma (the second trial edition)" [4]. On March 24, 2020, FDA approved the testing of convalescent plasmas for patients with serious or immediately life-threatening COVID-19 infections [5]. To date, thousands of convalescent plasmas have been collected and remarkable efficacy has been achieved in severely and critically ill COVID-19 patients in China. In order to standardize the treatment of COVID-19 Convalescent Plasma and share the clinical experience with the world, we summarized the therapeutic schedule as follows (Figure 1).

RESULTS

Recruit recovered COVID-19 patients

1. Inclusion Criteria (All six criteria must be met)
   - More than 3 weeks after the onset of symptoms of the COVID-19 and complete resolution of symptoms at least 14 days prior to donation.
   - In accordance with relieved isolation and discharge standards following the latest version of the therapeutic schedule.

- Age: 18 -55 years old.
- Weight: male≥50kg, female≥45kg.
- No history of blood-transmitted diseases.
- Eligible donors must be assessed by clinicians according to treatment.

2. Exclusion Criteria
   - With a history of pregnancy or transfusion whose HNA antibody and HLA antibody are positive.
   - Individual's physical condition is not eligible assessed by clinicians.
3. Verify the identities
4. Sign the informed consent
5. Health inquiry, physical examination, laboratory examination of blood samples (refer to technical operation procedures of blood station).

Collection and preparation of the convalescent plasma

1. Collection
   - Equipment and Operations: fully automatic apheresis machine or a fully automatic blood cell separator (refer to technical operation procedures of blood station).
   - Volume: 200-400ml (The exact volume should be assessed by clinicians).
   - The interval between plasma collection should be more than two weeks.
2. Storage
   - Follow the principle of sterility, repackaging the plasma 100-200ml each.
   - Store at 2-6° C for 48 hours. For long-term storage, it should be rapidly frozen to -20° C.

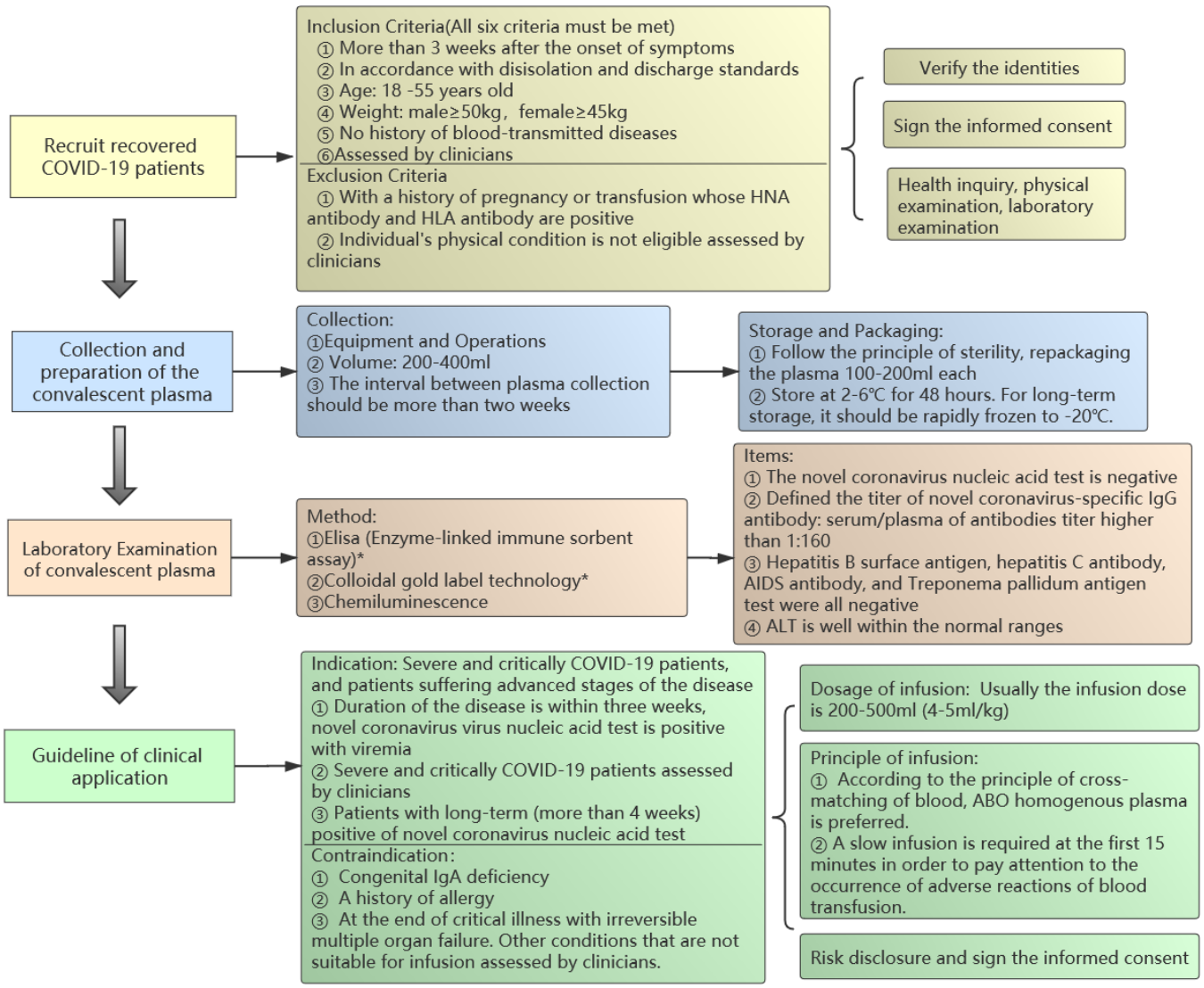

Figure 1. The standardized flow chart of the convalescent plasma transfusion.

3. Packaging: Labelling Requirements: refer to technical operation procedures of blood station.

Laboratory examination of convalescent plasma

1. Method: Elisa (Enzyme-linked immune sorbent assay)*, Colloidal gold label technology*, Chemiluminescence.
   *Note: We recommend using ELISA to detect the novel coronavirus antibody titer since the colloidal gold method was not suitable for titer detection and the false negative rate was high. The analysis of 17 samples showed that the positive rate and sensitivity of ELISA were significantly better than colloidal gold (The specific data is shown in Supplementary Table 1).
2. Items
   - The novel coronavirus nucleic acid test is negative.
   - Defined the titer of novel coronavirus-specific IgG antibody: serum/plasma of antibodies titer higher than 1:160.
   - Hepatitis B surface antigen, hepatitis C antibody, HIV antibody, and Treponema pallidum antigen test were all negative.
   - Alanine aminotransferase is well within the normal ranges.

Guideline of clinical application

1. Indication: Severe and critically COVID-19 patients, and patients suffering advanced stages of the disease.
   - Duration of the disease is within three weeks, novel coronavirus virus nucleic acid test is positive with viremia.
   - Severely and critically ill COVID-19 patients assessed by clinicians.
   - Patients with long-term (more than 4 weeks) positive of novel coronavirus nucleic acid test (for details please refer to patient 2 in Figure 2).
2. Contraindication
   - Congenital IgA deficiency.
   - A history of allergy including plasma infusion, human plasma protein products, sodium citrate. Plasma inactivated by methylene blue virus is strictly prohibited in patients with methylene blue allergy. Other history of severe allergies and contraindications.
   - At the end of critical illness with irreversible multiple organ failure. Other conditions that are not suitable for infusion assessed by clinicians.
3. Dosage of infusion: According to the clinical status and the patient's weight. Usually the infusion dose is 200-500ml (4-5ml/kg).
4. Principle of infusion
   - According to the principle of cross-matching of blood, ABO homogenous plasma is preferred.
   - A slow infusion is required at the first 15 minutes in order to pay attention to the occurrence of adverse reactions of blood transfusion.
5. Risk disclosure and sign the informed consent

The retrospective cohort study of the convalescent plasma transfusion

The individual CP therapeutic process and outcomes of the 19 patients treated with CP transfusion were shown in Figure 2A. It can be clearly seen that symptoms in the 19 patients were all improved according to the different shades of color, which signifies the severity of the disease. Four critically ill patients with negative detection of viral nucleic acid (ID: 2, 6, 7, 17) also showed a good response to the CP treatment. By analyzing the titer of neutralizing antibody from the donors and the therapy response of the COVID-19 patients, we found that the plasma from the donors with a higher neutralizing antibody titer had a better treatment response ($p=0.0017$) (Figure 2B). Consistent with previous studies, CP treatment could improve the clinical outcomes through neutralizing viremia and decrease the viral load. According to the latest treatment guidelines, two consecutive negative viral nucleic acid tests can be regarded as the standard of discharge. Our results showed that the viral nucleic acid tests turned negative immediately after the CP treatment in the critically ill patients (ID: 4, 5, 8, 9, 10, 12, 18) and the moderately/severely ill patients with persistently positive detection of viral nucleic acid for more than three weeks (ID: 3, 13, 14, 15, 16, 19) (Figure 2A). All the 19 patients treated with CP transfusion in our study were survived, and showed a significantly lower case-fatality rate compared to the control group (0% vs. 19%, $p=0.031$). The survival curves of the exposure group and control group were shown in Figure 2C.

DISCUSSION

The use of convalescent plasma has a long history. At the end of the 19th century, researchers found that recovery patients' plasma was effective in diphtheria and tetanus patients. Use of convalescent plasma has been studied in outbreaks of other respiratory infections, including the 2003 SARS-CoV-1, 2009-2010 H1N1 influenza virus pandemic and the 2012 MERS-CoV epidemic [6]. Previous studies showed a shorter hospital stay and lower mortality with no adverse events or complications in patients who treated with convalescent plasma treatment than those who were not [7]. Additionally, viral load after convalescent plasma treatment was significantly lower on

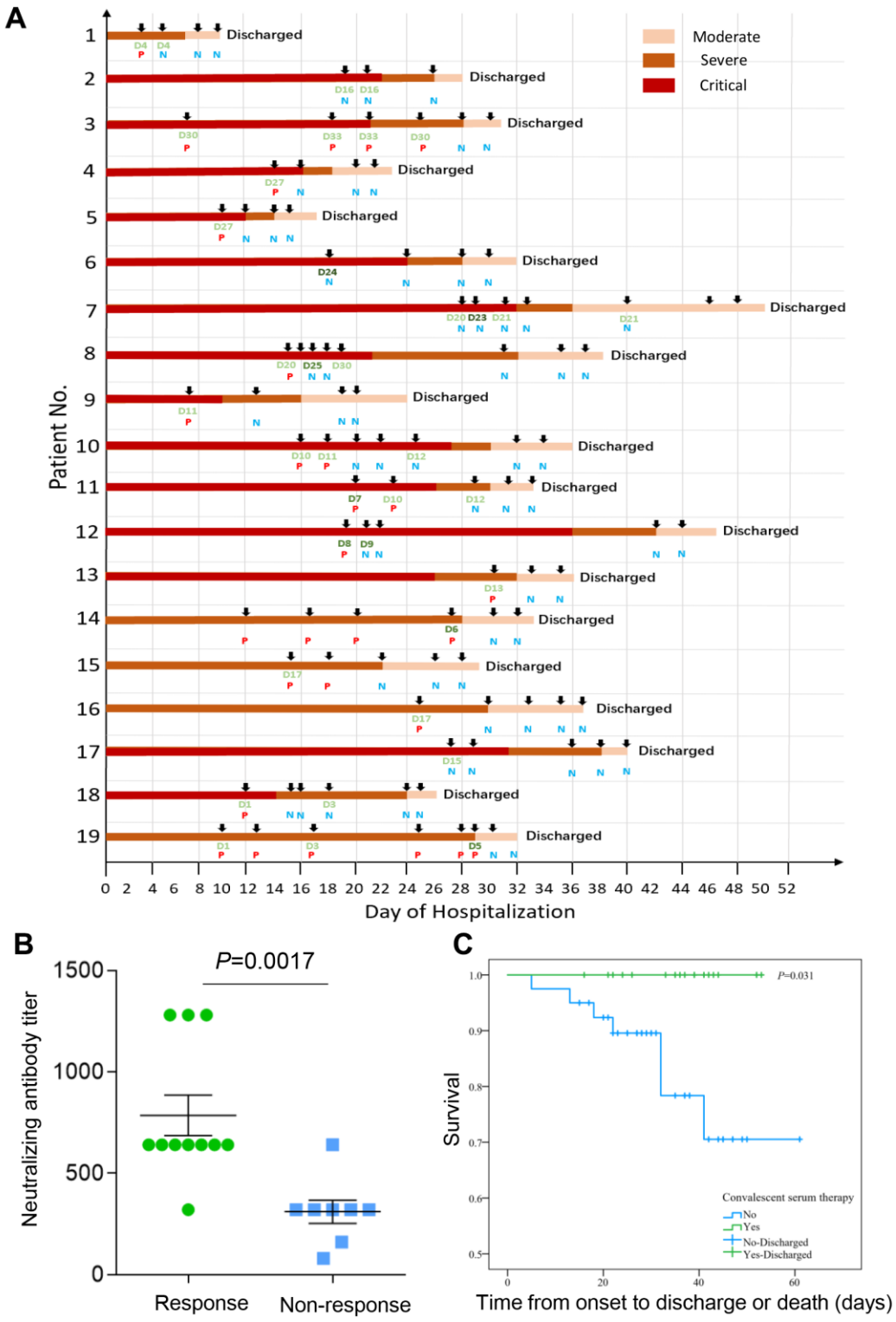

Figure 2. (**A**) Outcomes for individual patients included in 19 cases. Donor and receiver detail information see Supplementary Table 1; P, Nucleic acid test positive; N, Nucleic acid test negative; D, Donor patient (200ml); D, Donor patient (400ml). (**B**) The relationship between titer of neutralizing antibody from the donors and the therapy response of the COVID-19 patients. The plasma from the donors with a higher neutralizing antibody titer had a better treatment response (p=0.0017). The clinical symptoms were significantly improved and viral nucleic acid tests turned negative within five days after CP treatment was defined as "Response", otherwise it was "Non-response". (**C**) The survival curves of the exposure group and control group. All the 19 patients treated with CP transfusion in our study survived, and showed a significantly lower case-fatality rate compared to the control group (0% vs. 19%, *P*=0.031).

days 3, 5, and 7 after intensive care unit admission [8]. What's more, we found that patients with long-term positive nucleic acid test of novel coronavirus turn negative earlier after convalescent plasma treatment than those who without convalescent plasma treatment. Furthermore, asymptomatic patients with hypoimmunity, such as the elderly, children and patients with underlying diseases such as diabetes, hepatitis, AIDS, heart disease, tuberculosis, malignant tumor, etc., preferred to use convalescent plasma once the nucleic acid test was positive.

In our study, 19 COVID-19 patients were treated with CP and recovered quickly from the disease. The clinical symptoms were significantly improved as the individual critical/severe illness condition turned to moderate after CP treatment. The viral shedding was minimized and the clinical conditions of these patients improved as indicated by the viral nucleic acid test. We also found that the CP treatment was more effective in the patients with a higher neutralizing antibody titer transfusion. More importantly, all the patients in exposure group were survived and discharged, suggesting that the CP treatment was associated with a better outcome and a lower fatality.

In the war of fighting against emerging and pandemic COVID-19, the advantages of the convalescent plasma have been validated by the practice and clinical outcome in China. Therefore, it provided an unprecedented opportunity to perform clinical studies and trials of the efficacy of convalescent plasma transfusion during the pandemic of COVID-19. Meanwhile, the convalescent plasma transfusion is worthy of application and promotion in SARS-CoV-2-infected patients globally due to its safety and efficacy.

MATERIALS AND METHODS

A retrospective cohort study was conducted from January 22, 2020 to February 28, 2020. 19 patients with COVID-19 in Hunan Province confirmed by real-time viral RNA test and clinical manifestation were hospitalized and received transfusions from the CP donors. To compare the effectiveness of treatment defined by fatality rate, a control group comprised of 43 COVID-19 patients who did not receive CP treatment was selected from Hunan (23 cases) and Hubei (20 cases) provinces using a stratified random sampling method by age, gender and severity of the disease. Baseline characteristics (age, gender, severity, admission date, time from onset to hospitalization, length of days among survivors and time from hospitalization to death) of patients between the exposure group and control group were shown in Supplementary Tables 2, 3. The study was approved by the ethics committees of Xiangya Hospital, Central South University (approval #202002024) and other local hospitals. Written informed consent was obtained from each patient.

AUTHOR CONTRIBUTIONS

Xi Yuan, Zhimin Zhang collected the data. Shiyao Pei and Mingzhu Yin drafted the manuscript. Run Yao, Yubin Xie, Minxue Shen contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. Bijuan Li, Xiang Chen were involved in data cleaning, and verification. All authors have read and approved the final manuscript.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Tables

Supplementary Table 1. Detection of novel coronavirus antibody: Colloidal gold label technology and ELISA.

Sample No.	Colloidal gold label technology		ELISA	
	IgG	IgM	IgG	IgM
1	+	-	640	1280
2	+	-	80	640
3	+	-	210	1280
4	±	-	80	320
5	+	-	160	2560
6	+	-	160	2560
7	±	-	80	-
8	+	-	80	2560
9	-	-	40	320
10	+	-	320	-
11	+	-	80	-
12	±	-	40	-
13	+	-	160	650
14	+	-	160	2560
15	+	-	160	1280
16	±	-	40	-
17	+	±	640	2560
Positive rate	94.1%	5.9%	100%	70.6%

Coincidence Rate: IgG 94.12%.
IgM 29.41%.

Dilution Ratio: Colloidal gold 1:8.
ELISA 1:10.

Supplementary Table 2. Characteristics of the patients of exposure group and control group.

Characteristics	Exposure group	Control group		*P*
	Hunan (N=19)	Hunan (N=23)	Hubei (N=20)	
Age (age), mean±SD	66.3±15.3	57.3±15.0	69.1±14.3	0.030
Sex, n (%)				
Male	11 (57.9)	13 (56.5)	12 (60.0)	0.964
Female	8 (42.1)	10 (35.1)	8 (40.0)	
Severity, n (%)				
Mild-to-moderate	0 (0.0)	0 (0.0)	0 (0.0)	0.053
Severe	6 (31.6)	1 (4.3)	6 (30.0)	
Critical	13 (68.4)	22 (95.7)	14 (70.0)	
Admission date				
First case	22 Jan 2020	20 Jan 2020	3 Feb 2020	
Last case	28 Feb 2020	6 Feb 2020	24 Feb 2020	
Time from onset to hospitalization (days), median (IQR)	4.5 (3.0–7.7)	5.0 (3.0–8.0)	13.0 (8.5–15.0)	<0.001
Length of stay (days) among survivors, median (IQR)	32.5 (24.5–37.7)	20.0 (17.0–21.0)	29.0 (26.0–31.3)	<0.001
Time from hospitalization to death (days), median (IQR)	N/A	10.0 (3.3–22.7)	22.0 (19.0–22.0)	0.157

Supplementary Table 3. The demographic characteristics of the donors.

Donors no.	Blood center	Gender	Age	Donated plasma volume, ml	Blood type	IgM/IgG	Neutralizing antibody titer
D1	Department of Blood Transfusion Laboratory of Changsha Blood Center	Male	40	400	A	Negative/ Positive	80
D3	Department of Blood Transfusion Laboratory of Changsha Blood Center	Male	41	400	A	Positive/ Positive	320
D4	Department of Blood Transfusion Laboratory of Changsha Blood Center	Male	30	400	B	Negative/ Positive	1280
D5	Department of Blood Transfusion Laboratory of Changsha Blood Center	Male	29	400	A	Positive/ Positive	640
D6	Department of Blood Transfusion Laboratory of Changsha Blood Center	Male	38	400	A	Positive/ Positive	1280
D7	Department of Blood Transfusion Laboratory of Zhuzhou Blood Center	Male	47	400	A	Positive/ Positive	320
D8	Department of Blood Transfusion Laboratory of Zhuzhou Blood Center	Male	30	400	A	Positive/ Positive	640
D9	Department of Blood Transfusion Laboratory of Zhuzhou Blood Center	Female	26	400	O	Positive/ Positive	160
D10	Department of Blood Transfusion Laboratory of Zhuzhou Blood Center	Female	41	400	A	Did not detected	Did not detected
D11	Department of Blood Transfusion Laboratory of	Female	44	400	A	Negative/ Positive	640

	Zhuzhou Blood Center						
D12	Department of Blood Transfusion Laboratory of Zhuzhou Blood Center	Female	23	400	AB	Negative/ Positive	640
D13	Department of Blood Transfusion Laboratory of Zhuzhou Blood Center	Male	31	400	AB	Positive/ Positive	320
D15	Department of Blood Transfusion Laboratory of Yueyang Blood Center	Female	49	300	B	Positive/ Positive	640
D16	Department of Blood Transfusion Laboratory of Yueyang Blood Center	Male	43	400	O	Positive/ Positive	640
D17	Department of Blood Transfusion Laboratory of Yueyang Blood Center	Male	22	400	O	Positive/ Positive	640
D20	Department of Blood Transfusion Laboratory of Loudi Blood Center	Female	36	400	O	Positive/ Positive	1280
D21	Department of Blood Transfusion Laboratory of Loudi Blood Center	Female	38	400	A	Weekly Positive/ Positive	320
D23	Department of Blood Transfusion Laboratory of Loudi Blood Center	Male	41	400	O	Weekly Positive/ Weekly Positive	80
D24	Department of Blood Transfusion Laboratory of Loudi Blood Center	Male	42	400	A	Positive/ Positive	320
D25	Department of Blood Transfusion Laboratory of Loudi Blood Center	Male	40	400	B	Weekly Positive/ Positive	320
D27	Department of Blood Transfusion Laboratory of Xiangtan Blood Center	Male	47	400	O	Positive/ Positive	640
D30	Department of Blood Transfusion Laboratory of Shaoyang Blood Center	Male	24	300	A	Did not detected	Did not detected
D33	Department of Blood Transfusion Laboratory of Shaoyang Blood Center	Female	22	200	A	Negative/ Positive	320