Effect of Convalescent Plasma Therapy on Viral Shedding and Survival in COVID-19 Patients

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Summary (38 words):

Six COVID-19 subjects with respiratory failure received convalescent plasma at a median of 21.5 days after first detection of viral shedding, all subjects tested negative for SARS-CoV-2 RNA by 3 days after infusion, and 5 subjects died eventually.

Abstract

Currently, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19) has been reported in almost all countries globally, and no effective therapy has been documented for COVID-19 and the role of convalescent plasma therapy is unknown. In current study, 6 COVID-19 subjects with respiratory failure received convalescent plasma at a median of 21.5 days after first detection of viral shedding, all tested negative for SARS-CoV-2 RNA by 3 days after infusion, and 5 died eventually. In conclusion, convalescent plasma treatment can discontinue SARS-CoV-2 shedding but cannot reduce mortality in critically end-stage COVID-19 patients, and treatment should be initiated earlier.

Keywords: Convalescent plasma therapy; Coronavirus disease 2019 (COVID-19); Fatality; Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Survival rate; Viral shedding

BACKGROUND

From December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) has spread from Wuhan to worldwide currently [1, 2]. As of April 23, 2020, a total of 2544792 COVID-19 patients has been reported and 2544792 of them died globally, i.e., the mortality rate is as high as 6.9% [3]. However, no specific antiviral therapy is recommended [1], which enhances the difficulty for the pandemic containment and leads to empirical treatment for COVID-19 patients [4, 5]. Additionally, recent study demonstrated that lopinavir/ritonavir had no benefit for COVID-19 compared with supportive treatment [6].

Previous studies showed that the use of convalescent plasma collected from recovered SARS individuals can shorten the hospital stay and decrease the mortality for SARS patients [7-9]. Additionally, clinical benefits by transfusions of corresponding convalescent plasma were also observed in patients infected with Ebola virus, Middle East respiratory syndrome coronavirus, and influenza A H1N1 [9, 10]. However, no related data are available for COVID-19. In current study, we retrospectively collected the clinical data and analyzed the efficacy of the contemporaneous patients who received and not received convalescent plasma collected from recovered COVID-19 individuals.

METHODS

Design and Study Population

This retrospective, observational study was performed mainly in The First Affiliated Hospital of Zhengzhou University and The Sixth People's Hospital of Zhengzhou City, which are the highest referral hospitals for COVID-19 in Henan Province. Laboratory confirmed COVID-19 was diagnosed according to World Health Organization interim guidance [11]. Real-time reverse transcriptase polymerase chain reaction (PCR) tests for SARS-CoV-2 RNA were performed using nasopharyngeal swabs (Novel Coronavirus [2019-nCoV] Nucleic Acid Detection Kit [PCR Fluorescence Probing], Shanghai BioGerm Medical Biotechnology).

We extracted the epidemiological, demographic, clinical, laboratory, management, and outcome data of the contemporaneous COVID-19 patients who received and not received convalescent plasma. Clinical outcomes were followed up until April 1, 2020. The primary endpoint of current study was the fatality or recovery (discharge), and the secondary endpoint was SARS-CoV-2 RNA clearance. The criteria of recovery were the same as described by Lan et al [12]. Shortly, it is based on the recovery of symptoms and signs, consistent clearance of SARS-CoV-2, and absorption of lung inflammations (such as ground-glass opacities and/or consolidations).

The performance of the SARS-CoV-2 RNA Detection Kit

The technical performances of aforementioned detection kit are as following. (1) Limit of detection: 1×10^3 copies/ml. (2) Repeatability: A reference sample with the same precision was tested in five days by two persons with three batches of reagents, and the coefficient of variation of the intra-batch and inter-batch precision, intra-day and inter-day precision, intra-operator and inter-operator precision, and intra-laboratory and inter-laboratory precision is no more than 5.0% in all instances. (3) Positive/negative reference compliance rate within the company: The compliance rate is 100% for five positive reference samples and 12 negative reference samples. (4) Specificity: There is no cross-reaction with the pathogens like influenza A virus H1N1, H1N1 (2009), H3N2, H5N1 and H7N9; Influenza B virus (BV and BY); human coronavirus 229E/ HKU1/OC43/NL63/SARS/MERS; parainfluenza virus (types 1, 2, and 3); rhinovirus A/B /C; boca virus; respiratory syncytial virus; Epstein-Barr virus; measles virus; human cytomegalovirus; human cytomegalovirus; rotavirus; norovirus; varicella-zoster virus; mumps virus; chlamydia pneumoniae; chlamydia pneumoniae; legionella; pertussis; haemophilus influenzae; staphylococcus aureus; streptococcus pneumoniae; mycobacterium tuberculosis; streptococcus pyogenes; klebsiella pneumoniae; aspergillus fumigatus; candida albicans; candida glabrata; cryptococcus neoformans; and adenovirus 1, 2, 3, 4, 5, 7 and 55. Additionally, the reaction is not interfered by addition of the endogenous and exogenous interference substances. (5) The clinical evaluation is compared with the confirmed/excluded results obtained with

the recommended methods of “Technical Guidelines for Laboratory Testing of Pneumonia Infected with a Novel Coronavirus” and “Surveillance of Pneumonia Cases with Novel Coronavirus Infection”. The actual usage data in clinical were collected from Beijing Centers for Disease Control and Prevention, Guangdong Provincial Center for Disease Control and Prevention and other five institutions for statistical analysis. Upon preliminary evaluation, it has been basically confirmed that the performance of this kit can meet the clinical needs of epidemic emergency. The specimen types for clinical evaluation include nasopharyngeal swabs, oropharyngeal swabs and sputum. Because of only positive control and negative control were included in this kit, it was designed only for the qualitative (not quantitative) detection of SARS-CoV-2 RNA, and the results indicate the presence of SARS-CoV-2 RNA and can be used to support the diagnosis of SARS-CoV-2 infection; and it was widely used during the epidemic of COVID-19 in China [13].

Source of Convalescent Plasma

Convalescent plasma was obtained from individuals who had recovered from COVID-19. Young adult individuals who recovered from COVID-19 for one week to two weeks were eligible for further estimations as the blood donors. Additionally, all the donors were negative for the testing of SARS-CoV-2 RNA and IgM, and positive for IgG testing before donations. Informed consent was obtained from all donors who should be seronegative for hepatitis B and C, human immunodeficiency virus, and syphilis. From the mid-February, 2020, first recovered COVID-19 individual has donated the blood sample. A 200 to 400 ml plasma sample was harvested from each donor, the blood collection and storage process were performed in Henan (Provincial) Red Cross Blood Center. Gold immnnochromatography for SARS-CoV-2 IgM and IgG tests were performed using blood sample (New Coronavirus [2019-nCoV] Antibody Detection Kit, Shanghai Outdo Biotech and Tangshan Innovita Biotech).

Statistical Analysis

Continuous and categorical variables were presented as median (interquartile range) and n (%), respectively. Mann-Whitney U test, Chi-square test, or Fisher's exact test were used to compare the differences between various subgroups where appropriate. Analyses were carried out using SPSS statistical software, version 25.0 (IBM, Chicago, IL, USA). A p value of < 0.05 was set as the threshold for statistical significance.

Ethical Aspects

The protocol of this retrospective study was approved by the Institutional Review Board of The First Affiliated Hospital of Zhengzhou University, and the written informed consents were obtained from all the family members of patients who received plasma infusions.

RESULTS

Patients Characteristics

A total of 21 contemporaneously critical COVID-19 patients were enrolled in current study (Table 1), all patients required intensive care unit admission. Of them, 6 patients received convalescent plasma treatment based on the limited convalescent plasma availability and ABO compatibility. There are 5 and 11 male patients in convalescent plasma treatment group and non-convalescent plasma treatment (control) group, respectively. The median ages were 61.5 and 73 years respectively. The treatment group and control group had the comparable characteristics, clinical parameters, and management strategies (Table 1).

Safety and Efficacy of Convalescent Plasma Treatment

The median volume of plasma infused was 300 ml (Table 2). No immediate and noticeable adverse effects were observed with convalescent plasma infusions. The mortality in the treatment group was 5/6 and in the control group was 14/15 ($p = 0.184$). Each group had one recovered patient, respectively. All the 6 patients in treatment group obtained viral clearance after convalescent plasma transfusions, and 100% (5/5) and 21.4% (3/14) of fatal patients had undetectable SARS-CoV-2 before death in treatment and control groups, respectively ($p = 0.005$). Additionally, the survival period of treatment group was longer than control group ($p = 0.029$).

First Patient Received Convalescent Plasma Therapy in Henan Province

In Henan Province, the first available convalescent plasma was transfused for a 30 years postpartum woman on February 19, 2020 (Supplementary Figure 1). She was engaged in beef and mutton selling business in Wuhan and came to Henan Province at 29 gestational weeks plus 3 days on January 21, 2020, and initiated the illness with fever and dry cough on January 24. Then, she visited the hospital for testing and management, her SARS-CoV-2 was first detectable from January 28. On February 2, she had the caesarean section because of fetal destress, fortunately, the premature newborn was living and negative for SARS-CoV-2 for one month after birth.

Meantime and unfortunately, her bilateral pneumonia were gradually severe from the onset of illness (Supplementary Figure 2), and respiratory failure was diagnosed and invasive mechanical ventilation was required from February 9, and extracorporeal membrane oxygenation was needed from February 10. On February 19 and 21, she was received 400 ml and 200 ml of convalescent plasma transfusions, respectively. Notably, previously detectable SARS-CoV-2 was undetectable from February 21 to eventual fatality on March 6, 2020.

DISCUSSION

Recent study indicated that SARS-CoV-2 was detectable until death in non-survivors [14], and whether undetectable SARS-CoV-2 before death can regress fatalities is unknown. In current study, to the best of our knowledge, we firstly indicate that the convalescent plasma treatment contributes to the discontinuation of SARS-CoV-2 shedding and longer survival duration in COVID-19 patients with respiratory failure; however, it cannot reduce the mortality in critically end-stage COVID-19 patients.

As the neighbor Province of Hubei and nearest outside Province of Wuhan City, Henan Province was affected by the SARS-CoV-2 infection severely. It is known that Henan Province had the third largest number of COVID-19 patients and second largest number of fatal patients in China [15]. However, in mid-February, 2020, the epidemic was closing to the end, therefore, the enrolled patients were the most critical and last series of COVID-19 cases in Henan Province.

Commonly, viraemia peaks in the first week after infection in most acute viral diseases, and patient usually develops a primary immune response by day 10 to 14, which is followed by virus clearance [8]. In the third week, and clinical deterioration is occurred to be the result of inflammatory or hyperimmune attacks rather than direct viral-induced tissue damage [8, 14, 16]. Hence, convalescent plasma should theoretically be more effective when given in the early course of disease (i.e., before day 14, or during the viremic and seronegative stage) [8]. The failure of reduce the mortality may be attributed to the late transfusion of convalescent plasma, which were given on median day 21.5 during viral shedding. On the contrary, one critical patient in treatment group infused on day 11 during viral shedding was finally recovered.

Based on the current findings, the convalescent plasma treatment should be given to the COVD-19 patients with right phase or severity at the right timepoint. It is known that most mild COVD-19 patients can be self-recovered, and convalescent plasma may be improper therapy for them. And for end-stage COVD-19 patients, the convalescent plasma treatment may unable to regress the poor outcome as demonstrated by current study. Hence, the convalescent plasma treatment should probably

be used in potentially critical COVD-19 patients at their early stage. Thus, the early recognition of the COVD-19 patients who would subsequently and potentially develop to critically illness is the key question before the convalescent plasma treatment.

Our study has several limitations. First, limited number of patients. It is important to note that the COVID-19 outbreak was nearly finished outside Wuhan in China at the convalescent plasma available date, i.e., February 19 in Henan Province. Then, the vast majority of patients were discharged and new cases were few, and these 21 patients were almost all the critically ill cases at that moment. Conversely, mild patients did not need plasma. Second, the amounts of viral antibodies given to each patient was unknown and not standardized, which may lead to different clinical outcomes. Nevertheless, all plasma receiving patients underwent viral clearance after transfusions. Third, it was not randomized. However, the limited plasma availability, timeliness, ABO compatibility resulted in almost only one suitable patient existed at each certain circumstance. Last, the SARS-CoV-2 RNA was not quantitated because of the technic limitation of testing.

In conclusion, the current study firstly suggests that convalescent plasma therapy can discontinue the viral shedding and contribute longer survival duration in COVID-19 patients with respiratory failure, although it cannot reduce the mortality in critically end-stage patients. Additionally, we suggest that convalescent plasma treatment should be infused for potentially critical COVD-19 patients at their early phase based on the current study. Future large-scale studies are needed to investigate whether early phase infusion of convalescent plasma in proper receiving populations can prevent clinical deterioration and improve survival rate.

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Table 1. Comparison of demographic and clinical characteristics between treatment (convalescent plasma) group and control (non-convalescent plasma) group.

Demographic and clinical features	Treatment (n = 6)	Control (n = 15)	p value
Sex, male	5 (83.3)	11 (73.3)	1.0
Age, years	61.5 (31.5-77.8)	73 (60-79)	0.381
Chronic comorbidity			
Diabetes	1 (16.7)	5 (33.3)	0.623
Hypertension	1 (16.7)	3 (20)	1.0
Chronic liver diseases	0 (0)	2 (13.3)	1.0
Cardiovascular diseases	1 (16.7)	0 (0)	0.286
Respiratory system diseases	0 (0)	1 (6.7)	1.0
Chronic kidney disease	0 (0)	1 (6.7)	1.0
Main signs and symptoms			
Fever	5 (83.3)	13 (86.7)	1.0
Cough	5 (83.3)	14 (93.3)	0.5
Fatigue	4 (66.7)	10 (66.7)	1.0
Shortness of breath	4 (66.7)	12 (80)	0.598
Dyspnoea	3 (50)	8 (53.3)	1.0
Chest CT findings			
Bilateral pneumonia	6 (100)	14 (93.3)	1.0
Multiple mottling/ground-glass opacity	5 (83.3)	14 (93.3)	0.5
Laboratory parameters upon ICU admission			
Leucocytes ($\times 10^9$ /L; NR 3.5-9.5)	6.9 (4.7-15.8)	6.6 (5-11.3)	0.733
Neutrophils ($\times 10^9$ /L; NR 1.8-6.3)	5.3 (3-14.6)	5.6 (4.9-10.4)	1.0
Lymphocytes ($\times 10^9$ /L; NR 1.1-3.2)	1.2 (0.9-1.5)	0.9 (0.6-1.3)	0.34
C-reactive protein (mg/L; NR 0-5)	46.7 (8.5-111)	66 (24.5-94)	0.47
Procalcitonin (ng/mL; NR 0-0.046)	0.09 (0.07-0.12)	0.17 (0.08-0.47)	0.154

Alanine aminotransferase (U/L; NR 0-40)	41.5 (18.5-67.5)	30 (21-54)	0.91
Creatine kinase (μmol/L; NR 20-115)	192 (49-389)	97 (60-237)	0.733
Lactate dehydrogenase (U/L; NR 75-250)	423 (268-510)	331 (254-469)	0.622
D-dimer (mg/L; NR 0-0.55)	1.7 (0.2-4.26)	2.6 (0.33-3.9)	0.677
Main complications			
Respiratory failure	6 (100)	15 (100)	1.0
Acute respiratory distress syndrome	5 (83.3)	13 (86.7)	1.0
Secondary infection	4 (66.7)	12 (80)	0.598
Septic shock	3 (50)	8 (53.3)	1.0
Management			
Intensive care unit admission	6 (100)	15 (100)	1.0
Antibiotics	6 (100)	15 (100)	1.0
Antiviral therapy	4 (66.7)	12 (80)	0.598
Traditional Chinese medicine	3 (50)	8 (53.3)	1.0
Intravenous immunoglobulin therapy	5 (83.3)	14 (93.3)	0.5
Glucocorticoid pulse therapy	4 (66.7)	12 (80)	0.598
High-flow nasal cannula oxygen therapy	6 (100)	15 (100)	1.0
Mechanical ventilation	5 (83.3)	13 (86.6)	1.0
Extracorporeal membrane oxygenation	4 (66.7)	12 (80)	0.598
Continuous renal replacement therapy	3 (33.3)	10 (66.7)	0.631

Data are presented as median (IQR) or n (%). Abbreviation: NR, normal range.

Table 2. Safety and efficacy of treatment (convalescent plasma) group and control (non-convalescent plasma) group.

Parameters	Treatment (n = 6)	Control (n = 15)	p value
Duration of viral shedding, days	23.5 (19.5-24.5)	20 (19-24)[†]	0.381
Duration of illness[‡], days	45.5 (37.8-59)	31 (30-36)	0.029
Duration of viral shedding before treatment, days	21.5 (17.8-23)	-	-
Convalescent plasma volume, ml	300 (200-600)	-	-
Two times of convalescent plasma therapies	3 (50)	-	-
One time of convalescent plasma therapy	3 (50)	-	-
SARS-CoV-2 clearance after treatment	6 (100)[§]	-	-
Adverse events of convalescent plasma therapy			
Anaphylaxis	0 (0)	-	-
Fever	0 (0)	-	-
Clinical outcomes			
Remained in hospital	0 (0)	0 (0)	-
Discharge	1 (16.7)	1 (6.7)	-

Fatality	5 (83.3)	14 (93.3)	0.500
SARS-CoV-2 clearance in all patients			
Yes	6 (100)	4 (26.7)	0.004
No	0 (0)	11 (73.3)	-
SARS-CoV-2 clearance before death in fatal patients			
Yes	5/5 (100)	3/14 (21.4)	0.005
No	0/5 (0)	11/14 (78.6)	-

Data are presented as median (IQR), n (%) or n/N (%), where N is the total number of cases with available data. [†]Data were censored in 11 fatal patients who had detectable SARS-CoV-2 RNA till death. [‡]Duration of illness was calculated from the onset of illness to the date of discharge or fatality. [§]Four SARS-CoV-2 clearance occurred on the second day of transfusion, one occurred on the same day of transfusion, one occurred on the third day of transfusion. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.