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NEWS

Covid-19: FDA approves use of convalescent plasma to treat critically ill patients

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The US Food and Drug Administration has approved the use of plasma from recovered patients to treat people who are critically ill with covid-19, provided that doctors get approval over the telephone.[1]

The method has been used in the past to treat diseases such as polio, measles, and mumps, in the 1918 flu epidemic, and in previous outbreaks of respiratory infections similar to covid-19.

The FDA's decision came a day after the New York state governor, Andrew Cuomo, said that the state's health department would begin to treat critically ill patients with convalescent plasma. New York officials said they would recruit patients who have recovered from covid-19, probably from the New York City suburb where the state's outbreak began, NBC News reported.[2]

Cuomo said it was a trial to treat people in a serious condition. He said the state's health department had been working on it, and "We think it shows promise, and we're going to be starting that this week."[2]

Plasma from people who have recovered from covid-19 may contain antibodies to the virus that causes the disease and might be effective against the infection, the FDA said. Convalescent plasma has been studied in outbreaks of other respiratory infections, such as H1N1 influenza, SARS, and MERS. "Although promising, convalescent plasma has not been shown to be effective in every disease studied" and therefore clinical trials were needed to see if it was useful in covid-19, the FDA cautioned.

The FDA told doctors wanting to study the use of convalescent plasma to follow the usual system for an investigational new drug (IND) application.

The plasma must be collected from recovered patients who can donate blood, have had no symptoms for 14 days, and have had negative results on covid-19 tests.

However, given the current public health emergency, the FDA said it was providing emergency access to convalescent plasma for patients "with serious or immediately life threatening covid-19 infections."

Severe disease is defined as dyspnoea, respiratory frequency ≥30 breaths per minute, blood oxygen saturation ≤93%, ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300, or lung infiltrates >50% within 24 to 48 hours.

Life threatening disease is defined as respiratory failure, septic shock, or multiple organ dysfunction or failure.

In such cases, doctors can submit a form online or call FDA's hotline telephone number (1-866-300-4374) to get verbal approval for treatment, which is promised within four to eight hours.

Jeffrey Henderson of Washington University School of Medicine in St Louis, Missouri, told National Public Radio, "The FDA just opened the floodgates. Our institution is scrambling to be ready to use this. There are many others, I'm sure."[3]

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Treatment with convalescent plasma for critically ill patients with SARS-CoV-2 infection

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Treatment with convalescent plasma for critically ill patients with SARS-CoV-2 infection

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Abstract

As of March 24, 2020, novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for 379,661 infection cases with 16,428 deaths globally and the number is still increasing rapidly. Herein, we presented four critically ill patients with SARS-CoV-2 infection who received supportive care and convalescent plasma. Although all the four patients (including a pregnant woman) recovered from SARS-CoV-2 infection eventually, randomized trials are needed to eliminate the effect of other treatments and investigate the safety and efficacy of convalescent plasma therapy.

Keywords: SARS-CoV-2; Convalescent plasma; Critically illness

Introduction

Since late December 2019, an outbreak of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection first appeared in Wuhan, China (1), and rapidly spread to 171 countries. As of March 24, 2020, the virus has been responsible for 379,661 confirmed cases and 16,428 deaths worldwide. To date, no specific treatment was recommended for SARS-CoV-2 infection except for meticulous supportive care (2). Numerous therapeutics were explored or developed during the outbreak. A recent trial showed lopinavir-ritonavir has no treatment benefit for severe illness caused by SARS-CoV-2 (3). Immunotherapy with virus-specific antibodies in convalescent plasma had been used as a last resort to improve survival rate of patients with serious infectious diseases, such as SARS, middle east respiratory syndrome coronavirus, Ebola virus disease, pandemic influenza A, and avian-origin influenza A (4). Previous reports showed treatment with convalescent plasma collated from recovered patients could reduce the hospital stay and mortality of patients (5). However, the efficacy of convalescent plasma in critically ill patients with SARS-CoV-2 infection remains unclear. Herein, we reported the disease course on four critically ill SARS-CoV-2-infected patients treated with supportive care and convalescent plasma.

Case reports

Figure 1 showed the clinical course of the four critically ill patients infected with SARS-CoV-2. Our first case was a 69-year-old female with a history of hypertension who

presented with fever for two days and clear sputum for four days. On January 30, the patient was admitted to Dongguan Ninth People's Hospital because of positive reverse transcriptase polymerase chain reaction (RT-PCR) test of throat swab by Dongguan Center for Disease Control (CDC). A chest CT revealed bilateral ground-glass opacities primarily distributed along the pleura. Treatment with arbidol (200 mg three times daily), lopinavir-ritonavir (400 mg twice daily), interferon alpha inhalation (50 μg twice daily), and other supportive therapies was started. At 4 p.m. on February 4, the patient's oxygen pressure (pO_2) decreased to 56.5 mmHg with OI of 94 mmHg. Significantly increased consolidation was observed in the right lung. The patient was transferred to the intensive care unit (ICU) of Dongguan People's Hospital (a designated center for critically illness treatment) on February 5 and received invasive mechanical ventilation. Apart from antiviral drugs (lopinavir-ritonavir, oseltamivir, and interferon alpha), human albumin, zadaxin and immunoglobulin, antibacterial and antifungal drugs were administrated because of co-infection with bacteria and aspergillus. At 6:30 p.m. on February 11, the patient's pO_2 was 58 mmHg. She experienced septic shock with blood pressure of 89/44mmHg five hours later. Hypohemoglobin (92 g/L) and bloody sputum under bronchoscopy suggested pneumorrhagia. A bedside chest radiography showed obvious progression of disease. Although the patient was successfully rescued, follow-up chest radiographs showed continuous progression of pneumonia. A total of 900 ml O-compatible convalescent plasma were transfused to the patient in three batches; the first batch was given at 8 a.m. on February 17 (200 ml), the second one was at 8 a.m. on February 27 (400 ml), and the last one was at 8 a.m. on February 28 (300 ml). The virus load of the patient on February 18 was 55×10^5 copies per milliliter, which significantly decreased to 3.9×10^4 copies per milliliter on February 28, and further decreased to 180 copies per milliliter on March 5. The patient was extubated and non-invasion ventilation was given on March 3. Chest CT obtained on February 27, March 6, and March 15 showed persistent absorption of consolidation. The results of two repeat RT-PCR tests of oropharyngeal swabs (with at least one day interval) performed on March 9 and 11 were negative. The patient was discharged on March 13.

Our second case was a 55-year-old male with a history of chronic obstructive pulmonary disease who was admitted to a fever clinic of Xiangtan Central Hospital on February 5, 2020. He had nausea, poor appetite, and a cough with clear sputum for four days. The results of RT-PCR assay of throat swab was positive for SARS-CoV-2 infection. A chest CT obtained on February 6 revealed interlobular septal thickening with honeycombing change in the right upper lung. The patient started to receive antiviral treatment, including arbidol (200 mg three times daily), lopinavir-ritonavir (500 mg twice daily), and interferon alfa-2b (5 million units twice daily). After two days, he complaint of shortness of breath and the pO_2 decreased to 50 mmHg with oxygenation index (OI: pO_2/FiO_2) of 135 mmHg. The patient was thus diagnosed with acute respiratory distress syndrome and began to receive noninvasive mechanical ventilation and oxygen therapy through high-flow nasal cannula alternately. However, the conditions of the patient continued to deteriorate despite treatment with pulsed methylprednisolone. His pO_2 oscillated between 46 and 83 mmHg and the symptoms were not improved. Follow-up chest CT obtained on February 9 to 16 showed interstitial pneumonia extend to both lungs. At 3 p.m. on February 16, 200 ml convalescent plasma obtained from a patient recovered from SARS-CoV-2 infection in January, 2020 was transfused to the patient. No adverse reactions were observed. One day later, his pO_2 increased to 97 mmHg with OI of 198 mmHg. All drugs were discontinued except for methylprednisolone. Chest images obtained on February 17 to 21 showed obvious absorption of interstitial pneumonia. Three repetitive RT-PCR test results were negative from February 20 to 22. The patient recovered and was discharged on February 23. He was asked to continue the quarantine at home for 14 days and receive home oxygen therapy.

Our third case was a 73-year-old male who was admitted to Dongguan Ninth People's Hospital on February 2 because of self-reported dry cough for 4 days. He had a history of hypertension and chronic renal failure. On February 3, this patient was confirmed as being infected with SARS-CoV-2 by a virus RNA detection kit. At 23: 30, the patient developed acute respiratory failure with pO_2 of 53 mmHg and OI of 124 mmHg,

high-flow oxygen through face mask was given. He was then transferred to the isolation wards of ICU of Dongguan People's Hospital for further treatment. A chest radiograph showed bilateral infiltrative shadows. The viral load of the patient was as high as 85 × 10^5 copies per milliliter. The patient was treated with arbidol (200 mg three times daily), lopinavir-ritonavir (400 mg twice daily), oseltamivir (75 mg twice daily), and ribavirin and interferon alpha-2b (5 million units twice daily). On February 5, the patient was given tracheal intubation because of dyspnea and consistent decrease of oxygen saturation. On February 11, continuous renal replacement therapy (CRRT) started to given to the patient. Laboratory tests obtained on February 14 showed significantly increased white cells of 33.93 × 10^9/L and neutrophils of 31.08 × 10^9/L. He was diagnosed with multiple organ failure by clinical examination. On February 15, the patient developed septic shock and his blood pressure decreased to 90/68 mmHg with heart rate of 149 beat/min and respiratory rate of 30 breaths/min. A chest radiography showed bilateral "white lung". At 12:55 p.m. on February 15, the patient started to receive veno-venous extracorporeal membrane oxygenation (V-V ECMO), while the oxygenation index was unstable and the symptoms were not improved. High-throughput DNA sequencing of sputum suggested aspergillus infection. The patient was therefore treated with caspofungin and voriconazole. Eight transfusions of B-compatible convalescent plasma (2400 ml) were given to the patient from February 16 to March 13. On February 21, the patient was confirmed with active pneumorrhagia, cystorrhagia and gastrointestinal bleeding. Antibody testing on February 27 indicated positive anti-SARS-CoV-2 IgG. The viral load was reduced (detailed values were not available). Follow-up chest x-rays showed absorbed infiltrative lesions but pneumothorax. Two repeat RT-PCR test of sputum in deep lung on March 16 and 17 (with at least one day interval) showed negative and the serum IgM level decreased to normal range. On March 22, the patient was transferred to unfenced ICU for further treatment of underlying diseases and multiple organ failure.

Our fourth case was a 31-year-old pregnant woman (35 weeks plus 2 days) who was admitted to Xiaolan People's Hospital of Zhongshan on February 1 because of

pharyngalgia for 4 days and fever (39.3□) and difficulty breathing for half-day. The patient was confirmed as being infected with SARS-CoV-2 by Zhongshan CDC. A chest CT showed opacities in the lower lobe of the left lung. After admission, the patient developed severe acute respiratory distress syndrome, multiple organ dysfunction syndrome, and septic shock. Invasive ventilation and caesarean section were therefore given to the patient. Unfortunately, the newborn died of endouterine asphyxia. After the conditions turned stable, she was transferred to the Second People's Hospital of Zhongshan (a designated hospital for SARS-CoV-2 treatment) at 1:04 a.m. on February 2. Amounts of frothy sputum was observed under bronchofiberscope. Cardiac ultrasound suggested left ventricular enlargement with decreased systolic function. The patient received invasive ventilation and CRRT. Treatment with lopinavir-ritonavir (400 mg twice daily) and ribavirin (500 mg every 12 hours) was started on February 2. Gram-positive bacteria were detected by blood culture and imipenem and vancomycin were given to this patient. A chest radiograph showed increased consolidation and extended opacities. Oxygen saturation oscillated between 85% and 92% with OI of between 60 mmHg and 75 mmHg. At 12 a.m. on February 6, the patient started to receive V-V ECMO (flow rate: 3L/h). Her OI was significantly improved (with a maximum of 200 mmHg). Follow-up chest radiographs showed partial absorption of opacities. Left ventricular systolic function returned to normal. At 11:30 on February 19, a 300 ml transfusion of convalescent plasma were given to this patient. On February 27, CRRT and ECMO were removed. On March 11, trachea cannula was removed and nasal oxygen was given to the patient. On March 6, 8, and 11, anti-SARS-CoV-2 IgM changed from positive to weakly positive to negative, while anti-SARS-CoV-2 IgG was persistently positive. Follow-up chest CT showed near-complete absorption of opacities. The results of two continual RT-PCR tests of broncholveolr lvge fluid on March 11 and 14 were both negative. The patient recovered from SARS-CoV-2 infection and was discharged on March 17.

Discussion

A recent retrospective review of 72,314 SARS-CoV-2-infected cases by the China CDC

showed that 5% of the cases were critically illness characterized by respiratory failure, septic shock, and/or multiple organ dysfunction or failure. Around 48% of SARS-CoV-2-infected patients had comorbid conditions, commonly cardiovascular diseases and diabetes (9). Elderly people with underlying diseases were more likely to have higher Sequential Organ Failure Assessment score and higher risk of death. The treatment of SARS-CoV-2 infection faces compelling challenges. To date, no therapeutics have yet been proven effective for the treatment of critically illness except for supportive care, including treatment with antiviral drugs, corticosteroids, immunoglobulins, and noninvasive or invasive mechanical ventilation. Most critically ill patients infected with SARS-CoV-2 had elevated levels of infection-related biomarkers and inflammatory cytokines, indicating potential bacterial co-infection caused by dysregulated immune system (10). Antibacterial drugs are therefore given to these patients. Management of critical SARS-CoV-2 infection is not different from management of most viral pneumonia causing respiratory failure. The principal feature of patients with critical illness is the development of ARDS. ECMO is recommended by WHO interim guidelines to support eligible patients with ARDS, while the use of which is restricted to specialised centres globally and technology challenges (11). In this study, two patients were treated with ECMO, but the efficacy was mixed. Apart from ARDS, other life-threatening conditions including septic shock and multiple organ dysfunction or failure may occur in a substantial proportion of patients with SARS-CoV-2-related critically illness, the management of which was according to current evidence based guidelines (12). In China, if the current therapeutic strategies are not satisfactory for critically ill patients, physicians might turn to convalescent plasma transfusion based on the Pneumonitis Diagnosis and Treatment Program for SARS-CoV-2 infection (Trial Version 7). Convalescent plasma had been used as a last resort to improve survival rate of patients with SARS-infection. Previous evidences proved that convalescent plasma treatment can significant reduce the relative risk of mortality of patients (13), which might because that antibodies from convalescent plasma might suppress viraemia. The level of SARS-CoV-2 neutralizing antibodies in donor plasma could be important for the

effectiveness of intervention. However, the level of neutralizing antibodies in donor plasma before transfusion cannot be determined. In this study, three patients were tested either virus load or antibodies IgM and IgG. In the first case, SARS-CoV-2 virus load after convalescent plasma transfusion significantly dropped (from 55×10^5 to 3.9×10^4 to 180 copies per milliliter). Among the four patients, the time from the transfusion to negative RT-PCR test results ranged from 3 to 22 days. The third and fourth cases produced anti-SARS-CoV-2 IgG approximately 14 days after convalescent plasma transfusion. Patients who survived critically ill diseases might mount higher antibody responses, which can persist for longer periods as compared with those with non-severe disease (14). The antibody levels, however, were confounded by other treatments, such as antiviral drugs, steroids and intravenous immunoglobulin (15). A recent animal model indicated that antibodies produced from SARS-CoV-2 infection could protect from subsequent exposures (16).

Conclusions

Our results indicated convalescent plasma might be a potential therapy for critically ill patients infected with SARS-CoV-2. We observed no serious adverse reactions associated with the transfusion of convalescent plasma. However, the relative contributions of supportive care, investigational therapies, and patient's immune-response on survival could not be determined. Whether convalescent plasma and/or supportive care provide any clinical benefit is unknown. The safety and efficacy of convalescent plasma transfusion in SARS-CoV-2-infected patients should be studied within the context of a well-designed clinical trial.

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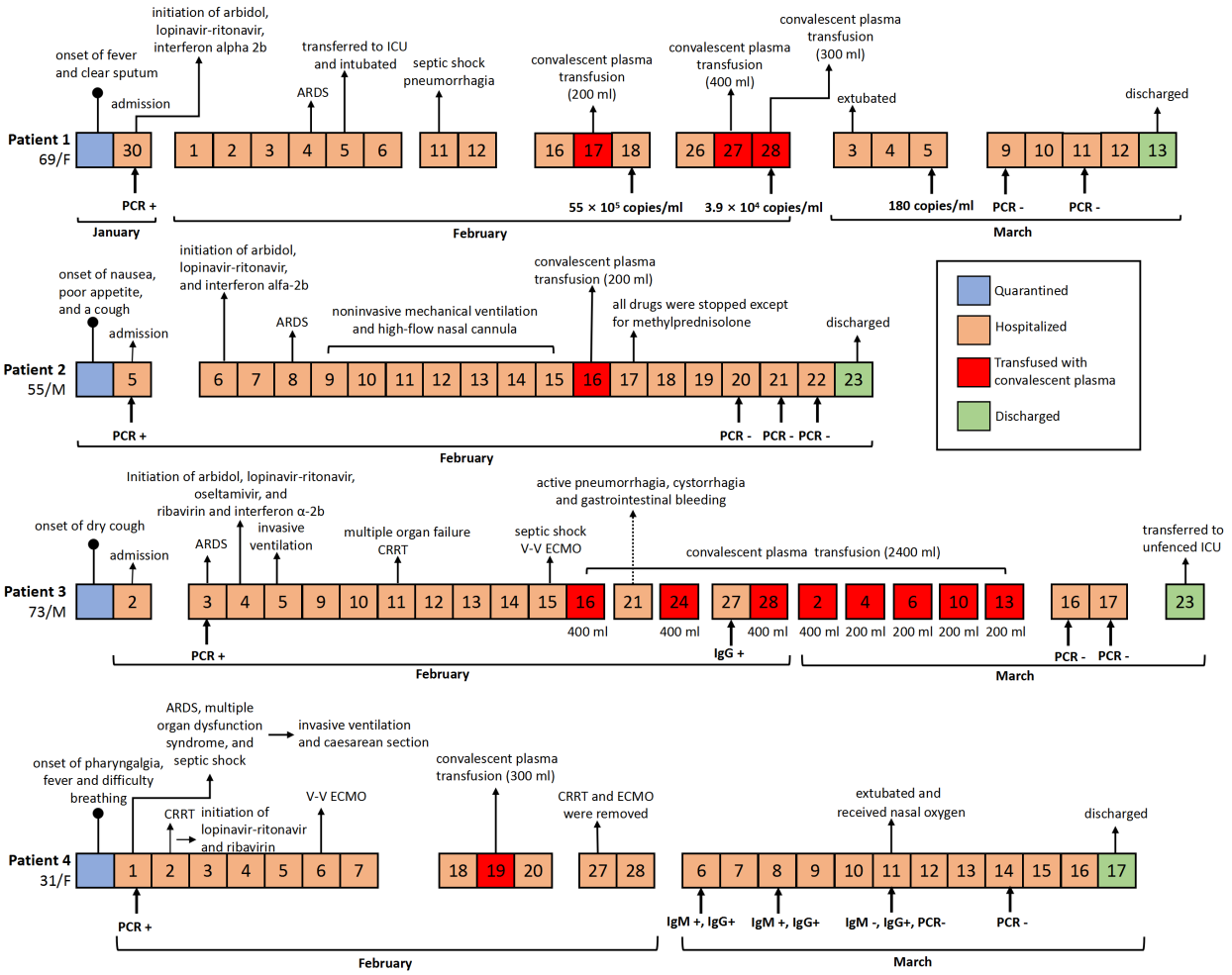
Figure legend

Figure 1: Timeline of symptom onset, RT-PCR testing, antiviral therapies, severe complications, convalescent plasma transfusion, levels of virus load and antibodies after transfusion, and outcomes of the four critically ill patients with SARS-CoV-2 infection. Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, reverse transcriptase polymerase chain reaction; CRRT, continuous renal replacement therapy; V-V ECMO, veno-venous extracorporeal membrane oxygenation; ICU, intensive care unit; ARDS, Acute Respiratory Distress Syndrome.


Patient 1
69/F
onset of fever and clear sputum
admission
initiation of arbidol, lopinavir-ritonavir, interferon alpha 2b
ARDS
transferred to ICU and intubated
septic shock pneumorrhagia
convalescent plasma transfusion (200 ml)
convalescent plasma transfusion (400 ml)
convalescent plasma transfusion (300 ml)
extubated
discharged
PCR +
55 × 10⁵ copies/ml
3.9 × 10⁴ copies/ml
180 copies/ml
PCR -
PCR -
January
February
March
Patient 2
55/M
onset of nausea, poor appetite, and a cough
admission
initiation of arbidol, lopinavir-ritonavir, and interferon alfa-2b
ARDS
noninvasive mechanical ventilation and high-flow nasal cannula
convalescent plasma transfusion (200 ml)
all drugs were stopped except for methylprednisolone
discharged
PCR +
PCR -
PCR -
PCR -
February
Quarantined
Hospitalized
Transfused with convalescent plasma
Discharged
Patient 3
73/M
onset of dry cough
admission
Initiation of arbidol, lopinavir-ritonavir, oseltamivir, and ribavirin and interferon α-2b
ARDS
invasive ventilation
multiple organ failure
CRRT
septic shock
V-V ECMO
active pneumorrhagia, cystorrhagia and gastrointestinal bleeding
convalescent plasma transfusion (2400 ml)
transferred to unfenced ICU
400 ml
400 ml
400 ml
400 ml
200 ml
200 ml
200 ml
200 ml
PCR +
IgG +
PCR -
PCR -
February
March
Patient 4
31/F
onset of pharyngalgia, fever and difficulty breathing
ARDS, multiple organ dysfunction syndrome, and septic shock
invasive ventilation and caesarean section
CRRT
initiation of lopinavir-ritonavir and ribavirin
V-V ECMO
convalescent plasma transfusion (300 ml)
CRRT and ECMO were removed
extubated and received nasal oxygen
discharged
PCR +
IgM +, IgG+
IgM +, IgG+
IgM -, IgG+, PCR-
PCR -
February
March

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Commentary

Could Intravenous Immunoglobulin Collected from Recovered Coronavirus Patients Protect against COVID-19 and Strengthen the Immune System of New Patients?

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Abstract: The emergence of the novel coronavirus in Wuhan, China, which causes severe respiratory tract infections in humans (COVID-19), has become a global health concern. Most coronaviruses infect animals but can evolve into strains that cross the species barrier and infect humans. At the present, there is no single specific vaccine or efficient antiviral therapy against COVID-19. Recently, we showed that intravenous immunoglobulin (IVIg) treatment reduces inflammation of intestinal epithelial cells and eliminates overgrowth of the opportunistic human fungal pathogen *Candida albicans* in the murine gut. Immunotherapy with IVIg could be employed to neutralize COVID-19. However, the efficacy of IVIg would be better if the immune IgG antibodies were collected from patients who have recovered from COVID-19 in the same city, or the surrounding area, in order to increase the chance of neutralizing the virus. These immune IgG antibodies will be specific against COVID-19 by boosting the immune response in newly infected patients. Different procedures may be used to remove or inactivate any possible pathogens from the plasma of recovered coronavirus patient derived immune IgG, including solvent/detergent, 60 °C heat-treatment, and nanofiltration. Overall, immunotherapy with immune IgG antibodies combined with antiviral drugs may be an alternative treatment against COVID-19 until stronger options such as vaccines are available.

Keywords: coronavirus; IVIg; immunotherapy; nCoV-2019; virus

The emergence of the novel coronavirus in Wuhan, China, which causes severe respiratory tract infections in humans (COVID-19), has become a global health concern. Most coronaviruses infect animals but can evolve into strains that can also infect humans. Recently, we showed that intravenous immunoglobulin (IVIg) treatment reduces inflammation of intestinal epithelial cells and eliminates overgrowth of the opportunistic human fungal pathogen *Candida albicans* in the murine gut in association with downregulation of proinflammatory mediators combined with upregulation of anti-inflammatory cytokines [1].

Coronaviruses are enveloped positive-stranded RNA viruses belonging to the family Coronaviridae [2]. An envelope-anchored spike protein promotes coronavirus entry into host cells by first binding to a host receptor and then fusing viral and host membranes [2]. Whole-genome sequencing of viral RNA has revealed that the virus causing COVID-19 is phylogenetically related to the SARS-related coronaviruses first isolated in Chinese horseshoe bats during 2015-2017 [3,4]. Researchers in Guangzhou, China, have recently suggested that pangolins are the probable animal source of the COVID-19 outbreak [5]. In terms of the interaction between the virus and its host, Lu et al.

have reported that angiotensin-converting enzyme 2 (ACE 2) is most probably used by the spike protein of the COVID-19 virus as a receptor similar to that SARS-CoV [6].

Recently, Tang et al. showed that the COVID-19 has evolved into two major lineages—dubbed 'L' and 'S' types. The older 'S-type' appears to be milder and less infectious, while the 'L-type', which emerged later, spreads quickly and is currently more aggressive than the S-type [7]. Current symptoms reported for patients with COVID-19 have included mild to severe respiratory illness with fever, fatigue, cough, myalgia, and difficulty breathing [8]. Tyrrell et al. showed that infected respiratory epithelial cells by coronavirus become vacuolated and show damaged cilia that lead to production of inflammatory mediators, which increase nasal secretion and cause local inflammation and swelling [9]. These responses in turn stimulate sneezing, obstruct the airway, and raise the temperature of the mucosa [9].

Currently, there is no single specific vaccine or effective antiviral therapy against COVID-19. Several pharmaceutical and biotechnological companies are working on vaccine development and estimate that this vaccine will take years to develop and test before it can reach a large population. Additionally, there are currently no approved treatments for any coronavirus disease, including COVID-19. Several antiviral drugs are being tested, and initial findings are expected soon. Individuals with weakened immune systems appear to be at greater risk of developing complications associated with COVID-19. Immunotherapy using IgG in combination with antiviral drugs could be used to treat or prevent COVID-19 and to strengthen our immune response against this virus [10,11]. IgG antibodies include two functional portions: the $F(ab')_2$ fragment, which is responsible for antigen recognition, and the crystallizable fragment (Fc), which is important for activation of the immune response by interacting with Fcγ receptors on B-cells and other innate immune cells [12]. The Fc fragment also plays an important role in the activation of complement and in the clearance of microorganisms [12].

IVIg is a pool of IgG from thousands of healthy donors, and exposure of individual donors to endemic infectious diseases, vaccines, and ubiquitous microorganisms participates in the production of IgG antibodies against different microorganisms and their products [13–15].

IVIg has been used to treat patients with autoimmune and chronic inflammatory diseases, such as dermatomyositis, Kawasaki disease, multiple sclerosis, lupus, chronic lymphocytic leukemia, and idiopathic thrombocytopenic purpura [16–18]. Furthermore, IVIg has also been used as an anti-infectious agent against viruses, bacteria, and fungi in human patients and experimental models [13,19–21]. IVIg treatment may result in some adverse events, which are associated with specific immunoglobulin preparations and individual differences, but many clinical and experimental studies show that switching from IVIg to subcutaneous immunoglobulin can minimize these adverse events [22–24].

IVIg plays an important role in the prevention of infectious episodes in primary immunodeficient patients, and the beneficial effects of these antibodies in the treatment of infectious diseases goes beyond simple neutralization of microorganisms or their toxins. Anti-inflammatory pathways are also critical for protection against infection [25].

IVIg may modulate the immune response via multiple mechanisms, including blocking a wide array of proinflammatory cytokines, Fc-gamma receptors (FcγRs), and leukocyte adhesion molecules, suppressing pathogenic Th1 and Th17 subsets, and neutralizing pathogenic autoantibodies [26–28]. IVIg can also expand regulatory T-cells by induction of cyclo-oxygenase-2-dependent prostaglandin E2 production in dendritic cells [29].

In our study, IVIg treatment reduced intestinal inflammation and decreased *Escherichia coli*, *Enterococcus faecalis*, and *C. albicans* populations in the gut of mice [1]. Overgrowth of *E. coli* and *E. faecalis* populations is known to be involved in dysbiosis of the gut microbiota in inflammatory bowel diseases (IBDs), which are chronic inflammatory conditions of the gastrointestinal tract [30,31]. We also showed that the beneficial effects of IVIg were associated with suppression of inflammatory cytokine IL-6 and enhancement of anti-inflammatory cytokine IL-10 in the gut [1]. Additionally, IVIg

therapy also led to increased expression of PPARγ, a ligand-activated transcription factor that mediates anti-inflammatory functions and resolution of inflammation, while TLR-4 expression, which mediates the inflammatory response, was reduced.

In general, sera from virtually all healthy adults contain anti-coronavirus antibodies [32]. Pyrc et al. showed that human sera from healthy adults inhibited HCoV-NL63 infection [10]. Additionally, they reported that IVIg can also neutralize HCoV-NL63 [10]. Boukhvalova et al. showed that, in contrast to commercially available polyclonal therapeutic IgG products, IVIg obtained from donors with high-titer antibodies against respiratory syncytial virus (RSV) have great potential to improve the outcome of RSV infection in immunocompromised subjects, not only by controlling viral replication but also by reducing damage to the lung parenchyma and epithelial airway lining [33,34].

Currently, all efforts to prevent the spread of COVID-19 so far have been inadequate. Immunotherapy with IgG can be employed to neutralize the virus causing COVID-19. The efficiency of IgG would be better if these immune IgG antibodies were collected from patients recovered from COVID-19 in the same city, or the surrounding area, as these donor subjects have naturally been confronted with the virus.

Immune IgG collected in Europe or the USA may be different from that collected in China as lifestyle, diet, and the environment play an important role in the development of specific antibodies against the virus. Recently, researchers at the Sacco University Hospital in Milan, Italy, have announced that they have isolated a new strain of coronavirus from an Italian patient that showed genetic differences when compared to the original strain isolated in China.

The idea is to treat infected patients with immune IgG collected from the same city in order to increase the chance of neutralizing the virus. Different procedures may be used to remove or inactivate any possible pathogens from the plasma of recovered coronavirus patient derived immune IgG, including solvent/detergent, 60 °C heat treatment, and nanofiltration (20 nm) [35–38]. Terpstra et al. showed that a 15 nm filtration step, combined with pepsin, and solvent-detergent treatment contribute to virus-elimination from liquid intravenous immunoglobulin [38].

Overall, immunotherapy with immune IgG combined with antiviral drugs could provide alternative treatment against COVID-19. These immune IgG antibodies collected from the recovered patients will be specific against COVID-19 by boosting the immune response in newly infected patients. Although a vaccine for COVID-19 is currently not available, the combination of the immune IgG antibodies with antiviral drugs can offer short-term and medium-term solutions against COVID-19.

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JAMA | Preliminary Communication

Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma

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IMPORTANCE Coronavirus disease 2019 (COVID-19) is a pandemic with no specific therapeutic agents and substantial mortality. It is critical to find new treatments.

OBJECTIVE To determine whether convalescent plasma transfusion may be beneficial in the treatment of critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

DESIGN, SETTING, AND PARTICIPANTS Case series of 5 critically ill patients with laboratory-confirmed COVID-19 and acute respiratory distress syndrome (ARDS) who met the following criteria: severe pneumonia with rapid progression and continuously high viral load despite antiviral treatment; PaO_2/FiO_2 <300; and mechanical ventilation. All 5 were treated with convalescent plasma transfusion. The study was conducted at the infectious disease department, Shenzhen Third People's Hospital in Shenzhen, China, from January 20, 2020, to March 25, 2020; final date of follow-up was March 25, 2020. Clinical outcomes were compared before and after convalescent plasma transfusion.

EXPOSURES Patients received transfusion with convalescent plasma with a SARS-CoV-2–specific antibody (IgG) binding titer greater than 1:1000 (end point dilution titer, by enzyme-linked immunosorbent assay [ELISA]) and a neutralization titer greater than 40 (end point dilution titer) that had been obtained from 5 patients who recovered from COVID-19. Convalescent plasma was administered between 10 and 22 days after admission.

MAIN OUTCOMES AND MEASURES Changes of body temperature, Sequential Organ Failure Assessment (SOFA) score (range 0-24, with higher scores indicating more severe illness), PaO_2/FiO_2, viral load, serum antibody titer, routine blood biochemical index, ARDS, and ventilatory and extracorporeal membrane oxygenation (ECMO) supports before and after convalescent plasma transfusion.

RESULTS All 5 patients (age range, 36-65 years; 2 women) were receiving mechanical ventilation at the time of treatment and all had received antiviral agents and methylprednisolone. Following plasma transfusion, body temperature normalized within 3 days in 4 of 5 patients, the SOFA score decreased, and PaO_2/FiO_2 increased within 12 days (range, 172-276 before and 284-366 after). Viral loads also decreased and became negative within 12 days after the transfusion, and SARS-CoV-2–specific ELISA and neutralizing antibody titers increased following the transfusion (range, 40-60 before and 80-320 on day 7). ARDS resolved in 4 patients at 12 days after transfusion, and 3 patients were weaned from mechanical ventilation within 2 weeks of treatment. Of the 5 patients, 3 have been discharged from the hospital (length of stay: 53, 51, and 55 days), and 2 are in stable condition at 37 days after transfusion.

CONCLUSIONS AND RELEVANCE In this preliminary uncontrolled case series of 5 critically ill patients with COVID-19 and ARDS, administration of convalescent plasma containing neutralizing antibody was followed by improvement in their clinical status. The limited sample size and study design preclude a definitive statement about the potential effectiveness of this treatment, and these observations require evaluation in clinical trials.

Editorial

Audio and Video and Supplemental content

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The epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originating in Wuhan, China, has rapidly spread worldwide.[1] As of March 24, 2020, China had reported 81 767 cases with 3281 deaths, and the World Health Organization declared coronavirus disease 2019 (COVID-19) a pandemic. As of March 18, 2020, cases were reported in approximately 195 countries.[2]

No specific therapeutic agents or vaccines for COVID-19 are available.[3] Several therapies, such as remdesivir and favipiravir, are under investigation,[3,4] but the antiviral efficacy of these drugs is not yet known. The use of convalescent plasma was recommended as an empirical treatment during outbreaks of Ebola virus in 2014, and a protocol for treatment of Middle East respiratory syndrome coronavirus with convalescent plasma was established in 2015.[5] This approach with other viral infections such as SARS-CoV, H5N1 avian influenza, and H1N1 influenza also suggested that transfusion of convalescent plasma was effective.[6-10] In previous reports, most of the patients received the convalescent plasma by single transfusion.[9-11] In a study involving patients with pandemic influenza A(H1N1) 2009 virus infection, treatment of severe infection with convalescent plasma (n = 20 patients) was associated with reduced respiratory tract viral load, serum cytokine response, and mortality.[10] In another study involving 80 patients with SARS, administration of convalescent plasma was associated with a higher rate of hospital bxdischarge at day 22 from symptom onset compared with patients who did not receive convalescent plasma.[12] Accordingly, these findings raise the hypothesis that use of convalescent plasma transfusion could be beneficial in patients infected with SARS-CoV-2.

The purpose of this study was to describe the initial clinical experience with convalescent plasma transfusion administered to critically ill patients with COVID-19.

Key Points

Question Could administration of convalescent plasma transfusion be beneficial in the treatment of critically ill patients with coronavirus disease 2019 (COVID-19)?

Findings In this uncontrolled case series of 5 critically ill patients with COVID-19 and acute respiratory distress syndrome (ARDS), administration of convalescent plasma containing neutralizing antibody was followed by an improvement in clinical status.

Meaning These preliminary findings raise the possibility that convalescent plasma transfusion may be helpful in the treatment of critically ill patients with COVID-19 and ARDS, but this approach requires evaluation in randomized clinical trials.

Methods

This study was conducted at the infectious disease department, Shenzhen Third People's Hospital, Shenzhen, China, from January 20, 2020, to March 25, 2020, and the final date of follow-up was March 25, 2020. The study was approved by the ethics committees from Shenzhen Third People's Hospital, and each patient gave written informed consent.

Patients

Patients with laboratory confirmed COVID-19, diagnosed using quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) (GeneoDX Co, Ltd)[13] were eligible to receive convalescent plasma treatment if they fulfilled the following criteria: (1) had severe pneumonia with rapid progression and continuously high viral load despite antiviral treatment; (2) PaO_2/FiO_2 of <300 (PaO_2 measured in mm Hg and FiO_2 measured as fraction of inspired oxygen)[14]; and (3) were currently or had been supported with mechanical ventilation. The serum of each recipient was obtained and enzyme-linked immunosorbent assay (ELISA) and neutralizing antibody titers were tested one day prior to the convalescent plasma transfusion. The ABO blood types of the patients were determined for potential compatibility with the convalescent plasma donor, and each received 2 consecutive transfusions of 200 to 250 mL of ABO-compatible convalescent plasma (400 mL of convalescent plasma in total) on the same day it was obtained from the donor. The patients received antiviral agents continuously until the SARS-CoV-2 viral loads became negative.

Disease Severity Classification

Patients with laboratory-confirmed COVID-19 infection who had any of the following were considered in critical condition: (1) respiratory failure requiring mechanical ventilation, (2) shock, identified by the use of vasopressor therapy and elevated lactate levels (>2 mmol/L) despite adequate fluid resuscitation, or (3) failure of other organs requiring admission to the intensive care unit (ICU).

Donors

The 5 donors of convalescent plasma were between the ages of 18 and 60 years. The donors had recovered from SARS-CoV-2 infection and were invited to donate their convalescent plasma after written informed consent was obtained. All donors had been previously diagnosed with laboratory-confirmed COVID-19 and subsequently tested negative for SARS-CoV-2 and other respiratory viruses, as well as for hepatitis B virus, hepatitis C virus, HIV, and syphilis at the time of blood donation. The donors had been well (asymptomatic) for at least 10 days, with a serum SARS-CoV-2-specific ELISA antibody titer higher than 1:1000 and a neutralizing antibody titer greater than 40. Following donation, 400 mL of convalescent plasma was obtained from each donor by apheresis, and the plasma was immediately transfused to the recipients on the same day it was obtained.

Clinical Information

Clinical information for the 5 patients before and after convalescent plasma transfusion was obtained from a review of the hospital computer medical system and included the following: demographic data, days of admission from symptom onset, and presenting symptoms; data about various treatments, including mechanical ventilation, antiviral therapies, and steroids; clinical data, including body temperature, PaO_2/FiO_2, and Sequential Organ Failure Assessment (SOFA) score (range 0-24, with higher scores indicating more severe

illness); laboratory data, including white blood cell count, lymphocyte count, chemistry panels assessing liver and kidney function, cycle threshold value (Ct), inflammatory factors C-reactive protein (CRP), procalcitonin, and IL-6, and serum antibody titer (IgG, IgM, and neutralizing antibodies); data from chest imaging studies; and information on complications, such as acute respiratory distress syndrome (ARDS), bacterial pneumonia, and multiple organ dysfunction syndrome.

Quantitative RT-PCR

The qRT-PCR for SARS-CoV-2 was assessed as described previously.[13] Nasopharyngeal specimens collected during hospitalization were sent to the laboratory in a viral transport case. Total nucleic acid extraction from the samples was performed using the QIAamp RNA Viral Kit (Qiagen), and qRT-PCR was performed using a commercial kit specific for 2019-nCoV detection (GeneoDX Co) approved by the China Food and Drug Administration. Each RT-PCR assay provided a Ct value, which is the number of cycles required for the fluorescent signal to cross the threshold for a positive test: a higher Ct value is correlated with a lower viral load. The specimens were considered positive if the Ct value was 37.0 or lower and negative if the results were undetermined. Specimens with a Ct value higher than 37 were repeated. The specimen was considered positive if the repeated results were the same as the initial result and between 37 and 40. If the repeated Ct was undetectable, the specimen was considered negative. All procedures involving clinical specimens and SARS-CoV-2 were performed in a biosafety level 3 laboratory. The Ct values of the 5 recipients were obtained on day −1, day 1, day 3, day 7, and day 12 after the transfusion.

ELISA

Microtiter plates (Sangon Biotech) were coated overnight at 4 °C with 4 μg/mL recombinant SARS-CoV-2 RBD (receptor binding domain) proteins (50 μL per well) expressed by our laboratory through 293-T cells. The plates were washed 3 times with phosphate-buffered saline (PBS) containing 0.1% vol/vol Tween-20 (PBST) and blocked with blocking solution (PBS containing 2% wt/vol nonfat dry milk) for 2 hours at 37 °C. The plates were then washed with PBST. The serum samples were diluted to 200-fold into PBS as initial concentration, and serial 3-fold dilutions of serum was added to the wells and incubated at 37 °C for 60 minutes. After 3 washes, 100 μL of horseradish peroxidase–conjugated goat anti-human IgG (for IgG antibody titer detection) and IgM (for IgM antibody titer detection) antibodies solution (Sangon Biotech) were added to each plate, respectively, and incubated at 37 °C for 60 minutes. After 5 washes, 100 μL of tetramethylbenzidine substrate (Sangon Biotech) was added at room temperature in the dark. After 15 minutes, the reaction was stopped with a 2 M H_2SO_4 solution (sulfuric acid). The absorbance was measured at 450 nm. All samples were run in triplicate. The ELISA titers were determined by end point dilution.

Serum Neutralization Assay

Vero cells (10^4) were seeded 24 hours before the infection in a 96-well plate (Costar). On the day of infection, the cells were

Table 1. Clinical Characteristics of SARS-CoV-2-Infected Patients Who Received Convalescent Plasma

	Patient				
	1	2	3	4	5
Sex	Male	Male	Female	Female	Male
Age, y	70s	60s	50s	30s	60s
Weight, kg	55	85	60	41.5	87
Smoking	No	No	No	No	No
Blood type	B	B	B	A	B
Coexisting chronic diseases	None	Hypertension; mitral insufficiency	None	None	None
Disease presentation and course					
Estimated incubation period, d[a]	1	7	3	7	15
Interval between symptom onset and admission, d	2	4	2	2	3
Interval between admission and plasma transfusion, d	22	10	20	19	20
Complications prior to plasma transfusion	Bacterial pneumonia; severe ARDS; MODS	Bacterial pneumonia; fungal pneumonia; severe ARDS; myocardial damage	Severe ARDS	Severe ARDS	Severe ARDS
Most severe disease classification	Critical	Critical	Critical	Critical	Critical
Treatments					
Steroids	Methylprednisolone	Methylprednisolone	Methylprednisolone	Methylprednisolone	Methylprednisolone
Antivirals	Lopinavir/ritonavir; interferon alfa-1b; favipiravir	Lopinavir/ritonavir; arbidol; darunavir	Lopinavir/ritonavir; interferon alfa-1b;	Interferon alfa-1b; favipiravir	Lopinavir/ritonavir; interferon alfa-1b

Abreviations: ARDS, acute respiratory distress syndrome; MODS, multiple organ dysfunction syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a] Estimated incubation period defined as interval between estimated exposure to SARS-CoV-2 and symptom onset.

Table 2. Comparison of Viral Load, Clinical Indexes, and Laboratory Results Before and After Convalescent Plasma Transfusion

	Patient				
	1	2	3	4	5
Clinical characteristics					
Body temperature, °C					
Just before transfusion	38.6	39.0	37.6	38.3	39.0
Day 1 posttransfusion	38.5	36.8	37.7	37.9	39.0
Day 3 posttransfusion	38.1	36.6	37.0	36.6	36.8
Day 7 posttransfusion	37.8	37.2	36.5	37.9	36.8
Day 12 posttransfusion	37.0	36.8	36.6	36.8	37.9
SOFA score[a]					
Just before transfusion	5	10	3	3	2
Day 1 posttransfusion	4	12	4	3	2
Day 3 posttransfusion	6	10	3	2	2
Day 5 posttransfusion	5	11	2	2	2
Day 7 posttransfusion	3	7	2	2	1
Day 12 posttransfusion	2	4	2	1	1
P_{AO_2}/F_{IO_2}[b]					
Just before transfusion	276	209	172	188	205
Day 1 posttransfusion	300	134	184	242	292
Day 3 posttransfusion	220	230	164	233	304
Day 7 posttransfusion	245	206	220	290	230
Day 12 posttransfusion	284	316	342	322	366
Ct value[c] (viral load proxy)					
On admission to hospital	23.0	19.7	18.9	38.0	28.0
Lowest value during hospitalization[d] (highest viral load)	19.2	19.7	18.9	26.6	26.5
Just before plasma transfusion	28.5	22.0	33.0	26.6	35.9
Day 1 posttransfusion	30.0	23.7	38.5	28.0	Negative
Day 3 posttransfusion	34.4	25.0	Negative	Negative	Negative
Day 7 posttransfusion	38.0	32.0	Negative	Negative	Negative
Day 12 posttransfusion	Negative	Negative	Negative	Negative	Negative
Mechanical ventilation					
Onset, days before transfusion	11	2	12	9	2
Extubated, days posttransfusion	Intubated	Intubated	2	9	9
ECMO					
Onset, days before transfusion	Not received	1	Not received	Not received	Not received
Removal, days posttransfusion	NA	5	NA	NA	NA
Laboratory findings					
C-reactive protein, mg/L (normal range, <8)					
Before transfusion	163.4	242.8	65.	156.0	173.1
Day 1 posttransfusion	146.2	223.0	108.3	NT	186.8
Day 3 posttransfusion	115.1	75.2	78.7	160.8	233.7
Day 5 posttransfusion	31.3	10.4	74.7	NT	260.4
Day 7 posttransfusion	31.2	13.9	6.2	9.6	5.5
Day 12 posttransfusion	5.3	33.1	NT	5.8	3.2
Procalcitonin, ng/mL (normal range, <0.1)					
Before transfusion	1.2	7.3	0.1	0.2	0.2
Day 1 posttransfusion	1.3	19.7	0.1	0.08	0.4
Day 3 posttransfusion	1.6	13.9	0.09	0.07	1.5
Day 5 posttransfusion	0.9	1.8	0.08	NT	0.9
Day 7 posttransfusion	1.1	0.1	0.04	0.04	0.09
Day 12 posttransfusion	0.4	0.2	NT	0.04	0.07

(continued)

Table 2. Comparison of Viral Load, Clinical Indexes, and Laboratory Results Before and After Convalescent Plasma Transfusion (continued)

	Patient				
	1	2	3	4	5
IL-6, pg/mL (normal range, 0-7)					
Before transfusion	70.5	438.2	63.9	79.1	87.8
Day 1 posttransfusion	74.9	NT	118.5	39.3	NT
Day 3 posttransfusion	34.5	1045.0	67.0	25.8	797.9
Day 5 posttransfusion	24.1	334.1	590.5	NT	NT
Day 7 posttransfusion	30.8	29.8	174.3	34.0	69.9
Day 12 posttransfusion	6.1	31.8	NT	2.7	54.9
Length of hospital stay, d	Remains hospitalized	Remains hospitalized	53	51	55
Current status as of March 25, 2020	Stable, still receiving mechanical ventilation	Stable, still receiving mechanical ventilation	Discharged home	Discharged home	Discharged home

Abbreviations: Ct, cycle threshold; ECMO, extracorporeal membrane oxygenation; NT, not tested.

[a] The SOFA score is calculated using 6 systems: respiratory, coagulation, hepatic, cardiovascular, central nervous system, and kidney. A score of 0 is given for normal function through to 4 for most abnormal for each system. The worst values on each day are recorded, and the final SOFA score is the sum of the scores of each system.

[b] P_{AO_2}/F_{IO_2} ratio was defined as the ratio of the partial pressure of arterial oxygen to the percentage of inspired oxygen.

[c] Cycle threshold is the number of polymerase chain reaction cycles required for gene amplification. A higher Ct value is correlated with a lower viral load.

[d] Lowest value (highest viral load) between hospital admission and plasma transfusion.

washed twice. Serum samples from patients were incubated at 56 °C for 30 minutes and then diluted 2-fold in cell culture medium (modified eagle medium). Aliquots (40 µL) of diluted serum samples (from 2-fold to 2056-fold) were added to 50 µL of cell culture medium containing 50 times the tissue culture infective dose ($TCID_{50}$) of the BetaCoV/Shenzhen/SZTH-003/2020 strain virus (isolated from this hospital, GISAID access number: EPI_ISL_406594)[15] on a 96-well plate and incubated at 37 °C for 2 hours in CO_2 5% vol/vol. Virus antibody mix was then added to cells in 96-well plates and plates were incubated at 37 °C with microscopic examination for cytopathic effect after a 5-day incubation. The highest dilution of serum that showed inhibition activity of SARS-CoV-2 was recorded as the neutralizing antibody titer. Assays were performed in triplicate with negative control samples from healthy volunteers.

Results

Five patients (age range, 36-73 years; 2 women) were treated with convalescent serum. None were smokers, and 4 of 5 had no preexisting medical conditions. All 5 had received various antiviral agents and steroids (**Table 1**). Convalescent plasma was administered between 10 and 22 days after admission.

The Ct value at the time of admission ranged from 18.9 to 38.0, and on the day of plasma transfusion from 22.0 to 35.9 (**Table 2** and **Figure 1**A). It increased (improved) within 1 day after transfusion. The Ct value of patient 5 became negative on posttransfusion day 1, patient 3 and patient 4 became negative on day 3, and patient 1 and patient 2 became negative on day 12 after the transfusion (Table 2).

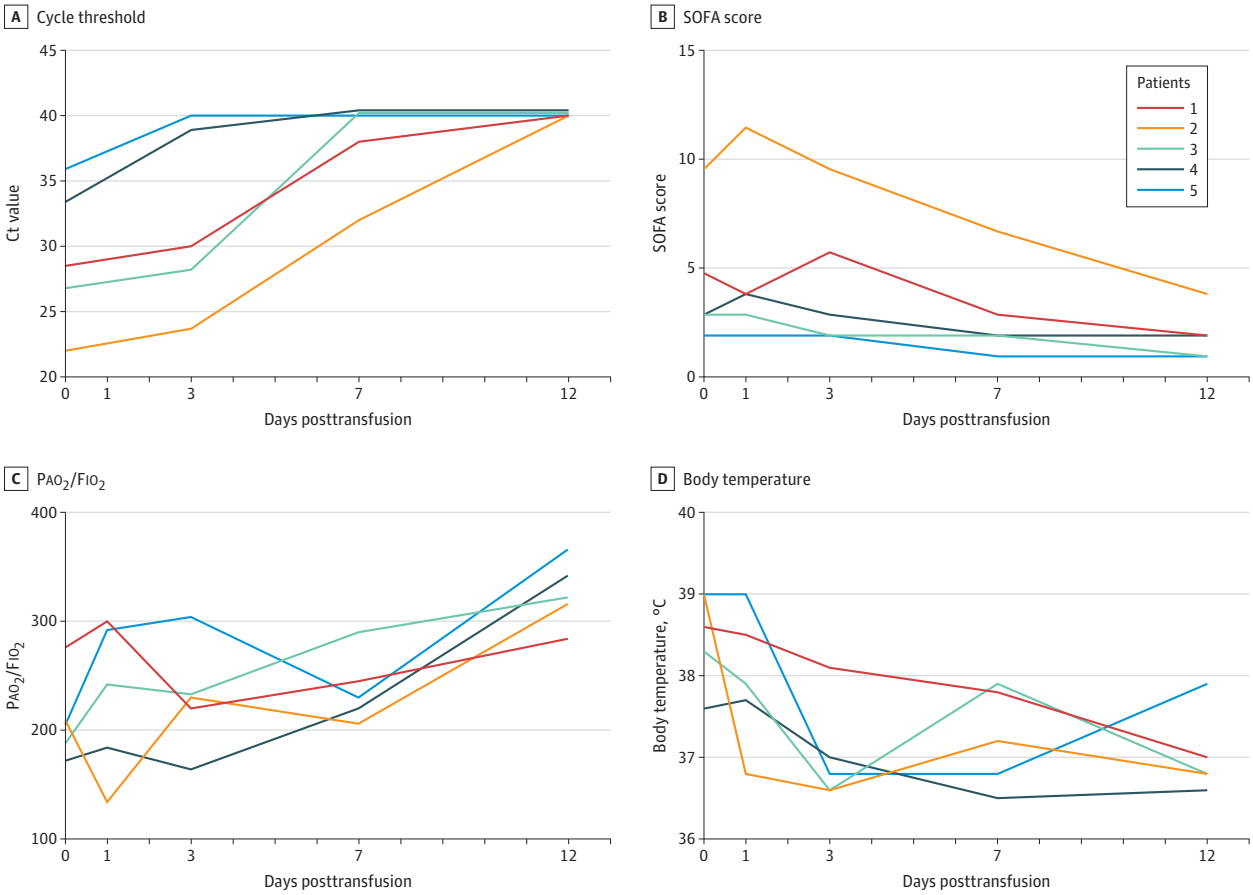
The SOFA score ranged from 2 to 10 prior to plasma transfusion, and decreased to a range of 1 to 4 at 12 days following transfusion (Table 2 and Figure 1B). The P_{AO_2}/F_{IO_2} ranged from 172 to 276 prior to transfusion, and increased (improved) for 4 of 5 patients within 7 days after transfusion (overall range, 206-290), and increased substantially (range, 284-366) on the 12th day after the plasma treatment (Table 2 and Figure 1C). Body temperature ranged from 37.6 to 39.0 °C before plasma transfusion and declined to the normal range on the third day after the transfusion (Table 2 and Figure 1D).

After the treatment, the values of the inflammatory biomarkers CRP, procalcitonin, and IL-6 of patients 1, 2, 4, and 5 decreased; the values of CRP and procalcitonin of patient 3 decreased (Table 2).

The computed tomography scans of the lungs of these patients all demonstrated severe pneumonia prior to plasma transfusion and showed improvement of the pulmonary lesion of patient 1 on the third day after the plasma transfusion (eFigure 1 in the Supplement) and gradual resolution of pulmonary lesions of other patients at 3 days after the plasma treatment (eFigures 2, 3, 4, and 5 in the Supplement).

One day prior to convalescent plasma administration, the RBD-specific IgG and IgM ELISA titers of the donors ranged between 1800 and 16 200 (ELISA end point dilution titers) (**Table 3**). The neutralization titers against SARS-CoV-2 ranged between 80 and 480 (neutralizing end point dilution titers). The RBD-specific IgG ELISA titers of 5 recipients ranged between 1800 and 48 600 and the IgM titers between 5400 and 145 800 a day prior to the convalescent transfusion (eTable in the Supplement). After the transfusion of convalescent plasma, the titers of IgG and IgM in the sera of these patients increased in a time-dependent manner. The IgG titers of the

Figure 1. Temporal Changes of Cycle Threshold Value, PaO_2/FiO_2, SOFA Score, and Body Temperature in Patients Receiving Convalescent Plasma Transfusion



A, Change in cycle threshold (Ct) value in nasopharyngeal swabs of infected patients at day 0, day 3, day 7, and day 12 after the plasma transfusion. A Ct value of 40 was defined as undetectable. B, Change in Sequential Organ Failure Assessment (SOFA) score of the patients with convalescent plasma treatment (range 0-24, with higher scores indicating more severe illness; see footnote to Table 2 for more complete definition). C, Change in PaO_2/FiO_2 ratio of the treated patients from day 0 to day 12 after treatment. D, Change in body temperature of the 5 patients following plasma transfusion.

Table 3. Characteristics and Antibody Titer of Convalescent Plasma Donors

	Donors[a]				
	1	2	3	4	5
Blood type	B	B	B	A	B
Donated plasma volume, mL	400	400	400	400	400
Interval between symptom onset and discharge, d	11	11	13	13	11
Interval between discharge and plasma donation, d	11	11	13	11	12
RBD-specific IgG ELISA titer[b]	16 200	1800	1800	5400	16 200
RBD-specific IgM ELISA titer[c]	16 200	1800	5400	5400	5400
Neutralizing antibody titer[d]	240	80	120	240	480

Abbreviation: RBD, receptor binding domain.

[a] Donors-patients were matched by number (donor 1 gave plasma to patient 1, etc).

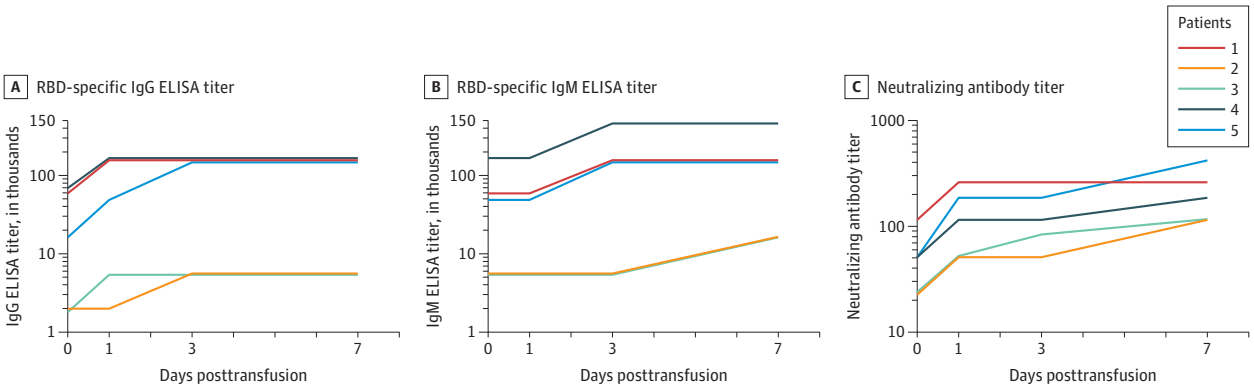
[b] ELISA end point dilution titers (IgG antibody). The expected titer of negative control from a healthy person is ≤200.

[c] ELISA end point dilution titers (IgM antibody). The expected titer of negative control from a healthy person is ≤200.

[d] Neutralization end point dilution titers. The expected titer of negative control from a healthy person is ≤10.

treated patients increased to 145 800, 5400, 5400, 145 800 and 145 800, and the IgM titers increased to 145 800, 5400, 5400, 437 400 and 145 800, respectively, at 3 days after transfusion. These IgG and IgM titers maintained a high level at 7 days after transfusion (**Figure 2**A and 2B; eTable in the Supplement). The neutralizing antibody titers of the 5 recipients

Figure 2. Changes of Receptor Binding Domain–Specific IgG and IgM ELISA and Neutralizing Antibody Titers Before and After Convalescent Plasma Transfusion in Patients



Higher titer values indicate greater protection. A, Variation of RBD-specific IgG ELISA titer. B, Variation of RBD-specific IgM ELISA titer. C, Variation of neutralizing antibody titer against SARS-CoV-2 in recipients in day 0, day 1, day 3, and day 7 following transfusion. The identical line segments were adjusted slightly to avoid superimposition. RBD indicates receptor binding domain.

ranged between 40 and 160 before transfusion; one day after transfusion, the titers increased to 320, 80, 80, 160, and 240; on day 7, they were 320, 160, 160, 240, and 480, respectively (Figure 2C; eTable in the Supplement).

All 5 patients were receiving mechanical ventilation at the time of transfusion, and 3 patients (patients 3, 4, and 5) were weaned from mechanical ventilation (Table 2). Patient 2 was receiving ECMO at the time of plasma treatment but did not require ECMO on day 5 after transfusion (Table 2). Patients 3, 4, and 5 were discharged from the hospital (length of stay: 53, 51, and 55 days, respectively). As of March 25, 2020, patients 1 and 2 remained hospitalized, with lengths of stay of 37 days each.

Discussion

In this case series, 5 patients who were critically ill with COVID-19 were treated with convalescent plasma. As assessed by Ct, viral load declined within days of treatment with convalescent plasma, and the clinical conditions of these patients improved, as indicated by body temperature reduction, improved PaO_2/FiO_2, and chest imaging. Four patients who had been receiving mechanical ventilation and ECMO no longer required respiratory support by 9 days after plasma transfusion.

Previous studies have reported the use of convalescent plasma transfusion in the treatment of various infections.[6,10,16] For example, patients (n = 50) with SARS had a significantly higher discharge rate by day 22 following onset of illness (73.4% vs 19.0%; $P<.001$) and lower case-fatality rate (0% vs 23.8%; $P = .049$) in the convalescent plasma treatment group (n = 19 patients) when compared with steroid treatment group (n = 21).[17] In another study of 93 patients with influenza A(H1N1), patients who received convalescent plasma treatment (n = 20) compared with those in the control group (n = 73) had significantly fewer deaths (20% vs 54.8%; $P = .01$) and a lower median lymphocyte count on ICU admission.[10]

In this study, collection and transfusion of the plasma were done as previously reported.[10] In addition, plasma was obtained from the donors and transfused in the recipients on the same day, which helps preserve the natural activity of the plasma.

Studies have shown that viral loads are highly correlated with disease severity and progression.[18] Fatal outcome of human influenza A(H5N1) has been associated with high viral load and hypercytokinemia.[19] Apart from antiviral treatment, virus-specific neutralizing antibody, which could accelerate virus clearance and prevent entry into target cells, serves as the main mechanism for the restriction and clearance of the viruses by the host.[20-22] In the current study, SARS-CoV-2 was still detectable in all 5 patents even though antiviral treatment had been given for at least 10 days, although viral load decreased and became undetectable soon after convalescent plasma treatment. As determined by ELISA, all plasma from the donors had high virus-specific IgG and IgM ELISA titers. Moreover, the neutralizing antibody titers, vital for the restriction of viral infection of the 5 recipients, significantly increased after plasma transfusion. The results highlight the possibility that antibodies from convalescent plasma may have contributed to the clearance of the virus and also the improvement of symptoms. In addition to viral neutralizing antibodies, acceleration of infected cell clearance by antibodies has also been found in an in vivo study of HIV-1 virus.[23] In the current study, all patients received antiviral agents, including interferon and lopinavir/ritonavir, during and following convalescent plasma treatment, which also may have contributed to the viral clearance observed.

Limitations

This study has several limitations. First, this was a small case series that included no controls. Second, it is unclear if these patients would have improved without transfusion of

convalescent plasma, although the change in Ct and PaO_2/FiO_2 represent encouraging findings. Third, all patients were treated with multiple other agents (including antiviral medications), and it is not possible to determine whether the improvement observed could have been related to therapies other than convalescent plasma. Fourth, plasma transfusion was administered 10 to 22 days after admission; whether a different timing of administration would have been associated with different outcomes cannot be determined. Fifth, whether this approach would reduce case-fatality rates is unknown.

Conclusions

In this preliminary uncontrolled case series of 5 critically ill patients with COVID-19 and ARDS, administration of convalescent plasma containing neutralizing antibody was followed by improvement in the patients' clinical status. The limited sample size and study design preclude a definitive statement about the potential effectiveness of this treatment, and these observations require evaluation in clinical trials.

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Control and Nursing in Guangdong Second Provincial General Hospital, have undergone intensive training to become familiar with the requirements for infection control in the negative pressure isolation wards. Herein, cameras cover the entire ward except for the privacy area. The infection control observer monitors medical staff in real time via computer monitors in a separate area (figure). The main responsibilities of the observer are to maintain the normal operation of the negative pressure isolation wards, supervise the implementation of disinfection, ensure a sufficient supply of protective materials, arrange specimens for inspection, and relieve anxiety of the medical personnel while treating patients.

The observers pay attention to the medical staff not only during their time in the negative pressure ward, but also during the putting on or taking off of protective equipment when they enter or leave the ward. Although the health-care providers have attended multiple training sessions and emergency drills, in operation (especially in high-stress negative pressure wards) some steps might be omitted or overlooked, thus incurring potential exposure to nosocomial infection. For example, when a nurse helped an elderly patient pull up a zipper in the negative pressure ward, the zipper unexpectedly ripped the nurse's glove. The nurse became nervous, and anxious to continue her procedures. Discovering this situation on screen, the observer immediately soothed the nurse and sent another staff member into the ward to assist. Following the occupational exposure process, the observer then instructed the nurse to remove her gloves carefully, disinfect her hands, and dispose of the ripped gloves. The observer also systematically assessed the risks for the nurse and arranged a quarantine room for medical observation to ensure full safety before she was allowed to return to the negative pressure ward.

The observing system, as a proactive infection control tool, provides immediate prevention against nosocomial infection in negative pressure isolation wards, which offers creative assistance to combat the COVID-19 outbreak. Guangdong Second Provincial General Hospital plans to incorporate artificial intelligence image recognition into the observing system, aiming to enhance the sensitivity and accuracy of instant detection. Implementing and improving the observing system might be a promising endeavor for controlling nosocomial infection of the COVID-19 outbreak and other acute infectious diseases.

We declare no competing interests.

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Convalescent plasma as a potential therapy for COVID-19

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The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which originated in Wuhan, China, has become a major concern all over the world. The pneumonia induced by the SARS-CoV-2 is named coronavirus disease 2019 (COVID-19). By Feb 22, 2020, this virus has affected more than 77 700 people worldwide and caused more than 2300 deaths. To date, no specific treatment has been proven to be effective for SARS-CoV-2 infection. Apart from supportive care, such as oxygen supply in mild cases and extracorporeal membrane oxygenation for the critically ill patients, specific drugs for this disease are still being researched. In the USA, the first patient infected with SARS-CoV-2 was treated by supportive care and intravenous remdesivir, before the patient recovered and was discharged.[1] However,

randomised clinical trials are needed to evaluate the safety and efficacy of remdesivir in the treatment of COVID-19.

Convalescent plasma or immunoglobulins have been used as a last resort to improve the survival rate of patients with SARS whose condition continued to deteriorate despite treatment with pulsed methylprednisolone. Moreover, several studies showed a shorter hospital stay and lower mortality in patients treated with convalescent plasma than those who were not treated with convalescent plasma.[2–4] In 2014, the use of convalescent plasma collected from patients who had recovered from Ebola virus disease was recommended by WHO as an empirical treatment during outbreaks.[5] A protocol for the use of convalescent plasma in the treatment of Middle East respiratory syndrome coronavirus was established in 2015.[6] In terms of patients with pandemic 2009 influenza A H1N1 (H1N1pdm09) virus infection, a prospective cohort study by Hung and colleagues showed a significant reduction in the relative risk of mortality (odds ratio 0·20 [95% CI 0·06–0·69], p=0·01) for patients treated with convalescent plasma.[7] Additionally, in a subgroup analysis, viral load after convalescent plasma treatment was significantly lower on days 3, 5, and 7 after intensive care unit admission. No adverse events were observed. A multicentre, prospective, double-blind, randomised controlled trial by Hung and colleagues showed that using convalescent plasma from patients who recovered from the influenza A H1N1pdm09 virus infection to treat patients with severe influenza A H1N1 infection was associated with a lower viral load and reduced mortality within 5 days of symptom onset.[8] A meta-analysis by Mair-Jenkins and colleagues showed that the mortality was reduced after receiving various doses of convalescent plasma in patients with severe acute respiratory infections, with no adverse events or complications after treatment.[9] Another meta-analysis by Luke and colleagues identified eight studies involving 1703 patients with 1918 influenzapneumonia from 1918 to 1925 who received an infusion of influenza-convalescent human blood products, which showed a pooled absolute reduction of 21% (95% CI 15–27; p<0·001) in the overall crude case-fatality rate at low risk of bias.[10]

One possible explanation for the efficacy of convalescent plasma therapy is that the antibodies from convalescent plasma might suppress viraemia. Schoofs and colleagues reported that 3BNC117-mediated immunotherapy, which is a broad neutralising antibody to HIV-1, enhances host humoral immunity to HIV-1.[11] An in vivo trial also showed that the effects of this antibody were not only limited to free viral clearance and blocking new infection, but also included acceleration of infected cell clearance.[12] Viraemia peaks in the first week of infection in most viral illnesses. The patient usually develops a primary immune response by days 10–14, which is followed by virus clearance.[4] Therefore, theoretically, it should be more effective to administer the convalescent plasma at the early stage of disease.[4] However, other treatments might have an effect on the relationship between convalescent plasma and antibody level, including antiviral drugs, steroids, and intravenous immunoglobulin.[10]

According to WHO,[13] management of COVID-19 has mainly focused on infection prevention, case detection and monitoring, and supportive care. However, no specific anti-SARS-CoV-2 treatment is recommended because of the absence of evidence. Most importantly, the current guidelines emphasise that systematic corticosteroids should not be given routinely for the treatment of COVID-19, which was also the recommendation in a a Commnt in *The Lancet*.[14] Evidence shows that convalescent plasma from patients who have recovered from viral infections can be used as a treatment without the occurrence of severe adverse events. Therefore, it might be worthwhile to test the safety and efficacy of convalescent plasma transfusion in SARS-CoV-2-infected patients.

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COVID-19: combining antiviral and anti-inflammatory treatments

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Both coronavirus disease 2019 (COVID-19) and severe acute respiratory syndrome (SARS) are characterised by an overexuberant inflammatory response and, for SARS, viral load is not correlated with the worsening of symptoms.[1,2] In our previous Correspondence to *The Lancet*,[3] we described how BenevolentAI's proprietary artificial intelligence (AI)-derived knowledge graph,[4] queried by a suite of algorithms, enabled identification of a target and a potential therapeutic against SARS coronavirus 2 (SARS-CoV-2; the causative organism in COVID-19). We identified a group of approved drugs that could inhibit clathrin-mediated endocytosis and thereby inhibit viral infection of cells (appendix). The drug targets are members of the numb-associated kinase (NAK) family—including AAK1 and GAK—the inhibition of which has been shown to reduce viral infection in vitro.[5,6] Baricitinib was identified as a NAK inhibitor, with a particularly high affinity for AAK1, a pivotal regulator of clathrin-mediated endocytosis. We suggested that this drug could be of use in countering SARS-CoV-2 infections, subject to appropriate clinical testing.

See Online for appendix

To take this work further in a short timescale, a necessity when dealing with a new human pathogen, we re-examined the affinity and selectivity of all the approved drugs in our knowledge graph to identify those with both antiviral and anti-inflammatory properties. Such drugs are predicted to be of particular importance in the treatment of severe cases of COVID-19, when the host inflammatory response becomes a major cause of lung damage and subsequent mortality. Comparison of the properties of the three best candidates are shown in the table. Baricitinib, fedratinib, and ruxolitinib are potent and selective JAK inhibitors approved for indications such as rheumatoid arthritis and myelofibrosis. All three are powerful anti-inflammatories that, as JAK–STAT signalling inhibitors, are likely to be effective against the consequences of the elevated levels of cytokines (including interferon-γ) typically observed in people with COVID-19.[2] Although the three candidates have similar JAK inhibitor potencies, a high affinity for AAK1 suggests baricitinib is the best of the group, especially given its once-daily oral dosing and acceptable side-effect profile.[7] The most significant side-effect seen over 4214 patient-years in the clinical trial programmes used for European Medicines Agency registration was a small increase in upper respiratory tract infections (similar to that observed with methotrexate), but the incidence of serious infections (eg, herpes zoster) over 52 weeks' dosing was small (3·2 per 100 patient-years), and similar to placebo.[7] Use of this agent in patients with COVID-19 over 7–14 days, for example, suggests side-effects would be trivial.

Other AI-algorithm-predicted NAK inhibitors include a combination of the oncology drugs sunitinib and