Special Article

Utility of convalescent plasma for COVID-19 treatment

Prasanta Kumar Ghosh

Ex-Adviser, Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, India

Abstract

The novel coronavirus identified and designated as SARS-CoV-2 has brought unprecedented suffering to people across the globe. There is yet neither an effective therapeutic substance nor a vaccine to treat the disease. Intensive research is being carried out globally to combat the menace. The road to success is miles away as effective drugs and vaccines (when available) are to be produced in adequate quantities and supplied at affordable prices to contain the disease. The observation that deployment of already-known technique of using convalescent plasma in the treatment of microbial diseases has yielded encouraging results in treating several deadly diseases, encourage the use of convalescent plasma collected from consented donors recently recovered from COVID-19 by plasmapheresis and using those for treating critically ill patients. The upgraded facilities for such purposes need to be in place along with the regulatory requirements for ensuring donor safety and judicious entitlement of convalescent plasma in patient care.

Keywords: Convalescent plasma, SARS-Co V, SARS-CoV-2

Address for correspondence: Dr. Prasanta Kumar Ghosh, Ex-Adviser, Department of Biotechnology, Ministry of Science and Technology, Government of India, Block: C2B, Flat: 5A, Janakpuri, New Delhi 110058, India.
E-mail: gprasanta2008@gmail.com

INTRODUCTION

The novel coronavirus SARS-CoV-2 causing COVID-19 disease was reported for the first time from China[1] during December 2019. SARS-CoV disease was described in the literature causing human disease by the Chinese scholars in 2002 from the Guangdong province of southern[2] China, where the first infected human was identified and described. The current infection from the novel coronavirus SARS-Co V-2 turned out to be pandemic soon after December 2019. Presently, the whole world is treating a mega number of infected individuals suffering from the disease. As of June 26, 2020 (07.00 PM) the total number of coronavirus cases[3] infected the world over were 9635,935 with deaths of 489,990(5.1%). The 10 most affected countries in the world were the USA, Brazil, the UK, Italy, France, Spain, Mexico, India, Iran, and Belgium. These 10 countries had 5707,393 infected cases (59% of the total) but registered 375,443 deaths (77% of the total deaths).

The purpose of this paper was to ascertain the utilities of convalescent plasma (CP) to neutralize the virus as a possible treatment option for severely ill patients with COVID-19. The use of CP in the treatment of microbial diseases is known. However, its use in the present situation of a pandemic for treating critically ill patients requires current evidence of usage success, the infrastructure in place, and the regulatory requirements in terms of donor

Received: 06-08-2020
Accepted: 10-08-2020
Published: 09-12-2020

Access this article online	
Quick Response Code:	Website: www.mgmjms.com
	DOI: 10.4103/mgmj.mgmj_74_20

How to cite this article: Ghosh P. Utility of convalescent plasma for COVID-19 treatment. MGM J Med Sci 2020;7:166-75.

safety and patient care. If industries get involved in the supply of CP in commercial projects, there would be need to spell out collection and dispersal norms as also, the current good manufacturing practices to be established under the Drugs Act for such establishments.

MATERIALS AND METHODS

Scientific information was collected from the published literature on how convalescent human plasma has been used for treating SARS-Co V infection and to treat COVID-19 disease. The meaningfulness of the work emanates from the fact that at present there is no effective therapeutic substance to treat the disease caused by the novel coronavirus and no vaccine is yet available.

COVID-19: broad treatment methods

In a COVID hospital/ward, which essentially means an isolated hospital/ward to ward off infectious materials, the suspected individual is examined clinically by the attending doctors and after being clinically satisfied about the disease in the individual, the suspect is required to undergo a serological test and if found positive, the suspect may be admitted for further treatment.

Every country has hospitals, healthcare establishments, and procedures to take care of their patients. On admission, the patients are treated by the doctors symptomatically in accordance with the general practices meant for the treatment of COVID-19 disease. Patients being admitted to hospitals usually come with symptoms such as dry cough, fever, fatigue, sore throat, headache, sometimes with slight dyspnoea, and also with gastrointestinal issues. Some may complain of diminished taste and smell characteristics and gastrointestinal issues. Some may have conjunctivitis too. The diagnosis has to be by real-time PCR using a nasal swab, tracheal aspirate, bronchoalveolar lavage samples, or even blood samples. As there is yet no effective agent for the treatment for COVID19, the treatment carried out is essentially symptomatic; paracetamol is given for bringing the body temperature to normal levels; pain killers for providing symptomatic relief; cough syrup to provide relief from cough; and adequate fluid intake[4] ensues. For controlling the virus[5,6] load using antiviral substances, thus far used/planned include remdesivir; oseltamivir (Trade name Tamiflu); lopinavir–ritonivir combination; lopinavir–ritonivir combination with interferon-α-2b; ritonivir with ASC09 combination; galidesvir; umefinovir–NCT04260594 combination; darunavir–NCT04252274 combination; abidol hydrochloride with interferon, abidol hydrochloride with oseltamivir and lopinavir/ritonivir combination; lopinavir with ritonavir and arbidol combination; and darunavir and cobicistat together. For controlling secondary bacterial infection empirically (often without a test of the nasal or throat swab), antibacterial agents such as azithromycin, moxifloxacin, ceftriaxone, teicoplanin, azithromycin, and some more have been used. Certain Chinese traditional[7] medicines and mesenchymal stem[8] cells among others have also been used. Steroids[9] such as prednisolone, methylprednisolone, and dexamethasone have been used in severely ill patients to minimize inflammation and the risks of death in acute respiratory distress syndrome (ARDS) patients. Multiple numbers of anticoagulants/blood thinners have also been used.

None of these agents including the antiviral drugs could adequately control the viral multiplication. The antiviral agents were effective to a limited extent only. Over a period of 10 days, usually, the surviving patients get considerable relief, as the body would fight back and enough cloned B cells are anticipated to be produced to neutralize the virus and the activated macrophages would engulf and opsonize the immunoglobulin-viral complexes. Activated T cells would destroy the infected cells and concurrently engulf the viruses along with the macrophages. Concurrently, there would be the generation of activated and cytotoxic CD4+T cells in the Th-1 pathway to destroy the infected host cells and to engulf the viral particles.

All the recovered patients are anticipated to have elevated clonal antibodies which are responsible for neutralizing the SARS-CoV-2 virus. The hospital stay after admission is about 10–14 days. Some patients, especially the older ones need to stay for more time and may be as long as 3 weeks, sometimes even a little more. A small number of patients especially the aged people and those having comorbid conditions such as the diabetics, cardiovascular diseases, obesity, high blood pressure may become critically ill and are treated specially in the intensive care units (ICUs). For such patients, the need for and the utility of CP might be the last resort of treatment along with the use of antivirals and other supportive agents. The clinical outcome of the treatment of patients with COVID-19 is summarized with certain examples.

Clinical outcome of treatment in COVID-19 globally: a few examples

The first epidemiological, clinical, laboratory, and radiological characteristics as also the treatment and clinical outcomes of 41 laboratory-confirmed patients with COVID-19 were described[10] on February 15, 2020. In this study, the median age was 49 years (interquartile range: 41–58), of which men were 73% (30 numbers) and

less than half had comorbid diseases including diabetes (8 numbers), cardiovascular diseases (6 numbers) and hypertension (6 numbers). The disease symptoms of all the 41 at the beginning were fever (40 numbers), cough (31 numbers), and fatigue (18 numbers). Other symptoms were sputum production (11 numbers), hemoptysis (coughing up blood in 2 numbers), headache (3 numbers), and diarrhea (1 number). All 41 patients had pneumonia. Over a period of 8 days from the onset of the disease, 22 numbers had breathing troubles (dyspnoea), and 26 numbers had a reduction of lymphocytes (lymphopenia – lymphocyte count less than 1.0×10^9/L). The computed tomography (CT) scan of the chest showed pneumonia with abnormal scan pictures of the lungs. Sooner by about 9 days after the onset of disease, ARDS was observed in 13 number of patients who were transferred to ICU where 6patients (15%) died. The levels of cytokines and chemokines such as the levels of IL-2, IL-7, IL-10, GSCF, IP-10, MCP-1, MIP1A, and TNF-α were measured intermittently and it was found that the ICU patients had higher plasma levels of these substances than the non-ICU patients. Elevated levels of these substances are indicative of the development of elevated actions of the immune cells, described as cytokine storms. Cytokine storm characterizes a hyperactive immune response. It signalizes the release of multiple mediators let out to attack and clear infectious agents that invade the body. Excessive release of such cytokines is injurious to the host cells. In COVID-19 disease there is an increase of multiple of such cytokines and excessive release of certain specific ones like IL-6 (22) has been thought to be correlating with the severity of the infection. In such conditions the immune cells flood and attack the organs such as the lungs, which result in blood vessel leakage, blood clotting, fall in the blood pressure, failure of the organs, and death. The treatments carried out were with antiviral therapy (oseltamivir) along with empirical antibiotics, used to treat pneumonia patients; corticosteroids were used in patients in severe illness to reduce the hyper activities of immune cells. CP was not used as a therapy. Post mortem studies of patients with COVID-19 were not carried out.

In another[11] study, published online on February 7, 2020 on a confirmed COVID-19 patient population of 138, the results were as follows. Of the 138 hospitalized confirmed patients with COVID-19, where the median age was 56 years (range 22–92 years), 75 numbers were men (54.3%). Among these 138 patients, the hospital-associated transmission of professionals was 40 (29%) and hospitalized patients were 17 (12%). The common symptoms in the admitted patients were fever (136 numbers), fatigue (96 numbers), and dry cough (82 numbers). Another lab-assisted results of a certain parameter such as lymphocyte counts, prothrombin time and lactate dehydrogenase measurement of the patients indicated conditions of lymphopenia in 97 patients, prolonged prothrombin time of 13 s (range 12.3–13.7 s) in 80 patients and elevated lactate dehydrogenase of 261 U/L (182–403 U/L) in 55 patients, indicating liver impairment. The CT scan of all the patients had shown ground-glass opacity shadows in the lungs of all the patients. As the therapy, most of the patients (126 numbers) received oseltamivir (Trade name Tamiflu, a neuraminidase inhibitor antiviral drug); antibacterial drugs such as moxifloxacin (89 numbers), ceftriaxone (34 numbers), azithromycin (25 numbers) and glucocorticoids (62 numbers). The patients, who developed complications including ARDS in 22 patients and/or arrhythmia (16 patients) and/or shock syndrome (11 patients) thereby requiring care in an ICU were in total 36 (26%). The patients treated in the ICU (36 numbest) were older in age (median age 66 years) (when compared to those treated in non-ICU units (median age 51 years). The analysis of results of the hospital-admitted patients for the period from January 01, 2020 up to February 3, 2020 (34 days) of these 138 patients was elaborated in the paper. As of February 3, 2020, the number of patients discharged (cured) was 47 (34%) and the number of deaths was 6 (4.3%). The remaining 85 were still in the hospital. The hospital stay of the discharged patients was 10 days-interquartile range (IQR) was 7–14. The gist of the findings was that the COVID-19 confirmed cases usually manifest fever, fatigue and dry cough; lab-assisted blood parameters would show lymphopenia, prolonged prothrombin time, and elevated lactate dehydrogenase levels indicating liver impairment. The antiviral drugs used and the antibacterial drugs for containing the virus did not show positive results. The treatment was essentially symptomic in order to provide relief to the infected. Hospital professionals and admitted patients were at high risk of contracting the disease through man to man contact and therefore utmost care was warranted by the professionals while handling patients with COVID-19. Aged individuals were at higher risk of contracting the disease and when contacted, the recovery chances were lower than younger individuals. The average hospital stay for the recovered was about 10 days. CP was not used in this study.

In another paper published[12] online on April 22, 2020 from New York City, USA, the information of confirmed COVID-19 cases of 5700 hospitalized patients was published. The median age was 63 years (interquartile range 52–75 years and range 0–107 years). The outcome was assessed for 2634 patients. The common co-morbidity conditions were hypertension (3026, 53%); diabetes (1808, 32%) and obesity (1737, 31%). Outcomes of 2634 patients who were either discharged or had died were brought out,

whereas the remaining 3066 patients were still admitted in the hospitals. The number of patients who died was 282. Only 373 patients (6.5% of the total and 14% of the 2634 numbers) required care in ICU. ICU care included massive mechanical ventilation and renal artery bypass procedure. The death of patients worked out to 4.95% on the pool of 5700 patients, which was on the high side; perhaps among the co-morbidity conditions, obesity was one of the main reasons. From among the discharged patients, a total of 45 patients were readmitted and the median time for readmission was 3 days. The endpoint of discharge requires a relook as it is assumed that the discharged patients were cured of COVID-19 and that their co-morbid conditions (except obesity) were medically controlled and were stable before discharging, and yet a sizable number required readmission. The other parameters reported in the study were similar to the previously published information such as more deaths of older patients having multiple co-morbid conditions. CP was not used in the study.

POSTMORTEM STUDIES

As the patients with COVID-19 often die because of life-threatening respiratory problems, it is appropriate to analyze the lung tissues of dead patients died of COVID-19 disease so as to understand the pathogenesis and to enable treatment based of the new findings if any. The lungs tissues of as many as 38 cases of patients[13] died of COVID-19 in Italy were systematically analyzed using hematoxylin–eosin staining. The results were published on April 22, 2020. The immunohistochemistry for the inflammatory infiltrate and the cellular components were performed. Electron microscopy (ECM) of the cells was also carried out. It was observed that there was capillary congestion, necrosis of pneumocytes, the formation of hyaline membrane, pulmonary edema in the interstitial tissues, pneumocytes hyperplasia, platelet-fibrin thrombi, and changes in the lungs tissues caused due to inflammation or injury. The results revealed the features of the diffuse alveolar disease (DAD) in the lungs causing disruption of the blood-air barrier which would lead to oxidative edema and fibrosis. Such conditions result in severely impaired blood and tissue oxygenation. The ECM photographs had shown the viral particles in the cytoplasm of pneumocytes. It was concluded that patients with COVID-19 develop DAD, the formation of hyaline membrane, and pneumocytes atypical hyperplasia. The presence of platelet-fibrin thrombi observed in the small arterial vessels was suggestive of using blood thinners as one of the main components of therapy.

In another study[14] based on autopsy findings of 21 patients with COVID-19 died at the University Hospital Basel and at the Cantonal Hospital Baselland, Switzerland showed that the primary cause of death was a respiratory failure with exudative diffuse alveolar damage with massive capillary congestion. The report was published on May 4, 2020. Deaths were often accompanied by microthrombi despite anticoagulation. Ten cases showed bronchopneumonia. Other pathologic conditions included pulmonary embolisms, alveolar hemorrhage, and vasculitis, signs of generalized thrombotic microangiopathy and senile cardiac amyloidosis. The comorbid conditions and old age were unfavorable factors to fight the disease.

Lessons from clinical outcome and postmortem studies

The use of palliative treatment along with the use of selected antiviral drugs and empirical use of antibacterials had been the mainstay thus far. Corticosteroids were used in critically ill patients with benefits in many cases. The published information on the symptoms of the disease and the line of treatment as depicted above as also the postmortem studies of the patients with COVID-19 revealed that this disease does not adequately respond to the known therapeutic substances and that excessive clotting of blood at the airways prevent or even stop the oxygen exchange with the blood. As a result, maximum deaths occur from respiratory failures. Along with the palliative treatments, the use of blood-thinning agents in appropriate dosages seems to provide a better outcome. Further, patients with comorbid conditions, especially individuals having high blood pressure, diabetes, asthma, obesity, and age above 65y, seem to be less competent to fight the disease.

USE OF CONVALESCENT PLASMA AS A THERAPY

CP against SARS-CoV-2 virus causing COVID-19 disease refers to the plasma received from the recovered COVID-19 fever who has recovered to health. Such CP contains immunoglobulins which have been produced by the cured individuals in response to SARS-CoV-2 viral infection. CP can be collected from recovered donors (on their consent) through a process called plasmapheresis or apheresis, using a special machine that separates the blood into different components; plasma in some quantities are collected and used for therapy, whereas the rest of the blood components are returned into the donor's body. The total volume of donated plasma should not exceed 15% of the total volume of blood of the donor at any stage of the procedure.[15-19] Many countries have resorted to using the CP to limit the multiplication of SARS-Co V-2 virus. Chronologically, the main initial global findings are summarized below:

The results of five critically-ill patients who were confirmed COVID-19 cases with ARDS, who were treated with CP

transfusion at the infectious disease department, Shenzhen Third People's Hospital in Shenzhen, China, from January 20, 2020, to March 25, 2020, were published online[20] on March 27, 2020, in JAMA. Between 10 and 22 days after admission, these patients received CP. Each patient received CP, obtained from five other patients who recovered from COVID-19. The brief particulars about the treatment received by the patients with their age were as follows: the five patients had age of 70, 60, 50, 30, and 60 years; all the patients received therapeutic doses of methylprednisolone; the antivirals received were Lopinavir/ritonavir, interferon α-1b, favipiravir; Lopinavir/ritonavir, arbidol, darunavir; Lopinavir/ritonavir, interferon α-1b; Interferon α-1b, favipiravir; and Lopinavir/ritonavir plus interferon α-1b, respectively. Each of this critically ill patient with COVID-19 received two consecutive transfusions of 200–250 mL of ABO compatible plasma and in a total of 400 mL plasma on the same the day when it was taken out from the donors. The administration of CP was between 10 and 22 days after admission. The five donors of plasma were between the age of 18 and 60 years. The major outcome of the treatment was: viral load declined within days of treatment with CP; the clinical conditions such as body temperature improved, PaO_2/FiO_2 improved (Horowitz index), chest imaging pictures also improved. Among the five patients, four were receiving mechanical ventilation and ECMO no longer required respiratory support by 9 days after the transfusion of CP.

On March 31, 2020, in a more detailed study, another group had reported[21] the disease course and the treatment results using CP along with supportive care of four critically ill patients who were suffering from COVID-19 disease. The work was done in China and the patients were admitted and quarantined on January 20, 2020. The age and sex of the four patients were as follows: patient—1, female, 69 years; patient—2, male, 55 years; patient—3, male, 73 years; and patient—4, female, 31 years. The initiation of treatment for all the patients were: the first patient was initiated from January 30, 2020, with arbidol, lopinavir-ritonavir, interferon α-2b; the second one with arbidol, lopinavir-ritonavir, and interferon-α-2b from February 6, 2020; the third one from February 4, 2020 with arbidol, lopinavir-ritonavir, oseltamivir, and ribavirin and interferon-α-2b; and the fourth one from February 1, 2020, with lopinavir-ritonavir and ribavirin. All the patients received methylprednisolone.

The first patient developed ARDS on February 4, 2020, and was therefore transferred to ICU on February 5, 2020, and received invasive mechanical ventilation. The patient developed septic shock pneumorrhagia on February 11, 2020, and on February 17, 2020, she received 200 mL of CP; her viral load on February 18 was measured at 55×10^5 copies per mL which was very high. However, after receiving the CP, whereas there was no adverse reaction, the disease symptoms started to recede although there was no change in the viral load. On February 27, 2020 she received another 400 mL of CP and another 300 mL on February 28, 2020 (in three shots a total of 900 mL of CP). On February 28, 2020, her viral load in plasma reduced to 3.9×10^4 number of copies/mL and thereafter, on March 5, 2020 the viral load was reduced to 180 copies /mL. She tested negative to viral load on March 09, 2020, onwards and was discharged on March 13, 2020.

The second patient developed ARDS on noninvasive mechanical ventilation and high-flow nasal cannula February 8, 2020, and received noninvasive mechanical ventilation and high-flow nasal cannula from February 9, 2020, up to February 15, 2020. On February 16, 2020, he received 200 mils of CP. The disease symptoms started to recede. Therefore, all the medicines except methylprednisolone were discontinued from February 17, 2020. The viral load was negative by RT-PCR from February 20, 2020, onwards and he was discharged on February 23, 2020.

The third patient reached the stage of ARDS of February 03, 2020. The condition further deteriorated on day February 15, 2020, and there was a septic shock requiring the patient to support through extracorporeal membrane oxygenation known as V-V-ECMO (veno-venous extracorporeal membrane oxygenation). Fortunately, on February 16, 2020 CP transfusion could be initiated. Four hundred milliliter of plasma was infused on February 15, 2020. On February 25, 2020 the patient went into conditions of active pneumorrhagia, cystorrhagia, and gastrointestinal bleeding. On each day of February 24 and 28, the patient received 400 mL on each day of CP. Thereafter the patient received again 400 mL of CP on March 2, and further 200 mL on each day of March 2, 4, 6, 10, and 13. In total, the patient received 2400 mL of CP and was alive! The RT-PCR samples on March 16 and 17 were negative to the SARS-CoV-2 virus. On March 23, 2020, the patient was transferred to an unfenced ICU.

The fourth patient developed the conditions of onset of pharyngalgia with fever and had difficulty breathing on January 31, 2020. On February 1, 2020, the patient developed symptoms of ARDS, multiple organ dysfunction syndrome, and septic shock. The patient required invasive ventilation care. On February 2, 2020 continuous renal replacement therapy (CRRT) was resorted to and antiviral drugs such as lopinavir-ritonavir and ribavirin were started.

On February 6, 2020 the patient required V-V ECMO and the condition was critical. On February 19, 2020 the patient received 300 mL of CP. Thereafter, the condition of the patient started to improve. On February 27, 2020, the CRRT and ECMO could be removed. On March 11, 2020, the patient was extubated and received nasal oxygen. On March 12 and 14, 2020, the patient tested negative on RT-PCR for the SARS-Co V-2 virus. The patient was discharged on March 17, 2020.

The conditions of each of the four patients were unique and had shown complex forms of manifestations requiring rigorous applications of multiple types of machines and instruments which can be deployed only in highly sophisticated medical care establishments. Interestingly, although the antiviral and corticosteroid therapies (methylprednisolone and others in the group) along with the required medical instruments and machine-based supportive therapy is extended, the effective therapy seems to have been provided by the use of CP. Though the level of neutralizing antibodies in donor plasma before transfusion cannot be determined because of time limitations, it seems obvious that neutralizing antibodies in donor plasma seem to be effective for intervention; it further appears that once the body receives the time to fight back through its own immune system resulting from the minimization of viral load, effected through the donor plasma, the macrophages, the natural killer cells, and the residual resting T cells and the matured B-cells start getting activated in the right direction switching off the regulators of the cytokine storm and directing towards opsonizing the viruses and clearing up the damaged cells and tissues. There appears to be the need for some time for the immune system to reorganize and to redeploy the Th-1 and Th-2 pathways in the balanced but vigorous fight-back syndrome even though the viral load starts getting neutralized as soon as the patient starts receiving CP. The neutralized but alive virus-immunoglobulin complex starts getting optsionized by the activated lymphocytes as soon as the immune system is reactivated in the structured manner.

In another study published on April 6, 2020, the investigators had reported from Korea[22] the case studies of two COVID-19 confirmed cases. Both the patients had severe pneumonia with ARDS.

The first case described was of a 71-year-old man. The individual visited the Community Health Center on February 22, presenting 12 days of fever and cough. On examination by real-time reverse transcription-polymerase chain reaction (RT-PCR), he was found to suffer from COVID-19 disease. In the hospital, he received initially 400 mg of hydroxychloroquine once daily. The chest radiograph obtained on day 2 showed mild opacities in the right lower lung. Thereafter, lopinavir/ritonavir 400 mg/100 mg twice daily was added to his therapeutics. On day 3, his condition deteriorated and his oxygen demand increased. He was therefore transferred to the tertiary-care hospital. His respiratory rate was over 30 times per minute. A blood test indicated lymphopenia (lymphocyte count was $0.4 \times 10^3/\mu L$), C-reactive protein (CRP) and lactic dehydrogenase (LDH) elevated up to 59.7 mg/L and 814 IU/L, respectively; the aspartate transaminase values were slightly on the higher size. Serial bacterial culture and polymerase chain reaction (PCR) for other respiratory viruses were negative. Antivirals such as lopinavir/ritonavir and hydroxychloroquine were continually used along with empirical antibiotics. But the patient remained febrile with aggravated oxygenation profiles and chest images. The CRP values and the IL-6 levels were showing elevated levels (CRP 172.6 mg/L and IL-6 208.2 pg/mL). The arterial blood gas analysis showed PaO_2/FiO_2 of 86 on day 9 confirming ARDS syndrome. Methylprednisolone at the rate of 1 mg/kg/day daily was started. On day 10, CP was obtained from a male donor in his 20s who had completely recovered from COVID-19 for 21 days who donated 500 mL of his plasma. The plasma of the donor measured an OD of 0.586 (cutoff value was 0.22) for IgG by ELISA. The plasma was administered to the patient at 12-h interval in two divided doses. No adverse reaction occurred. From day 11, the recipient started showing improvement as his fever subsided and oxygen demand decreased. The CRP and IL-6 levels became normal (5.7 mg/L and 1.5 pg/mL, respectively, for CRP and IL-6), the chest X-ray showed resolution of both lungs infiltrates on day 18, and his viral load detected negative by RT-PCR on day 26. The investigators reported that the patient underwent a tracheostomy and was successfully weaned from the mechanical ventilator.

The second case was of a woman, 67 years with co-morbid conditions such as a history of hypertension. She developed fever and myalgia and was diagnosed to be suffering from COVID-19 fever, based on RT-PCR results of March 6, 2020. She was initially admitted to a local public medical center and received hydroxychloroquine 400 mg once daily and lopinavir/ritonavir 400 mg/100 mg twice daily with empirical antibiotics from the day of her admission to the medical center. On day 3, she was transferred to the tertiary-care hospital as her conditions deteriorated; her oxygen demand increased and there were worsening infiltrative shadows in the left lower lung. Her venous oxygen saturation registered at 93% on 4 L/min oxygen flow via nasal cannula and her respiratory rate of 24 times

per minute. Routine blood tests showed mild leukocytosis ($12.67 \times 10^3/\mu L$) with lymphopenia ($0.7 \times 103/\mu L$), elevated CRP, IL-6, and LDH (131.1 mg/L, 474.7 pg/mL, 344 IU/L, respectively). The Bacterial cultures and the PCR for other respiratory viruses were all negative. Intubation and mechanical ventilator care were to be resorted to from day 4 as her conditions deteriorated. Intravenous methylprednisolone (0.5 mg/kg/day daily) was added. She had sustained high fever with rapidly increasing CRP (314 mg/L), WBC ($21.79 \times 10^3/\mu L$), and persistent lymphopenia ($0.5 \times 103/\mu L$). PaO_2/FiO_2 fell to 76 showing severe ARDS. With the use of steroids, chest images and the oxygen demand began to be improved. On day 6, CP was obtained from a male donor in his 20s who had recovered from COVID-19 for 18 days. The OD ratio for IgG of the donor plasma was 0.532 and the plasma was administered to the patient in the same way as Case 1. The quantity of CP collected was not reported; it is assumed that similar quantities (500 mL) might have been collected. The transfusion of plasma did not manifest in any adverse reaction. On transfusion of plasma, the conditions of leukocytosis and lymphopenia were immediately recovered. On day 9, the density of bilateral infiltration on chest X-ray improved very much with increased PaO_2/FiO_2 to 230. The level of CRP and IL-6 also returned to the normal ranges. The presence of the virus SARS-CoV-2 was substantially reduced as measured by RT-PCR on day 9 and was negative on day 20. The patient was discharged on day 24 after she became RT-PCR negative on day 20.

Intravenous use of methylprednisolone was started just before the CP infusion in both cases. Although the use of systemic corticosteroids has its benefits, the adverse reactions that are likely to be manifested later in the recipient patients should be kept in mind and the use should be restrained unless there are no alternate tools in the hands of the physicians dealing with the patients directly in the wards. Although CP was administered after 22 days from the onset of symptoms in Case 1, and 7 days in Case 2, respectively, doubts may arise that the decrease in the viral loads shown in both cases could be due to natural pathology of COVID-19. However, the fast disappearance of the disease symptoms after the application of CP tends to score higher on the use such plasma as another better alternative to treating the last-stage cases especially the patients who have co-morbid conditions and when the patients are more aged.

In another study conducted in Wuhan, China the group had investigated the efficacy of CP therapy in six COVID-19 confirmed patients.[23] The results were published on April 15, 2020. CP was used on each of the six patients. The other supportive therapy was continued. The points of observation for the usefulness of CP were determined by the alleviation of symptoms, changes in radiologic abnormalities, and laboratory tests. The investigators did not notice any adverse effect by the use of CP. It was found that the treatment led to a resolution of ground-glass opacities of the chest X-ray pictures in five patients and in two of the six patients there was the complete elimination of the virus. It was concluded that the treatment was effective.

In yet another study conducted in China on 10 confirmed patients with COVID-19 received,[24] each one received a dose of 200 mL of CP derived from recently recovered donors were transfused; these patients were receiving necessary supportive care and known antiviral agents. The plasma had antibody neutralizing titers above 1:640. With the intention of assessing the utility of the CP, the primary endpoint was safety and secondary endpoints were to assess the improvement of clinical symptoms and laboratory parameters within 3 days after the transfusion of CP. The median time of transfusion of CP was 16.5 days after the onset of the disease. It was observed that the clinical symptoms were significantly improved: there was an increase in lymphocyte counts, a decrease in C-reactive protein, and increase of oxyhemoglobin saturation. The viral load was undetectable in seven patients. There was no adverse reaction in the recipients. Overall, the results were highly encouraging. The results were published on April 28, 2020.

In a first systematic review published[25] on May 1, 2020, the usefulness of convalescent plasma transfusion (CPT) as a therapy in patients with COVID-19 was conducted based on published literature. The main findings were: increase in neutralizing antibody titers and concomitant disappearance of SARS-CoV-2 virus in almost all the patients; and reduction in the mortality of critically ill patients. There was also the appearance of beneficial clinical symptoms after the administration of CP.

The utility of CP, from the results of the above studies and the review, stand undisputed. It is to be remembered that there is yet no effective established therapy; physicians all over the world are using essentially antiviral drugs deploying multiple of them that are presently known and available. Along with antiviral therapy, empirically multiple numbers of antibacterial drugs that invade the lungs are also being tried. The postmortem studies of a sizable number of patients had shown evidence of blood clots in the alveoli of patients, thereby necessitating the use of blood thinners as an empirical therapy. The use of *N*-acetyl cysteine and

heparin has also been suggested without adequate medical evidence. Of all the medicaments used, the sensitized plasma received from the recently recovered COVID-19 individuals has shown elevated prospects in critically ill patients with COVID-19 as has been narrated above. A most recent publication which appeared while writing this review had also concluded the same way.[26]

EARLIER STUDIES ON CONVALESCENT PLASMA

In earlier studies on the use of CP for treating SARS disease were also supportive. In March 2003, there was an outbreak of severe acute respiratory syndrome (SARS) in 29 countries, caused by SARS-associated corona virus (SARS-Co V). There were reported infection[27] of 8096 patients in 29 countries; 774 people in all these 29 countries died. The fatality rate was 9.6%, much higher than the present COVID-19 fever[28] (around 3.4%). Most patients[29] were from Mainland China, Hong Kong, Taiwan, Singapore and Canada. Ribavirin and corticosteroids were used as the first line of treatment in most parts of Hong Kong and around. Ribavirin is an antiviral drug used to treat infection from multiple RNA viruses such as human respiratory syncytial virus (RSV), Hepatitis-C and certain other viral hemorrhagic fevers (VHFs) caused by Ebola and Marburg, Lassa fever and yellow fever viruses. However, the use of ribavirin and methylprednisolone could not produce adequately satisfactory outcome. CP, obtained from patients who recovered from SARS, had therefore been used as a desperate last resort. Interestingly, the results were very much encouraging and the patients who received CP along with ribavirin and corticosteroids had a shorter hospital stay and lower mortality than those patients who received the treatment of ribavirin and pulse methylprednisolone.[30,31]

From the above information, it appears almost certain that the use of CP shall be the most useful therapy to treat COVID-19 disease in the present circumstances and needs to be resorted to by the medical profession the world over till an alternate treatment option such as the development of an effective vaccine or an effective therapeutic substance is in hand. As the use of CP requires the consent of the COVID-19-cured donor to draw plasma and as the therapeutic agent requires careful handling within a short period of usefulness of the CP, rules and procedures require to be brought out by the governments so as to ensure effective use of such an important life-saving substance in different regions. Till effective therapeutic substances and/or vaccines are in place this option of saving lives need to be explored for critically ill patients; implementation of this option shall no doubt require in place promulgation of executive orders, setting out rules and guidelines, installation and implementation of best medical practices for evaluation, collection, storage and use of potent CP for saving lives.

The Indian Council of Medical Research(ICMR) has initiated[32,33] a multicenter clinical trial, titled "A Phase II, Open Label, Randomized Controlled Trial to Assess the Safety and Efficacy of Convalescent Plasma to Limit COVID-19 Associated Complications in Moderate Disease," in 64 hospitals throughout the country. It is a safety studies at the moment.

In Indian context, it is now prudent to come up with guidelines for the collection, preservation and safe use of CP for treating COVID-19 disease as the infection rate has increased phenomenally high. The US FDA came[34] out on May 1, 2020 with the US FDA guidelines for the health care providers and investigators on the administration and study of investigational CP collected from individuals. Besides multiple conditions, the guidelines require the consent from the donors of CP. Later during the month,[35] the US FDA guidelines were brought out for the industry on the subject matter.

Organizing the production of CP in the industrial scale requires the active participation of the industry. Industry can be encouraged to come up for handling this important area if guidelines and statutes are also in place. In the face of acute national problem, a well-balanced policy needs to be spelt out so that there is adequate availability of the right quality of CP which is used for treating the critically ill patients at affordable prices.

DISCUSSION AND CONCLUDING REMARKS

Although there has been considerable scientific, medical, technological, social and political endeavor to find an enduring solution to the COVID-19 disease menace and while the social and the political will of all the powerful countries have conjoined to fight the disease, there has not yet been in sight, any effective treatment either as a therapeutic substance or a vaccine. The emergence of a couple of effective SARS-Co V-2 vaccines to protect human from the COVID-19 disease is however in sight[36] and may come out by the beginning of 2021. Even when vaccines are available, the value of CP shall remain to treat certain COVID-19 critically ill patients. It is now considerably clear that SARS-Co V-2 kills by taking advantage of copious infection of the respiratory tract epithelial cells expressing more of ACE2 receptors, emanated and promoted by the cytokines. Cytokines are released by the immune cells in the initial phase of response to the profusely infected cells, thereby promoting cytokine storm and causing prolific and

unrestrained inflammation around the infected tissues in the respiratory tract and the lungs. The virus also causes destabilization in blood clotting by modulating the blood clotting characteristics, which result in fast clotting of blood at the sites of pneumocytes in the alveoli, which disrupts oxygen transfer across the alveolar membrane in the lungs, causing oxygen shortage in the blood and manifesting symptoms of breathlessness or dysponea and finally death. The promotion of cytokine storm by the immune cells is probably linked to a critical viral load above which the process is accelerated. This hypothesis needs verification however.

Based on the limited published data available from multiple sources as depicted above, it appears that CP shall be an important option for saving lives of critically ill patients. The limitations on the applications may be in the availability of enough quality plasma, to be recovered from cured and healthy patients who had recently suffered from COVID-19. There has to be methods for the fast determination of the viral load in blood as also equally reliable method for the fast evaluation of the titer of CP so as ascertain its SARS-CoV-2 neutralization capacity. With such information in hand, the attending physicians may be able to take faster decision on the treatment options of critically ill patients. To contain the misuse or over-exploitation of CP in not-so-deserving cases, detailed government guidelines need to be brought out by countries that wish to adopt this mode of therapy. International efforts would be a rightful gesture.

Once the immune system gets respite from the viral load using CP, multiple life-saving biochemical events are anticipated to be revived in the body of the patient, most eventful of which is a gain in time for reactivation of the entire chain of the immune train starting from more efficient presentation of the viral antigens by the dendritic cells to the lymphocytes, followed by balanced activation of the immune T cells in both the Th-1 and Th-2 pathways, thereby producing more clonal B-plasma cells for copious production of sensitized immunoglobulins, facilitating the viral neutralization process followed by opsonization of the immunoglobulin-viral complex by the macrophages and sensitized lymphocytes on one hand in the Th-2 pathway and on the other hand the activation of the resting $CD8^+$ T cells through the activated T-cells and the liberated cytokines in the Th-1 pathway, thereby producing a very large number of activated cytotoxic $CD8^+$ T lymphocytes to destroy the infected cells which are acting as the depots for viral multiplication, and devouring the liberated viral particles by the activated macrophages, cytotoxic T lymphocytes and NK cells.

It is assumed that by using CP the bodily inflammation at the disease-affected sites such as the lungs in particular, is minimized from the gain in time by the immune system not only to reorganize but also to minimize the cytokine storm, which starts subsiding, implying thereby that there is a threshold limit of viral load above which the phenomenon gets activated resulting in the hyperactivation of the immune system. Use of certain corticosteroids such as prednisolone, methylprednisolone, and dexamethasone, which does not kill the virus but can influence the minimization of the cytokine storm may probably act by competing with the immune receptors that the virus might be using to accelerate the cytokine storm. Corticosteroids are known to down-regulate the activated immune cells.

There had been evidence of life-saving manifestations on the use of CP in multiple cases of otherwise hopeless conditions of SARS-CoV-2 infected patients; this fact needs to be flagged in the current situation. National and even international efforts need to be mounted to come out with socially acceptable safe procedures under a regulatory umbrella to evaluate, collect and preserve safe CP from consented donors, and use such plasma for saving lives till such time as effective medicines (vaccines or therapeutics or both) are available. Such an effort shall go a long way, especially in poorer settings to save many precious lives at comparatively much lesser costs.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Zhou Y. Three things the Chinese government tried to hide during the novel coronavirus outbreak, QUARTZ. The changing coronavirus outbreak narrative pushed by China. Available from: https://qz.com/1801985/the-changing-coronavirus-outbreak-narrative-pushed-by-china/. [Last accessed on 2020 Feb 14].
2. World Health Organization. SARS (Severe Acute Respiratory Syndrome). Geneva, Switzerland: WHO; 2020.
3. COVID-19 | SARS-CoV-2 Coronavirus Portal. Available from: https://globalbiodefense.com/novel-coronavirus-covid-19-portal/, then go to Global Case Count Dashboard Johns Hopkins CSSE, then go to Global Case Count Dashboard Johns Hopkins CSSE https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6.
4. Pascarella G, Strumia A, Piliego C, Bruno F, Del Buono R, Costa F, *et al*. COVID-19 diagnosis and management: A comprehensive review. J Intern Med 2020;288:192-206.
5. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther 2020;14:58-60.
6. Wu R, Wang L, Kuo HCD, Shannar A, Peter R, Chou PJ, *et al*. An update on current therapeutic drugs treating COVID-19. Curr Pharmacol Rep 2020:1-15. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211915/.

7. Yang Y, Islam MS, Wang J, Li Y, Chen X. Traditional Chinese medicine in the treatment of patients infected with 2019-new coronavirus (SARS-cov-2): A review and perspective. Int J Biol Sci 2020;16:1708-17.
8. Golchin A, Seyedjafari E and Ardeshirylajimi A. Mesenchymal stem cell therapy for COVID-19: Present or future. Stem Cell Rev Rep 2020:1-7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7152513/.
9. Li H, Chen C, Hu F, Wang J, Zhao Q, Gale RP, *et al*. Impact of corticosteroid therapy on outcomes of persons with SARS-cov-2, SARS-cov, or MERS-cov infection: A systematic review and meta-analysis. Leukemia 2020;34:1503-11.
10. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
11. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
12. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, *et al*. Presenting characteristics, co morbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 2020;323:2052-9.
13. Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, *et al*. Pulmonary post-mortem findings in a large series of COVID-19 cases from Northern Italy: A two-centre descriptive study. Lancet 2020 June 8. doi: https://doi.org/10.1016/S1473-3099(20)30434–5. Available from: https://www.medrxiv.org/content/10.1101/2020.04.19.20054262v1 (This article is a preprint and has not been peer-reviewed. It reports new medical research that has yet to be evaluated and so should not be used to guide clinical practice.)
14. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, *et al*. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. Histopathology 2020;77:198-209. doi: 10.1111/his.14134. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1111/his.14134.
15. Malani AN, Sherbeck JP, Malani PN. Convalescent plasma and COVID-19. JAMA 2020. Available from: https://doi.org/10.1001/jama.2020.10699.
16. US Food and Drug Administration. Recommendations for Investigational COVID-19: Convalescent Plasma. Available from: https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma. [Last accessed on 2020 Jan 5].
17. American Red Cross. Plasma Donations from Recovered COVID-19 Patients. Available from: https://www.redcrossblood.org/donate-blood/dlp/plasmadonations-from-recovered-covid-19-patients.html.
18. COVID-19 Expanded Access Program: Plasma Donors Needed for Treatment Protocol. Available from: https://www.uscovidplasma.org/.
19. NCBI. General donor assessment – blood donor selection. In: Blood Donor Selection: Guidelines on Assessing Donor Suitability for Blood Donation. Available from: https://www.ncbi.nlm.nih.gov/books/NBK138219/.
20. Shen C, Wang Z, Zhao F,Yang Y, Li J, Yuan J, *et al*. Treatment of 5 critically Ill patients with COVID19 with convalescent plasma. JAMA 2020;323:1582-9.
21. Zhang B, Liu S, Tan T, Huang W, Dong Y, Chen L, *et al*. Treatment with convalescent plasma for critically ill patients with severe acute respiratory syndrome coronavirus 2 infection. Chest 2020;158:e9-13.
22. Ahn JY, Sohn Y, Lee SH, Cho Y, Hyun JH, Baek YJ, *et al*. Use of convalescent plasma therapy in two COVID-19 patients with acute respiratory distress syndrome in Korea. J Korean Med Sci 2020;35:e149.
23. Ye M, Fu D, Ren Y, Wang F, Wang D, Zhang F, *et al*. Treatment with convalescent plasma for COVID-19 patients in Wuhan, China. J Med Virol 2020. doi: 10.1002/jmv.25882. Online ahead of print. Available from: https://pubmed.ncbi.nlm.nih.gov/32293713/.
24. Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, *et al*. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci USA 2020;117(17):9490-6. doi:10.1073/pnas.2004168117. Available from: https://pubmed.ncbi.nlm.nih.gov/32253318/.
25. Rajendran K, Krishnasamy N, Rangarajan J, Rathinam J, Natarajan M, Ramachandran A. Convalescent plasma transfusion for the treatment of COVID-19: Systematic review. J Med Virol 2020:1-9. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1002/jmv.25961.
26. Fischer JC, Zänker K, Griensven MV, Schneider M, Milles DK, Knoefel WT, *et al*. The role of passive immunization in the age of SARSCoV2: An update. Eur J Med Res 2020;25:16.
27. Lai ST. Treatment of severe acute respiratory syndrome. Eur J Clin Microbiol Infect Dis 2005;24:583-91.
28. Worldometer. Coronavirus (COVID-19) Mortality Rate. Available from: https://www.worldometers.info/coronavirus/coronavirus-death-rate/. [Last accessed on 2020 May 14].
29. Wikipedia Contributors. 2002–2004 SARS outbreak. In: Wikipedia, The Free Encyclopedia; May 30, 2020. Available from: https://en.wikipedia.org/w/index.php?title=2002%E2%80%932004_SARS_outbreak&oldid=959728106. [Last accessed on 2020 May 31].
30. Soo YO, Cheng Y, Wong R, Hui DS, Lee CK, Tsang KK, *et al*. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. Clin Microbiol Infect 2004;10:676-8.
31. Cheng Y, Wong R, Soo YOY, Wong WS, Lee CK, Ng MHL, *et al*. Use of convalescent plasma therapy in SARS patients in Hong Kong, Eur J ClinMicrobiol Infect Dis 2005;24:44-6.
32. New Delhi: Indian Council Medical Research. Fact news:ICMR initiates multi-centre clinical trial to assess safety of convalescent plasma to limit coronavirus related complications. Available from: https://www.aninews.in/news/national/general-news/icmr-initiates-multi-centre-clinical-trial-to-assess-safety-of-convalescent-plasma-to-limit-coronavirus-related-complications20200507010451/.
33. New Delhi: Indian Council of Medical Research. Information on COVID-19. Website:https://main.icmr.nic.in/content/covid-19, then go to: List of approved institutions for PLACID Trial (dated 22/05/2020). Available from: https://main.icmr.nic.in/sites/default/files/upload_documents/PLACID_List_22052020.pdf
34. Recommendations for Investigational COVID-19 ... - FDA, Recommendations for Investigational COVID-19 Convalescent Plasma, May 1, 2020. Available from: https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma.
35. Investigational COVID-19 Convalescent Plasma | FDA, GUIDANCE DOCUMENT: Investigational COVID-19 Convalescent Plasma-Guidance for Industry, MAY 2020. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/investigational-covid-19-convalescent-plasma.
36. Ghosh S, Ghosh PK. Effective SARS-CoV-2 vaccines on the horizon. J Vaccines Vaccin 2020;11:428. doi:10.35248/2157-7560.20.11.428. Available from: https://www.longdom.org/open-access/effective-sarscov2-vaccines-on-the-horizon.pdf.