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Convalescent Plasma – Is it Useful for Treating SARS Co-V2 infection?

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Abstract

The world is challenged with the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic. Although preventive measures such as social distancing, personal protective equipment and isolation would decrease the spread of the infection, a definitive treatment is still under way. Antivirals, immunisation, convalescent plasma (CP) and many more modalities are under trial, and there has been no definite answer to the management of COVID-19 infection. All patients so far have received the standard and symptomatic care. It is shown that the SARS-CoV 2 is a respiratory pathogen, and 80% of the infected patients would recover from the illness and it is the 20% of the infected patients require hospitalisation and even critical care. CP has been used to treat recent epidemic respiratory infections such as Middle East respiratory syndrome and severe acute respiratory syndrome (SARS) infections with promising results. The CP of a recovered individual contains antibodies which neutralise the virus and decrease the viral replication in the patient. It is a classic adaptive immunotherapy and has been applied in the prevention and treatment of many infectious diseases. CP is plasma taken from a person who has recovered from an infection, which contains neutralising antibodies against the said infection. Giving CP to susceptible individuals or infected patients is a form of passive antibody therapy and in the case of SARS-CoV-2, is expected to provide protection by viral neutralisation and antibody-dependent cytotoxicity and phagocytosis. The adaptive response is to a specific antigen-binding array of molecules that are foreign to the host. The human response to viruses uses both the innate and the adaptive arms in its attempt to rid the host of the invading pathogen. The humoral response is a component of the adaptive immune response that allows for antibodies to bind to foreign invading pathogens, marks the pathogens and their toxins for phagocytosis and recruits further phagocytic cells to the site via the activation of the complement system and eventually prevents the pathogen from infecting target cells. Studies from Wuhan from various institutions during the research on COVID-19 infections during December 2019 have also shown promising results. Till date, randomised controlled studies for the use of CP in SARS-CoV-2 infection are lacking, and many countries have invited institutions to participate in clinical trials. The Indian Council of Medical research and the Central Drugs Standard Control Organisation, Government of India, have allowed the use of CP as an investigational drug under a trial basis. Internationally, agencies such as the USFDA, American Association of Blood Banks, European Blood Safety and British Blood Transfusion Society have also come out with various guidelines for the use of CP in COVID-19 infection. This article will review the current guidelines for the use of CP and compare the various guidelines of different agencies.

Keywords: Convalescent plasma, neutralising antibodies, plasmapheresis, SARS-CoV-2

INTRODUCTION

COVID-19 infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pandemic posing a significant global health crisis. The WHO in its COVID-19 situation report 144 dated 12 June 2020,^[1] has stated that around 7,410,510 patients are infected with the COVID-19 virus. The report also states that community transmission is established in about 85 countries, with 418,294 people dying of the disease in the pandemic at the time of writing this document. The issues at hand with the current pandemic

of COVID-19 are the high R_0 with an average of 2.65 (1.97–3.09),^[2] the lack of proven antiviral therapy to combat this viral infection and the vaccine for the infection being in

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its infancy. Prevention and good infection control practices such as frequent hand hygiene, respiratory etiquette, rational use of personal protective equipment, social distancing and effective screening and isolation of patients though in place, may not halt the transmission of infection from one individual to another, both in hospitals and in the community.

Pathogenesis and immunology

It becomes important and prudent to discuss and bring out salient features of the pathophysiology as it may impact the application of convalescent plasma (CP) in the management of COVID-19 patients in the future. The pathogenesis of the virus and the associated immunology occurs in stages or phases.^[3,4]

SARS-CoV-2 is transmitted as a droplet infection, contact with infected individuals and also has a potential for faecal–oral route of transmission. There are two stages of viral replication, an initial phase of replication in the mucosa of the upper respiratory tract and later on in the lower respiratory tract as well as the gastrointestinal mucosa, giving rise to a mild viraemia.^[3] The invasion and pathogenesis of COVID-19 is closely associated with the immune response of the host. Entry into the respiratory tract cells is said to occur via binding of the S glycoprotein of the virus to the angiotensin-converting enzyme-2 (ACE-2) receptors found on human cells. This further happens due to the direct contact of the receptor-binding domain (RBD) of the S proteins of the virus with the ACE-2 receptors on human cells, these being chiefly localised to the lung, heart, kidney and the intestinal cells.^[4] The affinity of the S protein of the SARS-CoV-2 is 10–20 times higher than that of the SARS virus, though structurally the S proteins of the two viruses may be similar.^[5]

Two types of immune responses are met with as a first line of defence against virus invasion namely, innate and adaptive immunity. Innate immunity against most viral infections involves the recognition of pathogen-associated molecular patterns causing cytolysis, killing the virus along with the cells, through the natural killer cells and the interferons. The innate response in the patient becomes manifest as the infection comes down to the lower respiratory tract, and it may be useful to measure the level of an immune response cytokine such as CXCL10.^[3] The measurement of these markers may be predictive of the course of the illness in the next stage. Adaptive immunity on the other hand plays an important role in clearing the infecting virus, either through the cell-mediated immunity via activated cytotoxic T cells (CD8+ and the CD4+ subset) that destroy the virus-infected cells and/or the activation of the B cells with antibody production.^[5] These antibodies may be neutralising antibodies with a specific activity in clearing the virus via neutralisation or non-neutralising antibodies which in turn could prove deleterious, leading to antibody-dependent enhancement (ADE) described later on in the review. An aberrant immune response leading to overproduction of pro-inflammatory cytokines such as interleukin-16 (IL-16) and tumour necrosis factor (TNF) could cause a cascade of cytokines (also called a cytokine storm) with subsequent

tissue damage, dysfunction of many organ systems leading to multiorgan failure and acute respiratory distress syndrome (ARDS).^[5]

Treatment of COVID-19 infection

At the time of writing, there are no proven specific antiviral drugs or vaccines available for the control of the spread of COVID-19 infection. The infection is controlled by symptomatic treatment.^[4] The potential therapeutic agents available to us at the present time are the use of antivirals^[6] (specifically the use of ribavirin, lopinavir, remdesivir and hydroxychloroquine)^[7] and CP. Of these, the present article would focus on the use of CP, with its evaluation having exhibited promising results.^[8,9]

Principles of the use of convalescent plasma in severe acute respiratory syndrome COVID-19 infection

CP has emerged as one of the promising options in the management of patients with COVID-19 infection. This was initiated in China earlier on, however the use of plasma has been in vogue for the earlier outbreaks of Middle East respiratory syndrome (MERS) and SARS infections.

CP refers to the plasma collected from individuals following resolution of infection when they develop antibodies. This may also be called passive antibody therapy through transfusion of CP as this may blunt the clinical severity of the disease. The use of CP in COVID-19 infection is based on historical accounts^[10,11] and classical interventions^[12-16] done previously in infections such as SARS and MERS CoV as well as the Ebola virus infection. It has been used as a therapeutic option when there is a total lack of drugs and vaccines to treat or manage patients. Human plasma has natural proteins, clotting factors and anti-inflammatory cytokines that induce an immunomodulatory effect to reduce the inflammatory response in the patient.^[16] Patients who recover from the COVID-19 infection have antibodies to SARS-CoV-2 that can neutralise the virus in the infected individual. This is almost similar to the setting of passive immunisation used to treat patients with other infectious diseases.^[17]

Neutralising antibodies are very important for the clearance of the virus. Antibodies to the spike (S) glycoprotein, also called the anti-S-specific antibodies, block the binding of the S protein to the cellular receptor h ACE2 that mediates binding of the virus and entry to the target cells.^[18] These neutralising antibodies are present in CP that help contain the severity of the infection. The efficacy of plasma therapy supposedly depends on the concentration of neutralising antibodies present in the plasma of recovered donors. There are variations in the neutralising antibodies in patients who have recovered from COVID-19 infection, particularly with respect to the titre of antibodies, and around 30% of the patients may not have neutralising antibodies in their plasma.^[19] This variation may depend on age, lymphocyte counts and the C-reactive protein in the blood of individuals. Hence, it becomes clear that there are other components of the plasma that contribute to recovery in these patients.^[17] Antibodies could be of the IgM, IgG and

IgA types directed at the spike (S) protein. There is a sequential switch of the antibody idiotype that binds to the RBD of the S protein of the virus. The earliest antibody belongs to the IgM isotype which later switches to the IgG isotype. The class switching may also happen to the IgA isotype which is largely produced by the plasma cells in the lamina propria of the mucosal surfaces. The IgA antibodies play a very important role in the immune defence of mucosal surface of the respiratory tract, the point of entry of the virus, making it an important immunoglobulin with respect to mucosal immunity. IgA could neutralise the SARS-CoV-2 before they reach and bind to the epithelial cells. IgA may have a role in a mucosal vaccine.^[20]

Immunomodulation and convalescent plasma

In addition to the direct antiviral effect of CP, it has also been shown that patients with COVID-19 infection who are critically ill exhibit anti-cardiolipin IgA antibodies as well as the anti- β -2 glycoprotein I IgA and IgG antibodies. Thus, the plasma of such patients recovering from COVID-19 infection would contain these antibodies that would neutralise the autoantibodies, reducing the incidence of thrombotic events such as the antiphospholipid syndrome-like disease, particularly in critically ill patients.^[21,22] In addition, some antibodies inhibit the complement cascade (C3a and C5a) and limit the formation of immune complexes. The systemic inflammation, secretion of inflammatory cytokines and migration of neutrophils occur to the lung with tissue damage. Hence, infusion of CP could neutralise cytokines such as TNF- α and IL-1 β .^[23]

Overview of collection of convalescent plasma in blood banks in India

Traditionally, plasma is collected from healthy blood donors at blood bank centres, either by a whole blood donation or by plasmapheresis. Plasma collected from a whole-blood donation is 200 to 250 ml in volume and can be stored for a period of 1 year at a temperature of $\leq -40^{\circ}\text{C}$. This is called fresh frozen plasma (FFP). On the other hand, plasmapheresis is a procedure carried out with the help of a cell separator wherein the plasma from the donor alone is collected and all other components such as red cells, platelets and white blood cells are sent back to the donor's circulation. This is carried out as an automated procedure in a sterile manner with a disposable kit. Blood banks have a choice of collecting CP by either of the two procedures, however plasmapheresis is preferred, as the advantage of collecting plasma by this method being, a volume of 500 ml can be collected in a single procedure and the same donor can be requested to donate plasma once a fortnight. This is achieved well if the plasma protein levels are maintained well within limits. In contrast, plasma obtained from whole-blood donation is 200–250 ml (lesser volume compared to that obtained from plasmapheresis) and the donation can be made once in 3 months.^[24]

The Indian Council of Medical Research (ICMR) and the Central Drugs Standard Control Organisation have proposed that CP can be used as a drug on a clinical trial basis activity.

The general plan involves counselling and motivation of all COVID-19 patients at the time of discharge from the hospital towards donation of CP after 28 days from the time of onset of symptoms if the patient continues to remain asymptomatic. The utility of CP in treating and managing moderate and severe COVID-19 disease is explained to these patients, who might at a later date come back as plasma donors. The same patients are contacted to come back by day 25 of their discharge from the hospital. At this time, they are encouraged to visit the blood bank centre for counselling and evaluation for their eligibility to donate plasma.

While it is felt that CP would be of benefit to patients suffering from COVID-19 infection, the safety and efficacy of the CP therapy as a treatment modality for COVID-19 remains to be proven with time. The Organising Committee of the International Society of Blood Transfusion Working Party on Global Blood Safety in April 2020^[25] brought out a document with points which one should consider in the preparation and transfusion of COVID-19 plasma in low- and middle-income countries (LMICs) of the world. This largely revolves around the resource limitations in the donation, preparation, quality assurance and safety evaluation and that of the efficacy of the plasma therapy in LMICs, the need to protect both the donor and the recipient in the whole process of collection and transfusion of the product to patients with COVID-19 infection under the supervision of regulatory authority and systems in the LMICs. However, India does have a robust regulatory system to oversee the functioning of blood banks and accreditation systems are in place such as the National Accreditation Board for Hospitals & Healthcare Institutions which also accredits clinical transfusion of patients in hospitals and healthcare institutions.

Donor recruitment and eligibility for convalescent plasma donation

The essential criteria for plasma donation involves a patient who has made complete recovery from COVID-19 infection. This would not be too difficult a task, given the magnitude of the problem as it exists in India at the present time. However, a question that comes to one's mind is 'What constitutes a CP donor?'. Total reliance in recruiting the potential donor based on symptoms may be misleading. Hence, there is a need for documentation of the COVID-19 infection and recovery from the same. Contacting potential donors could be done by telephone, electronic media or by using outreach methods such as advertising and communication through social media. The flip side of this approach could be a breach of patient confidentiality and privacy which must be protected at all times.

The most optimal algorithm for the pre-donation screening of potential donors is still evolving at the time of writing this document. Hence, one does expect heterogeneity in approach and testing protocols and strategies at the present time. It is proposed that potential donors are screened with a questionnaire for a history of COVID-19 infection suffered in the recent past,

which is confirmed with a reverse transcription-polymerase chain reaction (RT-PCR) assay on a nasopharyngeal swab. This patient should have passed 14 days after the complete resolution of symptoms such as fever, cough and shortness of breath and a negative molecular test for SARS-CoV-2. This is important as the potential donor must be free of the viral active infection at the time of plasma collection.^[24,26] This is because they pose a risk to the blood centre staff during the procedure. The donor criteria for donation of plasma are as follows (criteria laid down by the ICMR).^[24]

1. Prior diagnosis of COVID-19 documented by a laboratory test and
2. Complete resolution of symptoms and at least one negative lab test for COVID-19 at 28 days prior to donation
3. If plasma is collected prior to 28 days after full recovery from illness, then confirmation of the resolution of the infection should be obtained through demonstration of two non-reactive nucleic acid tests (NAT) for SARS-CoV-2 performed at an interval of at least 24 h on nasopharyngeal swabs
4. Titration of anti-COVID-19 (both IgG and IgM) antibodies and SARS-CoV-2 neutralising antibodies may be done depending on the availability of facilities at the time of testing (desired titres for IgG antibodies >1024 or neutralising antibodies > 40) doubling dilution of donor serum will be done and titration will be done using ELISA. If not done at the time of plasma collection, the donor samples will be stored in aliquots at <-80°C to be tested at a later date
5. Only males and nulliparous female donors of weight > 55 kg will be included
6. Donor eligibility criteria for whole-blood donation as per the departmental standard operating procedure (SOP) will be followed in accordance to the Drugs and Cosmetics Act 1940 and rules 1945 therein (as amended till March 2020)^[27]
7. Donor will be screened, followed by a brief physical examination
8. Donors not fit to donate blood based on the history and examination will be deferred and excluded from plasma donor pool for a time period specified by country

regulation and departmental SOPs

9. Blood group and antibody screening – Antibody screen positive donors will be deferred
10. Complete blood count including haemoglobin (Hb), haematocrit, platelet count and total leucocyte count (TLC) and differential leucocyte count
11. Donors with Hb >12.5 g/dl, platelet count >150,000/µl of blood and TLC within normal limits will be accepted
12. Screening for HIV, HBV and HCV by serology and NAT, syphilis and malaria by serology. Negative donors will be included
13. Total serum protein. Donors with total serum protein >6 g/dl will be accepted
14. Presence of IgG antibodies to COVID-19 by rapid test as per manufacturer’s instruction. Donors negative for these will be deferred.

A comparison of donor recruitment criteria as proposed by different expert groups across the world is summarised in Table 1.

Antibodies to the SARS CoV-2 of clinical significance belong to the IgG class. These start to increase around 10 days after the onset of symptoms, and most patients are expected to have achieved seroconversion within the first 3 weeks. The level of antibodies against the internal nucleoprotein (NP) and the S glycoprotein is said to correlate well with the neutralising antibodies and the neutralisation of the virus. Hence, the timing of antibody seroconversion appears to be crucial for collection of serum specimens of patients for a diagnosis and selecting potential CP donors.^[31] Titration of anti-COVID-19 antibodies and SARS-CoV-2 neutralising antibodies may be done depending on the availability of facilities at the time of testing (desired titres for IgG antibodies >1024 or neutralising antibodies >40) doubling dilution of donor serum will be done, and titration will be done using ELISA. If not done at the time of plasma collection, the donor samples will be stored in aliquots at <-80°C to be tested at a later date. A study by Kevin To *et al.* from Hong Kong focussed on determining serial viral loads, antibody kinetics and analysis of the viral genome of patients with SARS-CoV-2. They found the antibodies to rise significantly after 10 days of the onset of symptoms; the serum

Table 1: Donor recruitment criteria

	ICMR ^[24,27]	US FDA ^[28,29]	EU ^[25,26]	NHS UK ^[30]
Weight	>55 kg	>45 kg	>50 kg	>50 kg, >35 years
Gender	Male Nulliparous female	Male Nulliparous female If multiparous, negative for HLA	Male Nulliparous female If multiparous Negative for HLA/anti-HPA HNA ab	Male Nulliparous female If multiparous Negative for HLA/anti-HPA HNA ab
Donation interval	14 days	4 weeks for serial plasmapheresis	14 days	15 days
Collecting dose	500 ml	>68 kg 800 ml<68 kg 600 ml	600-800 ml	600 ml

All guidelines state that the donor must have recovered from COVID-19 at least 14 days prior and the IgG antibody must be positive and nasopharyngeal RT-PCR for SARS-CoV-2 must be negative. RT-PCR: Reverse transcription-polymerase chain reaction, SARS-CoV: Severe acute respiratory syndrome-coronavirus

IgG antibody levels to rise at the same time or even earlier than the IgM antibodies and the anti-S, anti-RBD IgG and the anti-NP antibody levels to correlate well with the virus neutralisation titre of $R^2 > 0.9$.^[32] The anti-RBD IgG antibodies in particular are purported to be useful as they measure the extent to which viral entry into the cells is blocked. Hence, these antibodies may be specifically useful in selecting potential plasma donors. On the other hand, it has also been found that non-RBD-specific anti-S antibodies may not neutralise the virus and could also prove to be a reason for antibody-dependent immune enhancement (ADE), contributing to increased severity of the infection.^[33]

Whilst NATs detect the SARS-CoV-2 RNA and aid the diagnosis of the infection, developments in serology assays would allow identification of individuals with a robust antibody response capable of neutralising the virus. This would help identifying those individuals who have suffered the infection before, who could now serve as CP donors for patients with active infection.^[34]

The neutralisation assay for specific antibodies in the donor

The principle behind using CP in SARS-CoV-2 infection is the ability of the plasma to neutralise the virus in the patient. Various assays are being used for identifying the neutralising antibodies exemplified by plaque reduction neutralisation assays. All micro-neutralisation assays require a minimum of Biosafety level III. However, they are not amenable to high-throughput testing, incur a long turnaround time and results are expected after 5–7 days. The assays are technically demanding, handled by dedicated virology laboratories, yet have variations between results. The efficacy of the plasma therapy is directly related to the concentration of the neutralising antibodies in the donor plasma. The usual procedure for the measurement of neutralising antibodies involves the use of Vero 6 cells coated onto wells and then a mix with the serum and the live virus to a calculated quantity.^[35,36] However, this method involves the use of live virus which may be pathogenic and requires to be done under containment level III and is not within the realms of the clinical laboratory. Another assay that has been developed without the need for a live virus is the pseudotyped – lentiviral-vector-based neutralisation assay. Also called the PsV neutralisation assay, this is a sensitive, reproducible assay and does not involve the use of a highly pathogenic virus, hence can be used in a laboratory with a Biosafety Level-2.^[37] Given the issues and technical complexities of neutralisation tests, fewer laboratories handle these assays. Increasingly, CP pre-transfusion testing would depend on assay to determine antibodies in the donor's serum and quantitative measurement of the same when this is possible. There are various formats for analysing the antibody response such as ELISA,^[34] Biotin-avidin assay^[38] or the iFlash Chemiluminiscent immunoassay.^[39] Fatima *et al.*^[34] were able to demonstrate a strong seroconversion as evidenced by high titres of antibodies (close to an area under the curve value of 1:1000 in their ELISA) and a good correlation of these with a neutralisation assay titre of 1:160. They along with another investigative group^[40] also reported a number of such specimens with high antibody titres on ELISA to correlate with strong

neutralising activity. There have also been improvements in the ELISA technique to include a number of biotinylated spike antigens and detect antibodies in convalescent patient's sera that specifically bind to ACE-2 spike RBD interface.

Various agencies and regulatory bodies appear to recommend different levels of antibodies as adequate for plasma therapy [Table 2].

However, if the neutralisation assay is not available for some reason at the time of plasma collection and transfusion, then the next best alternative would be to aliquot 10 ml × 0.5 ml of the plasma taken at the time of donation, stored at –80°C for testing in the future at a location where facilities are available. This could be used for correlation with the clinical response or the lack of it in patients with COVID-19 who were transfused with the plasma. This has been well brought out in the European programme of CP collection and Transfusion.^[41]

The process of donation and collection of plasma

Plasma is collected from the donor who is eligible by plasmapheresis. Around 500 ml is collected from each procedure. The plasma is split as 200 ml in two different bags and one bag with 100 mL of plasma. The donor is placed under observation for the usual adverse reactions such as vasovagal syncope, haematoma and hypocalcaemia. Once the plasma units are collected, there must be a process of labelling with a specific code or an identifier which would indicate that the plasma unit has been collected and is intended for use only in COVID-19 patients. This assures complete traceability, which is important in inventory management.

Selection of patients for transfusion

There is no conclusive evidence to establish indications for transfusion of plasma to patients with COVID-19 infection. The indications cited here are based on a number of observations made by different studies and researchers around the world. Most of the consensus of experts have been from Asia, hence the same may have been adapted by India and the ICMR. A risk stratification has been done based on many clinical studies,^[42] and the parameters brought forth are as follows by the ICMR:^[24]

- Age >18 years
- ABO-compatible recipient with the donor
- RT-PCR-confirmed COVID-19 infection in the patient
- Written informed consent

Table 2: Product criteria

	ICMR ^[24,27]	US FDA ^[28,29]	EU ^[25,26]	NHS UK ^[30]
Neutralising antibodies	>1:40	>1:160	>1:160	>1:100
Neutralising antibodies to be done	When facility is available	Before transfusion	Before transfusion	Before transfusion
Storage	<–40°C	<–18°C	<–20°C	<–20°C
Shelf life (year)	1	1	1	1
Pathogen inactivation	Not required	Not required	Required	Not required

- Partial pressure of arterial oxygen (PaO₂)/fraction of inspired oxygen (FiO₂) <300 mmHg in oxygen
- Respiratory rate of >24/min
- Blood oxygen saturation <93%.

Exclusion criteria while selecting patients for transfusion are as follows:

- Pregnant women
- Breastfeeding women
- If there is a known hypersensitivity to blood products
- If the recipient has received pooled immunoglobulin in the last 30 days
- If the patient is critically ill with (a) P/F ratio <200 (indicates moderate-to-severe ARDS) (b) shock (requiring vasopressors to maintain a mean arterial pressure of ≥65 mmHg or below 65)
- If the patient is participating in another clinical trial
- If the clinical status of the patient precludes infusion of blood products.

Criteria as laid down by the International Society of Blood Transfusion working party while selecting patients for transfusion^[43]

Inclusion criteria

- Laboratory-confirmed COVID-19 infection
- The patient must be suffering from a severe or a life-threatening COVID-19 infection.

The severe disease is defined as:

- Dyspnoea
- Respiratory frequency >30/min
- Blood oxygen saturation of <93%
- PaO₂ to FiO₂ ratio <300
- Lung infiltrate >50% within 24–48 h.

Life-threatening disease is defined as

- Respiratory failure requiring mechanical ventilation
- Septic shock
- Multiple organ failure including receipt of renal replacement therapy or extracorporeal life support
- Age of the patient ≥18 years
- The patient must be able to provide an informed consent.

Exclusion criteria

- Negative RT-PCR from respiratory secretions
- History of allergic reaction to blood or plasma products (as judged by the investigator)
- Medical conditions in which receipt of 500 ml intravascular volume may be detrimental to the patient (e.g., actively decompensated congestive cardiac failure)
- Severe multiorgan failure and haemodynamic instability
- Other documented uncontrolled infection
- Severe Disseminated Intravascular Coagulation requiring factor replacement, FFP, cryoprecipitate
- If the expected survival is <48 h.

Transfusion process, dose and the frequency

The volume, dose and the schedule are based on what has been described in previous epidemics and on a consensus of opinion

from experts. This is because there is no conclusive evidence for the actual dose. The actual dose and the schedule may depend on the indication (prevention vs. treatment of patients). A study in China^[44] used a single unit of plasma of 200 ml. However treatment protocols may use at least two units of plasma. The titre of neutralising antibodies based on a neutralisation assay also influence the dosing and the volume of plasma used in patients.^[44] The steps that may be followed would be as follows:

- Request from the clinical department managing the case
- An ABO-compatible plasma unit of 200 ml would be thawed and issued as per the blood bank issue protocols
- The patient is transfused and monitored for any adverse reactions and clinical response
- Transfusions must be carried out using transfusion sets
- The second plasma unit would be issued based on need (severity of the disease, response and tolerance of the patient)
- A maximum of two units of plasma is transfused to the patient in 24 h. Higher volumes exceeding 600 ml/day are contraindicated due to the risk of transfusion-associated circulatory overload (TACO).

The second plasma unit should preferably be from a different donor to the first one, as that may have the chance of higher antibody titre. However, this would depend on the availability of an ABO-compatible plasma unit. If this is not available, the plasma from the first donor is given. The product and patient criteria as proposed by different expert groups are summarised in Tables 2 and 3.

Risks of plasma transfusion

The risk of plasma transfusion to recipients is expected to be no different from those of routinely done plasma transfusion in hospitals. While there is always a risk of transmission of blood-borne viruses as pathogens, there are non-infectious hazards of plasma transfusion such as allergic transfusion reactions, anaphylaxis, TACO and transfusion-related acute lung injury (TRALI). TRALI though uncommon in occurrence and frequency, is still important as a risk in the COVID-19 setting on account of the previous damage to the pulmonary alveolar cells and the endothelial cell lining of the lung capillaries. Volume management remains an important challenge in this setting due to the risk of TACO.^[45]

A largely theoretical risk of transmission of the COVID-19 virus is through plasma transfusion. because < 1% of the patients with the SARS-CoV-2 infection are reported to have detectable virus in their blood. However, no study till date has reported the transmission of this viral infection through blood and blood products. In addition, it is not a common practice to include screening for respiratory viruses such as influenza, parainfluenza and respiratory syncytial virus as a part of donor blood screening before transfusion of the product.

Antibody Dependent Immune Enhancement (ADE)

ADE is a known immunological phenomenon with some of the viruses such as Dengue virus infection where the intensity of the current infection increases with the presence of pre-existing poorly neutralising antibodies (which would

Table 3: Patient criteria

	ICMR ^[24,27]	US FDA ^[28,29]	EU ^[25,26]	NHS UK ^[30]
Age	>18	All ages	All ages	All ages
Respiratory rate	>24/min	>30/min	>30/min	Positive RT-PCR of SARS-CoV-2 ICU admission
P/F ratio Hg in oxygen	<300	<200	<300	Decided by the treating physician
O ₂ saturation	<93%	<93%	<93%	Decided by the treating physician
dose	200 ml followed by 200 ml after 24 h	270-300 ml	8-10 ml/kg with a maximum of 600 ml/dose for a maximum of 3 days	270 ml followed by another 270 ml after 12 h
When to transfuse	Early disease	Early and progressive	Within 7 days of disease	Early and progressive

P/F: PaO₂/FiO₂, ICU: Intensive care unit, RT-PCR: Reverse transcription-polymerase chain reaction, SARS-CoV: Severe acute respiratory syndrome-coronavirus, PaO₂: Partial pressure of arterial oxygen, FiO₂: Fraction of inspired oxygen

have developed in response to a previous infection). These would help replication of the virus within macrophages and other cells through interaction with F_c and complement receptors. *In vitro* assays have demonstrated that this will be mediated in SARS-CoV-2 through antibodies to the spike protein, hence apoptosis in these cells.^[46,47] This is a postulated mechanism to explain the difference in severity of the infection in various regions of the world. The quality and amount of antibody response in a patient would help decide the final outcome of the patient. Antibodies may be protective when they are able to neutralise the virus or could be pathogenic when they produce deleterious effects and contribute to enhanced pathogenicity and inflammation in the host. Multiple factors would determine the nature of the antibodies such as concentration, specificity, affinity and isotype of the antibody. Antibodies to the two major proteins namely S and N proteins are described. In one study carried out in immunised mice, the anti-N antibodies showed a significant upregulation of pro-inflammatory cytokine secretion with increased lung parenchymal infiltration by neutrophils and eosinophils and a severe lung pathology.^[47] However, this process when extrapolated to human physiology, though causing an enhancement of the disease process, does not contribute to increased transmissibility of the infection as the ADE process is independent of ACE-2 expression, no productive viral replication or shedding.

A risk-benefit analysis model was constructed with a stochastic age-specific susceptible-exposed-infected-removed model of COVID-19 transmission that reflected the demography of Baltimore city. This analysis showed a definite benefit from treatment or prophylaxis with CP with conservative estimates of efficacy applied in the model under study.^[44]

Grey areas for the use of convalescent plasma in COVID-19 – A cause for concern

CP is not a proven treatment for COVID-19, and it is under investigation. There is a chance that passive transfer of antibodies might enhance hyperinflammation in some patients. A theoretical risk related to virus-specific antibodies, which when

transferred with CP, can cause ADE of infection. Neutralising antibodies in the CP have to be done prior to transfusion as there are reports that about 30% of patients have very low titre of neutralising antibodies or even undetectable. Furthermore, the transfer of coagulation factors present in plasma products is potentially harmful for people with COVID-19, who are already at an increased risk of thromboembolic events.^[48] There is no established guideline on what one does with the collected plasma, in situations when patients no longer require the product. The severe life-threatening reactions such as TRALI, TACO and anaphylaxis during or within few hours of transfusion must also be taken into consideration. At the present time, most of the published studies on the use of CP for COVID-19 infection are based on small study groups from individual institutions and there are no randomisation, no control groups to compare and no concurrent treatment with antivirals and steroids.^[49,50] There has been one randomised trial published recently on the effect of plasma therapy with respect to time to clinical improvement in patients with severe and life-threatening COVID-19 infection. This randomized clinical trial found that CP therapy when added to standard treatment, does not significantly change the time to clinical improvement within 28 days.^[50] The assays used to estimate antibodies in the donor plasma have a high degree of variability with very little standardization. It is a debatable point as to what constitutes an acceptable threshold for donation, given the fact that specific antibody profiles are not well correlated with clinical outcomes of patients.^[51] Hence, the use of CP does require high-calibre studies without which the efficacy for its use in COVID-19 infection will continue to be in question.

CONCLUSION

Human plasma from recovered COVID-19 patients appears at the present time to be a promising therapeutic option, which, in combination with other measures, may help reduce the morbidity of the infection and the number of ICU and hospital days for the patient. However, the efficacy of this therapeutic modality can be adjudged best with the help of well-designed clinical trials in this country, which would hopefully cover for confounding variables in these patients and help us arrive at some useful conclusions.

The advent of the newer investigational antiviral agents such as remdesivir and favipiravir does place the role of CP in a position where it may complement other forms of therapy.

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Conflicts of interest

There are no conflicts of interest.

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