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Convalescent plasma – An insight into a novel treatment of covid-19 ICU patients

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ABSTRACT

Introduction: Various therapies have been tried for Covid disease including the use of antivirals, steroids, monoclonal antibodies and convalescent plasma. Method: The study was conducted on convalescent plasma transfused ICU patients. Part A of the study involves clinical outcomes based on gender, age, comorbidities, blood group,and the average length of stay. Part B investigates clinical outcomes in patients transfused with convalescent plasma before and after the November 2021 guidelines. Part C of the study includes patients in cytokine storm and the efficacy of tocilizumab in these patients.Result: Out of the 326 ICU patients transfused with convalescent plasma the overall mortality was 152 (53.3 %). On comparing blood groups and clinical outcomes, a clinically significant result was found. A clinically significant association was also seen on comparing the clinical outcome of 18–50 years and 61–70 years age group and in female gender patients. The average number of ICU days had a positive impact on the overall patient survival. Out of the patients in 'cytokine storm' (n = 109), on day 20, the survival percentage in the non-Tocilizumab group showed a downward trend throughout. However, in the Tocilizumab group, the survival percentage remained stable throughout till around day 50. Conclusion: Amongst the convalescent plasma transfused ICU patients, females, having blood group B, and an average length of stay of fewer than 20 days had a better chance of survival. The patients given tocilizumab and convalescent plasma had a better chance of survival compared to tocilizumab alone.

1. Introduction

On 11th of March 2020, the World Health Organization declared the Covid-19 as a pandemic. Emerging from Wuhan, the capital city of Hubei province in central China, in December of 2019, it has caused more than 3.5 million deaths worldwide as of June 2021 [1]. The first case recorded in India was in January 2020 [2]. COVID-19 is caused by a single-stranded +ss RNA beta coronavirus. It may present mild, moderate, or severe illness. Among the severe clinical manifestations, there is severe pneumonia, ARDS, as well as extrapulmonary manifestations, and systemic complications such as sepsis, and septic shock. The treatment for the coronavirus disease is uncertain, ever-evolving, and empirical in the form of multivitamins, steroid cover, antivirals, and convalescent plasma (CP).

Due to the lack of evidence for the treatment of COVID-19 and vaccines, classical and historical interventions remerged as options for the control of diseases [3]. CP therapy has been used as a strategy of passive immunization in the prevention and management of infectious diseases since the early 20th century [4]. CP is obtained using apheresis in survivors with prior infections caused by pathogens of interest in whom antibodies against the causal agent of disease are developed. The major target is to neutralize the pathogen for its eradication [5]. CP has been considered an emergency intervention in several pandemics, including the Spanish flu, SARS-CoV, West Nile virus, and Ebola virus [6–10]. CP when administered early showed a reduction in mortality compared with placebo or no therapy in severe acute respiratory

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infections of viral etiology like influenza and SARS-CoV. However, a similar response to the Ebola virus disease was not observed [11]. The United States Food and Drug Administration (FDA) approved CP as a treatment modality for ICU patients on March 24, 2020 [1].

CP therapy in the initial phase of the pandemic had several unanswered questions. These included lack of experience in this novel therapy, optimum utilization of the product, an adequate volume of the product, usage based on the severity of disease, the interval between transfusions, the titer of the patients and the donors, the role of demographics in CP therapy and optimal donation time.

This study was done to have a better understanding of this novel therapy and to find the optimal usage within the evolving guidelines.

2. Materials and methods

Patient and donor population.

This is a single-center, retrospective, observational study conducted on CP transfused ICU patients in a tertiary care hospital in north India from March 2020 to July 2021. All patients detected to be SARS-CoV-2 RNA positive by nasal and pharyngeal swab sample using a SARS-CoV-2 nucleic acid detection kit (Argene RT-PCR Kit, Biomeuriex) and whose medical health records were retrievable were included in the study. Donor assessment was done according to the guidelines issued by the government of India for donor eligibility criteria for COVID-19 convalescent plasma [12]. All donors were screened for HBsAg, anti-HCV, anti-HIV, malaria, and syphilis. Approximately 500 mL of plasma was collected from donors by therapeutic apheresis procedure using Haemonetics MCS plus - Extended storage plasma apheresis set REF 995 E after taking informed consent. The CP was labeled, separated into 2 aliquots, and stored at or below −20 °C in deep freezers. The CP was selected on the basis of 'first in, first out policy 'after ensuring group compatibility between the patient and donor. The 2 aliquots were issued on consecutive days to the same patient. Informed and written consent was obtained from the patient or attendant before issuing CP. The clinical decision to transfuse the CCP was based on the existing guidelines and amendments thereof. The study population included ICU admitted COVID-19 patients, positive for SARS-Co-2 detected by real time polymerase chain reaction (RT-PCR), with written informed consent and should fulfill any of the two criteria i.e.

a. PaO2/ FiO2: 200–300.

b. Respiratory Rate > 24/min and SaO2 < 93 % on room air.

However, the patients with known hypersensitivity to blood products, receipt of Pooled Immunoglobulin in last 30 days, having contraindications to blood products, pregnant or breast feeding women,

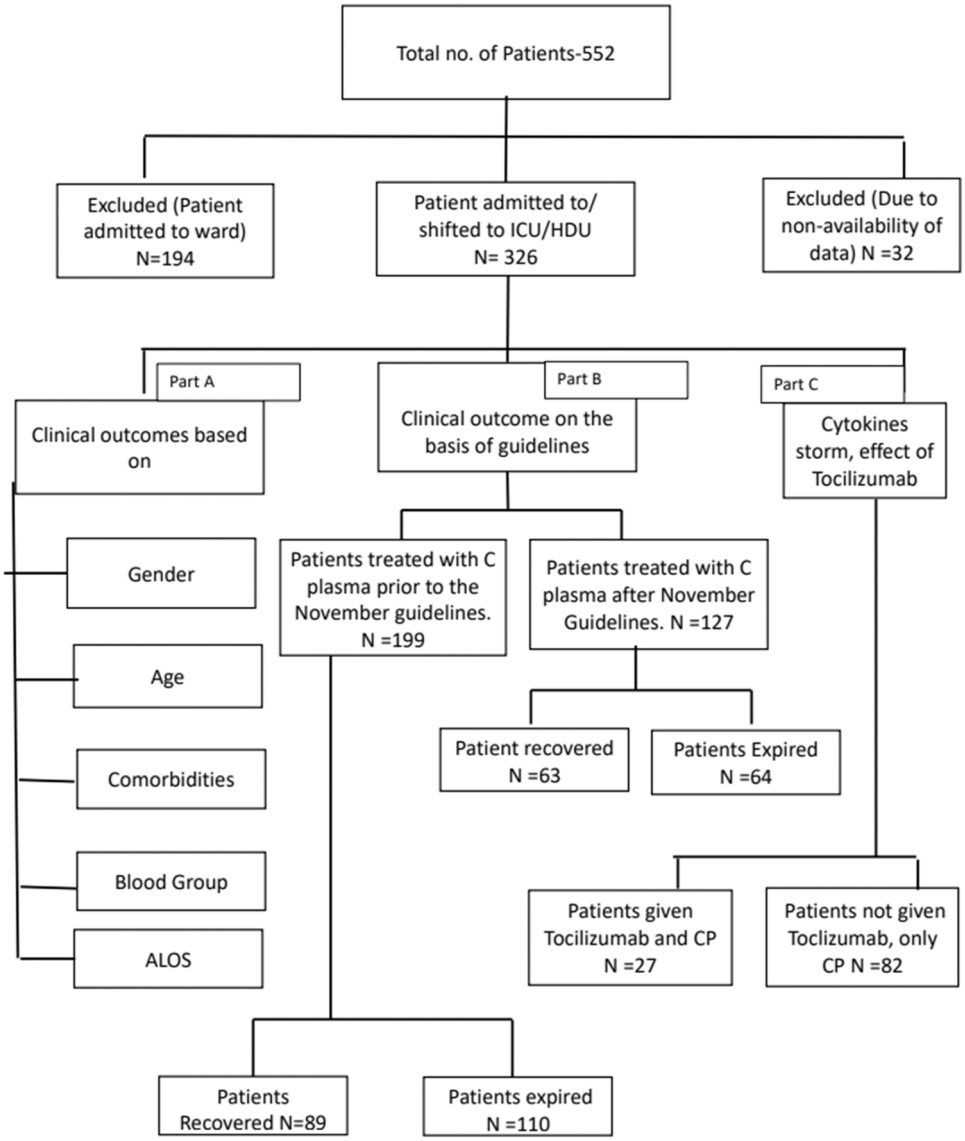

Fig. 1. Flow chart of study design.

respiratory failure caused by illness other than SARS-CoV-2, with other documented uncontrolled infection, severe DIC, TTP, or antithrombin III deficiency needing factor replacement, FFP, cryoprecipitate, active intracranial bleeding, and clinically significant myocardial ischemia are excluded from the study.

Study design.

The study was done on ICU patients transfused with CP and was divided into 3 parts viz. Part A, B, and C (Fig. 1). In part A of the study, the clinical outcome was assessed based on gender, age, comorbidities, blood group, and the average length of stay (ALOS) of the CP transfused ICU patients. In November 2020, based on the existing literature and experience on Covid-19, the Government of India, Ministry of health and family welfare issued the amended guidelines specifying the patient and donor criteria for transfusion of CP [12]. In part B of the study, the CP transfused patients were divided based on guidelines issued in November 2020, into pre-guidelines and post guidelines and the clinical outcome of these patients was studied to assess the effectiveness of the new guidelines. Recipient criteria for transfusing the CP according to the pre and post-November guidelines are summarized in Table 1.

'Cytokine storm' syndrome was defined as cutoff values of IL-6 level > 80 pg/mL followed by CRP level > 97 mg/L[13]. 'Recovered patient' was defined as a patient being shifted out from ICU to ward or being discharged directly. In part C of the study the patients who were in 'cytokine storm' were compared for the clinical outcome of tocilizumab and CP Vs CP alone. However, few patients in 'cytokine storm' could not be given this drug and were therefore excluded from this part of the study. These patients were those with active hepatic disease, absolute neutrophil count < 500 and platelet count $< 50{,}000$, and patients who refused the drug due to personal reasons [13]. All patients were followed up for 1 month after discharge by teleconsultation.

Inclusion criterion.

All patients admitted to the ICU with complete documents and demographic details who received CP therapy at our hospital were collected and tabulated.

Statistical analysis.

Table 1
Comparison of pre and post-November COVID.

	Pre-Guidelines (17/11/2020)	Post Guidelines (17/11/2020)
• Donor	> 18 years of age Males or nulliparous female donors of weight > 55Kg Prior diagnosis of COVID-19 documented by a laboratory test (RT-PCR) with symptomatic disease with at least fever and cough and Complete resolution of symptoms at least 28 days before donation OR Complete resolution of symptoms at least 14 days before donation & two negative real-time PCR test for COVID-19 collected 24 h apart.	Age group of 18–60 year Only Men, Women who have never been pregnant were allowed to donate c plasma of Appropriate Body Weight > 50 kg Diagnosis COVID-19 RT-PCR positive or Rapid Antigen Test positive Normal physical Status After 14 days of symptom resolution (testing negative for COVID-19 is not necessary)
• C plasma Antibody titer	• Antibodies should be present. • No cut off titer.	Required Concentration - IgG antibody against COVID-19 Titre of 1:640 (ELISA) OR - 13 AU (Arbitrary Unit)/mL9 (CLIA) OR - Neutralizing Antibody Titres of 1:80 (PRNT/MNT)
• Patient criteria	• Day of administration not defined. • Usually given as the last treatment.	3–7 days from onset of symptoms, but not later than 10 days,
• Patient IgG against Covid-19	• Patient's IgG titer levels were not taken into account	• Should not be present

R-4.1.1 software was used for the analysis. In the descriptive analysis odds ratio was expressed as numerical counts. Odds ratio (OR) which is a measure of association between exposure and an outcome was calculated. P-value is a measure of the probability that an observed difference could have occurred just by random chance, the lower the p-value the greater the statistical significance of the observed difference. P-value < 0.05 was considered to be statistically significant.

3. Results

The total number of patients given CP during this period was 552. Out of these 31 were excluded due to non-availability of data and 194 were excluded as they were admitted to wards. Thus, the final analysis was performed on 326 patients admitted to ICU. No major complications occurred during or after the apheresis procedure or during the transfusion of the product. The overall mortality in the ICU patients transfused with CP was 152 (53.3 %) out of 326 patients.

On comparing different blood groups and their clinical outcome, a statistically significant association was observed ($p < 0.05$) (Table 2) implying that the blood groups had an association with the overall outcome of the patient transfused with CP.

On comparing the clinical outcome of individual blood groups i.e. A Vs AB, A vs O, A vs B, AB Vs O no clinically significant association was seen (p-value >0.05). However, on comparing the clinical outcome of B blood group patients Vs O blood group patients and B blood group patients with AB blood group patients clinically significant association was seen (p-value <0.05) (Table 3). The donors of blood group B with anti-A antibodies had a better outcome than these individual blood group patients transfused with CP.

Out of 326 ICU patients that received CP therapy, 268 patients were presented with comorbidities and 59 patients did not have any comorbidities. The clinical outcome based on comorbidities was statistically insignificant ($p > 0.05$). The most common comorbidities seen in our study were diabetes ($n = 141$), hypertension ($n = 160$), and chronic kidney disease (CKD) ($n = 57$). Having comorbidity compared to not having comorbidity, had an odds ratio of 0.83 (Table 6).

ALOS was determined for patients in ICU. The average number of ICU days in the CP transfused patients had a positive impact on the overall patient survival (Fig. 2) ($p < 0.05$). Furthermore, on comparing the survival benefit with number of days in ICU, it was found that clinical outcome was better in CP transfused ICU patients when the patient was discharged/ recovered in less than 20 days ($p < 0.05$). A stay of more than 20 days was not associated with a significant survival benefit ($p > 0.05$).

In Part B of the study, patients treated with C plasma before ($n = 199$) and after the establishment of guidelines ($n = 127$) were analyzed for the clinical outcome (Table 7). The recovery rate improved albeit insignificantly after the establishment of new guidelines, incorporating the new criteria for patients and donors.

In part C of the study (Fig. 3), out of the patients in 'cytokine storm' ($n = 109$), On day 10, 85 % survival was seen in both the groups i.e., Tocilizumab and Non-Tocilizumab. On day 20, the survival percentage in Tocilizumab group decreased to 51 % and remained stable throughout till around day 50. In Non-Tocilizumab group, on day 20, the survival percentage decreased to 60 %, on day 30–48 % and declined to 45 % on day 50, i.e., showed a downward trend throughout.

Table 2
Comparison of all Blood Groups and their clinical outcome in CP transfused patients.

	Recovered	Expired	***Total***	The p-value is 0.028529 The result is significant at $p < 0.05$.
A	24	34	58	
B	82	66	148	
AB	12	24	36	
O	34	50	84	
Total	152	174	**326**	

Table 3
Comparison of B Blood Group and O blood, B Blood Group and AB blood.

Comparison of B Blood Group and O blood group and their clinical outcome in CP transfused ICU patients					Comparison of B Blood Group and AB blood group and their clinical outcome in CP transfused ICU patients				
Blood Group	Recovered	Expired	*Total*	*P value*	*Blood Group*	Recovered	Expired	*Total*	*P value*
B	82	66	148	0.028836 Significant at $p < 0.05$.	B	82	66	148	0.017501. Significant at $p < 0.05$.
O	34	50	84		AB	12	24	36	
Total	116	116	232		Total	94	90	184	

The clinical outcome of CP transfused patients based on age group was not clinically significant (p-value > 0.05) (Table 4). On comparing the clinical outcome of different age groups i.e., 18–50 yrs Vs 51–60 yrs, 18–50 yrs vs > 70 yrs, 51–60 yrs vs 61–70 yrs, 51–60 yrs Vs > 70 yrs, 61–70 yrs Vs > 70 yrs no clinically significant association was seen (p-value >0.05). However, on comparing the clinical outcome of the age group 18–50 yrs Vs 61–70 yrs age group patients clinically significant association was seen (p-value <0.05) i.e. the patient recovery in age group 61–70 years was significantly more than in the age group 18–50 years.

Table 4
Patients belonging to different age groups and their clinical outcome.

	Recovered	Expired	***Totals***	The p-value is 0.241579. The result is not significant at $p > 0.05$.
18–50	16	29	45	
51–60	50	60	110	
61–70	47	40	87	
> 70	39	45	84	
Total	152	174	**326**	

Out of 326 ICU patients that received CP therapy, the total males in the study were 260, out of which 146(56.15 %) expired and 114(43.85 %) recovered and the total females were 66, out of which 28(42.42 %) expired and 38(57.58 %) recovered. The clinical outcome on the basis of gender was clinically significant ($p < 0.05$) implying that the recovery rate was significantly better in CP transfused females than in males.

Table 5
Clinical outcome on the basis of gender.

	Recovered	Expired	*Totals*
Male	114	146	260
Female	38	28	66
Total	152	174	326

The *p*-value is 0.04585. Significant at $p < 0.05$.

Table 6
Outcome on the basis of comorbidities.

	Recovered	Expired	*Total*	*The p-value is 0.314109. Not significant at $p > 0.05$*
Comorbidities present	121	146	267	
Comorbidities absent	31	28	59	
Total	152	174	326	

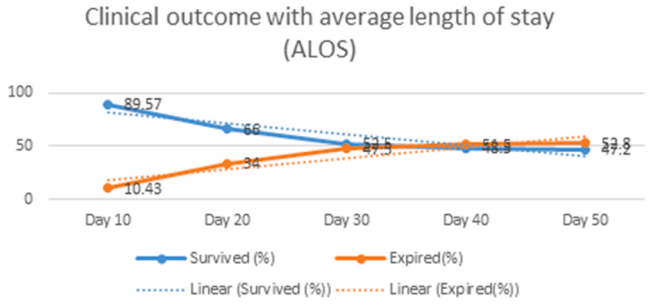

Fig. 2. Clinical Outcome with ALOS.

4. Discussion

Patients of covid disease can be divided into mild, moderate, and severe categories based on their symptoms and oxygen requirement, and

Table 7
Recovery rate before and after the introduction of November 2020 guidelines.

	Recovered	Expired	*Total*	The *p*-value is 0.388806. *Not* significant at $p > 0.05$.
Prior to November guidelines	89	110	199	
After November guidelines	63	64	127	
Total	152	174	326	

chest imaging [14]. Asymptomatic or Pre-symptomatic patients are the individuals who test positive for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test [NAAT] or an antigen test) but who have no symptoms that are COVID-19-related. Patients with mild illness are the individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging. Patients with moderate illness are individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO2) ≥ 94 % on room air at sea level. Severe illness patients are individuals who have SpO2 < 94 % on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) < 300 mm Hg, a respiratory rate > 30 breaths/min, or lung infiltrates > 50 %. Patients with a critical illness are individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction [14]. The current study was undertaken to evaluate the effectiveness of CP in patients with severe COVID 19 diseases. This is a retrospective case-control study undertaken in North India.

The definite mechanism of action of CP has not been defined yet. CP utilization is based on historical evidence, where convalescent plasma collected from recovered patients was used to fight the new virus causing outbreaks and pandemics. It was the lack of specific therapy that prompted the need for an empirical nonspecific therapy to provide passive immunization. The possible mechanisms of action include direct neutralization of the virus leading to suppression of viremia, antibody-dependent cellular cytotoxicity (ADCC), reducing inflammation, and restoring coagulation factors and other plasma proteins [15]. A recently published 'proof-of-principle 'study of CP revealed rapid viral clearance by neutralizing antibodies. It explained that these antibodies had a multifaceted action and were involved in rapid viral clearance supports beyond IgG-driven neutralization, including antibody effector functions such as antibody-dependent cellular cytotoxicity, and phagocytosis (16). The purpose of the CP is to provide immediate antibody-mediated immunity (AMI). Antibody-mediated immunity consists of neutralizing antibodies that bind to the virus directly and halt the viral replication cycle leading to a reduction in viral load and infectivity. Antibody-mediated immunity also consists of other non-neutralizing antibodies which contribute to enhanced recovery [17]. The constant fragment (Fc) of the antibody confers the biological properties of the immunoglobulin molecule. The Fc mediated antibody effect was also noted in the Ebola virus and respiratory syncytial virus infection as a

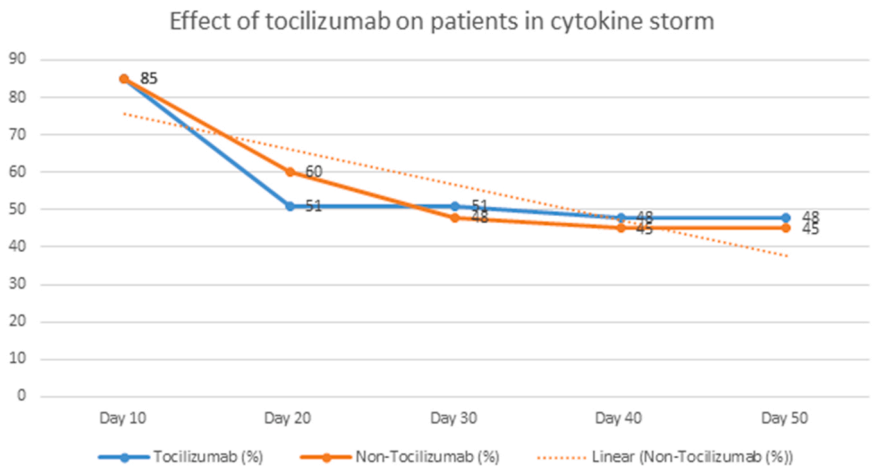

Fig. 3. Effect of Tocilizumab on patients with cytokine storm.

part of antibody-mediated protection [18]. The interaction with Fc-receptors propagates the antibody-dependent cellular phagocytosis (ADCP) and leads to complement activation, thereby removing virus-infected cells from the body.

Many factors can affect the response to CP, such as the antibody titer of the product, the severity of the disease, and the time of administration from the day of disease onset. Viremia peaks in the first week in most viral diseases and seroconversion occurs around the time of about 2 weeks from disease onset, implying CP to be more effective if administered in the early stages of the disease [19].

There is an apparent disparity of results of COVID-19 convalescent plasma trials. The serological tests may differ in their ability to detect relevant antibody activity, and this because different centers use different serological assays for assessment. The lack of standard assay and uniformity in the protocol are a major reason for the disparity even within the same geographical region. We have compared our results with several studies including one of the major studies in our region i.e. The PLACID trial [20]. We observed several differences in comparison. This trial investigated the effectiveness of using CP to treat moderately affected covid-19 patients in 464 adults in 39 centers across India. It concluded that CP was not associated with a reduction in progression to severe covid-19 or all-cause mortality. However, in our study, we have taken 326 critically ill ICU patients who received C plasma and compared their clinical outcomes in various settings. An attempt was also made to check for the efficacy of evolving guidelines and the effect of the drug 'tocilizumab' on the cytokine syndrome.

In part A of our study, we compared the clinical outcome based on blood groups, age groups, gender, ALOS, and comorbidities in CP transfused ICU patients. A similar study was done by Budhiraja et al. [21] who included 694 ICU patients amongst whom 333 were transfused CP and 361 were given supportive care only.

There have been various studies that have suggested a predictive effect of blood group on the COVID-19 patients. However, the role of CP and its effectivity according to the blood groups hasn't been explored much, especially in this region. In our study, comparing different blood groups and their clinical outcome in transfused patients, a statistically significant association was observed ($p < 0.05$). In a similar study done on critically ill and laboratory-confirmed COVID-19 patients admitted to ICU who have transfused CP in addition to the anti-viral therapy, the role of different blood groups and their clinical impact was ascertained. Similar to our findings they elucidated that blood groups did have a role in the outcome of the patients transfused with CP. In our study we further compared the clinical outcome between different blood groups and found B blood group patients had a better outcome. This is according to the study conducted by Hacibekiroğlu et al.[22] who concluded that plasma without anti-A antibody (i.e., except blood group B and O) had lower efficiency when compared with that of plasma containing anti-A antibody (blood group B and O). They further stated that it would be worthwhile to know the titer of anti-A1 antibody in CP with a clear benefit in CP having high anti-A1 antibody titers. A letter to the editor by Focasi also suggests similar findings and supports our study [23]. Anti-A antibody has been shown to benefit CP in various ways.

There have been studies in the literature that have found the maximum benefit of CP in ICU patients, belonging to an elderly age group. Budhiraja et al. showed that the mortality in the plasma group was 26.7 % versus 43 % in the no plasma group ($p = 0.004$) [21]. Even Mehew et al. [24] in their study on 330 CP donors observed that the neutralizing antibody levels were considerably higher in the elderly, men, and those hospitalized earlier. Similarly, in our study, among patients belonging to the age group 61–70 years, 54 % showed maximum recovery compared with other age groups.

In our study, the clinical outcome in CP transfused patients based on gender revealed that recovery in females was clinically significant as compared to recovery in males. Other studies have reported similar findings where recovery was found to be higher in females [20]. Min Jin et al. have also reported that the number of men who died from COVID-19 is 2.4 times that of women [25]. In the studies done on neutralizing titers in donor populations based on gender, it has been shown that the titers are higher in males, especially the elder males, thereby making them better donors [24]. This would mean that selecting elderly male donors would be an effective strategy for high neutralizing titer CP. In our study, due to the government guidelines, only male donors and female donors with no pregnancy (to prevent transfusion-associated acute lung injury (TRALI)) were recruited.

In our study the overall cohort of 326 ICU patients, 59 were those without any known comorbidities. We report that having comorbidity decreased the odds of recovery of the patient by 0.93. This could be a chance occurrence and needs to be further substantiated with other studies.

In another study a subgroup analysis of patients who received CP was taken; it was reported that mortality between specific comorbidities (HTN, DM & CAD) was not different. However, they've reported a significant decrease in mortality in hypothyroidism patients [21].

The average length of stay was determined for patients in ICU. The average number of ICU days in the CP transfused patients had a positive impact on the overall patient survival. Furthermore, on comparing the survival benefit with the number of days in ICU, it was found that clinical outcome was better in CP transfused ICU patients when the patient was discharged/ recovered in less than 20 days ($p < 0.05$). A stay of more than 20 days was not associated with a significant survival benefit ($p > 0.05$).

Initially, in June 2020 with the discovery of plasma therapy as a

probable and possible treatment for the Covid-19 disease, its 'off label' use was advocated in patients with the moderate disease who were not improving (progressively increasing oxygen requirement) despite the use of steroids [26]. However, in November 2020 guidelines [13] were established which laid out the indications for use of the plasma therapy. The November guidelines issued by ICMR stated several important features signifying the efficacy of CP. The first important feature associated with the CP efficacy is the neutralizing titer of the antibody. The guidelines specifically address that IgG antibody against COVID-19 Titre of 1:640 (ELISA) OR - 13 AU (Absorbance Unit)/mL9 (CLIA) OR – Neutralizing Antibody Titres of 1:80 (PRNT/MNT) is required for CP. The specifics of neutralizing titer ensured the uniformity and quality of the CP product. All potential donors with titers below the expected level were deferred. This was even more important as the antibody titer levels tend to decrease with time. Cao et al. [27] in their study found that the level of specific neutralizing antibodies to SARS-CoV decreased gradually 4 months after the disease process, reaching undetectable levels in 25.6 % (IgG) and 16.1 % (neutralizing antibodies) of patients at 36 months after disease status. Begin P et all in their study suggested that not only the CP with high levels of viral neutralization and high levels of Fc-mediated function were associated with a reduced risk for intubation or death whereas high levels of IgG antibodies against the whole transmembrane spike protein were associated with an increased risk of intubation or death i.e., might be harmful[28]. As per the guidelines, the potential recipient should have no IgG antibody against COVID-19 by an appropriate test. In our study, before the establishment of these guidelines, CP was transfused irrespective of IgG titers of the patient, i.e., after determining qualitatively if the antibodies were present or not. However, after guidelines, routine checking of antibody titers (SARS-CoV-2 IgG II, Quantitative, Abbott, Ireland) of the patients and donors was done and only the patients with negative IgG titers were given CP from the donors with titers above the 'cut off' levels.

The second important feature associated with the efficacy of CP and addressed in the guideline was the treatment time point. The guidelines specified that the potential recipient of CP would be a patient in the early stage of COVID-19 disease, who need to be transfused CP 3–7 days from onset of symptoms, but no later than 10 days. A better treatment outcome was observed among SARS patients who were given CP before 14 days (58.3 % vs. 15.6 %; $P < 0.01$), highlighting the importance of timely rescue therapy [29]. In our study, in the initial phase, CP was infused only as a last resort of treatment in moderate to severe cases who were not responding to the conventional treatment strategies. Consistent with the new guidelines, all patients in our study were then transfused within 10 days of onset of symptoms. A meta-study by Stephen et al. from 10 randomized clinical trials, 20 matched control studies, 2 dose-response studies, and 96 case reports or case series between January 1, 2020, to January 16, 2021, showed that the mortality reduction associated with CP transfusion was greater in studies that transfused patients within 3 days of hospital admission (OR, 0.44; 95 % CI, 0.32–0.61) compared with studies that transfused patients more than 3 days after hospital admission (OR, 0.79; 95 % CI, 0.62–0.98 [30]. In our study, patients treated with C plasma before ($n = 199$) and after the establishment of guidelines ($n = 127$) were analyzed for the clinical outcome. Out of the 199 patients, 90 (45.2 %) patients recovered after the transfusion of CP whereas, after the early introduction of CP, 63 (49.6 %) out of 127 patients recovered. Here, the early introduction of CP post-November 2020 ICMR guidelines increased the odds of recovery by 1.10. The recovery rate improved albeit insignificantly after the establishment of new guidelines, incorporating the new criteria for patients and donors. Marconato M et al. have argued that CP neutralizing antibodies can have an impact even in already seroconverted individuals, underlining that application for antibody therapeutics should generally not be viewed as restricted to the earliest, pre-seroconversion infection phase [16]. This implied the evolving guidelines and better understanding of this novel disease may pave the way for a better quality CP product that would benefit patients.

The term "cytokine storm" refers to a group of immune dysregulation illnesses marked by constitutional symptoms, systemic inflammation, and multiorgan dysfunction, which can lead to multiorgan failure if not treated properly [31]. Various studies have elaborated on the exact definition and cut-off values for IL 6 and CRP. In one such study conducted in Germany; the authors calculated optimal cutoff values of IL-6 level > 80 pg/mL followed by CRP level > 97 mg/L. They further concluded that these values were highly predictive of the need for mechanical ventilation in covid 19 [32]. Another large prospective cohort ($n = 501$) concluded that among 15 variables measured at hospital admission, CRP at a cutoff of 87.5 mg·L− 1 was the most sensitive (0.97), and IL-6 at a cutoff of 86 pg·mL− 1 was the most specific (0.89) for predicting death [33]. It has been demonstrated that cytokine storms may play an important role in severe respiratory failure and mortality caused by COVID-19 [34].

Therapies directed at the cytokine storm target dysregulated inflammation in patients with COVID-19. There have been studies that suggest cytokine-targeted therapies like tocilizumab decrease the hyperactivated immune response in patients of COVID-19. Tocilizumab is a monoclonal antibody against interleukin-6 receptor [34] used in severely ill covid 19 patients.

Part C of our study comprises all those patients who were in a cytokine storm($n = 109$). Out of these 27(24.7 %) were given tocilizumab along with CP and 82 were treated with C plasma only. In part C of the study, out of the patients in 'cytokine storm' ($n = 109$), On day 10, 85 % survival was seen in both the groups i.e. Tocilizumab and Non-Tocilizumab. On day 20, the survival percentage in the Tocilizumab group decreased to 51 % and remained stable throughout day 50. In the non-Tocilizumab group, on day 20, the survival percentage decreased to 60 %, on day 30–48 %, and declined to 45 % on day 50, i.e., showed a downward trend throughout ($p > 0.05$). In a study conducted by Gupta et al. [35] it was reported that out of 4116 patients treated with tocilizumab all-cause mortality within 28 days of random assignment, occurred in 35 % of patients allocated to usual care and 31 % of patients allocated to tocilizumab (rate ratio 0.85; 95 % CI, 0.76–0·95; $p = 0·0028$). Patients who received tocilizumab were more likely to be discharged from the hospital within 28 days than those who received standard care [25]. The finding that the combination of CCP and Tocilizumab may be beneficial relative to Tocilizumab alone points towards the beneficial effects of CCP. CCP has been reported to have anti-inflammatory effects and perhaps this combination introduced the antibody which modifies inflammatory response, reduced inflammation and neutralized virus. Plasma components can further provide other beneficial actions, such as restoring coagulation factors. However, this finding and the beneficial effect of the combination therapy needs to be followed up in subsequent studies.

Limitations

It is a retrospective study. During the study duration, patients received several treatments such as hydroxychloroquine, remdesivir, ivermectin, azithromycin, and steroids (dexamethasone or methylprednisolone) depending on prevailing guidelines at those time points and the treating physician's discretion.

The recovery of the patients could not be ascertained and compared in the no plasma group as this was not a part of our study design.

The sample size for tocilizumab was small, making it difficult to determine if recovery was a true finding.

5. Conclusion

The blood groups had an association with the overall outcome of the patient transfused with CP. The use of CP offers benefits to individuals who have blood group B when compared to other blood groups. The ICU patients in the elderly age group had a significant benefit in the overall mortality as compared to other age groups. The average number of ICU

days in the CP transfused patients had a positive impact on the overall patient survival. The clinical outcome was even better in CP transfused ICU patients when the patient was discharged/ recovered in less than 20 days. A stay of more than 20 days was not associated with a significant survival benefit. The recovery rate improved, albeit insignificantly, after the establishment of new guidelines, incorporating the new criteria for patients and donors. In 'cytokine storm' patients, the survival percentage in the Tocilizumab group remained stable throughout till around day 50 whereas in the non-Tocilizumab group, showed a downward trend throughout. The use of tocilizumab for Covid 19 disease should also be further studied. The limitations of CP notwithstanding, we were able to identify a subset of patients with severely ill COVID-19 who are likely to benefit from CP. Further research is needed to better understand the effectiveness of CP in covid disease.

CRediT authorship contribution statement

Dr. Mohit Chowdhry,Dr. S.Minu Lekshmi, Dr.Prachi Singh, Dr. Maryam Hussain Designed, Supervised and planned the research, Dr. Prachi Singh,Dr. Maryam Hussain, Dr. S.Minu Lekshmi collected the data, Dr. Mohit Chowdhry, Dr. S.Minu Lekshmi, Dr.Prachi Singh, Dr. Maryam Hussain took the lead in writing the manuscript and draft preparation, Dr. Mohit Chowdhry Dr.Prachi Singh Dr. Maryam Hussain were involved in reviewing and editing the document, Dr.M.S.Kanwar Dr. Rajesh Chawla,Dr. Soma Agarwal, Dr Avdesh Bansal, Dr. Vini Kantru, Dr. Nikhil Modi, Dr. Roseleen Bali, Dr Aakanksha Chawla were involved in Final manuscript editing, Mr Manoj Kumar & Mr Zaigham Ali Khan were involved in doing statistics.

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