

Original Research Article

COVID-19 convalescent plasma donor characteristics: correlation with blood group, age, gender, symptoms and their association with SARS CoV-2 antibody levels: A bi-centric study from India

Abstract

Background: Understanding demographics and collection characteristics of Convalescent Plasma (CP) donors is the corner stone for maintaining a viable inventory of high titre CP products in an acute-crisis setting. Specific objectives of this study were to find a correlation between SARS-CoV-2 antibody level with age, gender and blood group in CP donors.

Material and method: An observational, retrospective study was conducted at blood centres of two multispecialty hospitals in Delhi from 1st November 2020 to 31st January 2021.

Results: 400/ 750 (53.3%) CP donations were from centre A and 350/750 (46.7%) were from centre B. Majority were males (96.93%) and mean age was 35.09±8.7 years. 41-50 years showed higher median SARS-CoV-2 antibodies. Mean deferral rate was 19.87%. Commonest reason (15.05%) was absence of detectable/low SARS CoV-2 antibodies. Group-wise median antibody levels were AB>B>A>O at both centres. Moderate correlation between number of symptoms and SARS CoV-2 antibody levels was observed; high symptom scores showed high SARS CoV-2 antibodies values (correlation coefficient=0.721; $p < 0.00001$). The individual symptom-wise predictors like fever ($p= 0.002$), GE or GE-like syndrome ($p < 0.001$) were positive predictors for high SARS CoV-2 antibodies. Cold ($p = 0.006$) and absence of symptoms ($p = 0.060$) were negative predictors.

Comment [h1]: There should be an inclusion of how the symptoms and health outcomes were collected/ measured in the abstract

Comment [h2]: Please Add Keywords

Comment [h3]: Please Add: The

Comment [h4]: Materials and Method

Comment [h5]: The number of samples/participants should be mentioned and instruments for data collection

Comment [h6]: The total number should be included in the materials and method. Then start this result with a proper sentence: For example: "The result of this study revealed"

Comment [h7]: Please include information about the female participants

Comment [h8]: No meaning; Please rephrase or remove

Comment [h9]: Insert the values correlation coefficient of the moderate correlation.

Comment [h10]: Make this a new sentence

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Conclusion: Awareness of CP donor characteristics, post-screening deferrals, and correlates of antibody values with age, symptoms and blood group will help improve collection outcomes and give better preparedness for future recurrent waves due to evolution of more virulent SARS-CoV-2 strains and fill treatment gap till availability of more specific treatment.

Comment [h12]: The results should be summarized before stating the significance and recommendations

1. Introduction

Late December 2019, the world was introduced to this century's worst pandemic, "the COVID-19 pandemic", caused by the novel coronavirus - SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). Healthcare systems across the globe was crippled under this huge challenge and with no definitive standard or approved treatments for COVID-19. Passive immunisation using Convalescent plasma (CP) has long been used as a therapeutic tool in blood transfusion for achieving immediate short-term immunisation and have shown therapeutic potential against infectious agents.¹ CP products, is obtained by collecting whole blood or plasma from a patient who has survived a previous infection and developed humoral immunity against the pathogen responsible for the disease in question, are a possible source of specific antibodies of human origin.² The plasma thus extracted containing pathogen-specific antibodies (in this case-SARS CoV-2 antibodies) and has become a well-recognized emergency or empirical therapy,³ which has been successfully applied in the field of cell therapy and immunotherapy.⁴

Comment [h13]: Restructure this for Example: "The world was introduced to this century's worst pandemic-THE COVID-19 Pandemic in December, 2019

Comment [h14]: add

Comment [h15]: add

Comment [h16]: Please rephrase this sentence to true meaning.

Comment [h17]: You are trying to say that obtaining CP is a possible source of specific antibodies of human origin. However, the structure of the sentence does not fit into this meaning.

Comment [h18]: Syntax error: The plasma thus extracted contains pathogen-specific antibodies..... Consider merging the last two sentences to have a holistic meaning with precision and articulation.

Comment [h19]: add

US-FDA on April 13, 2020 authorized the use of convalescent plasma as a potentially effective treatment of the patients, as an investigational drug.⁵ The Ministry of Health and Family Welfare, Govt. of India on 13th June 2020, in their "Clinical Management Guidelines: COVID 19, Version 3", endorsed off-label use of convalescent plasma for COVID 19 patients with moderate disease who are not improving (oxygen requirement is progressively increasing) despite use of steroids.⁶ As the COVID-19 pandemic continues to rage, so does its devastation

and case counts continue to escalate steeply worldwide, CP currently remains the most accessible viral- specific therapy for hospitalized patients.^{7,8,9,10,11, 12} Measurements of antibody response among patients with COVID-19 show that the majority patients develop IgM and IgG within 2 weeks of symptom onset, with specificity towards receptor-binding domain (RBD) and spike protein viral epitopes correlating with virus neutralization.^{13,14} Identifying, recruiting, selecting, collecting, and preparing plasma from convalescent patients with adequate SARS-CoV-2-neutralizing Ab titres in catastrophic situation like that faced in recent times has become the biggest challenge well within the remit of most blood transfusion services across the globe. Thus understanding demographics and other collection characteristics of donors who qualify for CP donation is the corner stone for maintaining a viable inventory of high titre CP products in an acute crisis setting. Recent interim recommendations issued by the AABB, who have endorsed not only safety of CP, but have also recommended use of high-titer CP as close to symptom onset as possible as the main predictors of its effectiveness.¹⁵

Comment [h20]: meaning?

Comment [h21]: This is the justification and should be written on a separate paragraph

It is important not only to focus on the clinical endpoints of a CP therapy, but also to describe the impact of donor characteristics (age, gender, blood group, symptoms of COVID-19, interval between SARS-CoV-2 diagnosis and CP collection) on the antibody content in the CP products. CP collections might become more effective if donor characteristics correlating with higher functional antibody activity are better understood. This study is unique as till date, there is no study from India on COVID-19 convalescent plasma collection analysing major CP donor characteristics and SARS-COV-2 antibody response. Thus, our experience in CP collection is relevant for future waves; including CP donor characteristics on the quality of CP product and our analysis of reasons for donor deferrals in Indian population.

Comment [h22]: add

Comment [h23]: Rephrase this sentence as part of the justification

2. Aims and objectives

The aim of this study was to analyse the CP donor characteristics and SARS-COV-2 antibody response along with major reasons for donor deferral while recruiting donors for CP collection

with specific objectives to find out the relationship of SARS-CoV-2 antibody level with age, gender and blood group of donors. In one of the two centres we also analysed variation of antibody levels according to symptomatology, as we all know that efficacy of convalescent plasma therapy relies on robust antibody response in convalescent plasma donors.

Comment [h24]: Consider removing this; repetition. You have mentioned this before. You can also summarize this in one or two sentences.

3. Material and methods

This is an observational, bi-centric, retrospective, study done at blood centres of two multispecialty hospitals in Delhi, with dedicated COVID-19 management units. Donor characteristics data for both centres was retrieved from the blood bank donor management database and records from both the centres for a period of 3 months, from 1st November 2020 to 31st January 2021. The donor selection was based on standard blood bank donor selection requirements (Drugs and Cosmetics Second Amendment Rules, 2020)¹⁶ and additional inclusion criteria as per the emergency release authorization issued by the Ministry of Health and Family Welfare

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(MOHFW), Govt. of India”, for off-label use of convalescent plasma for COVID-19 in moderate disease¹⁷ with the CP collection donor selection and collection guidelines released by the Indian Council of Medical Research (ICMR), New Delhi.¹⁸ CP was collected from consenting COVID-19 recovered individuals meeting the above requirement.

Comment [h27]: Rephrase: This is not well structured

3.1. Study Design and Setting

Comment [h28]: Give a brief description of the Blood centres as it relates to your study

Study population was divided into two cohorts based on the collection centres where CP was collected:

Comment [h29]: Why did you use Cohort here: Did you conduct a cohort study. You should use “groups” instead.

Centre A- Blood centre (Department of Transfusion Medicine) of BLK Super Speciality Hospital, New Delhi, India.

Comment [h30]: Full meaning

Centre B –Blood centre (Department of Transfusion Medicine) of Max Super Speciality Hospital, New Delhi, India

3.2. Ethical Approval

The study was approved by the institutional ethics committees (IEC) of involved hospitals. As a convalescent plasma donor screening and registration protocol of both the blood centres, informed consent had been obtained prior to plasma donation for any additional testing on the plasma and samples collected and its subsequent use for study/ research purposes from all donors and the same are in place. In view of retrospective study design, and no risk of disclosure of the donor identity involved in this study, the requirement to obtain the blood donors' consent to review the donor records was waived off by the IECs. Confidentiality of the data was maintained, and the study procedures were performed in compliance with the 1964 Helsinki declaration and its later amendment.

Comment [h31]: Use Initial Cap Institutional Ethics Committees Attach reference number of the ethical approval.

Comment [h32]: Rephrase

Comment [h33]: Complex and compound sentence: Consider breaking the sentence to reveal true meaning

Comment [h34]: Add Reference

3.3. Donor selection

All CP donors meeting traditional allogeneic blood donor criteria per the standard blood bank donor selection requirements (Drugs and Cosmetics Second Amendment Rules, 2020) the following additional selection criteria as defined by MOHFW were included:

Comment [h35]: corrected

- Men or nulliparous women who were aged between 18 and 60 years
- Body weight greater than 55 kg.
- Prior diagnosis of COVID-19 documented by a confirmed by rRT-PCR test or a Rapid antigen test (RAT) by an ICMR approved assays. Additionally, the symptoms must have completely resolved for 28 consecutive days before donation or a period of 14 days before donation with rRT-PCR negative test results for SARS-CoV-2 from oro and nasopharyngeal swabs. In case of repeat CP donation and interval of at least 2 weeks of the first plasma donation was ensured.

Comment [h36]: complete: Oro-pharyngeal

Comment [h37]: add a comma punctuation

Donors fulfilling these requirements underwent a medical interview, with routine donor medical examination, and explained about donation of plasma via plasmapheresis.

Comment [h38]: not stated in the abstract

Data captured by Centre A, additionally included details pertaining to onset of disease, duration and type of symptoms [fever; cough; non-specific symptoms (body ache or headache); runny nose or cold; shortness of breath (SOB); acute gastroenteritis (GE) (GE-like syndrome): nausea, vomiting, or abdominal pain; anosmia or ageusia].

3.4. CP donor screening

All routine screening tests, including ABO blood grouping; Rhesus phenotype; complete blood counts (including Hemoglobin (Hb), Platelet count, Total and differential leucocyte count), antibody screening for clinically significant antibodies (Extended Rh, Kell, Duffy, Kidd, MNS) and serum protein estimation was carried out. Donors with Hb >12.5g/dl, platelet count >1.5x 10⁹/L, TLC within normal limits, total serum protein > 6gm/dl and antibody screening negative were accepted. Screening for HIV, hepatitis B or C virus, syphilis, and malaria were conducted according per regulatory guidelines [Drugs and Cosmetics (Second Amendment) Rules, 2020]¹².

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3.5. SARS-CoV-2 antibody test

Neutralizing antibodies levels have been shown to correlate best with anti-S1 IgG and anti-S1 total Ig levels.^{19,20,21}

At center A, donors were tested for Anti-SARS-CoV-2 IgG (S1) antibodies on VITROS-3600 chemiluminescent immunoassay (CLIA) in accordance with manufacturer instructions. CP was collected from donors. Institutional cut-off of 5 S/Co was taken for donor selection and titer of 12 S/Co was considered as high titer CP.^{22,23,24}

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Centre B Tested the donors on the LIAISON® XL Analyzer (DiaSorin S.p.A., Saluggia, Italy) applying the manufacturer's cut-off >15.0 AU/mL (borderline results 12.0 – 15.0, required a re-test algorithm)²⁵

3.6. Plasma Collection:

At centre A, CP was obtained by apheresis using Amicus™ (Fresenius Kabi) or Trima Accel® (Terumo BCT, Lakewood, CO) cell separators and at Centre B Apheresis was performed using Haemonetics MCS + LN90 00-220E blood cell separator (Haemonetics, Boston, MA, USA). 400-450 ml plasma was collected from each donor and divided into two 200-225-ml aliquots and stored at less than -30°C. Units were thawed at 37°C for issue. The mean volume of plasma collected was 405.34±14.09ml. The Average time taken was 30.49±6.49minutes. Only 5 donors suffered mild adverse reactions during the procedure. There were 2 small hematomas and 3 experienced minor citrate reactions in the form of mild dysesthesias, resolved by adjustment of the inlet pump flow rate and oral calcium supplements.

Comment [h42]: What? Meaning?

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3.7. *Statistical Analysis:* Summarized data are generally expressed as means ± SD or medians and overall ranges (minimum–maximum). Linear regression between 2 parameters was quantified by goodness of fit with r^2 . Correlations between 2 data sets were calculated using Pearson's correlation coefficient and the t distribution with n-2 degrees of freedom. P values < 0.05 were considered to indicate statistical significance while p values < 0.0001 were considered to indicate highly statistical significance. Statistical analysis was done using IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

Comment [h45]: This is wrong: Just state what you used and not stating general representation of statistics

Comment [h46]: What is R2: Meaning

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Comment [h49]: merge this:

4. Results

4.1. Donor characteristics

Donor characteristics data was retrieved from the blood bank donor management database and records from both the centres. 936 consenting COVID-19 recovered individuals were screened for eligibility for Convalescent plasma donation from both blood centres, of which

Comment [h50]: Overall. The results were comprehensive, The socio demographics should be summarized since the tables are presented; The tables should appear immediately after the result discussion. The referred figure or table should be indicated after each discussion.

750 qualified for CP collection. Deferral rate was defined as the total number of donors deferred at time of collection out of total number of presenting donors.

Comment [h51]: reference?

400/ 750 (53.3%) CP donations were from centre A and 350/750 (46.7%) were from centre B. Majority of the donors were males (96.93%) and the mean age of donors was 35.09±8.7 years with a median age of 34 (20-60) years. Female donors were younger than male donors ($p = 0.0001$). 606/750 (80.8%) of the donors were replacement donors. 19.2% voluntary donors were either hospital staff or walk in regular blood donor who had recovered from COVID-19 infection and volunteered to donate CP.

Comment [h52]: Start with a sentence?

Comment [h53]: What of female characteristics

Most of the donors were into professional service (35.5%), followed by semi-professional /self-employed (27.1%), government service (15.8%), student (11.6 %), and 10% into other activities. Of those involved in professional services and government services, 11.8% of them were healthcare workers and 3.2% were policemen. Among different blood groups, B donors were most common (44.13%). Other blood groups were O -24.8%, A positive-22%, AB 9.07%. Further demographic and clinical characteristics are presented in Table 1.

4.2. Deferral

Of the 936 donors screened, 186 donors got deferred due to various reasons, resulting in a mean deferral rate of 19.87%. The characteristics of deferred donors are shown in Table 2. On a gender basis, the deferral rate was higher in male donors (79.57% compared to 20.43% female donors, $p < 0.0001$). Commonest deferral reason was absence of detectable/low SARS CoV-2 antibodies (15.05%), inadequate time gap from last plasma donation (9.68%), low Hb (9.14%), low platelet count (8.60%), high blood pressure (7.53%) and poor venous access (5.91%). 5.37 % donors tested reactive for transfusion transmissible infections (HIV 1&2, HBV, HCV or Syphilis). 13.44% donors were rejected during screening as they not have a diagnostic test report (ICMR approved test) for COVID-19 infection (i.e. diagnostic evidence of COVID-19 infection) described in section 1.2 above.

Comment [h54]: Add a punctuation

4.3. Median SARS CoV-2 IgG (S1) levels comparative by blood donor characteristics: gender, age and blood group

Among the 750 donors who donated, donors with blood group AB compared with other groups had higher median levels of SARS CoV-2 antibodies at both centres [Figure 1 (a) & (b)]. Centre A: 48.59 S/Co for group AB, 41.2 S/Co for group B and 39 S/Co for group A and 35.5 S/Co for group O; similar trend was seen at Centre B. Centre B: 76 AU/ml for group AB, 69.1 AU/ml for group B and 58.6 AU/ml for group A and 47.4 AU/ml for group O. At Centre A, median levels of SARS CoV-2 antibody levels were higher in females compared to males (49.32 S/Co vs 38.09 S/Co) and similar trend was observed in the donors who donated at Centre B (female 63 AU/ml vs males 52.9 AU/ml) [Figure 2 (a) & (b)]. Age group of 41-50 years showed higher median SARS CoV-2 antibody levels at both the centres (Centre A: 51.36 S/Co for age group 41-50 vs 45.15 S/Co for age group 31-40 years, 38.5 S/Co for 51-60 years and 37.1 S/Co for 20-30 years and at Centre B: 82.1 AU/ml in 41-50 years, 59.9 AU/ml in 31-40 years, 54.85 AU/ml in 51-60 years and 44.9 AU/ml in 20-30 years age group) (Figure 3a & 3c). However, antibody levels showed weak correlation with age of the donors (coefficient of linear regression between donor and SARS CoV-2 antibody level in Centre A $r^2=0.0245$; correlation coefficient =0.49; Centre B $r^2 = 0.004$; correlation coefficient =0.06 Fig. 3b & 3d).

4.4. Additional characteristics of CP donors from Centre A

At centre A additional data regarding disease onset and symptoms experienced by the donors at the time of infection were captured. 94% of the donors had been symptomatic and 6 % were asymptomatic at the time when they were rRT- PCR positive.

Donors were categorized as mild, moderate and severe categories on symptomatology as per NIH guidelines²⁶.

1. Mild Disease: Individuals with signs and symptoms of COVID-19 viz. fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell but who do not have shortness of breath, dyspnea, or abnormal chest imaging.
2. Moderate Disease: 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.
3. Severe Disease: 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, respiratory frequency >30 breaths/min, or lung infiltrates >50%.

Comment [h55]: No need for this.

Overall, 73 % (292/400) of donors had mild symptoms, 27 % (108/400) had moderate symptoms and none had severe symptoms. 4 donors were known hypertensives under control on medication, 3 had diabetes mellitus controlled on medication and others did not have any co-morbid conditions. During the period of rRT-PCR positivity, most participants (89%) got home management. The rest of them were managed at COVID-19 dedicated hospitals (4% managed in wards and 7% at dedicated quarantine centres). No donors needed ICU management.

Data captured by Centre A, also included details pertaining to onset of disease, duration and type of symptoms [fever; cough; non-specific symptoms (body ache or headache); runny nose or cold; shortness of breath (SOB); acute gastroenteritis (GE) or (GE-like syndrome): nausea, vomiting, or abdominal pain; anosmia or ageusia). A symptom scoring was done according to number of symptoms. Each symptom was given a score of 1. Fever, cough, sore throat, rhinorrhoea& nasal congestion, GE or GE-like syndrome, non-specific symptoms (body ache

or headache) were the most common symptoms. 18% of the donors experienced 4 or more symptoms. The number of COVID-19 symptoms (symptom score) reported by the CP donors showed a moderate linear correlation with the SARS Cov-2 antibody levels ($r^2 = 0.5193$; correlation coefficient = 0.721; $p < 0.00001$) [Table 3 & Figure 4].

4.5. *Correlates of SARS CoV-2 Antibody levels symptom-wise*

Multivariable analysis performed on the subset of donors from Centre A for whom data on details of symptom experienced during COVID-19 infection was available. Table 4 shows the results of multiple linear regression analysis for the outcome of SARS CoV-2 IgG(S1) antibodies in S/CO units (on day of donation) and the following independent variables: No symptoms or symptoms like fever, cough/ sore throat/ shortness of breath, cold, symptoms GE or GE-like syndrome, headache/bodyache, anosmia, ageusia. Results of the multiple linear regression indicated that there was a collective significant effect between the symptoms and antibody levels, ($F(8, 391) = 20.50$, $p < 0.001$, $R^2 = 0.295$). The individual predictors were examined further and indicated that fever ($t = 3.085$, $p = 0.002$), GE or GE-like syndrome ($t = 10.433$, $p < 0.001$), cold ($t = -2.757$, $p = 0.006$) and no symptoms ($t = -1.888$, $p = 0.060$) were significant predictors in the model. Presence of fever as a symptom predicted a mean S/CO increase by 16.87 (6.12 - 27.62), $p = 0.002$. Donors with GE or GE-like syndrome had very high antibody levels and presence of GE or GE-like symptoms predicted a mean S/CO increase by 66.69 (54.13 - 79.26), $p < 0.001$. Absence of symptoms predicted a mean S/CO decrease by 16.58 (0.69 - 33.85), $p = 0.060$. Cold as a symptoms predicted a mean S/CO decrease by 15.80 (5.53 - 27.07), $p = 0.006$.

No significant correlation was seen when anosmia or ageusia as symptoms were analysed.

5. Discussion

Convalescent plasma is a simple immunologic therapy for the COVID-19 pandemic. Blood centres with plasmapheresis can implement a CP collection program, following basic recommendations.

Comment [h56]: This should be removed: Already stated in the previous sections

Here, we report a series of 750 convalescent plasma collections across two centres in Delhi. Of the 936 donors screened during this period, 13.44% got deferred as they did not have diagnostic laboratory report for confirming COVID-19 infection as per regulatory requirement.

Majority (52%) of the donors were first-time donors. Our findings are similar to those reported by Lasky B et. al who is their study too reported high number of first time donors and also a high deferral rate in CP donors.²⁷ Highest deferral rate (15.05%) was due to absence of detectable/low SARSCoV-2 antibodies, followed by parity (9.68%) in potential female donors.

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Comment [h60]: what is their deferral rate?

Comment [h61]: For which study?

Wendel S et. al. have reported absence of neutralizing antibodies at the time of screening in 21 of the 271 (7.75%) donor screened for CP donation in Brazil.²⁸ Dhiman Y et.al. in their study have cited multiparity (38%) as one of the main reasons for deferral among potential female CP donors.²⁹

Comment [h62]: Does this agree with you result: give reasons!!

Donors with blood group AB had higher median levels of SARS CoV-2 antibodies compared to other blood groups. Group-wise median antibody levels were AB>B>A>O at both centres. (Centre A: 48.59 S/Co group AB vs 41.2 S/Co group B vs 39 S/Co group A vs 35.5 S/Co group O; Centre B: 76 AU/ml group AB vs 69.1 AU/ml group B vs 58.6 AU/ml group A vs 47.4 AU/ml group O), Female donors showed a higher median SARS CoV-2 antibody levels at both the centres. (Centre A: 49.32 S/Co female vs 38.09 S/Co males; centre B 63 AU/ml female vs 52.9 AU/ml male). Though antibody levels were higher in females than males, the number of female donors was very small 23(3.07%) and difference was not statistically significant. Our findings are similar to those reported by Mehew J et.al in their study on association of gender, age and hospitalisation with neutralising antibody levels. They reported higher median levels of

neutralising antibodies with blood group AB compared with other groups (1:148 vs 1:104 for group B, 1:70 for group A and 1:47 for group O). However, contrary to our observation on gender association, they observed that median levels of neutralising antibodies to be higher in men compared to women (1:97 vs 1:47).³⁰ Singh P et.al. from India, have reported similarly observations on blood group association with neutralizing antibodies and have found neutralising antibodies in significantly higher percentage of people with blood group AB (0.36), followed by B (0.31), A (0.22) and lowest in people with blood group O (0.11). Difference between blood group AB versus O was highly significant ($p < 0.001$).³¹ Some studies have reported that non-O blood group individuals are at higher risk of developing COVID-19 in comparison to O blood group and they have postulated that this is either due to natural antibodies against blood group antigens acting as a part of innate immune response to neutralize viral particles or alternatively, blood group antigens maybe acting as additional receptors for the virus and individuals who are capable of expressing these antigens on epithelial cells, would possibly have a high propensity to be affected by SARS-CoV-2.^{32, 33,34} In our study, we found a higher median SARS CoV-2 antibody levels in donors in age group of 41-50 years compared with other age groups in both the centres. However, levels showed weak correlation with age of the donors (centre A correlation coefficient=0.49; centre B correlation coefficient =0.06). Other studies however suggest that people of male gender,³⁵ older age,³⁶ have higher SARS CoV-2 antibodies indicating that they may be susceptible to SARS CoV-2 infection.

Many studies have reported that titers of neutralizing antibodies are associated with disease severity.^{37, 38,39} We could not analyse this in our CP donors since none of the donors had severe COVID-19. Our study is in line with other studies which have also had similar cohort of CP donors and predominantly represented patients who had mild or moderate disease without hospitalization coming forward to donate CP.^{11,40,41} . However, when the patients with mild or

Comment [h63]: Similar:

Comment [h64]: Statistically significant

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moderate disease were given scoring according to the number of reported COVID-19 symptoms, we observed a correlation between number of symptoms and SARS Cov-2 antibody levels. The results revealed that a high symptom scores goes with high SARS CoV-2 antibodies values with moderate linear correlation ($r^2 = 0.5193$; correlation coefficient=0.721; $p < 0.00001$). Koper S. et. al. also reported a positive correlation of neutralization titres with the number of reported COVID-19 symptoms and with the time from SARS-CoV-2 diagnosis to plasmapheresis.⁴² Our results on multiple linear regression indicated that a collective significant effect exists between the symptoms and antibody levels. The individual predictors examined indicated that donors who had fever ($t = 3.085$, $p = 0.002$) and GE or GE like syndrome ($t = 10.433$, $p < 0.001$) showed significantly higher SARS Cov-2 antibodies and absence of symptoms ($t = -1.888$, $p = 0.060$) or cold as a symptom ($t = -2.757$, $p = 0.006$) and were significant negative predictors in the model. Association of higher SARS Cov-2 antibody levels with fever and GE or GE like syndrome have not been reported in any other study except a recent study by Amjadi MF et. al. who also demonstrated for the first time that COVID-19 symptoms such as fever, abdominal pain, diarrhea and low appetite, correlated consistently with higher anti-SARS-CoV-2 antibody levels. Our results and those reported by Amjadi MF et. al. provided new insights into anti-SARS-CoV-2 antibodies development based on these symptoms.⁴³

There are some limitations to this study. One of the limitation of our study is that our analysis for correlates of SARS CoV-2 antibody values are retrospective only and single point evaluation which requires follow-up to evaluate the dynamics of antibody levels and their symptom-wise dynamics and also to re-assess those cases who were deferred due to absence of detectable/low SARS CoV-2 antibodies. Collection and usage of CP is currently waning with increasing vaccination rates and availability of alternative early-stage treatments. Nevertheless, our data on CP donor characteristics, post-screening deferrals, and clinical correlates of

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antibody values and their correlation with age, symptoms and blood group will help blood centres to better prepare for future recurrent waves of COVID-19 infection due to evolution of more virulent SARS-CoV-2 strains and fill the gap until a more specific treatment options become available.

Comment [h81]: corrected

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CRedit author statement

Conceptualization: RS, MD, RY

CP collection: SP, TC, RS, MD, RY, GPT, AER

Methodology: RS, MD

Statistical analyses and interpretation: GPT, RS, MD

Resources: RY, GPT, AER

Writing- Original Draft: RS, MD

Writing- Review & Editing: RS, AH

Figure 1: Median SAR CoV-2 antibody levels, by blood donor blood group

Fig 1a

Fig 1b

UNDER PEER REVIEW

N	88	182	87	43	N	77	149	99	25
Median	39	41.2	35.5	48.59	Median	58.6	69.1	47.4	76

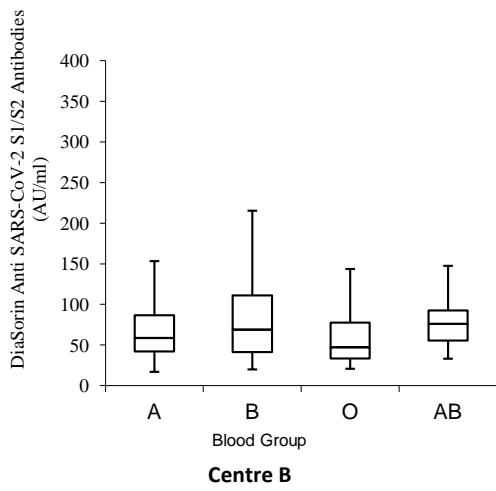
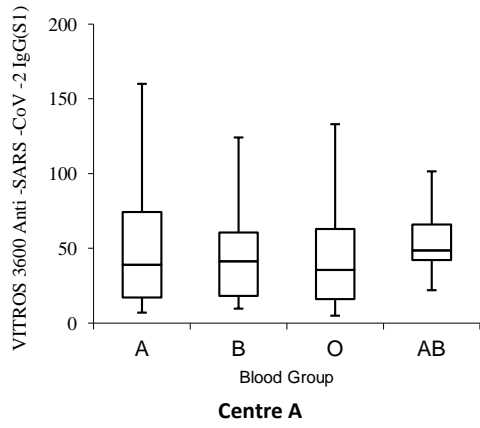
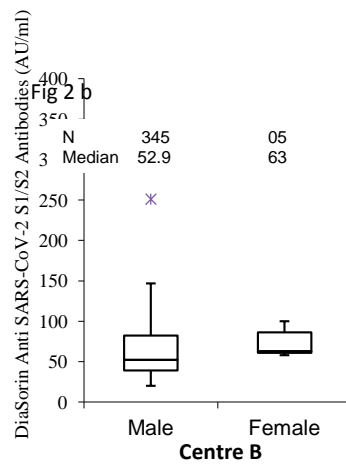
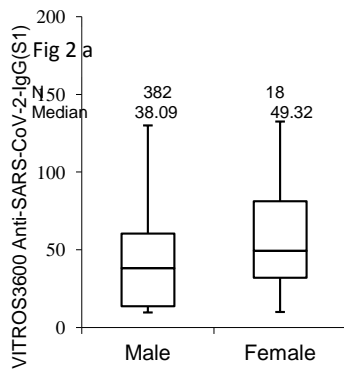


Figure 2: Median SAR CoV-2 antibody levels, by gender



UNDER PEER REVIEW

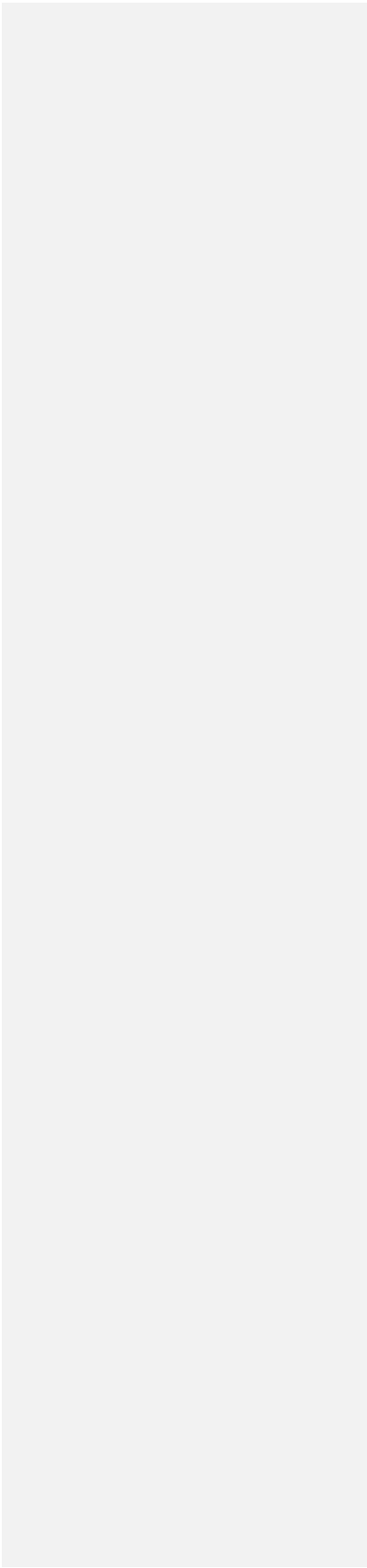
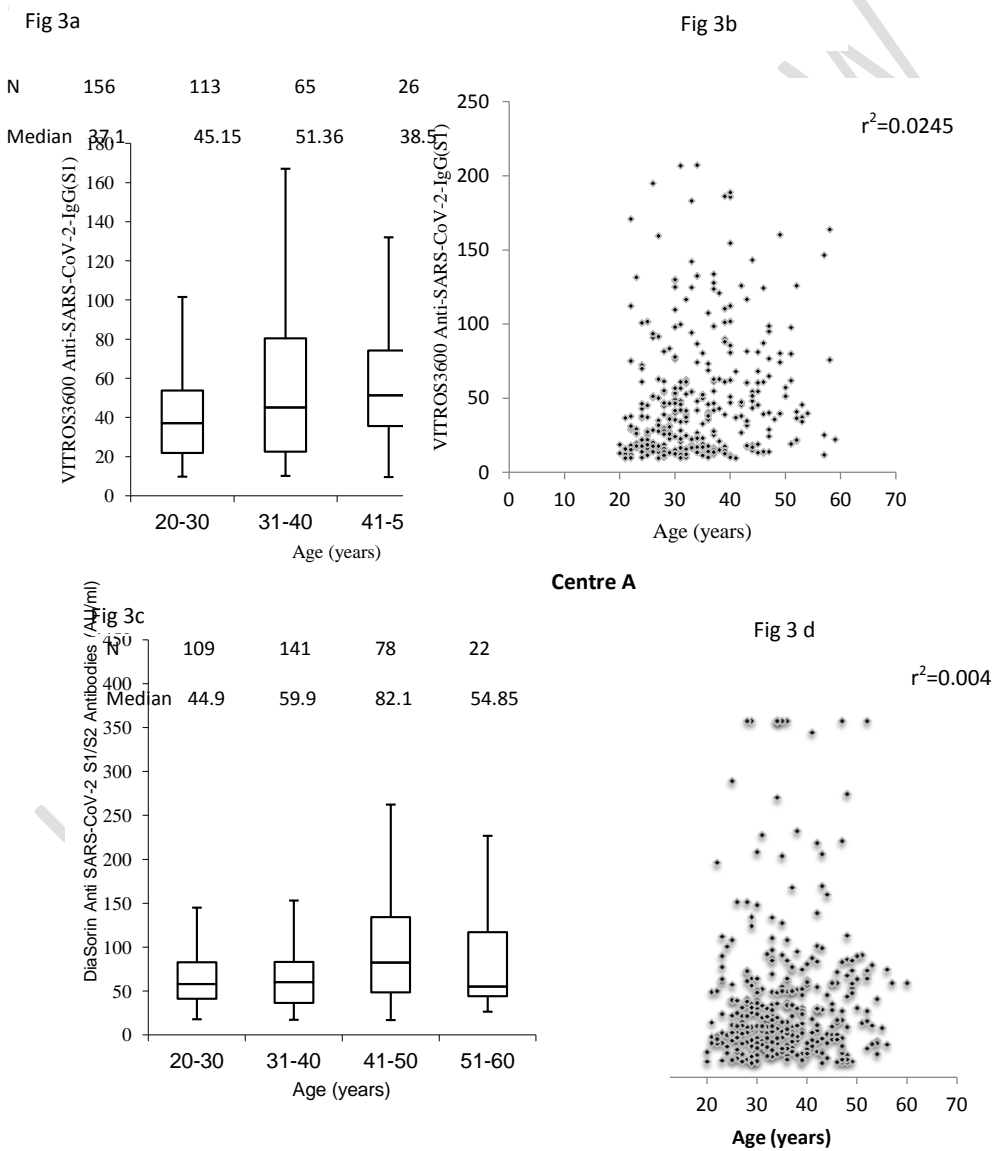
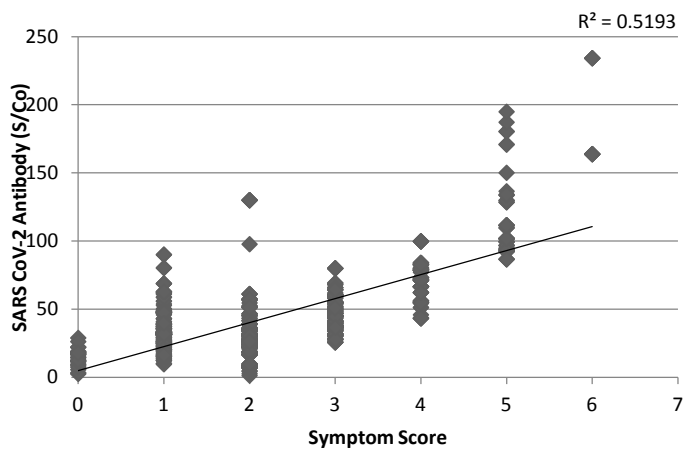


Figure 3: (a) Median SAR CoV-2 antibody levels, by age, centre A; (b) Linear regression between age and SARS CoV-2 antibody levels, Centre A; (c) Median SAR CoV-2 antibody levels, by age, centre B; (d) Linear regression between age and SARS CoV-2 antibody levels,



Centre B

Figure 4 Correlation between symptom score and SAR CoV-2 antibodies



COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation

but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

- 1 Garraud O., Heshmati F., Pozzetto B., Lefrere F., Girot R., Saillol A. Plasma therapy against infectious pathogens, as of yesterday, today and tomorrow. *Transfus Clin Biol.* 2016;23:39–44.
- 2 Burnouf T, Seghatchian J. Ebola virus convalescent blood products: where we are now and where we may need to go. *Transfus Apher Sci.* 2014;51:120–5.
- 3 Marano G, Vaglio S, Pupella S, Facco G, Catalano L, Liunbruno GM, Grazzini G. Convalescent plasma: new evidence for an old therapeutic tool? *Blood Transfus.* 2016
- 4 Garraud O. Passive immunotherapy with convalescent plasma against COVID-19? What about the evidence base and clinical trials? *Transfus Apher Sci.* 2020 Aug;59(4):102858. doi: 10.1016/j.transci.2020.102858. Epub 2020 Jun 27. PMID: 32631501; PMCID: PMC7320683.
- 5 US FDA. Investigational COVID-19 Convalescent Plasma: Guidance for Industry [Internet] 2020 [Accessed on 17th June 2021]. Available from: <https://www.fda.gov/media/13678/download>.

- 6 Ministry of Health & Family Welfare. Clinical Management Protocol: COVID-19 version 3, dated 13/6/20 www.mohfw.gov.in/pdf/ClinicalManagementProtocolforNormalCOVID19.pdf.
- 7 Casadevall A, Pirofski LA. The convalescent sera option for containing COVID- 19. *J Clin Invest*. 2020;130:1545–8.
- 8 Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, et al. Deployment of convalescent plasma for the prevention and treatment of COVID- 19. *J Clin Invest*. 2020;130:2757–65.
- 9 Focosi D, Franchini M. COVID-19 convalescent plasma therapy: hit fast, hit hard! *Vox Sang*. 2021 Apr 1. doi: 10.1111/vox.13091. Epub ahead of print. PMID: 33794556
- 10 Shenoy AG, Hettinger AZ, Fernandez SJ, et al. Early mortality benefit with COVID-19 convalescent plasma: a matched control study. *Br J Haematol*. 2021;192:706–713
- 11 Joyner MJ, Carter RE, Senefeld JW, et al. Convalescent plasma antibody levels and the risk of death from Covid-19. *N Engl J Med*. DOI: 10.1056/NEJMoa2031893
- 12 Salazar E, Christensen PA, Graviss EA, et al. Treatment of COVID-19 patients with convalescent plasma reveals a signal of significantly decreased mortality. *Am J Pathol* 2020; 190: 2290-303
- 13 Long, Q. X. et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat. Med.* **26**, 845–848 (2020).
- 14 Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann A, Cornaby C, Bartelt L, Weiss S, Park Y, Edwards CE, Weimer E, Scherer EM, Roupheal N, Edupuganti S, Weiskopf D, Tse LV, Hou YJ, Margolis D, Sette A, Collins MH, Schmitz J, Baric RS, de Silva AM. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci Immunol*. 2020 Jun 11;5(48):eabc8413. doi: 10.1126/sciimmunol.abc8413. PMID: 32527802; PMCID: PMC7292505.

- 15 Cohn CS, Estcourt L, Grossman BJ, et al. COVID-19 convalescent plasma: Interim recommendations from the AABB. *Transfusion*. 2021 Apr; 61(4):1313-1323. doi: 10.1111/trf.16328. Epub 2021 Mar 7. PMID: 33586160; PMCID: PMC8014606.
- 16 2020.18.03 Final G.S.R. 166(E) Amendment in Part X B & Part XII B pertains to Blood centre & blood components. [https://cdsco.gov.in/opencms/opencms/en/Notifications/Gazette-Notifications/Accessed January12, 2020](https://cdsco.gov.in/opencms/opencms/en/Notifications/Gazette-Notifications/Accessed%20January12,%202020).
- 17 Ministry of Health & family Welfare. Clinical Management Protocol: COVID-19 version 3, dated 13/6/20
www.mohfw.gov.in/pdf/ClinicalManagementProtocolfornormalCOVID19.pdf
- 18 <https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2018/UploadPublicNoticesFiles/MoHFW%20Convalescent%20Plasma%20in%20COVID%20draft.pdf> (accessed on 18th June 20221)
- 19 McAndrews KM, Dowlatshahi DP, Dai J, Becker LM, Hensel J, Snowden LM, et al. Heterogeneous antibodies against SARSCoV- 2 spike receptor binding domain and nucleocapsid with implications for COVID-19 immunity. *JCI Insight*. 2020;5.
- 20 Luchsinger LL, Ransegnola BP, Jin DK, Muecksch F, Weisblum Y, Bao W, et al. Serological assays estimate highly variable SARS-CoV-2 neutralizing antibody activity in recovered COVID-19 patients. *J Clin Microbiol*. 2020;58.
- 21 FDA. 2021. Revised COVID Convalescent Plasma EUA.
<https://www.fda.gov/media/141477/download>. Accessed March 2021.
- 22 . FDA. 2020. *COVID Convalescent Plasma EUA*. <https://www.fda.gov/media/141477/download>. Accessed September 2020
- 23 FDA. 2020. *FDA Issues Emergency Use Authorization for Convalescent Plasma as Potential Promising COVID-19 Treatment, Another Achievement in Administration's Fight Against Pandemic*. <https://www.fda.gov/news-events/press-announcements/fda-issues-emergency-use->

[authorization-convalescent-plasma-potential-promising-covid-19-treatment](#) Accessed December 2020.

24 FDA. 2021. *Revised COVID Convalescent Plasma*

EUA. <https://www.fda.gov/media/141477/download>. Accessed March 2021.

25 https://www.diasorin.com/sites/default/files/allegati_prodotti/liaisonr_sars-cov-2_s1s2_igg_m0870004366-d_lr.pdf

26 COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at <https://www.covid19treatmentguidelines.nih.gov/>. Accessed [19/6/2021]

27 Lasky B, Goodhue Meyer E, Steele WR, Crowder LA, Young PP. COVID-19 convalescent plasma donor characteristics, product disposition, and comparison with standard apheresis donors. *Transfusion*. 2021 May;61(5):1471-1478. doi: 10.1111/trf.16286. Epub 2021 Mar 7. PMID: 33458811; PMCID: PMC8013318.

28 Wendel S, Kutner JM, Machado R, Fontao Wendel R, Bub C, Fachini R, et al. Screening for SARS-CoV-2 antibodies in convalescent plasma in Brazil: preliminary lessons from a voluntary convalescent donor program. *Transfusion*. 2020 Dec; 60(12): 2938–51.

29 Dhiman Y, Coshic P, Pandey HC, Khatiwada B, Singh J, Mehta V, Gupta S. Deterrents in recruitment of COVID-19 convalescent plasma donors: Experience from a hospital-based blood centre in India. *Transfus Med*. 2021 Jun;31(3):149-154. doi: 10.1111/tme.12768. Epub 2021 Mar 21. PMID: 33749020.

30 Mehew J, Johnson R, Roberts D, Harvala H. Convalescent plasma for COVID-19: male gender, older age and hospitalisation associated with high neutralising antibody levels, England, 22 April to 12 May 2020. *Euro Surveill*. 2020 Nov;25(45):2001754. doi: 10.2807/1560-7917.ES.2020.25.45.2001754. PMID: 33183404; PMCID: PMC7667632.

- 31 Singh, P., Srivastava, A., Upadhyay, S., Singh, A., Gupta, P., Maurya, S., Pandey, R.K., Shrivastava, A., Dev, P., Singh, V., Mishra, R., Shukla, M., Chaubey, G., Kumar, P., Rai, V., Tripathy, Y.B., Pathak, A., Mishra, V., Mallick, C.B., & Shrivastava, P. (2021). The association of ABO blood group with the asymptomatic COVID-19 cases in India. medRxiv.
- 32 Shokri, P., Golmohammadi, S., Noori, M., Nejadghaderi, S.A., Carson-Chahhoud, K. and Safiri, S. (2021), The relationship between blood groups and risk of infection with SARS-CoV-2 or development of severe outcomes: A review. *Rev Med Virol*. <https://doi.org/10.1002/rmv.2247>
- 33 Aljanobi GA, Alhajjaj AH, Alkhabbaz FL, Al-Jishi JM. The relationship between ABO blood group type and the COVID-19 susceptibility in Qatif Central Hospital, eastern Province, Saudi Arabia: a retrospective cohort study. *OJIM* 2020; 10(02): 232- 238. <https://doi.org/10.4236/ojim.2020.102024>
- 34 Samra, S., Habeb, M. & Nafae, R. ABO groups can play a role in susceptibility and severity of COVID-19. *Egypt J Bronchol* 15, 9 (2021). <https://doi.org/10.1186/s43168-020-00051-w>
- 35 Vahidy FS, Pan AP, Ahnstedt H, Munshi Y, Choi HA, Tiruneh Y, et al. Sex differences in susceptibility, severity, and outcomes of coronavirus disease 2019: cross-sectional analysis from a diverse US metropolitan area. *PLoS One*. 2021;16:e0245556.
- 36 Davies NG, Klepac P, Liu Y, Prem K, Jit M, group CC-w, Eggo RM. Age-dependent effects in the transmission and control of COVID-19 epidemics. *Nat Med*. 2020;26:1205–11.
- 37 Mehew J, Johnson R, Roberts D, Harvala H. Convalescent plasma for COVID-19: male gender, older age and hospitalisation associated with high neutralising antibody levels, England, 22 April to 12 May 2020. *Euro Surveill*. 2020 Nov;25(45):2001754–7917.
- 38 Terpos E, Politou M, Sergentanis TN, Mentis A, Rosati M, Stellas D, et al. Anti-SARS-CoV-2 Antibody Responses in Convalescent Plasma Donors Are Increased in Hospitalized Patients; Subanalyses of a Phase 2 Clinical Study. *Microorganisms*. 2020 Nov;8(12):1885.

- 39 Bošnjak B, Stein SC, Willenzon S, Cordes AK, Puppe W, Bernhardt G, et al. Low serum neutralizing anti-SARS-CoV-2 S antibody levels in mildly affected COVID-19 convalescent patients revealed by two different detection methods. *Cell Mol Immunol*. 2020 Nov.
- 40 Jungbauer C, Weseslindtner L, Weidner L, Gansdorfer S, Farcet MR, Gschaider-Reichhart E, et al. Characterization of 100 sequential SARS-CoV-2 convalescent plasma donations. *Transfusion*. 2021 Jan;61(1):12–6.
- 41 Gniadek TJ, Thiede JM, Matchett WE, Gress AR, Pape KA, Fiege JK, Jenkins MK, Menachery VD, Langlois RA, Bold TD: SARS-CoV-2 neutralization and serology testing of COVID-19 convalescent plasma from donors with nonsevere disease. *Transfusion*. 2021 Jan;61(1):17–23.
- 42 Körper S, Jahrsdörfer B, Corman V, M, Pilch J, et.al. Donors for SARS-CoV-2 Convalescent Plasma for a Controlled Clinical Trial: Donor Characteristics, Content and Time Course of SARS-CoV-2 Neutralizing Antibodies. *Transfus Med Hemother* 2021;48:137-147. doi: 10.1159/000515610
- 43 Amjadi MF, O'Connell SE, Armbrust T, Mergaert AM, Narpala SR, Halfmann PJ, Bashar SJ, Glover CR, Heffron AS, Taylor A, Flach B, O'Connor DH, Kawaoka Y, McDermott AB, Sethi AK, Shelef MA. Fever, Diarrhea, and Severe Disease Correlate with High Persistent Antibody Levels against SARS-CoV-2. *medRxiv* [Preprint]. 2021 Jan 6:2021.01.05.21249240. doi: 10.1101/2021.01.05.21249240. PMID: 33442707; PMCID: PMC7805469.

Table 1: Convalescent plasma donor characteristics (Combined Centre A & B)

Total screened n (%)	
Male	875 (93.48)
Female	61 (6.52)
Total deferred n (%)	
	P < 0.0001
Male	148 (79.57)
Female	38 (20.43)
Total Donated n (%)	
Female	23 (3.07)
Male	727 (96.93)
Type of Donor	
Voluntary	144(19.2)
Replacement	606(80.8)
First time	390 (52)
Repeat	360(48)
Age, years	
Mean \pm SD (Range)	P = 0.0001
All	35.09 \pm 8.7 (20-60)
Female	27.8 \pm 3.86 (21-36)
Male	34.8 \pm 8.74 (20-60)
Weight, kg	
Mean \pm SD (Range)	p < 0.001
All	73.32 \pm 14.10
Female	60.22 \pm 9.75
Male	74.38 \pm 13.86

Blood group, n (%)

A	165 (22)
B	331(44.13)
AB	68 (9.07)
O	186 (24.8)

Hemoglobin g/dl(Mean \pm SD) 14.92 \pm 1.42

Platelet Count (x10⁹/l) (Mean \pm SD) 236.9 \pm 63.1

Total leukocyte count (x10⁹/l) (Mean \pm SD) 7.62 \pm 2.62

Occupation %

Professional service	36
Government service	16
Semi-professional /self-employed	27
Students	12
Other	10

Interval between rRT-PCR/RAT positive test and plasma donation (days)-mean \pm SD (min-max) 63.93 (21.52) (15-119)

SARS CoV-2 Antibody Mean \pm SD(range)

Centre A

Antibody S/CO 50.21 \pm 41.52 (9.59-207.2)

Centre B

Antibody AU/ml 84.01 \pm 76.31(16-400)

SARS CoV-2 Antibody from rRT-PCR positive result n (%) Mean \pm SD

Centre A P = 0.2336

45 days and less	49	45.13±33.93 S/Co
More than 45 days	351	52.49±41.27 S/Co
Centre B	P = 0.1218	
45 days and less	101	73.55±80.34 AU/ml
More than 45 days	249	88.25±80.34 AU/ml

Table 2: Deferred donor details (Combined Centre A & B)

Reason for deferral	N=186	%
Overage/underage	5	2.69
Low platelet count	16	8.60
Anaemia/Low haemoglobin	17	9.14
Underweight	5	2.69
Poor venous access	11	5.91
High blood pressure	14	7.53
High blood Sugar	2	1.08
Absence of detectable/Low SARS CoV-2 antibodies	28	15.05
On Medication	5	2.69
Skin allergy	1	0.54
Minor/ Major Surgery	5	2.69
Inadequate time gap	18	9.68
Hepatitis B	3	1.61
Hepatitis C	4	2.15

Syphilis positive	3	1.61
Coronary Artery Disease	4	2.15
H/o parity	18	9.68
No COVID-19 positive diagnostic test result	25	13.44
Anxiety before donation	2	1.08

Table 3 Correlation between symptom score and SAR CoV-2 antibodies (Centre A)

Score	n*(%)	Median (Range)	p < 0.00001
0	24(6)	16.95(9.31-79.5)	r2=0.5193
1	115(28.75)	32.09 (9.6-90)	
2	103(25.8)	27.9 (9.59-130)	
3	86(21.5)	44.6(25.73-79.93)	
4	40(10)	71.8 (12.89-99.8)	
5	26(6.5)	101.85(28.9-194.9)	
6	6(1.5)	163.8 (63.8-234.21)	

*The majority of donors had several symptoms

Table 4: Symptom wise multivariable analyses for correlates SARS CoV-2 antibody levels (Centre A)

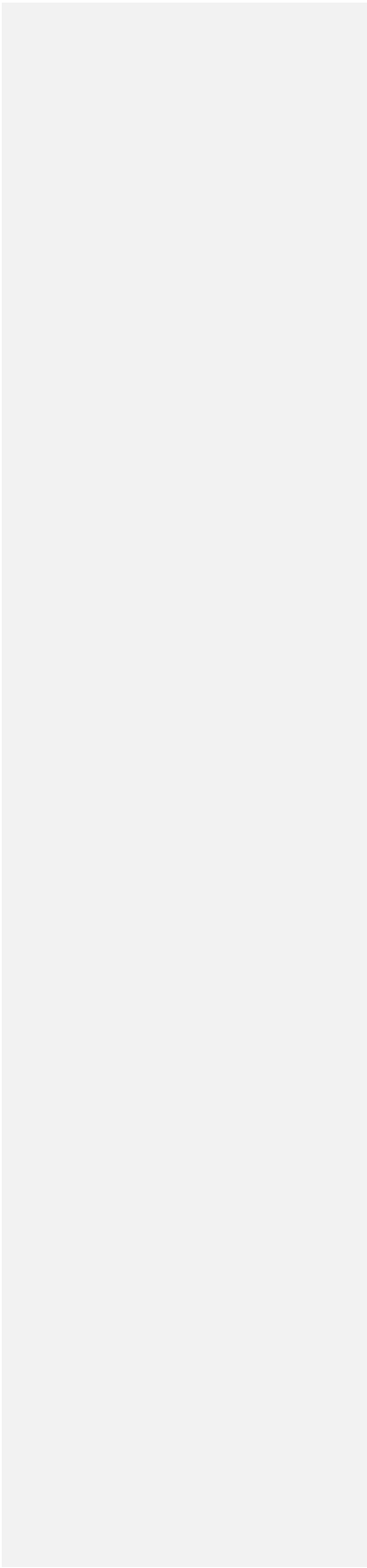
Multivariable analysis			
Symptoms	n	β (SE)	p-value
Asymptomatic	24.00	-0.10 (8.78)	0.06

Fever	332.00	0.16 (5.47)	0.000
GE or GE-like syndrome	33	0.46(66.69)	0.000
Anosmia	32.00	0.05 (11.45)	0.49
Ageusia	23.00	-0.10 (13.32)	0.21
Headache/Body ache	28.00	0.00 (6.68)	0.94
Cough/ Sore Throat/ SOB	179.00	0.06 (3.85)	0.23
Cold	49.00	-0.13 (5.73)	0.01

Significant correlates are shown in bold

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