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Clinical predictors of donor antibody titre and correlation with recipient antibody response in a COVID-19 convalescent plasma clinical trial

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Abstract. Madariaga MLL, Guthmiller JJ, Schrantz S, Jansen MO, Christensen C, Kumar M, Prochaska M, Wool G, Durkin-Celauro A, Oh WH, Trockman L, Vigneswaran J, Keskey R, Shaw DG, Dugan H, Zheng N-Y, Cobb M, Utset H, Wang J, Stovicek O, Bethel C, Matushek S, Giurcanu M, Beavis KG, di Sabato D, Meltzer D, Ferguson MK, Kress JP, Shanmugarajah K, Matthews JB, Fung JF, Wilson PC, Alverdy JC, Donington JS (University of Chicago, Chicago, USA). Clinical predictors of donor antibody titre and correlation with recipient antibody response in a COVID-19 convalescent plasma clinical trial (Original). *J. Intern. Med.* 2021; **289**: 559–573. https://doi.org/10.1111/joim.13185

Background. Convalescent plasma therapy for COVID-19 relies on transfer of anti-viral antibody from donors to recipients via plasma transfusion. The relationship between clinical characteristics and antibody response to COVID-19 is not well defined. We investigated predictors of convalescent antibody production and quantified recipient antibody response in a convalescent plasma therapy clinical trial.

Methods. Multivariable analysis of clinical and serological parameters in 103 confirmed COVID-19 convalescent plasma donors 28 days or more following symptom resolution was performed. Mixed-effects regression models with piecewise linear trends were used to characterize serial antibody responses in 10 convalescent plasma recipients with severe COVID-19.

Results. Donor antibody titres ranged from 0 to 1 : 3892 (anti-receptor binding domain (RBD)) and 0 to 1 : 3289 (anti-spike). Higher anti-RBD and anti-spike titres were associated with increased age, hospitalization for COVID-19, fever and absence of myalgia (all $P < 0.05$). Fatigue was significantly associated with anti-RBD ($P = 0.03$). In pairwise comparison amongst ABO blood types, AB donors had higher anti-RBD and anti-spike than O donors ($P < 0.05$). No toxicity was associated with plasma transfusion. Non-ECMO recipient anti-RBD antibody titre increased on average 31% per day during the first three days post-transfusion ($P = 0.01$) and anti-spike antibody titre by 40.3% ($P = 0.02$).

Conclusion. Advanced age, fever, absence of myalgia, fatigue, blood type and hospitalization were associated with higher convalescent antibody titre to COVID-19. Despite variability in donor titre, 80% of convalescent plasma recipients showed significant increase in antibody levels post-transfusion. A more complete understanding of the dose-response effect of plasma transfusion amongst COVID-19-infected patients is needed.

Keywords: convalescent plasma, COVID-19, antibody titre.

Introduction

Convalescent plasma therapy has historically been used as a treatment during epidemics [1]. In this

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therapy, neutralizing anti-viral antibodies, as well as non-neutralizing antibodies and other immunomodulators, are transferred via plasma transfusion from those who have recovered from disease to those currently infected [2-4]. For patients with severe COVID-19, convalescent plasma therapy has safely led to improvement in clinical and radiographic parameters [5-10]. Once adequate numbers of people convalesced and supply chain logistics were established, providing plasma therapy to a large number of patients has proven feasible [11].

Efficacy of convalescent plasma therapy relies on a robust antibody response in convalescent plasma donors. Measurements of antibody response amongst patients with COVID-19 demonstrate that the majority 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 [12-14]. Strikingly, a small proportion of recovered COVID-19-infected patients show no detectable antibodies to these epitopes [12, 15].

The relationship between host characteristics, disease course and variability in antibody response to COVID-19 is poorly understood. The aim of this study was to establish a translational convalescent plasma programme to investigate the relationship between clinical and serological parameters in convalescent plasma donors and define the antibody response of convalescent plasma recipients.

Methods

Study design

This was a prospective open-label clinical study to assess the feasibility, safety and immunological impact of delivering anti-SARS-CoV-2 convalescent plasma to hospitalized patients aged 18 years or older with severe or life-threatening COVID-19 disease within 21 days from the onset of their illness. This study was conducted at University of Chicago Medicine (UCM) from 10 April 2020 to 17 May 2020. The final date of follow-up was 25 May 2020.

Recruitment team

We used existing hospital infrastructure and personnel to build the convalescent plasma programme at a time when state-wide shelter-in-place orders were active, elective procedures were not being performed, and non-COVID-19-related research activities were halted. The donor enrolment team consisted of two surgeons, two surgical residents and three physician assistants. A dedicated study coordinator was present at the UCM Blood Donation Center to facilitate whole blood donation and collect research samples. Recipients were selected during daily videoconference with infectious disease. One surgeon visited the hospital COVID-19 unit daily to obtain consent and research samples.

Convalescent plasma donors

Plasma donors were age 18 or older, able to donate blood per standard UCM Blood Donation Center guidelines, had a documented COVID-19 polymerase chain reaction (PCR) positive test, and complete resolution of symptoms at least 28 days prior to donation. Recruitment occurred via social media, news outlets, word-of-mouth and announcements in university and community bulletins. The UCM infectious disease team provided an institutional list of patients with a positive PCR test for COVID-19, and their physicians were emailed to request permission to contact the patient for donor participation. Interested plasma donors were directed to fill out a short screening survey online. Potential donors meeting study criteria were screened for eligibility, reported symptoms and comorbidities, consented and were scheduled for donation at the UCM Blood Donation Center in a single telephone encounter. After meeting the UCM Blood Donation Center eligibility criteria, whole blood was collected and processed according to standard UCM Blood Donation Center procedures. Standard whole blood donation was used for plasma collection because it fit into pre-existing UCM Blood Bank infrastructure and workflow therefore facilitating rapid deployment of a collection process and allowing for red blood cell and unused plasma units to be used in the regular Blood Bank inventory. During blood donation, a single research sample was collected at the same time as blood samples for standard immunohaematology testing and infectious disease screening. Leucocyte filters used in separation of constituent blood parts were also collected for research.

Convalescent plasma recipients

Eligibility for convalescent plasma recipients included: age 18 or older, laboratory-confirmed

COVID-19, within 21 days from the start of illness and severe or life-threatening COVID-19 as defined by the United States Food and Drug Administration (FDA) [16]. Severe COVID-19 was defined as dyspnoea, respiratory frequency ≥ 30/min, blood oxygen saturation ≤ 93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300 and/or lung infiltrates >50% within 24–48 h. Life-threatening COVID-19 was defined as respiratory failure, septic shock and/or multiple organ dysfunction or failure. Patients who were pregnant received pooled immunoglobulin in the past 30 days or had a history of transfusion reaction were excluded from this study. Recipients had routine pretransfusion testing, in keeping with institution policies.

Convalescent plasma transfusion

On the day of enrolment, an emergency investigational new drug (eIND) application was filed and approved for each recipient by the FDA [16]. Subsequently, one ABO-compatible unit of convalescent plasma (~300 mL) was transfused over 4 h. Repeat administration of convalescent plasma occurred in one recipient (R7). Blood samples and nasopharyngeal swabs were obtained at day 0, 1, 3, 7 and 14 post-transfusion.

Outcomes

The primary outcome was feasibility as defined by the collection of convalescent plasma and its administration into hospitalized patients. Secondary outcomes included type and duration of respiratory support, cardiac arrest, transfer to intensive care unit (ICU), length of stay, mortality, complications of plasma administration, process outcomes and antibody titre of plasma donors and recipients.

Antibody test and real-time polymerase chain reaction (RT-PCR) detection of SARS-CoV-2

Levels of anti-RBD and anti-spike antibodies were measured by enzyme-linked immunosorbent assay (ELISA) in blood samples at time of donation and plasma recipients, as previously described [17]. Antibody measurement was not specific for a particular isotype but rather detected all isotypes. Nasopharyngeal specimens were obtained by flocked swabs in plasma recipients and analysed by RT-PCR to detect SARS-CoV-2 RNA.

Statistics

Study data were collected and managed using REDCap electronic data capture tools hosted at UCM [18, 19]. Donor patient characteristics were compared using the chi-squared test for categorical variables and the two-sample t-test for continuous variables. Univariate regression analysis for antibody titre (anti-RBD and anti-spike) was conducted against age, sex, body mass index (BMI), previous pregnancy, previous blood donation, blood type, symptoms (fever, cough, sore throat, dyspnoea, abdominal pain, ageusia, anosmia, fatigue, myalgia, headache), comorbidities (respiratory, cardiovascular, renal, diabetes, autoimmune disease, cancer, liver disease), smoking history, travel in the past 3 months to the United States, Asia or Europe, symptom duration, interval from symptoms resolution to plasma donation and hospitalization. Pairwise comparison using t-tests without adjusting for multiple comparisons was used to compare antibody titres amongst different ABO blood groups.

We conducted multivariable analyses to identify prediction models for anti-RBD and anti-spike antibody titres amongst convalescent plasma donors. Best subset variable selection method was chosen to identify the subset of predictors that maximizes the adjusted R-squared amongst all possible models. To compare daily change in recipient antibody response, we fit mixed-effects regression models with piecewise linear trend with a change point at 3 days after intervention for log-transformed antibody titres. We considered recipients on extracorporeal membrane oxygenation (ECMO) (R3 and R6) separately from recipients not on ECMO (R1, 2, 4, 5, 7, 8, 9, 10), because ECMO recipients had different baseline characteristics.

Data analysis was performed using software R, version 3.6.3. Mixed-effects regression models were fit using the lmer function of the lme4 package [20]. Data analysis was conducted within RStudio environment, and R markdown files with fully reproducible data analysis can be obtained from the authors upon request.

Study approval

This study was approved by the Institutional Review Board (IRB20-0523). All participants (plasma donors and plasma recipients) gave

written informed consent prior to inclusion in the study. Analysis was performed by MLM and MG. This clinical trial was registered at ClinicalTrials.gov with identifier NCT04340050.

Results

Clinical characteristics of convalescent plasma donors

697 potential plasma donors were recruited to our study over 35 days (Table 1). The average age was 43.5 years (range 18 to 87), the majority were female (63.1%), and 37% had never donated blood before. Potential donors with confirmed positive COVID-19 PCR (n = 384, 55%) were more likely to be male, have ageusia and anosmia, and lack cough, sore throat and dyspnoea compared to the 313 symptomatic patients who had clinical signs of COVID-19 but were never tested (Table 1). Amongst plasma donors (n = 103) who donated as of publication, average symptom duration was 11.9 ± 5.91 days, 9 (8.7%) had respiratory comorbidities such as asthma, chronic obstructive pulmonary disease or obstructive sleep apnoea and 8 (7.8%) had been previously hospitalized for COVID-19 (Table 1, Table S2). The average interval between symptom start and plasma donation was 45.1 ± 8.02 days.

Predictors of donor anti-RBD and anti-spike antibody titre

Donor antibody titres measured on day of plasma donation ranged from 0 to 1:3892 (anti-RBD) and from 0 to 1:3288.7 (anti-spike) (Table 1). In univariable regression analysis, higher average anti-RBD and anti-spike antibody titres were associated with plasma donors who were older, male, had higher BMI, had fever and had been hospitalized ($P < 0.05$, Table S1). In a pairwise comparison amongst ABO groups without adjusting for multiple comparisons, AB donors had higher anti-RBD titre than O negative donors ($P = 0.048$) and higher anti-spike titre than O negative ($P = 0.015$) or O positive ($P = 0.037$) donors.

To determine predictors of anti-RBD and anti-spike antibody titre, we performed best subset multivariable analysis including age, sex, blood type, history of previous blood donation, fever, cough, fatigue, myalgia, symptom duration, hospitalization and travel in the United States within the past 3 months. Significant predictors of anti-RBD antibody titre were age ($P = 0.02$), fever ($P < 0.01$), previous hospitalization ($P < 0.01$), lack of myalgia ($P = 0.01$) and fatigue ($P = 0.03$) (R-squared = 0.40, adjusted R-squared = 0.32, Table 2). Significant predictors of anti-spike antibody titre were age ($P = 0.02$), fever ($P = 0.01$), previous hospitalization ($P = 0.01$) and absence of myalgia ($P < 0.01$) (R-squared = 0.35, adjusted R-squared = 0.26, Table 2). O positive blood type was associated with lower anti-RBD ($P = 0.05$) but did not meet significance threshold for anti-spike ($P = 0.07$).

Clinical course of 10 convalescent plasma recipients

Ten hospitalized patients with severe or life-threatening COVID-19 received plasma on day 0 (Fig. 1, Table 3). Plasma recipients were on average 61.9 years old (range 30–86) and 40% women. The average time from start of symptoms to plasma transfusion was 12 days (range 2–21), and the average time from hospital admission to plasma transfusion was 6 days (range 2–17). At the time of plasma transfusion, two patients were on ECMO, one patient was mechanically ventilated, two patients were on high-flow nasal cannula (HFNC), four patients were on nasal cannula and one patient was on room air. Five patients had received other therapies for COVID-19 before transfusion, including remdesivir, tocilizumab, anakinra and hydroxychloroquine. Only one patient had no prior documented comorbidities. One patient had undergone bilateral lung transplantation for cystic fibrosis (R8), one patient had undergone stem cell transplant for myelodysplastic syndrome (R7) and one patient had end-stage renal disease on haemodialysis (R10).

Figure 2 shows selected clinical and laboratory parameters of convalescent plasma recipients. Only one recipient (R8) had fever prior to transfusion and this resolved by day 3 post-transfusion. R3 and R6 remained on ECMO throughout the study period. In the remaining 8 recipients, oxygen requirements improved to room air or nasal cannula. The Sequential Organ Failure Assessment (SOFA) score [21] was calculated for recipients on mechanical ventilation or ECMO and showed a general trend towards improvement; notably both ECMO patients were weaned off vasopressor and intra-aortic balloon pump support by 7 days post-transfusion. Levels of inflammatory marker C-reactive protein (CRP) were variable. CRP decreased in six recipients (R1, R2, R5, R6, R9, R10). SARS-CoV-2 NP swab PCR remained positive in 5 patients and turned

Table 1. *Characteristics of recruited convalescent donors (*n = *697)*

	Recruited potential donors (*n* = 697)	No COVID-19 test (*n* = 313)	Positive COVID-19 PCR test (*n* = 384)	*P* value	Plasma donors (*n* = 103)
Age (years)—mean (SD)	43.5 (14.8)	44.4 (14.6)	42.8 (14.9)	0.172	41.8 (13.9)
Female—*n* (%)	440 (63.1)	211 (67.4)	227 (59.4)	0.036	50 (48.5)
Previously pregnant—*n* (%)	253 (36.3)	120 (38.3)	132 (34.6)	0.341	26 (25.2)
Previous blood donor—*n* (%)	439 (63.0)	205 (65.5)	232 (60.7)	0.225	73 (70.9)
Symptoms					
Fever	487 (69.9)	220 (70.3)	266 (69.6)	0.917	81 (78.6)
Cough	508 (72.9)	243 (77.6)	263 (68.8)	0.012	75 (72.8)
Sore throat	317 (45.5)	170 (54.3)	146 (38.2)	<0.001	46 (44.7)
Dyspnoea	358 (51.4)	181 (57.8)	176 (46.1)	0.003	45 (43.7)
Abdominal pain	164 (23.5)	81 (25.9)	82 (21.5)	0.202	17 (16.5)
Ageusia	383 (54.9)	142 (45.4)	240 (62.8)	<0.001	64 (62.1)
Anosmia	375 (53.8)	145 (46.3)	229 (59.9)	<0.001	58 (56.3)
Fatigue	611 (87.7)	278 (88.8)	331 (86.6)	0.454	93 (90.3)
Myalgia	524 (75.2)	233 (74.4)	289 (75.7)	0.780	82 (79.6)
Headache	513 (73.6)	224 (71.6)	289 (75.7)	0.257	76 (73.8)
Symptom duration (days)—mean (SD)	14.8 (8.9)	14.9 (9.0)	14.8 (8.9)	0.908	11.9 (5.91)
BMI—mean (SD)					26.9 (5.6)
Comorbidities—*n* (%)					
Cardiovascular					6 (5.8)
Diabetes					1 (1.0)
Respiratory disease (asthma, COPD, OSA)					9 (8.7)
Liver disease					0
Kidney disease					1 (1.0)
Autoimmune disease					4 (3.9)
History of cancer					2 (1.9)
Smoking—*n* (%)					
Past or current smoker					26 (25.2)
Never smoker					77 (74.8)
Travel within the past 3 months—*n* (%)					
USA					55 (53.4)
Asia					3 (2.9)
Europe					9 (8.7)
Hospitalized—*n* (%)					8 (7.8)
Interval between symptom start and plasma donation (days)—mean (SD)					45.1 (8.02)
Interval between symptom end and plasma donation (days)—mean (SD)					33.3 (6.47)
ABO blood type—*n* (%)					
A positive					35 (35.7)
A negative					5 (5.1)
B positive					13 (13.3)

Table 1 (*Continued*)

	Recruited potential donors (n = 697)	No COVID-19 test (n = 313)	Positive COVID-19 PCR test (n = 384)	P value	Plasma donors (n = 103)
B negative					0
AB positive					4 (4.1)
AB negative					0
O positive					35 (35.7)
O negative					6 (6.1)
Anti-RBD antibody titre—mean (range, SD)					522.5 (0–3892.3, 637.3)
Anti-Spike antibody titre—mean (range, SD)					543.1 (0–3288.7, 564.8)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; OSA, obstructive sleep apnoea; RBD, receptor binding domain; SD, standard deviation. Plasma donors (n = 103) underwent additional questionnaire after consent.

Table 2. *Best subset multivariable regression model for predictors of anti-RBD antibody titre and anti-spike antibody titre*

	Reciprocal anti-RBD antibody titre estimate	P value	Reciprocal anti-spike antibody titre estimate	P value
Age	10.2	0.02	9.80	0.02
Male	190.62	0.07	138.27	0.18
Previous blood donation	−213.48	0.07	137.26	0.22
Fever	362.98	0.01	347.52	0.01
Cough	178.41	0.16	180.25	0.15
Fatigue	424.76	0.03	300.72	0.11
Myalgia	−434.68	0.01	−434.56	<0.01
Symptom duration	−15.36	0.11	−11.2	0.23
Hospitalization	757.60	<0.01	524.8	0.005
Travel in USA within 3 months	−149.72	0.16	−188.38	0.07
Blood type A negative	−275.97	0.29	−265.37	0.29
Blood type O positive	−218.78	0.05	−198.39	0.07

RBD, receptor binding domain; USA, United States of America. Anti-RBD, R-squared 0.40, adjusted R-squared 0.32. Anti-spike, R-squared 0.35, adjusted R-squared 0.26.

negative in 4 patients; 1 patient (R6) had been positive for SARS-CoV-2 17 days prior to plasma transfusion but was negative for SARS-CoV-2 on day of transfusion (Fig. 1). At last follow-up, 1 patient on ECMO remained in the hospital (R6), 1 patient on ECMO was transitioned to comfort care and died on day 30 after plasma transfusion (R3), 4 patients were discharged to rehabilitation facilities and 4 patients were discharged to their place of residence (Fig. 1).

Post-transfusion relationship between convalescent plasma donor and recipient antibody titre

On day of transfusion, anti-RBD antibody titres were undetectable in 3 recipients (R1, R2, R10) and anti-spike antibody titres were undetectable in 3 recipients (R1, R8, R10) (Table 3 and Fig. 3). Both patients on ECMO had very high antibody titre at day 0 which decreased in the days after transfusion (Fig. 3). The remaining plasma recipients

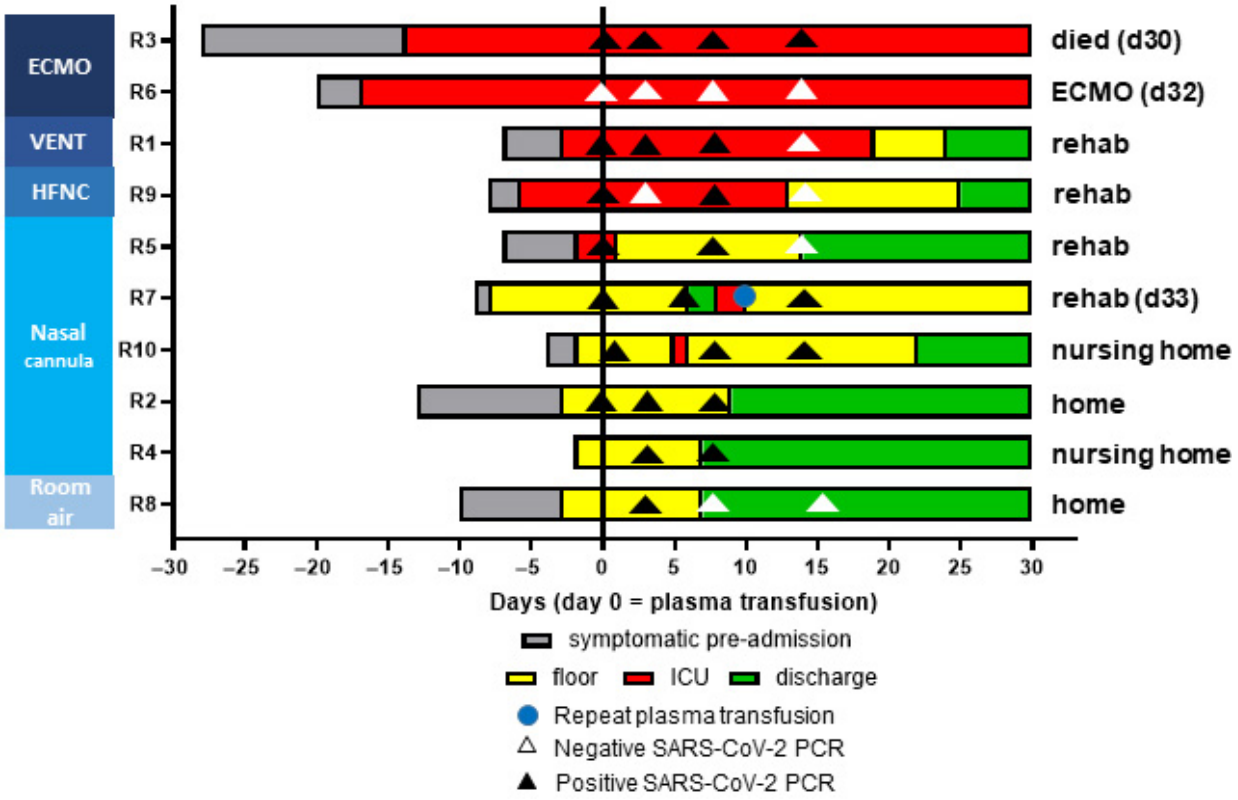


Fig. 1 *Recipient hospital course. Recipient clinical course before and after plasma transfusion (day 0). Number of days symptomatic prior to admission (grey) and recipient location in the intensive care unit (ICU, red), hospital floor (yellow) and home (green) shown by day of plasma transfusion. Positive SARS-CoV-2 NP swab PCR test indicated by black triangle and negative test indicated by white triangle. Repeat plasma dosing indicated by blue circle. Respiratory support at time of plasma transfusion indicated by left column (ECMO, extracorporeal membrane oxygenation; vent, mechanical ventilation; nasal cannula; room air).*

showed increase in antibody titre within the first three days after transfusion (R1, 2, 4, 5, 7, 8, 9) with the exception of R10 who did not show any antibody titre until day 7 (anti-spike) and day 14 (anti-RBD) after transfusion (Fig. 3).

We performed a mixed-effects model for log-transformed reciprocal antibody titre adjusting for donor antibody titre level looking at the first 3 days post-transfusion amongst the non-ECMO patients. After plasma transfusion, recipient anti-RBD antibody titre increased on average by 31% per day ($P = 0.01$) and recipient anti-spike antibody titre increased on average by 40.3% per day ($P = 0.01$; Fig. 4). Amongst the two ECMO recipients, recipient antibody response was not significantly changed until three days after plasma transfusion (decreasing by 9.2% per day for anti-RBD titre and 8.2% per day for anti-spike titre, $P < 0.01$; Fig. 4).

Safety of convalescent plasma transfusion

We monitored the clinical status of the recipients before, during and immediately after transfusion. No recipients experienced toxicity associated with plasma transfusion. There was no clinical deterioration or worsening of disease status immediately related to plasma transfusion.

Safety of convalescent plasma transfusion in high-risk populations

Patient R8 was a 30-year-old male with a history of cystic fibrosis who underwent bilateral lung transplantation 1 year prior. He presented with fevers, chills, ageusia and acute kidney injury

Table 3. *Clinical characteristics of recipients receiving plasma for this study*

Respiratory support prior to plasma transfusion	Recipient #	Age	Gender	BMI	Comorbidities	Symptoms	Previous COVID treatment	Interval from symptom start to plasma transfusion (days)	Interval from hospital admission to plasma transfusion (days)	Donor Anti-RBD titre (1/X)	Recipient Anti-RBD titre (1/X) on day 0 and day 1	Donor Anti-spike titre (1/X)	Recipient Anti-spike titre (1/X) on day 0 and day 1	Disposition
Veno-venous ECMO	R3	51	F	36.0	HTN, DM, PE, asthma	Fever, cough, dyspnoea	Remdesivir, tocilizumab	21	14	585.4	2732.1 1915.4	580.4	7666.7 5964.3	Died (day 30)
	R6	59	M	28.9	HTN, DM	Fever, chills, decreased appetite, dizziness	Remdesivir, tocilizumab	20	17	78.7	4301.6 4042.6	305.1	13833.6 11544.3	ICU (day 32)
Mechanical ventilation	R1	57	M	31.0	HTN, DM, NAFLD	Fever, cough, nausea	Tocilizumab	10	3	80.2	0 51	68.9	0 72.2	Rehab (day 24)
High-flow nasal cannula	R9	78	M	24.4	HTN, prostate cancer	Fever, cough	None	14	6	1312.8	879.7 1160.6	1179.2	2118.4 2996.1	Rehab (day 26)
Nasal Cannula	R5	66	F	28.2	HTN, PE/DVT, recent hospitalization for orthopaedic procedure	Altered mental status, dyspnoea	None	9	2	292.4	113.6 222.9	351.1	181.6 313.7	Rehab (day 14)
	R7[a]	57	M	–	HTN, Myelodysplastic syndrome s/p stem cell transplant	Dyspnoea	Tocilizumab, anakinra	9	8	2330.2	810 1540.8	1648	1056.6 1038.2	Rehab (day 33)
	R10	86	F	32.2	ESRD on HD, stroke, PVD s/p AKA, DM, PE/DVT, CHF	Dyspnoea, abdominal pain	None	6	2	434.8	0 0	756.4	0 0	Home (day 23)
	R2	61	M	26.4	none	Cough, weakness, hiccups, altered mental status	Hydroxy-chloroquine	16	3	72.6	0 50	92.7	446.2 1413.2	Home (day 9)
	R4	74	F	–	HTN, Alzheimer's disease	Fever, altered mental status	None	2	2	382.0	1461.3 1493.6	716.3	3154.4 3038.8	Home (day 8)

Table 3 (*Continued*)

Respiratory support prior to plasma transfusion	Recipient #	Age	Gender	BMI	Comorbidities	Symptoms	Previous COVID treatment	Interval from symptom start to plasma transfusion (days)	Interval from hospital admission to plasma transfusion (days)	Donor Anti-RBD titre (1/X)	Recipient Anti-RBD titre (1/X) on day 0 and day 1	Donor Anti-spike titre (1/X)	Recipient Anti-spike titre (1/X) on day 0 and day 1	Disposition
Room air	R8	30	M	23.2	Cystic fibrosis s/p bilateral lung transplant, DM	Fever, chills, fatigue, ageusia	None	13	3	3892.3	71.9 172.7	2921.1	0 138.3	Home (day 6)

AKA, above the knee amputation; CHF, congestive heart failure; DM, diabetes mellitus; DVT, deep venous thrombosis; ESRD, end-stage renal disease; HTN, hypertension; NAFLD, nonalcoholic fatty liver disease; PE, pulmonary embolism; PVD, peripheral vascular disease.
[a]R7 was admitted 2 weeks prior to the ICU with COVID-19 pneumonia and was on high-flow nasal cannula. He was discharged to home on 2L oxygen but presented several days later with acute dyspnoea.

with creatinine 3.6 mg dL^{-1}. He tested positive for COVID-19 5 days prior to transfusion. He continued on prednisone (5 mg daily) but tacrolimus (4 mg twice daily) and mycophenolate mofetil (250 mg daily) were reduced. On day 2 after plasma transfusion, he defervesced. His symptoms improved and he was discharged to home on day 6 after transfusion. At a follow-up clinic visit on day 9, his NP swab PCR was negative for COVID-19.

Patient R7 was a 57-year-old male with a history of myelodysplastic syndrome who underwent stem cell transplant 10 months prior. He presented with fever, cough and dyspnoea and tested positive for COVID-19 23 days prior to transfusion. He was on chronic prednisone (5 mg daily) and ruxolitinib (5 mg twice daily). On his first admission, he required ICU care and HFNC. He underwent treatment with stress-dose steroids, remdesivir, tocilizumab and anakinra and was discharged to home after 12 days on 2L of nasal cannula. He was readmitted to the hospital 3 days later with worsening dyspnoea and 6L oxygen requirement. During this second admission, he initially underwent empiric treatment for suspected graft-versus-host disease with tacrolimus and stress-dose steroids. He underwent convalescent plasma therapy on hospital day 8 and was discharged to home 4 days later on 2L nasal cannula. He presented a third time to the emergency room 3 days later with worsening dyspnoea with oxygen saturation 70% and was started on high-flow nasal cannula. He was given a second convalescent plasma transfusion 10 days after the first transfusion. He is currently less dyspnoeic on 4L nasal cannula. His CRP remained less than 3 mg L^{-1} after plasma transfusion. Symptoms improved by day 13, and he was discharged to a rehabilitation facility on day 33.

Patient R10 was an 86-year-old female with history of heart failure, pulmonary embolism, stroke, peripheral artery disease, gluteal abscess and end-stage renal disease on haemodialysis who presented with fevers, dyspnoea, altered mental status and abdominal pain. Plasma was transfused after dialysis to minimize the risk of volume overload. She was weaned off supplemental oxygen 3 days post-transfusion. She was briefly admitted to the ICU for three days with hypotension due to fluid removal from haemodialysis and poor oral intake. She was discharged to a long-term care facility on day 23.

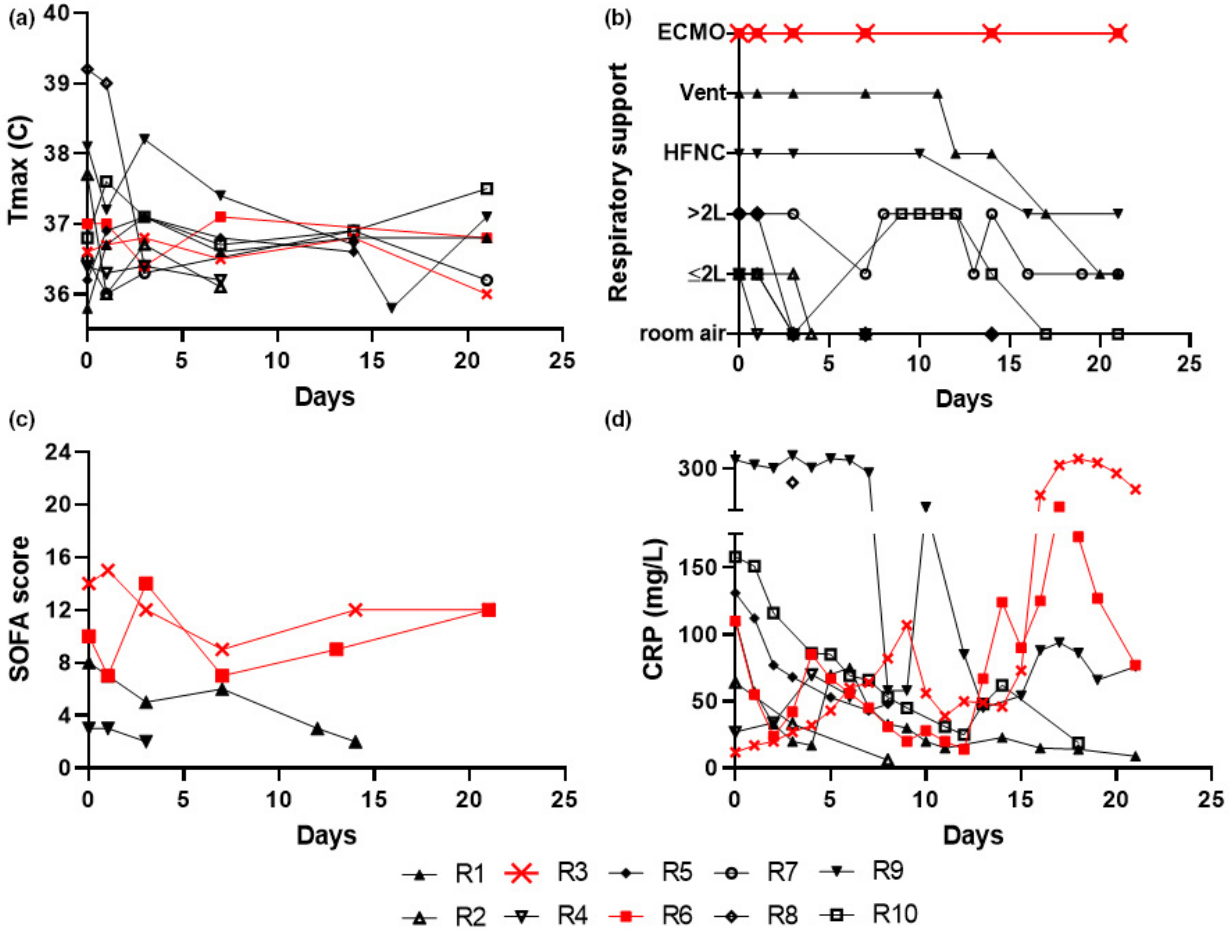
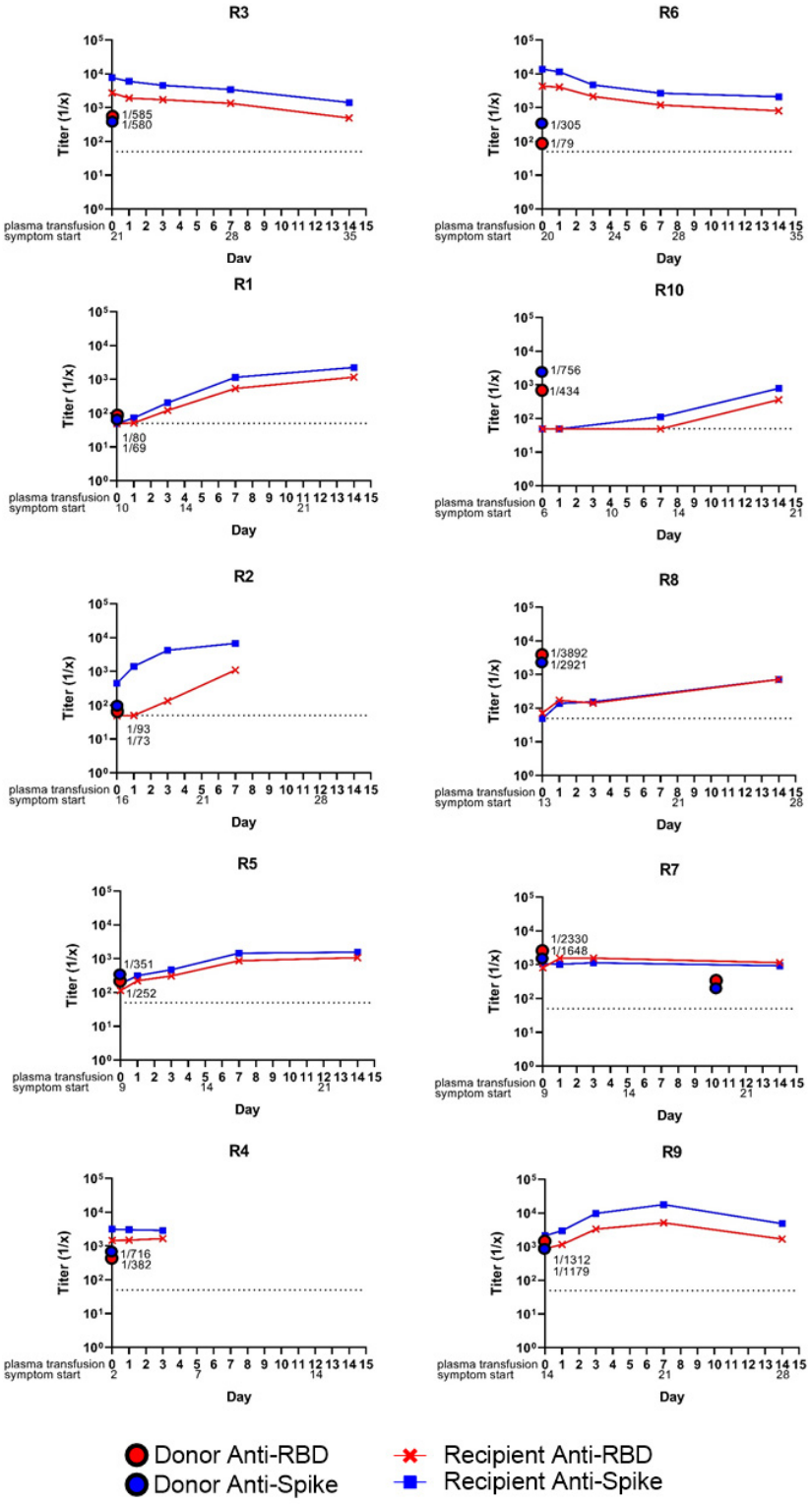


Fig. 2 *Recipient clinical and laboratory parameters after plasma transfusion. (a) Maximum daily temperature (T_{max}, c); (b) Type of respiratory support required (ECMO, extracorporeal membranous oxygenation; Vent, mechanically ventilated; HFNC, high-flow nasal cannula; L, number of litres of oxygen on nasal cannula); (c) Sequential Organ Failure Assessment (SOFA) score for recipients on mechanical ventilation or ECMO; (d) Inflammatory marker C-reactive protein (CRP). Data for patients on ECMO are in red.*

Discussion

We developed a translational convalescent plasma treatment programme within the existing hospital infrastructure during the COVID-19 pandemic that provided a new therapeutic option for patients whilst assessing the antibody profile of both convalescent and hospitalized patient populations. Our multivariable analysis demonstrated that clinical characteristics can predict serological response of antibodies associated with virus neutralization [12]. Higher anti-RBD and anti-spike antibody were more likely found in convalescents who were older, hospitalized, had fever and lacked myalgia. Fatigue also significantly predicted higher anti-RBD but not anti-spike antibody titre.

Fig. 3 *Recipient serology after plasma transfusion. Reciprocal donor plasma anti-RBD (red circle) and anti-spike (blue circle) antibody titre are plotted on the y-axis. Dotted line at 1:50 represents the limits of antibody detection. Reciprocal recipient anti-RBD (red line) and anti-spike (blue line) antibody titre are plotted on day 0 prior to transfusion and on days 1, 3, 7 and 14 post-transfusion.*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9 14 21
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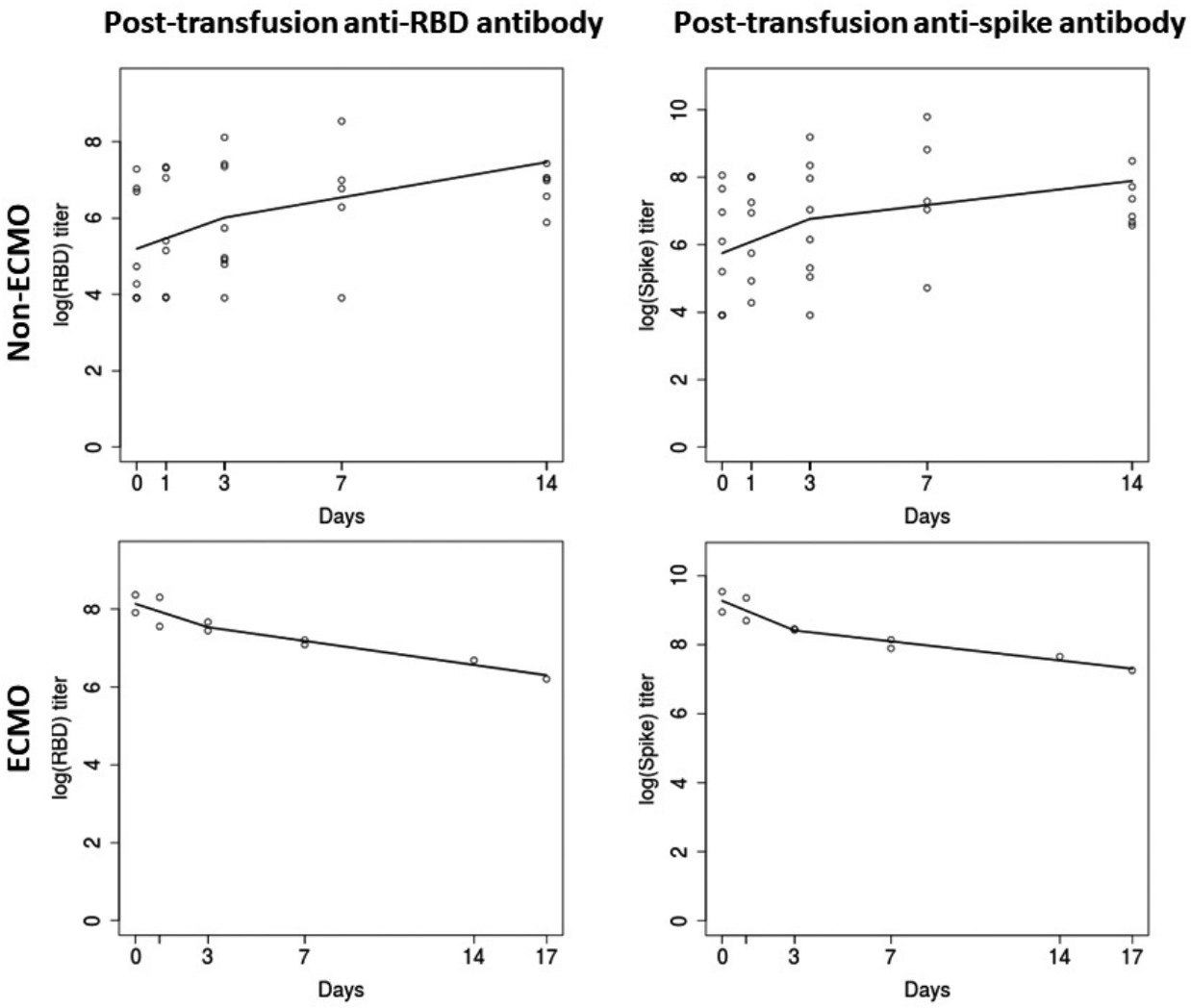

Fig. 4 *Antibody response curve in plasma recipients. Log-transformed rate of antibody titre change of anti-RBD and anti-spike antibodies in the non-ECMO (*n = *8) and ECMO (*n = *2) recipients were fitted in a mixed effects piecewise linear regression model.*

Variability in convalescent populations and immune response to viral infection may explain why recovery is not always marked by seroconversion [12, 15]. Indeed, in our study four plasma donors (as well as four plasma recipients) had undetectable antibody titres. Disparate plasma donor populations and geography may explain why symptom duration and elapsed time from symptom onset was associated with antibody response in New York City [13] but not amongst our patients in Chicago. Disparate plasma donor populations and geography may also explain antibody variability. These data highlight that the impact of variability in antibody type and titre on virus-neutralizing activity and long-term immunity is unknown.

Interestingly, we found that antibody titres significantly differed across ABO blood type groups, with O donors (who have natural anti-A and anti-B antibodies) demonstrating lower anti-RBD and anti-spike titres than AB donors. Previous studies showed that O blood type populations are less susceptible to infection with SARS-CoV [22] and

SARS-CoV-2 (COVID-19) [23]. Anti-A antibodies inhibited binding of the SARS-CoV spike protein to angiotensin-converting enzyme 2 receptors *in vitro* [24]. Further studies on the relationship between ABO polymorphism and antibody titre may uncover genetic determinants of the host response to COVID-19.

Recipients received plasma with a range of antibody titre from 1:73 to 1:3892 (anti-RBD) and 1:69 to 1:2921 (anti-spike). Despite this, 80% of recipients demonstrated a significant increase in anti-spike and anti-RBD antibody titre in the 3 days post-transfusion that was independent of donor antibody titre and were discharged after clinical improvement. Interestingly, recipient antibody titre continued to increase up to 14 days in four recipients (R1, 2, 8, 10); in contrast, the two most severely ill patients on ECMO who had the highest antibody titres (up to 1:13 833 anti-spike antibody in R6) showed a decrease in antibody titre after receiving plasma on day 20-21 of illness.

Importantly, we demonstrate the safety of transfusing convalescent plasma in immunosuppressed patients after lung transplantation and stem cell transplantation. None of the plasma recipients in this study deteriorated after convalescent plasma transfusion, consistent with the safety profile of other trials [5-9, 11]. Repeat plasma dose in recipient R7 was also well tolerated. Preclinical models of SARS-CoV and clinical experience of other viral illness had raised concern about the potential for non-neutralizing antibody to cause antibody dependent enhancement of disease, which was not seen here despite variable titres of donor antibodies [25-27].

The variability in post-transfusion recipient antibody titre and clinical response seen here and in other studies [5, 6, 28, 29] indicates that the therapeutic activity of convalescent plasma depends on the timing of treatment and composition of convalescent plasma. Indeed, plasma contains more than 1000 proteins, including albumin, immunoglobulins, complement and coagulation factors as well as organic compounds such as cytokines [4]. Convalescent plasma drawn shortly after natural infection [1, 5-8] may be enriched for populations of protective antibodies not present in plasma derived from long-recovered or rarely hospitalized donors studied here. Furthermore, immunomodulatory and non-virus-neutralizing antibody effects such as stimulation of the host humoral immune response and facilitating viral uptake into cells via Fc receptors to increase viral antigen presentation to other effector cells may contribute to disease recovery. Taken together, whilst randomized controlled efficacy trials for convalescent plasma therapy in COVID-19 are currently underway, establishing effective anti-COVID-19 plasma-based therapy will require both an understanding of the precise dose and type of virus-neutralizing antibody and in-depth characterization of plasma donor–recipient pairs.

The availability of a pre-existing hospital-based blood collection facility within our medical centre significantly eased the procurement of convalescent plasma and will allow us to assess immunological characteristics of donor–recipient pairs in future studies. Such hospital-based blood collection facilities have been declining in number across the United States for several decades [30]. Cultivating region-specific convalescent plasma inventory may potentially facilitate the identification and isolation of antibodies with specific activity against local virus strains and be a useful model for future outbreaks. In addition, convalescent plasma derived from whole blood collection is a rapidly scalable technique that requires basic phlebotomy and blood separation rather than a dedicated apheresis personnel and equipment. Furthermore, a significant proportion (36.3%) of our plasma donors had never donated blood before, indicating that a convalescent plasma donation programme can serve as important community outreach during a time when patients avoid hospitals that are perceived as unsafe [31].

In summary, development of a convalescent plasma programme is feasible, rapidly deployable and economical when existing resources of equipment, space and personnel are used. Establishing the clinical predictors of high antibody titre and understanding the serological post-transfusion response may guide patient selection and shed light on antibody response to COVID-19. Further work characterizing convalescent plasma donor and recipient pairs is needed to elucidate mechanisms of convalescent plasma therapy and demonstrate optimal viral epitope therapeutic targets.

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Conflict of interest statement

The authors have declared that no conflict of interest exists.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Univariate regression analysis for reciprocal anti-RBD and anti-spike antibody titer.

Table S2. Detailed characteristics of enrolled plasma donors (n = 221).■

