Journal of Infection 90 (2025) 106412

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Viruses and Viral Diseases

Individual patient and donor seroprofiles in convalescent plasma treatment of COVID-19 in REMAP-CAP clinical trial

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ARTICLE INFO

Article history:
Accepted 5 January 2025
Available online 9 January 2025

Keywords:
Infectious diseases
Transfusion medicine
Critical care
Virology
COVID-19
SARS-CoV-2
Convalescent plasma treatment
Serology
Antibodies

SUMMARY

Objectives: Convalescent plasma (CP) treatment of COVID-19 has shown significant therapeutic effect only when administered early. We investigated the importance of patient and CP seroprofiles on treatment outcome in REMAP-CAP CP trial.

Methods: We evaluated neutralising antibodies (nAb), anti-spike (S) IgM, IgG, IgG avidity, IgG fucosylation and respiratory viral loads in a sub-set of patients (n=80) and controls (n=51) before and after transfusion, comparing them to those in the CP units (n=157) they received.

Results: Most patients were SARS-CoV-2 seropositive pre-transfusion (72% nAb; 89% S-IgG seropositivity). The majority (80%) had higher pre-transfusion S-IgG levels (median 1.7×10^6 arbitrary units (AU); 56%) or S-IgG production rates (median 1.1×10^6 AU/day; 64%) than they received from CP (median 2.2×10^5 AU). Only 22% of the patients demonstrated significant (median 24-fold) increase in their S-IgG levels acquired from transfusion. Better outcomes, measured by organ support-free days, were associated with increase in S-IgM levels ($p=0.007$), decreased S-IgG fucosylation ($p<0.001$), lower patient age ($p<0.001$) but not with receiving CP ($p=0.337$).

Conclusions: Based on our data, increased S-antibody levels linked to transfused CP were only observed in pre-seroconversion or immunodeficient patients lacking their own SARS-CoV-2 antibodies, representing the groups where CP treatment has previously shown most benefit.

Introduction

SARS-CoV-2 is the causative agent of the recent pandemic of COVID-19.[1] SARS-CoV-2 antibodies, directed at the S-antigen induced by infection or vaccination mediate protective immunity.[2,3]

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https://doi.org/10.1016/j.jinf.2025.106412

Despite the successful development of targeted antiviral therapy and vaccines providing relatively good protection against severe disease, better interventions for vulnerable groups, such as the immunocompromised, are still needed. While immune protection relies on both cellular and humoral immunity, it was hypothesised that the administration of SARS-CoV-2-specific neutralising antibodies (nAb) might enhance host immunity. Therefore, high hopes were originally placed on convalescent plasma (CP) therapy using plasma collected from previously infected or vaccinated donors. The approach has been shown to be effective as prophylaxis of severe disease progression when administered early or before hospitalisation.[4–6] While several trials initially reported lack of benefit in treating hospitalised patients with CP,[6–9] a meta-analysis has since shown that early CP treatment can be effective regardless of hospitalisation.[10] Similarly, monoclonal anti-SARS-CoV-2 antibody therapy has shown to be effective, but only for seronegative patients.[6,11] These findings imply that CP administration is only able to change the course of disease before an endogenous antibody response has been mounted, or when there is lack of immune competence to mount such a response, mimicking the role of passive immunisation.

Even if initial serostatus of the patients seems crucial for successful CP treatment, the role of antibody characteristics to benefit or to cause harm has not been studied extensively. For example, clinical trials to date have not addressed antibody metrics of the patient and of the transfused CP at individual level. We hypothesised that transfusion of plasma with relatively low levels of neutralising antibodies but with high avidity or high effector functions may still be beneficial for a patient with low levels of protective antibodies but redundant for someone with high anti-viral titres. One well established mechanism for modulating antibody efficacy is its level of fucosylation,[12,13] and hypergalactosylation further enhances this effect. Antibody fucosylation differentially impacts cytotoxicity mediated by natural killer (NK) and polymorphonuclear (PMN) effector cells, and plasma with anti-S antibodies with low levels of fucosylation may enhance clearance of virus but provoke a greater inflammatory response with corresponding harm to the patient.[14–18]

In this study, we have generated detailed serological profiles of patients participating in the REMAP-CAP CP trial.[9] Serological reactivities of samples collected from treated subjects before and after receipt of CP, and of the CP used for treatment were compared. Antibody data were then combined with viral loads and outcome data to determine the efficacy of treatment in relation to post-transfusion antibody metrics and clinical outcome with a wider aim to identify potential serological markers of favourable outcomes.

Materials and methods

Patients and convalescent plasma donors

The study included an intensively sampled sub-cohort of the REMAP-CAP CP trial (Table 1; ClinicalTrials.gov: NCT02735707)[9] comprising 80 patients of CP treatment group and 51 patients of control group, treated at intensive care units (ICU) in 129 hospitals in UK between March 2020 and January 2021. Participating hospitals could either collect samples for the intensively sampled sub-cohort or opt-out from additional sampling. From each patient, a plasma or serum sample and a respiratory tract sample were collected before

Table 1
COVID-19 patients in REMAP-CAP trial and in its intensively sampled sub-cohort studied presently.

COVID-19 patients	REMAP-CAP UK		Intensively sampled REMAP-CAP sub-cohort	
	Treatment group	Control group	Treatment group	Control group
Trial start-completion	09/03/2020 - 18/01/2021			
Participants	Intensive care unit (ICU) patients hospitalised due to COVID-19			
No. participants	1078	909	80	51
Age, median (IQR[A]), years	61 (52–69)	61 (52–70)	59 (48–67)	57 (52–66)
Male / Female	67% / 33%	68% / 32%	70% / 30%	65% / 35%
Comorbidities				
Diabetes	31% (339/1078)	30% (268/907)	28% (22/80)	39% (20/51)
Respiratory disease	23% (245/1078)	24% (216/907)	19% (15/80)	24% (12//51)
Kidney disease[B]	11% (107/1000)	10% (83/837)	6% (5/77)	16% (7/45)
Severe cardiovascular disease[C]	9% (96/1053)	8% (67/890)	5% (4/80)	14% (7/51)
Immunosuppressive therapy[D] or disease	6.3% (67/1066)	6.6% (60/907)	6.3% (5/80)	0% (0/51)
APACHE[E] score at baseline, median (IQR)	13 (8–19)	12 (8–19)	14 (9–20)	13 (8–21)
nAb[F] negative at baseline	31% (271/874)	27% (149/558)	28% (22/78)	24% (12/50)
Respiratory support at baseline				
Invasive mechanical ventilation	33% (356/1078)	32% (289/909)	31% (31/80)	37% (19/51)
Non-invasive mechanical ventilation	46% (493/1078)	45% (407/909)	48% (38/80)	35% (18/51)
High-flow nasal cannula	21% (225/1078)	23% (211/909)	19% (15/80)	27% (14/51)
COVID-19 therapy (received within 48 h of randomisation)				
Glucocorticoids	94% (1014/1078)	93% (845/909)	93% (74/80)	92% (47/51)
Remdesivir	46% (491/1078)	44% (398/909)	49% (39/80)	57% (29/51)
Tocilizumab or sarilumab	39% (425/1078)	38% (348/909)	29% (23/80)	18% (9/51)
Time to randomisation from, median (IQR), hours				
Hospital admission[G]	43 (24–79)	42 (23–84)	31 (20–67)	29 (22–51)
ICU admission	18 (10–24)	17 (11–23)	19 (10–29)	20 (11–28)
Start of convalescent plasma (CP) treatment	≤48 (median 6) hours after randomisation; Median 48 h after hospital admission; Time since onset of symptoms was not recorded			
Dosage of treatment	550 ml CP	No infusion	550 ml CP	No infusion
Primary end-point	Organ support-free days; median (IQR), death coded as −1			
	0 (−1 to 16)	3 (−1 to−16)	7 (−1 to 16)	3 (−1 to 15)
Mortality rate	37%	38%	29%	33%

[A] Inter quartile range;
[B] Prior serum creatinine ≥130 μmol/L (males) or ≥130 μmol/L (females) or dialysis;
[C] New York Heart Association class IV;
[D] Recent chemotherapy, radiation, high-dose or long-term immunosuppressive medication;
[E] Acute physiology and chronic health evaluation II score;
[F] neutralising antibody;
[G] Including time in emergency department.

Table 2
SARS-CoV-2 convalescent plasma donors studied presently.

Convalescent plasma	REMAP-CAP	Vaccine		
		Post 1st dose	Post 2nd dose	Omicron
No. donors	157	33	66	14
Donor plasma collection	22/04/2020 - 12/05/2020	26/04/2021 - 28/07/2021	28/04/2021 - 14/08/2021	27/01/2022 - 17/02/2022
Donor plasma inclusion criteria	EUROimmun anti-spike IgG ratio ≥6	EUROimmun anti-spike IgG ratio ≥1, pre-vaccine		Prior omicron infection
Prior infecting SARS-CoV-2 variant	Ancestral	Ancestral (28; 85%) Alpha[A] (5; 15%))	Ancestral (50; 76%) Alpha[A] (16; 24%)	Omicron
Time since SARS-CoV-2 infection	≥28 days after resolution of symptoms	≥92–396 days (median 202)	≥170–473 days (median 354)	45–66 days (median 52)
Time since latest vaccine dose	Not vaccinated	33–79 days (median 55)	29–140 days (median 57)	16–227 days (median 60)

[A] alpha: based on estimated seroconversion date, possibly alpha, ancestral variant cannot be excluded.

(day 1) and after CP transfusion (median day 9, range 2–28). The control group was similarly sampled at day 1 and, median, day 9 (range 2–28). Samples were also obtained from 157 individuals who donated the 160 units of CP transfused to the 80 patients of the treatment group (Table 2; two units per patient, 250 ml each). Plasma was donated in the UK ≥28 days after resolution of symptoms of a prior ancestral SARS-CoV-2 infection with Wuhan non-variant strain and had EUROimmun S-IgG s/co ratio ≥6. Each plasma unit was traced to the patient who received it. Patient outcome was measured based on the number of organ support-free days during hospitalisation (days alive and free of ICU–based organ support during first 21 days since ICU admission; death was coded as −1). Survival was recorded at discharge from the hospital.

The vaccine panel included 113 immunised UK blood donors (Table 2). Most (n=99) were initially identified as potential CP donors, but their plasma had not been used clinically and were re-sampled later, post-vaccination. Pre-vaccine inclusion criterium was EUROimmun S-IgG s/co ratio of ≥1. The donors had a prior infection with ancestral SARS-CoV-2 (n=78) or possibly with the alpha variant (n=21) in 2020 or early 2021 (range: 92–473 days prior to sampling, median 310 days, estimated based on the earliest seropositive pre-vaccine sample) followed by vaccination (latest dose, range: 29–140 days before sampling, median 57 days).[19,20] At the time of sampling, 33 had received one and 66 had received two doses of vaccine. The omicron subpanel comprised 14 individuals who had received 2–3 doses of vaccine followed by SARS-CoV-2 omicron infection in December 2021 (range 45–66 days prior to sampling, median 52 days; Table 2).

SARS-CoV-2 testing

All donor and recipient blood samples (n=516) were subjected for SARS-CoV-2 serological testing including IgM and IgM titres, IgG avidity, neutralising antibody titres and IgG fucosylation as described below, whereas respiratory samples (n=262) were assayed by PCR as described.[21] Performance and reproducibility of the serological assays are described in Supplementary Figure 1.

Anti-Wuhan SARS-CoV-2 spike (S) IgM and IgG titres and IgG avidity were measured by ELISA as described[20,22,23] with the following modifications: IgG titre was normalised against 3 calibrator plasmas and the three normalised results were averaged, instead of using one calibrator and presented in arbitrary units (AU); IgG avidity was measured with 5 M urea, instead of 4 M, for better discrimination among contemporary samples showing higher avidities compared to samples collected early in the pandemic. Neutralising antibodies (nAb) against Wuhan type England/02/2020 SARS-CoV-2 isolate were measured (cause of ancestral infections); for REMAP-CAP CP donors and treatment group patients with a reporter cell assay [24]; and for REMAP-CAP control group patients and vaccinated CP donors with a live virus microneutralization assay.[25] The two nAb assays were harmonised using linear transformation based on 50 individuals assayed with both methods (Supplementary figure 2).

The method used for S-IgG fucosylation measurements was modified from.[26] Wuhan spike antigen[23] at 1 µg/ml in carbonate-bicarbonate buffer (pH 9.6) was coated onto microwell strips (Thermo Fisher 446442) at 50 µl/well and incubated overnight. The wells were washed once with PBS + 0.05% Tween 20 (PBST), blocked for 1 h with 10 mg/ml bovine serum albumin (BSA) in PBST (PBST-BSA), and washed twice afterwards. Samples were diluted in PBST-BSA and 50 µl/well was added and incubated for 1.5 h. The samples were tested in four-fold dilution series and compared with S-IgG assay, 8-fold lower dilution factors were used for the fucosylation assay (e.g., dilutions 1:100, 1:400, 1:1600, 1:6400 for fucosylation and 1:800, 1:3200, 1:12 800, 1:51 200 for S-IgG). Suitable dilutions were selected based on initial S-IgG screening. After washing the wells thrice, biotinylated FcγRIIIa[26] was diluted to 0.5 µg/ml in PBST-BSA, 50 µl/well was added and incubated for 1 h, followed by three additional washes. Peroxidase labelled streptavidin (Sanquin M2032) was diluted to 0.2 µg/ml in PBST-BSA, 50 µl/well was added, incubated for 30 min, and the wells were washed trice. Next, 100 µl/well of tetramethylbenzidine substrate (Thermo Fisher 34028) was added and incubated for 20 min. The reaction was stopped with 0.5 M sulfuric acid and absorbance measured at 450 nm. Titres of afucosylated S-IgG and total S-IgG, were obtained from corresponding titration a curves, fitted with a four-parameter logistic function:

$$\log(absorbance) = \frac{a}{1 + e^{b\times(\log(dilution\ factor)-c)}} + d$$

where a, b, c and d are fitting parameters, and normalised against three calibrator plasmas. Fucosylation level was obtained from the titres as described,[26] here parameter values were $y = -0.149 \times x + 14.4$ determined with five liquid chromatography-mass spectrometry assayed samples[26] confirmed by ELISA in three independent repetitions.

Data analysis and statistics

Pre-processing of data was conducted using Python 3.10, Pandas 2.1.4 and Scikit-Learn 1.3.2 libraries,[27,28] and comprised cleaning and feature selection. Cleaning consisted of label encoding and imputation. Data with missing IgG-related measurements were excluded. Feature selection was conducted using factor analysis, where inter-dependent variables and those with no predictive value for the dependent (whether patients received convalescent plasma) were excluded. This was determined quantitatively using the Kaiser criterion[29] at three components with Varimax rotation. Processed sample size (n) was 49 for test and 37 for control. Variables selected were follow-up sampling day, patient age, sex at birth, BMI, mortality, and mean daily difference in the following antibody metrics: nAb, S-IgM, S-IgG, S-IgG avidity, and S-IgG fucosylation percentage.

To model the influence of receiving convalescent plasma on survival, number of organ support-free days and blood antibody profiles, hypothesis testing was conducted with SPSS 29.0.1 (IBM Corporation, New York: USA) by fitting a binomial logistic generalized linear model (GLM)

via inverse probability of treatment weighting (IPTW) weighted maximum likelihood estimation followed by standardisation.[30,31] The model was fitted using the canonical (logit) link function, which ensures positive fitted values, and an intercept was incorporated as an indicator of baseline probabilities. A supplementary linear GLM was fit to predict organ support-free days, using a non-canonical log link function and intercept; mortality and sampling day were omitted from this model due to being collinear with the dependent variable.

No interaction terms were included, and bootstrapping was not performed in either model. Both model assumptions were verified by plotting residuals versus fitted values, versus each covariate in the model and versus each covariate not in the model. We assessed the residuals for temporal dependency and found none (data not shown).

Study approval

Signed consent was obtained from each donor at the time of donation. It included the use of data for the purpose of clinical audit to assess and improve the service provided by NHS Blood and Transplant as well as for research to improve our knowledge of the donor population. Approval for plasma samples collected from vaccinated donors was received from the West Midlands Solihull Research Ethics Committee, UK (REC-reference: 21/WM/0082, IRAS-project-ID: 296926). The REMAP-CAP convalescent plasma clinical trial was registered with an identifier: NCT02735707. The study, including the administration of CP, sampling and testing of recipients, was approved by London-Surrey Borders Research Ethics Committee London Centre (18/LO/0660). Written or verbal informed consent, in accordance with regional legislation, was obtained from all patients or their surrogates.

Limitations

The reporter cell neutralisation assay was adopted, and replaced the live virus assay, when it became available as it allowed for more precision and data quantification. All the samples could not be assayed with the same neutralisation assay as not enough sample was available for retesting.

The intensively sampled sub-cohort included 131 patients out of 1987 participants of the REMAP-CAP trial. There were only limited numbers of patients with low day 1 anti-SARS-CoV-2 titres and this limited analysis of clinical response to CP treatment, even if potency of each convalescent plasma transfusion was accounted for at individual level unlike in the previous REMAP-CAP trial analyses.

The date of onset of symptoms was not recorded in REMAP-CAP. As time since onset of symptoms may be an important factor in efficacy of CP treatment, the lack of this information limited comparability of findings with those from other CP efficacy trials.

Data availability

Data regarding the REMAP-CAP CP trial is available at[9] and at ClinicalTrials.gov: NCT02735707. Values underlying graphed data are presented in Supporting Data Values supplement. Further data supporting the findings of this study are available upon reasonable request from the authors.

Results

Intensively sampled recipients of CP (n=80) and controls (n=51), and the respective REMAP-CAP previously infected (n=157) and vaccinated (n=113) CP donors were assayed for nAb, S-IgM, S-IgG, S-IgG avidity, and S-IgG fucosylation percentage (Fig. 1). Most patients were already SARS-CoV-2 seropositive at trial onset on day 1, with 82% and 84% positive for S-IgM; 89% and 92% positive for S-IgG; 72% and 76% with detectable nAb in treatment and control groups, respectively. Follow-up samples taken on day 2–28 showed 95% and 92% S-IgM, 100% and 100% S-IgG and 95% and 98% nAb, seropositivity, respectively, with greatly increased titres compared with day 1 (Fig. 1A-C; Supplementary Figure 3). The median S-IgG baseline titres at day 1 were comparable between treatment and control groups (143 AU/ml and 95 AU/ml, respectively; Fig. 1B).

The patients of treatment group each received a CP transfusion comprising plasma from two donors. The 157 donors constituted 80 unique CP transfusion (donor pairs) for the 80 patients. As the patients were not sampled immediately after the transfusion, day 1 post-transfusion titres were calculated based on pre-transfusion and CP titres. CP transfusion was assumed to dilute 6-fold into bloodstream (≈550 ml of CP diluted into ≈3 L of patient's plasma) and Fig. 1A-C show the CP transfusion titres after accounting for the dilution. Antibody distribution outside of intravascular space was not modelled. REMAP-CAP CP donors showed slightly higher nAb and S-IgG titres than recipients on day 1 (median S-IgG titres of 715 AU/ml and 343 AU/ml; Fig. 1B and C). However, when comparing the average titre of each CP transfusion against patient day 1 pre-transfusion titre and accounting for the dilution of CP into bloodstream, the transfusion appeared less effective (Fig. 1A-C; Fig. 2A). Most patients (56%) already had more endogenous S-IgG at day 1 than they received through CP transfusion (< 1-fold relative increase compared to patient baseline titre; 68 AU/ml geometric mean absolute increase), whereas a small relative increase in S-IgG levels (1 to 9-fold; 94 AU/ml) was noted in 22% of patients and even higher relative increase (10 to 126-fold; 81 AU/ml) in further 22% of patients (Fig. 2A and B). Higher relative increase in S-IgG was mostly explained by low pre-transfusion S-IgG titre and to lesser extent by potency of the CP. REMAP-CAP CP, considered high titre at the time, showed median S-IgG of 384 AU/ml, while vaccinees sampled in 2021 showed median S-IgG of 1608 AU/ml and those sampled in 2022 median of 3213 AU/ml (Fig. 1B). Theoretically, if vaccinee CP had been used instead of the REMAP-CAP CP, 39% of the patients would have received 10 to 890-fold Increase in S-IgG. However, 38% of the patients would still have had more endogenous S-IgG at day 1 than provided by the transfusion (Fig. 2B). Day 1 post-transfusion antibody levels (S-IgM; S-IgG; nAb; S-IgG avidity; S-IgG fucosylation) in comparison to control levels without transfusion are further presented in Fig. 3 and in Supplementary figure 3.

Anti-SARS-CoV-2 antibody in post-transfusion sample (collected at day 2–28; Supplementary figure 3) thus comprised patient pre-transfusion antibodies (day 1 sample), antibodies received from CP (as calculated above) and additional patient antibodies elicited by infection within the sampling interval. Estimates for the latter endogenous S-IgG production following transfusion were obtained by subtracting the pre-transfusion and transfused S-IgG from the post-transfusion S-IgG (Fig. 2A and C). After transfusion there was 5.0-fold median (interquartile range, IQR 1.0 to 15) increase in S-IgG during follow-up due to endogenous IgG production (Fig. 2C). The patients with higher antibody titres pre-transfusion, relative to CP they received, showed 2.5-fold median (IQR 0.57 to 7.6) increase in S-IgG during follow-up and compared with day 1 post-transfusion titre, which corresponds to median 43% (IQR 5.3% to 110%) increase per day. The patients with lower titres pre-transfusion demonstrated higher increase in S-IgG from the CP showed also higher *de novo* antibody production, with a median 9.2-fold (IQR 4.0 to 18) increase in S-IgG, corresponding to median 120% (IQR 29% to 240%) increase per day. Only 9% of the patients demonstrated over 10-fold, median 30-fold, increase in their post-transfusion antibody levels which were not derived solely from endogenous IgG production; CP contributed a > 10-fold increase followed by median −0.1% (range −85% to 26%) daily increase due to endogenous production.

Characteristic of primary infection, S-IgG avidity was low in the baseline samples taken early during hospitalisation, with median values of 0.04 in the treatment group and 0.04 in the controls

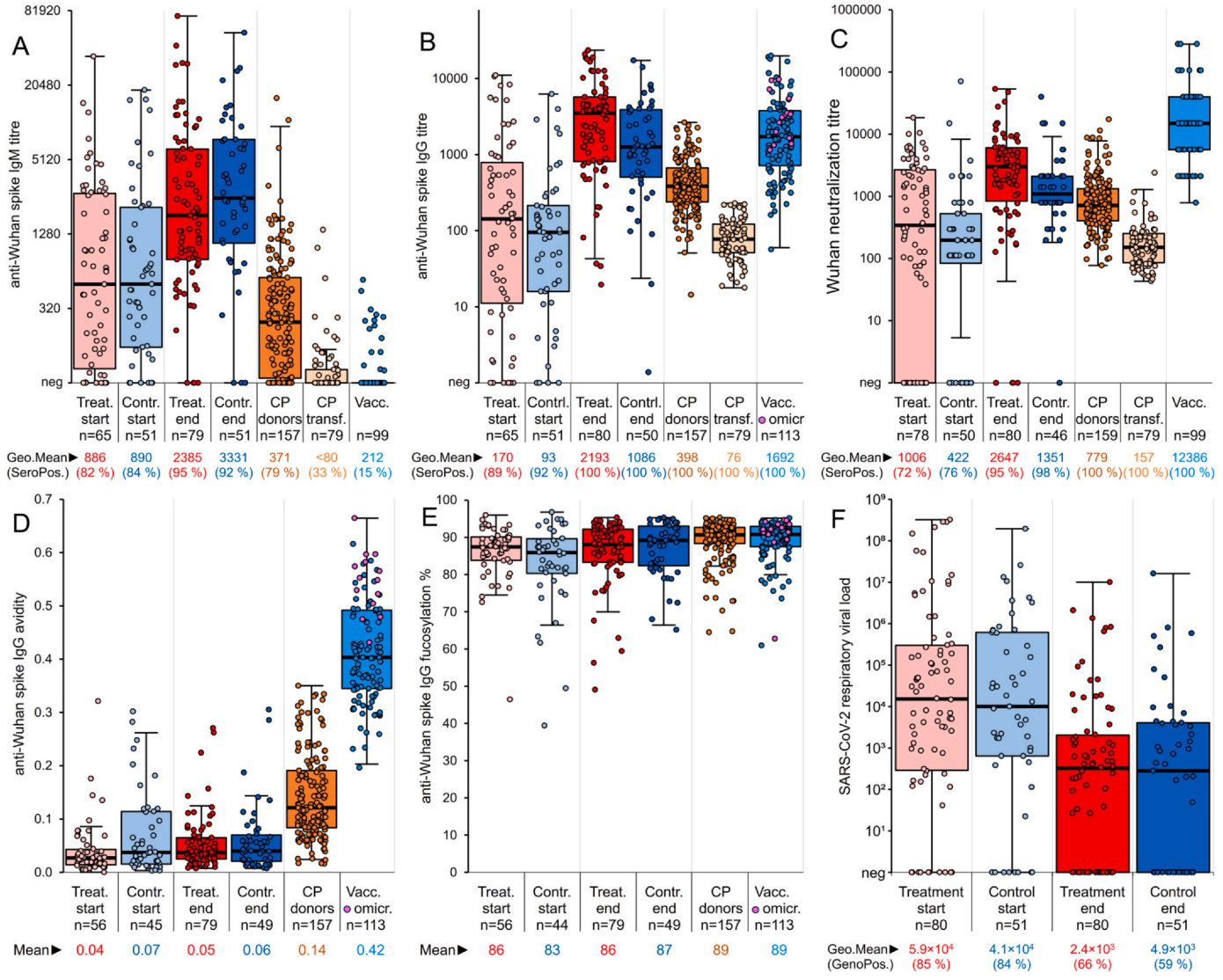

Fig. 1. Anti-Wuhan spike (S) IgM (A) and IgG titre (B), Wuhan neutralisation titre (C), S-IgG avidity (D), S-IgG fucosylation percentage (E), and SARS-CoV-2 respiratory viral load (F). Included groups were REMAP-CAP patients of treatment (Treat.) and control (Contr.) groups at trial start (day 1; pre-convalescent plasma, CP) and at end of follow-up (day 2–28), as well as REMAP-CAP CP donors, REMAP-CAP CP transfusions (transf.) each comprising plasma of two donors, and vaccinated CP donors not included in the trial some of which with past omicron (omicr.) infection. After transfusion CP antibodies dilute into patient plasma, and panels A-C show transfusion titre after accounting for this dilution, i.e. the average titre when two 250 ml plasma units are diluted into 3 litres (estimated patient plasma volume). Box plots range from 25th to 75th percentile with median line inside. Whiskers enclose data points ≤1.5×IQR from the 25th or 75th percentile. Mean or geometric mean (Geo.Mean) values as well as sero- and genopositivities (SeroPos; GenoPos) are shown below each panel. N.B. geometric means were calculated for positive samples as negative results cannot be reliably positioned on logarithmic scale.

(Fig. 1D). REMAP-CAP CP donors, sampled in 2020 at least 28 days after resolution of COVID-19 symptoms, showed a median avidity of 0.12. Vaccinees sampled in 2021, median 320 days after pre-vaccine SARS-CoV-2 infection, showed a median avidity of 0.40 and vaccinees sampled in 2022, after post-vaccine omicron infection, showed the highest median avidity of 0.55. As expected, avidity was much higher in convalescent plasma donors after SARS-CoV-2 infection or vaccination compared to the study patients (Fig. 1D). Patient samples showed relatively high S-IgG fucosylation levels, with median values of 87% in the treatment group and 86% in the control group at day 1 and 88% and 89% at follow-up, respectively (Fig. 1E). Fucosylation levels of 91% were observed in REMAP-CAP CP donors and vaccinees (Fig. 1E). However, in each group several patients and donors displayed levels below 80% of fucosylation. Respiratory viral loads decreased during hospitalisation equally in treatment and control groups, respectively: median 1.5×10^4 IU/ml and 1.0×10^4 IU/ml at day 1; 3.2×10^2 IU/ml and 2.8×10^2 IU/ml at follow-up (Fig. 1F).

Consistent with the REMAP-CAP trial analysis, receipt of CP was not found to lead to significantly different outcomes for mortality or number of organ support-free days. None of the antibody or viral load metrics were significantly associated with mortality between the treatment and control groups (Fig. 3) nor within all patients studied (treatment and controls grouped together; Supplementary Figure 4). The number of immunosuppressed REMAP-CAP patients in the intensively sampled cohort (n=5; 3.8%) was too low for analysis.

Changes (absolute difference per day) in nAb titre, S-IgG avidity and S-IgG fucosylation were all found to be significantly different between control and treatment groups (Table 3), with higher nAb titres and avidity in the treatment group but lower levels of fucosylation. Biological significance of higher nAb increase in the treatment group, compared to the controls, could not be confirmed as the nAb testing in the present study did have limitations (see methods chapter for details) and the corresponding change in S-IgG was not statistically significant. Mean avidity remained almost identical in

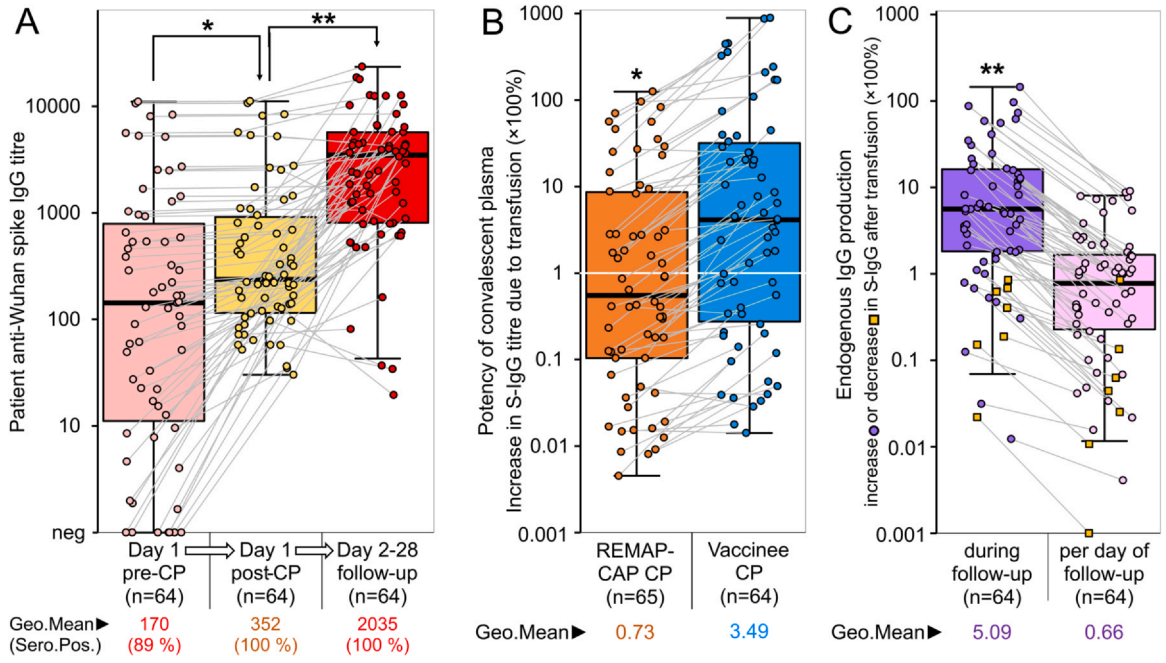

Fig. 2. Convalescent plasma (CP) treatment in relation to pre and post-transfusion anti-Wuhan spike (S) IgG titre in the treatment group of REMAP-CAP trial. Box plots range from 25th to 75th percentile with median line inside. Whiskers enclose data points ≤1.5×IQR from the 25th or 75th percentile. Geometric mean values (Geo.Mean) and seropositivities (SeroPos) are shown below the graphs. N.B. geometric means were calculated for positive values as negative results and values cannot be reliably positioned on logarithmic scale. (A) Distributions show S-IgG titres before CP treatment (Day 1 pre-CP), the resulting titres immediately after transfusion (Day 1 post-CP), and S-IgG titres in post-transfusion samples collected 1–27 days after the transfusion (Day 2–28 follow-up). Day 1 post-transfusion titre was calculated by adding transfused IgG (2×250 ml of CP, assumed to dilute into 3 litre plasma volume) to the pre-transfusion IgG titre. (B) Potency of CP in relation to pre-transfusion S-IgG titre (increase between day 1 pre- and post-transfusion titres, marked with * in panel A). The box plot on the left shows the REMAP-CAP trial, while the box plot on the right simulates transfusion with median 4.2-fold higher titre vaccinee CP. For the simulation, each REMAP-CAP patient was randomly assigned with two CP donations from the vaccine panel. (C) Endogenous S-IgG production during follow-up and per day of follow-up (increase between day 1 post-transfusion titre and day 2–28 follow-up titre, marked with ** in panel A). Patients whose S-IgG titre decreased during follow-up are marked with a yellow square and are not included in box plots, while those who showed increase in titre are marked with a circle.

the control group (median daily change -7.0×10^{-4} and slightly increased in the treatment group (median daily change 0.001), whereas conversely net fucosylation slightly decreased in the treatment group (median daily change −0.022) and increased in the control (median daily change 0.26). Patients of treatment group received highly fucosylated CP (median fucosylation 91%) yet the transfused IgG amount was small (median 35% of all post-transfusion S-IgG at day 1, and 3% at day 2–28) compared to endogenous IgG which also showed relatively high fucosylation already at day 1 (median fucosylation 87%; Fig. 1B and E). Change in net fucosylation thus mostly depended on endogenous IgG production. While some patients did show a decrease in S-IgG avidity, all who were sampled more than two weeks after day 1 showed increases. Few patients also showed avidities higher than the rest and higher than could be expected for a primary infection (Fig. 1D). Since avidity against other SARS-CoV-2 antigens, serology of other potentially cross-reactive coronaviruses or patient pre-COVID-19 samples were not available, the exact nature of these high avidity results could not be confirmed.

We then investigated whether the effect of CP treatment differed based on serostatus at trial onset by including an additional calculated binary variable in the model (with IPTW recalculation), in which patients were assigned to “high” or “low” serostatus groups, depending on whether their day one S-IgG and S-IgM titres were above or below threshold values (10 and 160 AU/ml, respectively). Serostatus was found to not be significantly different between treatment and control groups (p=0.590) and receipt of CP was not found to lead to significantly different outcomes in either “high” or “low” serostatus groups, albeit with a low number of patients in “low” serostatus group (n=29).

In the model fit against organ support-free days, better outcomes were significantly associated with higher daily increase in S-IgM titre and decrease in S-IgG fucosylation, regardless of CP treatment (Table 4). In contrast, higher patient age was significantly associated with lower number of organ support-free days (Table 4). Greater change in S-IgM was associated with low day 1 S-IgM and S-IgG titres, but the low day 1 titres were not associated with number of organ support-free days.

Discussion

It has been previously shown that timing in relation to SARS-CoV-2 disease progression is an essential factor for successful CP treatment. Early treatment of elderly outpatients within 3 days of onset of symptoms,[4] and similarly of adult outpatients within 5 to 9 days,[5] has shown to be beneficial. Trials and meta-analyses studying CP treatment of already hospitalised COVID-19 patients have shown results both in favour[10,32–35] and against[6–9] use of CP. A recent meta-analysis has shown, however, that CP treatment of hospitalised patients is indeed effective in reducing mortality, but only if administered within 7 days from symptom onset.[10] Majority of trials analysed administered their transfusions at 8 days from onset or later and the meta-analysis observed no benefit in them.[10] This discrepancy in timing of the treatment is likely a key factor explaining the differences in outcome between the various trials.

Since the time of onset of symptoms was not recorded in the REMAP-CAP trial,[9] we cannot directly compare the data with other studies. This limitation is carried from the original trial also to the present study. REMAP-CAP patient inclusion criterium of admission to intensive care within 2 days is inconclusive, and this may happen directly from emergency centre, or long after the onset of symptoms as disease progression can vary and patients may transfer to intensive care from the general ward, or even from another hospital. Additionally, global variation in healthcare systems is great and a delay of even one day may significantly diminish effectiveness of CP

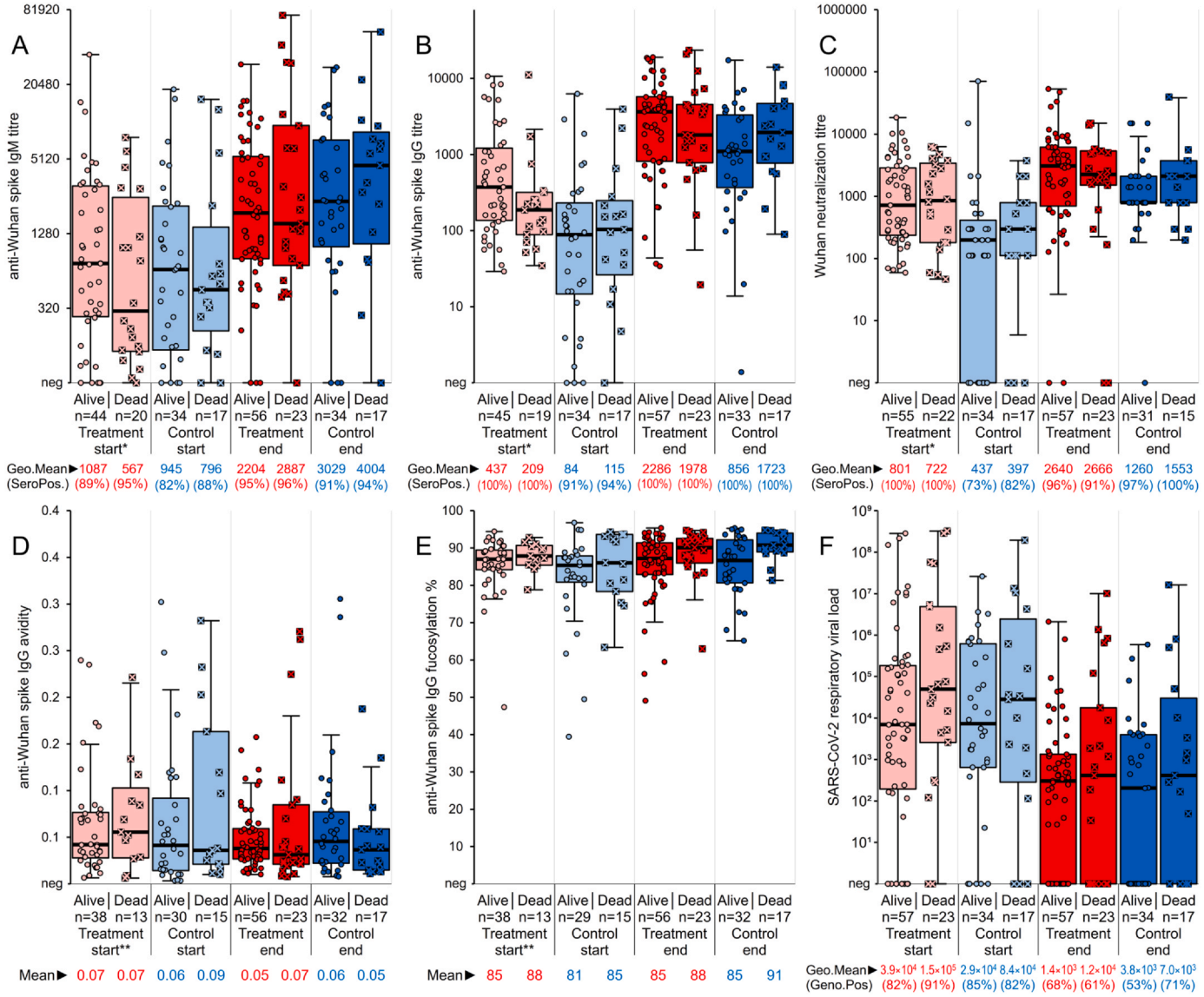

Fig. 3. Antibody and viral load results of alive and deceased REMAP-CAP patients of treatment and control group. Anti-Wuhan spike (S) IgM (A) and IgG titre (B), Wuhan neutralisation titre (C), S-IgG avidity (D), S-IgG fucosylation percentage (E), and SARS-CoV-2 respiratory viral load (F) at trial start (day 1; post-convalescent plasma, CP) and at end of follow-up (day 2–28). *Titres at treatment start were calculated by adding transfused antibodies (2×250 ml of CP, assumed to dilute into 3-litre plasma volume) to the patient pre-transfusion titre. **Avidity and fucosylation at treatment start were calculated by average of CP and pre-transfusion patient plasma, weighted by the respective titres and accounting for dilution of CP (e.g. 8 units of 100% fucosylated IgG added to 1 unit with 10% fucosylation results in plasma with 90% fucosylation). Mortality was recorded at hospital discharge. No statistically significant difference was observed in alive and deceased patients between treatment and control. Box plots range from 25th to 75th percentile with median line inside. Whiskers enclose data points ≤1.5×IQR from the 25th or 75th percentile. Mean or geometric mean (Geo.Mean) values as well as sero- and genopositivities (SeroPos; GenoPos) are shown below each panel. N.B. geometric means were calculated for positive samples as negative results cannot be reliably positioned on logarithmic scale.

Table 3
Parameter estimates for GLM fit against convalescent plasma receipt.

Parameter	B	Standard error	5% CI	95% CI	p
Intercept	−0.25752	1.721089	−3.63079	3.115757	0.881
Sampling day	−0.02908	0.043052	−0.11346	0.055302	0.500
Mean nAb[A] difference / day	−0.00252	0.001139	−0.00475	−0.00029	0.027*
Mean S[B]-IgM difference / day	0.000501	0.000303	-9.4×10^{-5}	0.001095	0.099
Mean S-IgG difference / day	−0.00068	0.000543	−0.00174	0.000383	0.210
Mean S-IgG avidity difference / day	−124.064	42.26975	−206.911	−41.2165	0.003*
Mean S-IgG fucosylation difference / day	0.544828	0.159978	0.231277	0.85838	< 0.001*
Patient age	0.016754	0.025891	−0.03399	0.067499	0.518
Sex	0.341527	0.610898	−0.85581	1.538866	0.576
BMI	−0.00016	0.028864	−0.05673	0.056415	0.996
Mortality	−0.97085	0.66348	−2.27124	0.329551	0.143

* = $p < 0.05$.
[A] neutralising antibody;
[B] anti-spike.

Table 4
Parameter estimates for GLM fit against organ support-free days.

Parameter	B	Standard error	5% CI	95% CI	p
Intercept	3.296	0.4371	2.440	4.153	< 0.001*
Mean nAb[A] difference / day	-4.907×10^{-5}	0.0001	0.000	0.000	0.729
Mean S[B]-IgM difference / day	-7.196×10^{-5}	2.6588×10^{-5}	0.000	-1.985×10^{-5}	0.007*
Mean S-IgG difference / day	0.000	0.0002	0.000	0.000	0.522
Mean S-IgG avidity difference / day	3.339	6.7920	−9.973	16.651	0.623
Mean S-IgG fucosylation difference / day	−0.103	0.0268	−0.155	−0.050	< 0.001*
Patient age	−0.025	0.0054	−0.036	−0.015	< 0.001*
Sex	0.039	0.2038	−0.361	0.438	0.849
BMI	0.004	0.0104	−0.016	0.024	0.690

* = $p < 0.05$.
[A] neutralising antibody;
[B] anti-spike.

treatment. This further emphasises the importance of recording the date of onset of symptoms as not only can it be used in evaluating the effectiveness of treatment but also to e.g. for comparison between countries.

Although treating with CP only SARS-CoV-2 seronegative COVID-19 patients appears a plausible strategy, this approach has not been utilised widely in CP trials. It may be that antibody testing is not readily available at the participating hospital, or that testing would introduce additional delays. Several trials have investigated CP efficacy based on baseline seropositivity and showed a trend favouring treatment of seronegatives, yet not with statistical significance.[7,9,34] Binary seronegative vs. positive classification may include confounding factors: low sample size after subgrouping; seronegativity may depend on the test used, e.g. a nAb negative patient may be positive for S-IgG or S-IgM; seropositive group may also include those with low levels anti-SARS-CoV-2 and who may still benefit from the treatment. In addition to patient baseline characteristics, CP of sufficiently high titre is required for beneficial outcome.[36] Antibody level needed is likely to depend on time since onset of symptoms and on whether the intent is to prevent severe disease in outpatients or exposed risk groups, or to reduce mortality in already severely ill patients. Efficacy may also depend on relative concentrations between patient plasma and CP. Use of even higher titre CP or highly concentrated antibody products, e.g. hyperimmunoglobulin, could possibly extend the time window from onset within which the treatment needs to be administrated.

In this study, we have analysed a subset of REMAP-CAP trial recipients together with the CP they received, further to improve our understanding of how virological benefits of CP treatment link to patient seroprofile and to quality of CP used. We show that over 90% of patients had S-IgG antibodies and 74% nAb already at the time of enrolment to REMAP-CAP trial. This confirms our earlier conclusions that the CP was generally administered late in the course of infection. We have previously shown that **nAb levels** and antibody qualities were no different between the CP used in our REMAP-CAP trial and that used in the successful early treatment trial in Argentina.[20] This suggests that even relatively low nAb levels can be therapeutically effective if provided at an early stage of infection. Indeed, assessment of the effect of CP used in the REMAP-CAP trial on recipient antibody status showed that in most cases the levels of nAb received in CP were minuscule compared to those produced endogenously.

Interestingly, data acquired from the monoclonal antibody trials on casirivimab and imdevimab have also shown their effectiveness only when treating seronegative but hospitalised, or immunocompromised patients,[11] further emphasising the importance of early treatment. Compared with CP, monoclonal antibody therapy can administer higher amounts of nAbs. E.g. 4 g of casirivimab and 4 g of imdevimab[11] is more than total antibody content of the REMAP-CAP CP transfusion, of which only a fraction is against SARS-CoV-2. Monoclonals may, however, quickly lose their efficacy due to antigenic change in the virus.[37] Immune system of CP donors, on the other hand, will adapt to the latest variants they have been infected with and polyclonality can also provide relatively long-lasting cross-neutralisation.[19] Blood donation services can also efficiently acquire, test and distribute CP, whereas monoclonals require lengthy development and medical licensing.

While the REMAP-CAP patients as a whole did not benefit from CP, those lacking antibodies against SARS-CoV-2 potentially could, yet this group was too small for analysis. The proportion of nAb-negative REMAP-CAP patients at trial onset was 28%, but many already had low but measurable levels of S-IgM or S-IgG antibodies and only 10% of all patients were seronegative in all assays, greatly reducing the statistical power to detect an effect of CP in this group. High level of variation in both antibody titres and quality (Fig. 1) may also create noise hampering detection of efficacy when analysing the patients without subgrouping. Furthermore, it should be noted that due to the intensive sampling and laborious laboratory analyses, only a small subset of the REMAP-CAP CP cohort was included in this study (7%; 131/1887). REMAP-CAP patients did also show high antibody production following transfusion, leading its effect to become transient also in patients who received the highest boost of anti-SARS-CoV-2 antibodies through the treatment. Thus, even higher titre contemporary CP or infusion of monoclonal antibodies (Abani et al., 2022) easily becomes redundant in immunocompetent patients due to endogenous antibodies, and the timing of treatment becomes the main requirement for successful CP therapy, yet CP potency may further improve the efficacy.

Better outcomes, measured by the number of organ support-free days, were not associated with receipt of CP (p=0.337), but with increase in S-IgM levels (p=0.007). This could suggest that as those at an **earlier state of infection** are able to mount an antibody response which improves their outcome, early administration of CP would also likely achieve the same. Those who are hospitalised at a later stage of infection may already have high levels of nAb or may be unable to mount an effective response; their severe COVID would be unlikely to be influenced through additional passive antibody administration. This is consistent with a previous study which demonstrated that those with elevated effector immune responses or exaggerated inflammation had worse clinical outcomes and were unlikely to respond to CP.[38]

Although low S-IgG fucosylation has been associated with severe COVID-19; and afucosylated IgG can promote harmful inflammation through binding to FcγRIIIa of immune cells, it was also originally postulated to be potentially protective especially in patient with relatively low viraemia.[14–16] Accordingly, REMAP-CAP patients did show lower fucosylation than CP donors, even if low fucosylation was not associated with worse outcome. We noted that a slight decrease in **fucosylation** levels was independently associated with better outcomes ($p < 0.001$), shown as increased number of organ support-free days. The level of afucosylated anti-SARS-CoV-2 IgG may predict the risk of hospitalisation, but evidence is lacking on

whether fucosylation similarly affects the outcome of patients already severely ill. S-IgG fucosylation increases rapidly during the first weeks after onset of symptoms[14,15] although the quantity of afucosylated S-IgG may increase even if their proportion decreases. The effect of afucosylated IgG can also vary according to the FcγRIIIa genotype of effector cells.[39] Interestingly, a study reported CP treatment of hospitalised COVID-19 patients to be harmful, with the adverse effect correlating with S-IgG titre of the CP.[8] Afucosylated antibodies in the CP could potentially mediate such an effect, yet high levels of antibody-dependent cellular cytotoxicity, suggestive of relatively low fucosylation, of the CP was shown to reduce the harm.[8]

Furthermore, in the present study, S-IgG **avidity** was not associated with outcome, although the median follow-up time of 9 days may be too short to observe significant antibody maturation. In a small study, association between avidity maturation of total S antibodies and resolution of COVID-19 has been reported, albeit the authors did not standardise the avidity measurement for S-IgG and S-IgM ratios.[40] The avidity against ancestral SARS-CoV-2 has been reported to enhance both homologous neutralisation and cross neutralisation of variants of concern,[41] yet in this regard CP collected for REMAP-CAP was of relatively low avidity.

While the SARS-CoV-2 pandemic has led to historically extensive research on CP treatment, the approach was already applied 100 years ago in treatment of patients hospitalised due to pandemic influenza A virus.[42] Passive immune therapy via administration intravenous immunoglobulins has likewise been implemented for decades in prevention or treatment of viral diseases, e.g. against hepatitis B virus.[43] Recent discoveries in CP treatment of COVID-19 thus partially seem re-learned history. Since new pandemics are likely to emerge, we should be better prepared also in terms of CP treatment. Early antibody therapy of sufficient quantity can be effective especially in prevention of severe disease as well as in treating hospitalised patients. Prophylactic treatment can reduce number of hospitalised patients, but good planning and testing strategies are needed to identify which patients are to be treated. Current facilities may also need to be adapted for outpatient transfusions. Healthcare system turnaround times may need to be faster for hospitalised patients to receive CP treatment early enough, and the correct patients need to be chosen swiftly, e.g. based on time since symptom onset or baseline serostatus.

The present study provides detailed serological profiles of patients participating in the REMAP-CAP CP trial and of the CP received, at individual level. The presence of endogenous antibodies against SARS-CoV-2 before transfusion and their production rate post-transfusion conceivably makes the treatment of hospitalised patients redundant, likely explaining observed lack of benefit. For CP treatment to be effective, it should be administered during early primary SARS-CoV-2 infection to prevent severe disease in risk groups or, in the current setting, applied in early treatment immunocompromised patients lacking their own antibodies. CP treatment may be beneficial in emerging infectious diseases and future pandemics, especially in low-income setting and early stages when other interventions are likely lacking, provided the extensive research on CP treatment of COVID-19 is correctly applied. Further insight is still needed on how qualitative properties of antibodies, including the measurements of fucosylation and avidity, may impact disease progression or efficacy of CP, and how to best select CP donors when treating immunocompromised patients afflicted by current or future SARS-CoV-2 variants.

Funding

This work was supported by SUPPORT-E Project (HH, DJR, LE); National Institute for Heath and Care Research, COVID-19-RECPLAS (DJR, LE); Research Council of Finland (JH); Finnish Society for Study of Infectious Diseases (VN); Finnish Foundation for Research on Viral Diseases (VN); NHS Blood and Transplant (HH, DJR, LE, AAL, HPT); Sigrid Jusélius Foundation (VN, KH); Magnus Ehrnrooth Foundation (VN, KH); Societatis Medicorum Fennicae - The Medical Society of Finland (VN, KH).

CRediT authorship contribution statement

HH: Conceptualization, Writing, Supervision. **PS:** Conceptualization, Resources, Writing, Supervision, Funding acquisition. **DJR:** Conceptualization, Funding acquisition. **VN:** Conceptualization, Methodology, Formal analysis, Investigation, Writing, Visualization, Funding acquisition. **CK:** Methodology. **TS:** Methodology, Writing. **RM:** Formal analysis, Writing. **CK:** Investigation. **HLAM:** Investigation. **SS:** Investigation, Writing. **LE:** Resources, Writing. **HPT:** Resources. **AAL:** Resources, Writing. **MSH:** Resources. **JH:** Resources, Writing, Funding acquisition. **GV:** Resources, Writing. **DKM:** Writing. **CES:** Writing. **KH:** Writing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank all of those who contributed to organising the vaccinated donor study at NHS Blood and Transplant including the Plasma for Medicine Programme, the Birmingham New Street Blood Donor Centre, and the NHSBT Clinical Trials Unit.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106412.

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