Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.
Supplement to: Convalescent Plasma for COVID-19. A randomized clinical trial

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1. Investigators

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2. Supplemental methods

Methods

Additional study procedure details

All the comorbidities were filled in as free text in the electronic case record form (eCRF). The comorbidities were assigned using the following definitions; hypertension was defined as hypertension reported in the medical history, including hypertension with or without end organ damage, and also both hypertension for which medication was given and for which no medication was given. Diabetes mellitus was defined as both type 1 and type 2. This also included diabetes with or without end organ damage. Cardiac history was defined as a chronic disorder of cardiac function, which made the subject eligible for flu vaccination according to the Dutch General Practitioner guideline. A history of pulmonary disease was defined as any concurrent pulmonary disease which requires medication or follow-up with a pulmonologist. A history of cancer was defined as any current invasive cancer or disease that might evolve into invasive in the previous five years (e.g. basal cell carcinoma was not included, since these types of cancer usually do not metastasize). Also, if a subject was treated with curative intention longer than five years ago, the subject was considered cured and not labeled with a medical history of cancer. A history of immunodeficiency was defined as any medical condition which would put the subject at increased risk for infection or any medication that would put the subject on increased risk for infection. A history of chronic kidney disease was defined as any kidney disorder with a GFR < 60ml/min. A history of liver cirrhosis was defined as liver cirrhosis due to any kind of condition.

Laboratory assessments (CRP, ferritin, LDH, lymphocytes and bilirubin) were only recorded if they were done so in agreement with the standard of care, and were not mandatory to measure. Serum tubes for ELISA and PRNT50 measurement were done so if the logistics were available to collect them and analyze them. PRNT50s were performed if enough serum was available after the ELISA.

Participants were assessed daily for the first 14 days if they were on the ward. For the WHO COVID-19 disease severity score on day 15 an in-person visited was preferred, but follow-up by phone was acceptable if an in-person assessment was not feasible.

The SAE that were registered in this study were limited to death from any cause or a life-threatening transfusion reaction. This limited SAE registration was approved by the institutional review board.

All sites collected and entered data into an eCRF (OpenClinica). A.G., C.J., B.R., C.R., G.P. extracted and analyzed the data.

Additional study population details

Acquisition of donors

To include as many donors as possible, a (social) media message was published asking people whom have had COVID-19 were needed to donate plasma. The subjects with a PCR proven disease could apply by e-mail. Afterwards a study member called the potential donor by phone for an informed consent conversation and afterwards an informed consent form was sent and signed. The potential donors were then assessed for plasma donation eligibility by Sanquin Blood Supply. Once the donors were deemed eligible a donation was planned. Donors were asked to donate a total of four times in four weeks, but not required to. On the day of first donation, a serum blood tube was drawn to do an ELISA and PRNT50. The donors were sent a detailed questionnaire by e-mail using Gemstracker.

Acquisition of patients

Patients admitted to hospital with a PCR proven COVID-19 disease that was less than 96 hours old and who were 18 years or older were assed for eligibility. The subjects were required to be not included in another intervention trial of COVID-19 to be included in this study. After the first 63 patients these criteria were modified to also include the exclusion criteria known IgA deficiency and >96 hours on invasive ventilation at time of screening. When the treating physician deemed it would better to not participate in the study (e.g. no benefit was expected from the plasma unit due to the patient being very close to dismissal from hospital) the patient would not be included. First, the sponsor was called to assess whether or not there was a unit of plasma available with adequate PRNT50 titers and ABO compability. Patients were then approached for informed consent. When written informed consent was obtained the patient was randomized for the study using ALEA.

Virological essay

We analyzed serum samples of donors and patients for the presence of neutralizing antibodies by performing a PRNT with the SARS-CoV-2 virus (German isolate; GISAID ID EPI_ISL 406862; European Virus Archive Global #026V-03883) as we have described previously. [1] We 2-fold serially diluted heat-inactivated samples and added 400 plaque-forming units to each well, then incubated at 37°C for one hour before placing the mixtures on Vero-E6 cells. After eight hours of incubation, we fixed and stained

the cells and counted the number of infected cells per well by using an ImmunoSpot Image Analyzer (CTL Europe GmbH, https://www.immunospot.eu). The serum neutralization titer is the reciprocal of the highest dilution resulting in an infection reduction of >50% (PRNT50). We considered a titer ≥1:20 to be positive.

Serum was also tested for the presence of anti-SARS-CoV-2 total Ig and IgM with the Wantai Enzyme Linked Immunosorbent Essay (ELISA) test (Wantai Biological, Beijing). We previously showed that a positive total Ig or a IgM with an optical density (OD) ratio >10 (which equals an OD of 2.0), correlates closely with PRNT50 of at least 1:80.[2]

Statistical analysis

Baseline descriptive statistics are provided as median with IQR or mean with 95% confidence intervals (CI) for continuous variables and as count with percentage for categorical variables. The effect of plasma therapy on overall mortality was estimated by logistic regression models adjusted for the independent factors at inclusion sex, age, intensive care unit admission, CRP, absolute lymphocyte count, bilirubin and FIO2. A 2-sided Wald test on the odds ratio (OR) with 95% CI of the treatment effect based on the multivariable model was planned to assess whether ConvP reduces mortality at the adjusted alpha-level of 0.0480. A proportional odds ordinal logistic regression model was used to estimate the odds of being improved on the 8-point WHO COVID-19 disease severity scale at day 15 of inclusion and adjusted for the seven factors mentioned above. This model tested the hypothesis that the treatment to control OR is equal to one. The impact of ConvP therapy on the length of hospital stay was analyzed with a proportional hazards model for the subdistribution of hospital discharge as proposed by Fine and Gray (1999).

3. Supplemental study results

Baseline characteristics

The number of comorbidities were filled in for all 86 subjects and 100 donors. Laboratory assessments on baseline were only recorded in the eCRF if this was done so in concordance with routine care. The CRP was not measured in one subject, ferritin was not measured in 29 subjects, LDH was not measured in three subjects, lymphocytes were not measured in eight subjects and bilirubin was not measured in seven subjects.

Serum was drawn for the subjects for PRNT50 analysis and ELISA testing on baseline, only if transport for the serum tubes was available or feasible. Due to these circumstances we were able to collect 66 out of 86 samples. Reasons for not obtaining samples: no transport available in 8 cases and transport logistically not feasible in 12 cases. On all 66 samples a Wantai ELISA was performed. In one of the 66 samples did not have enough material for both IgM and Ig total ELISA so just IgM ELISA was performed in this subject.We considered the tot Ig positive, because the IgM tested positive for antibodies. In 56 out of these 66 samples there was enough serum to perform a PRNT50.

Unadjusted and adjusted Odds Ratios for the primary endpoint

Table 2: Odds Ratios and 95% CIs from Unadjusted and Adjusted Logistic Regression for Overall Mortality			
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
ConvP	0.472 (0.148; 1.384)	0.948 (0.195; 4.670)	0.946*
Age	-	1.097 (1.034; 1.179)	0.005
Female Sex	-	0.717 (0.118; 3.932)	0.704
ICU on Admission	-	6.701 (0.209; 142.602)	0.219
CRP on Admission	-	1.000 (0.991; 1.009)	0.932
Lymphocyte Count on Admission	-	0.114 (0.013; 0.609)	0.023
FI02 on Admission	-	1.022 (0.994; 1.054)	0.134
Bilirubine	-	0.873 (0.727; 0.986)	0.078

*P-value from multivariable adjusted logistic regression

Unadjusted and adjusted Odds Ratios for secondary endpoint 8-point WHO score

Table 3: Odds Ratios and 95% CIs from Unadjusted and Adjusted Logistic Regression for 8-point WHO COVID disease severity scale			
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
ConvP	0.585 (0.273; 1.239)	1.300 (0.519; 3.318)	0.578*
Age	-	1.054 (1.017; 1.095)	0.006
Female Sex	-	0.991 (0.345; 2.834)	0.987
ICU on Admission	-	8.990 (1.566; 59.097)	0.017
CRP on Admission	-	1.004 (0.998; 1.010)	0.164
Lymphocyte Count on Admission	-	0.189 (0.060; 0.546)	0.003
FI02 on Admission	-	1.030 (1.012; 1.050)	0.001
Bilirubine	-	0.982 (0.930; 1.036)	0.501

*P-value from multivariable adjusted logistic regression

Unadjusted and adjusted Hazard Ratio for the secondary endpoint duration of hospital stay

Table 4: Hazard Ratios and 95% CIs from Unadjusted Fine & Gray Regression for Time-to-Discharge			
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	P-value
ConvP	1.332 (0.811; 2.187)	0.883 (0.487; 1.603)	0.684*
Age	-	0.965 (0.947; 0.985)	<.001
Female Sex	-	1.166 (0.593; 2.290)	0.656
ICU on Admission	-	0.218 (0.069; 0.694)	0.010
CRP on Admission	-	0.998 (0.994; 1.002)	0.271
Lymphocyte Count on Admission	-	3.028 (1.495; 6.135)	0.002
FI02 on Admission	-	0.980 (0.968; 0.992)	0.002
Bilirubine	-	1.025 (0.991; 1.061)	0.150

*P-value from multivariable adjusted logistic regression

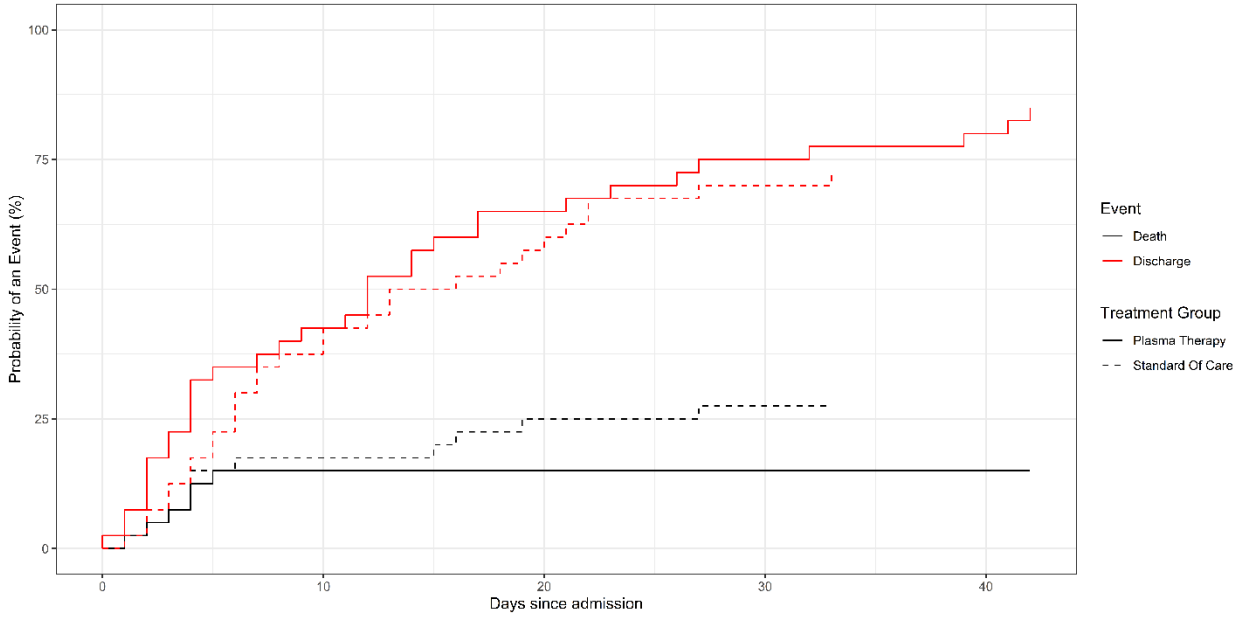
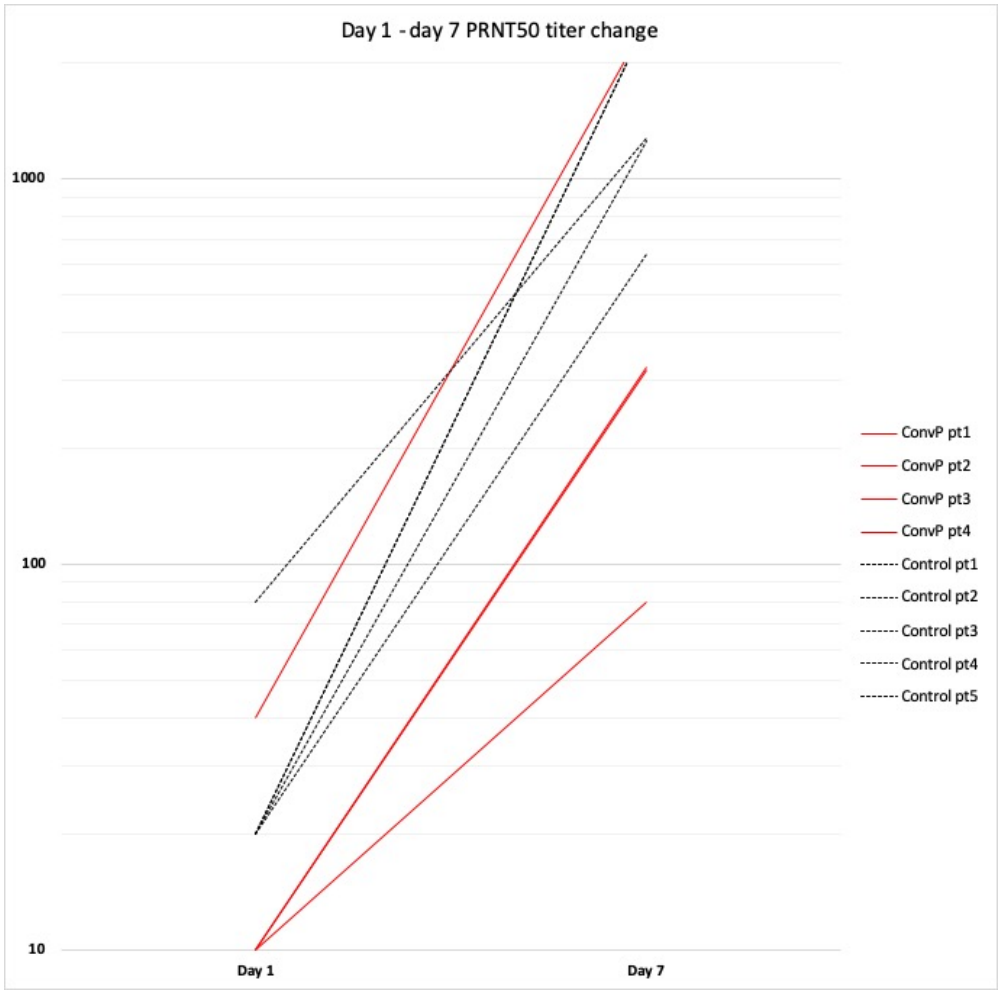


Figure 1c


Day 1 - day 7 PRNT50 titer change
1000
100
10
Day 1
Day 7
ConvP pt1
ConvP pt2
ConvP pt3
ConvP pt4
Control pt1
Control pt2
Control pt3
Control pt4
Control pt5

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2. GeurtsvanKessel CH, Okba NMA, Igloi Z, et al. Towards the next phase: evaluation of serological assays for diagnostics and exposure assessment. medRxiv 2020:2020.04.23.20077156.