Effects of Potent Neutralizing Antibodies from Convalescent Plasma in Patients Hospitalized for Severe SARS-CoV-2 Infection.

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Abstract

Convalescent plasma could be an inexpensive and widely available treatment for COVID-19 patients but reports on effectiveness are inconclusive. We collected convalescent plasma from donors with high titers of neutralizing anti-SARS-CoV-2 antibodies effectively blocking SARS-CoV-2 infection in vitro. In a randomized clinical trial of 86 COVID-19 patients, no overall clinical benefit of 300 mL convalescent plasma was found in patients hospitalized for COVID-19 in the Netherlands. Using a comprehensive translational approach, we unraveled the virological and immunological responses following plasma treatment which helps to understand which COVID-19 patients may benefit from this therapy and should be the focus of future studies. Convalescent plasma treatment in this patient group did not improve survival, had no effect on the clinical course of disease, nor did plasma enhance viral clearance in the respiratory tract, influence anti-SARS-CoV-2 antibody development or serum proinflammatory cytokines levels. The vast majority of patients already had potent neutralizing anti-SARS-CoV-2 antibodies at hospital admission and at comparable titers as the carefully selected plasma donors. Together, these data indicate that the variable effectivity observed in trials on convalescent plasma for COVID-19 may be explained by the timing of treatment and varying levels of preexisting anti-SARS-CoV-2 immunity in patients. It also substantiates that convalescent plasma should be studied as early as possible in the disease course or at least preceding the start of an autologous humoral response.

Trial registration: Clinicaltrials.gov: NCT04342182

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), continues to put a tremendous strain on healthcare systems despite the advances that were made regarding the management of these patients. *Anti-inflammatory* therapy with dexamethasone significantly decreased mortality. (1) Its beneficial effect is well documented after at least 7 days of symptoms and when patients need supplemental oxygen or admission to an intensive care unit. The role of direct antiviral therapy is less well established as a beneficial effect was observed in one but not in a second much larger randomized trial on remdesivir. (2, 3) Furthermore, even in resource rich countries, the drug has been out of stock repeatedly. All other repurposed antiviral drugs studied so far have failed to show any benefit. Clearly, there is an unmet need for antiviral therapy with well-established efficacy and global availability.

Anti-SARS-CoV-2 antibodies are considered a promising treatment for COVID-19, and highly potent monoclonal antibodies are being studied. (4–6) Convalescent plasma (ConvP) can contain high levels of SARS-CoV-2 neutralizing antibodies and could therefore be regarded as an antiviral alternative for monoclonal antibodies to treat COVID-19. ConvP is a potentially scalable option during viral outbreaks. During previous human outbreaks of SARS-CoV and MERS-CoV, ConvP was used as a therapy with some success according to several small studies. (7–10) In SARS-CoV-2, preclinical research indicates a

protective effect of human ConvP containing high levels of neutralizing antibodies when administered to hamsters prior to SARS-CoV-2 infection. (11)

Conclusive evidence for the effectiveness of ConvP as a treatment for human SARS-CoV-2 infection has however yet to be generated. (12–17) ConvP administration late in the disease at 30 days post onset of symptoms did not benefit severely ill COVID-19 patients from China.(15) Results from meta-analyses including also non-randomized observational cohorts suggested that ConvP may benefit only subsets of patients. (18–22) According to the NIH COVID-19 Treatment Guidelines panel statement, conclusive evidence in support of ConvP therapy in patients hospitalized for COVID-19 are lacking (23), despite its emergency use authorization by the FDA on the 23rd of August 2020. (24)

The goal of this study was to evaluate in a randomized trial, the efficacy of ConvP treatment in hospitalized COVID-19 patients. We hypothesized that the administration of ConvP with high titers of neutralizing antibodies would provide benefit to COVID-19 patients in terms of clinical symptoms, SARS-CoV-2 shedding and normalization of inflammatory markers. Our study demonstrates, however, that ConvP treatment fails to provide benefit in general. We were able to substantiate that autologous neutralizing antibodies already present at hospital admission may explain this finding. Our data provide guidance for future trials of antibody based therapy for COVID-19 as well as clinicians involved in COVID-19 patient care.

Results

Demographic characteristics of COVID-19 patients

The study enrollment period was from the 8th of April to the 14th of June 2020. During that period, a total of 204 patients from 14 Dutch hospitals with a RT-PCR confirmed SARS-CoV-2 infection and admitted for a moderate, severe or life-threatening COVID-19 infection were screened for eligibility. All had symptomatic COVID-19 disease as evaluated by study physicians according to the guidelines set by the Dutch National Institute for Public Health and Environment. (*25*) The most common reason for patients to decline participation was fear of adverse events (Supplementary figure 1). A total of 86 COVID-19 patients were enrolled and randomized to standard of care (n=43) or treatment with ConvP (n=43) (Table 1).

Overall, 72% of the patients were male, and median age was 63 years (IQR 56 – 74). At inclusion, they had COVID-19 related symptoms for a median of 10 days (IQR 6 – 15) and had been admitted to the hospital for 2 days (IQR 1 – 3) in line with what was previously reported as median duration of symptoms at hospitalization. (*26, 27*) A total of 13 patients were admitted to the ICU and mechanically ventilated, all for no longer than 96 hours at inclusion. Patients randomized to standard of care (SoC) experienced symptoms for a median of 2 more days at inclusion and more people had WHO disease severity scores ≥4 (98% versus 84%) or ≥6 (ICU admission (19% versus 12%). Four out of 5 blood biomarkers associated with an unfavorable COVID-19 disease course (CRP, ferritin, LDH, lymphocyte count) were slightly more disadvantageous in the SoC group. The total number of comorbidities were 56 and 57 respectively. From 66 of the patients, blood samples for additional immunological evaluations could be retrieved at baseline.

Of these, 34 were in the ConvP arm and their baseline characteristics were balanced with the overall group (Supplementary table 1).

Clinical outcomes

Of the 43 patients randomized to ConvP, 6 (14%) died while 11 of the 43 (26%) SoC patients died leading to an unadjusted odds ratio of 0.47 (95% CI: 0.15 – 1.38) for death. While numerically higher, the predefined primary endpoint in the study protocol was an adjusted analysis of the overall mortality at day 60 after enrollment for patients treated with ConvP of which the odds ratio was 0.95 (95%CI: 0.20 – 4.67, *p*=0.95) (Supplementary table 2). This adjusted analysis of mortality accounts for consistently reported predictors of death in COVID-19 patients. (*28-32*) By adjusting for the same confounders in a proportional odds ordinal logistic regression model we identified age specific probabilities for being scored in specific categories of the 8-point WHO COVID-19 disease severity score at day 15 (Figure 1A) and day 30 (Figure 1B) after randomization. Notably, these probabilities were comparable between both study arms for all scores at day 15 (*p*=0.58) and day 30 (*p*=0.67) throughout the study (Supplementary table 3 and 4). An identical number of 25 (58%) patients in the ConvP and 25 (58%) in the SoC group had improved by day 15 on the WHO COVID-19 disease severity score (adjusted odds ratio 1.30, 95%CI: 0.52 – 3.32, *p*=0.58). Treatment with ConvP was also not associated with earlier discharge in Cox Regression analysis (adjusted hazard ratio 0.88, 95%CI: 0.49 – 1.60, *p*=0.68), also when cumulative incidences were corrected for the competing risk of death (Figure 1C and Supplementary figure 2 and Supplementary table 5). The median duration of admission was 8 days (IQR 3 – 21) and 8 days (IQR 4 – 19) for ConvP versus SoC. Patients who died were 69 years (IQR 63 – 84) and 12 (71%) were men. Baseline data of patients who survived or died in each of the study arms are available in the supplementary table 6. No serious adverse events possible related to ConvP were observed.

Overall, no difference was observed between the highest measurements of CRP or ferritin (the 2 biomarkers included in the electronic case report form) 7 and 14 days after enrollment between groups (Figure 1D and Supplementary table 7). In a subset of 34 patients with data available, we noticed that the median absolute lymphocyte counts in the peripheral blood at these time points were comparable and followed similar trends in recovery in each treatment group within 2 weeks after enrolment.

Donor characteristics

Collectively, 3200 recovered COVID-19 donors volunteered in April 2020 as ConvP donors. The first 115 patients were selected who had RT-PCR proven COVID-19 disease, fulfilled the other inclusion criteria for ConvP collection, had a determination of anti-SARS-CoV-2 neutralizing antibody responses (Supplementary table 8) and completed the online questionnaire regarding donor characteristics. Hundred-five of the 115 donors were male, the median age was 43 years and they had been symptomatic for a median of 12 days (IQR 8 – 18). Their disease course had been generally mild reflected by a 12% admission rate for COVID-19. Overall, we detected virus specific total Ig and IgM antibodies against SARS-CoV-2 receptor binding domain (RBD) by ELISA in serum samples of 114 of 115 (99%) donors at median 34 days in their convalescent phase. The median total Ig and IgM optical density ratios in all donors were

respectively 15.08 (IQR 8.60 – 18.41) and 4.03 (IQR 0.96 – 14.33). Although optical density ratios from ELISAs correlate with neutralization capacity against SARS-CoV-2 substantial outliers with lower than expected neutralizing antibody titers are often observed and the correlation plateaus at increasing antibody levels leading to a loss of discriminative capacity to detect plasma with very high neutralizing antibody titers. (*33*) Therefore, a plaque reduction neutralization test (PRNT) using the whole SARS-CoV-2 virus was used for the selection of donors for the study. In 110 of 115 donors (96%) tested, neutralizing antibodies could be detected. The median PRNT50 titer was 1:160 (IQR 1:80 – 1:640) with 78% and 43% having a PRNT50 of at least 1:80 or 1:320 respectively. PRNT50 titers of 1:80 and 1:320 were previously shown to predict a <5% and <1% chance of demonstrating replication competent virus in the upper respiratory airway of COVID-19 patients. (*34*) A titer above 1:80 was defined as the minimum neutralizing capacity required for a donor to be eligible for ConvP donation. Of the 19 donors of whom ConvP was eventually used, all but 2 had a PRNT50 titer of at least 1:320. These 19 selected individuals all had mild disease without hospitalization and a more recent resolution of symptoms (20 days) than other donors.

Immunological analyses in COVID-19 patients

Serum was available from 66 subjects for PRNT50 and ELISA testing at inclusion. Logistical issues at the peak of the pandemic prevented the collection of serum from the remaining 20 patients. At inclusion, 80% tested positive with the total Ig anti-SARS-CoV-2 RBD antibody test with optical density ratios at 14.80 (IQR 1.84 – 18.41) while IgM anti-SARS-CoV-2 RBD antibodies were present in 77% with optical density ratios at 5.98 (IQR 0.86 – 25.19) (Figure 2A). Interestingly, the 19 selected donors had levels of anti-SARS-CoV-2 RBD total Ig (optical density ratio 18.39, IQR 14.19 – 18.39) that were comparable (p=0.78) with the baseline levels in hospitalized patients who had been symptomatic for a median of 10 days while anti-SARS-CoV-2 RBD IgM tended to be higher (optical density ratio 25.13, IQR 7.55 – 33.32; p=0.02). The optical density ratio of the total Ig (p=0.71) and IgM (p=0.83) anti-SARS-CoV-2 RBD antibody between patients in the ConvP and SoC group were however not meaningfully different (Figure 2B).

We further confirmed the presence of anti-SARS-CoV-2 antibodies in the serum of COVID-19 patients by measuring anti-nucleocapsid (N protein) IgM and IgG antibodies and comparing COVID-19 patients with a group of convalescent plasma donors. Fifty-one patients had anti-nucleocapsid IgM and IgG antibodies with a median of 5.65 units (IQR 2.59 – 11.73) and 16.19 units (IQR 6.91 – 30.48), respectively (Figure 2C). Donors (n=54) had anti-nucleocapsid IgM and IgG antibodies with a median of 3.62 units (IQR 2.20 – 6.28) and 20.25 units (IQR 13.74 – 30.69), respectively. Using a cut-off of 11 units, 14 patients were positive for IgM antibodies (27.5%) and 31 for IgG antibodies (60.8%) while from the donors 11 were positive for IgM antibodies (20.4%) and 45 for IgG antibodies (80.3%). None of 9 healthy controls had IgM or IgG antibodies against SARS-CoV-2 nucleocapsid above the cut-off.

Next, to explore the functionality of detected antibodies, we used viral neutralization tests with SARS-CoV-2 from the same serum samples of patients and ConvP donors in whom the presence of anti-SARS-CoV-2 RBD specific antibodies were determined. This was possible in 56 of 66 enrolled patients due to limited serum availability after antibody testing in 10 patients. To our surprise, in 44 (79%) patients, neutralizing

antibodies at PRNT50 of ≥1:20 were detected at median 1:160 (IQR 1:20 – 1:1280) (Figure 3). As expected, this titer correlated (r^2=0.36, *p*=0.07) with the duration of symptoms (Supplementary figure 3), and was comparable to the median titer observed in all 115 donors tested (*p*=0.4). The median PRNT50 of the 19 selected donors (1:640, IQR 1:320 – 1:1280) was however higher *p*=0.011, with 90% having a PRNT50 titer ≥1:320 compared to 46% (*p*=0.001) in patients. Only 2 of the 19 selected donors had titers <1:320 and these were administered to 2 patients with PRTN50 titers at >1:2560 and 1:640 respectively at baseline. These patients were admitted for 12 and 13 days and both survived throughout day 60.

To independently confirm the high virus neutralizing anti-SARS-CoV-2 antibody levels in the included patients, we additionally tested serum from 37 RT-PCR confirmed COVID-19 patients from the month preceding the start of the study from whom serum samples from <72h after hospital admission to a non-ICU ward were available at the Erasmus MC university medical center. With a median age of 65 years (IQR 56 – 74), 60% males and symptom duration of 9 days (IQR 4 – 13) these patients were comparable to the study population, as were their biomarkers that predict disease outcomes (Supplementary table 9). We found that 26/37 (70%) of these patients had anti-SARS-CoV-2 Ig antibodies including 23/37 (62%) at a ratio >10, indicating the presence of neutralization capacity based on our previous observation (Supplementary figure 4). (*34*)

Finally, to assess whether ConvP treatment had a more indirect effect on the COVID-19 disease course by potentially dampening the inflammatory response, we measured serum pro-inflammatory cytokines IL-6, TNFα, IFNγ, IL-1β, IL-2, IL-4, IL-10 and IL-12p70. We investigated 9 patients that received ConvP and 10 SoC for which we had a complete set of serum samples for the first 2 weeks post inclusion in the study. For these patients, we compared the cytokines levels at enrollment (day 1), and at days 7 and day 14 after enrollment. There was no difference between the treatment arms on day 1 for IL-6, TNFα and IFNγ. Importantly, the decrease in cytokine levels on day 7 and 14 were comparable between patients receiving ConvP and standard of care both for concentration (Figure 4A) and fold change from day 1 (Figure 4B). No differences were seen between groups for IL-1β, IL-2, IL-4, IL-10 and IL-12p70 (Supplementary table 10). The above provides further evidence that plasma therapy had no effect on the inflammation and course of COVID-19.

Virological analyses in COVID-19 patients

Nasopharyngeal swabs were taken of 51, 54, 53, 15 and 45 patients at enrollment (day 1) and day 3, day 7, day 10 and day 14 after enrollment respectively. In unadjusted analyses, the proportion of samples where SARS-CoV-2 genome was detectable by RT-PCR was higher in the SoC group at day 1 (82% vs 67%), day 3 (79% vs 46%), and day 14 (21% vs 9%) compared to patients in the ConvP group. The calculated median SARS-CoV-2 viral loads were higher in the SoC group at inclusion ($5{,}7x10^3$ copies/ml, IQR: $7{,}3x10^2$ – $4{,}7x10^4$ vs $1.4x10^3$ copies/ml, IQR: 0 – $1.7x10^4$) and day 3 ($1{,}1x10^3$ copies/ml, IQR: 0 – $8{,}2x10^3$ vs 0 copies/ml, IQR: 0 – $8.7x10^1$). Thus, the apparent lower virus shedding in the upper airway was already present before ConvP initiation and this remained so over time in the treatment group without major appreciable influence of ConvP therapy (Figure 5A). Indeed, after adjustment in a mixed model by

covariates associated with COVID-19 disease severity as we had pre-defined in the study protocol (Supplementary table 11), the slope of the viral load decay from day 1 to 14 was estimated to be less steep in the ConvP group at -0.4log copies/mL (95%CI -0.7 – -0.1) overall (Figure 5B) and comparable when considering only patients with detectable SARS-CoV-2 in nasopharyngeal swabs at enrollment (Figure 5C).

With regard to virus viability, we had 12 patients (6 in each arm, median 8 days of symptoms at inclusion) where we were able to do culture samples for SARS-CoV-2 replication, obtained after median 2 days following inclusion. Although a systematic collection of cultures before inclusion was not required per protocol and current knowledge indicates that viral cultures tend to become negative around 8 days of symptoms, (*34, 35*) none of the 6 patients from the SoC arm and 1 patient on ConvP had cytopathic effects in culture after 7 days of incubation, again signaling no added value of ConvP. This patient with a sample containing replication competent virus had 6 days of symptoms at inclusion, without antibodies detected then, and died during follow-up.

Discussion

Our study demonstrates that the administration of ConvP with high titers of virus neutralizing antibodies does not benefit patients who are hospitalized for COVID-19 after 10 days post symptom onset. Overall, we found no improvement in key clinical, immunological or virological parameters indicative of any effect favoring ConvP. This certainly does not exclude a possible beneficial effect in patients who have not yet started producing autologous neutralizing antibodies at the time of ConvP transfusion, but these patients were rare in our study population. When these data on the baseline antibody levels became available to us, we considered it highly unlikely that the current study design would allow for the detection of a significant clinical effect. After discussion with the DSMB, the decision was made to interrupt study recruitment. Our data agree with the recent data from the Placid trial. In this study of 464 patients, half were randomized to ConvP. The intervention did not decrease the risk of progression to severe disease or death nor did it decrease hospital stay. However, in contrast to our study, donors for the Placid trial were not screened for the presence of neutralizing antibodies before their plasma was used for the study. When this was done in retrospect, the PRNT50 titer in their donors turned out to more than 10-fold lower (1:40) than the median titer of 1:640 in our ConvP donors. (36)

When the ConCOVID study was designed, the timing of neutralizing antibody development during SARS-CoV-2 infection was not well established and certainly not common knowledge for those involved in COVID-19 care. We considered it unlikely that patients with severe disease requiring hospitalization would already have high titers of autologous neutralizing antibodies at the time of hospital admission. Although no formal stopping rule was reached when the study was discontinued, the study team as well as the DSMB members concluded that, considering the hypothesis of the study that was being tested, the chances of finding a significant difference in the primary endpoint even after full enrollment were too small to justify its continuation under its current design. Also, amending the study by excluding patients

with autologous antibodies at screening was considered no option either as it would leave too few eligible patients as approximately 80% would have to be excluded.

Our observations are relevant for studies that continue to enroll hospitalized patients, as well as for emergency access, and compassionate use programs on ConvP for COVID-19. The data strongly suggests that any effect elicited by ConvP is more likely to occur when ConvP is given as early as possible in the disease course, which was also suggested by the cohort of the Mayo Clinic-led Expanded Access Program. (14) However, the latter was an observational study without a formal control arm and data on the time since symptom onset were not reported. Using antibody based therapy as early as possible after exposure to maximize their therapeutic effects is similar to the use of anti-HBV or rabies immunoglobulin preparations. (37, 38) Given the current data on the development of humoral anti-SARS-CoV-2 responses starting after approximately 1 week of symptoms, the window of opportunity is likely to be before day 7 after symptom onset and rapidly decreases thereafter. (39, 40) Nevertheless, many ongoing trials are now focusing on hospitalized patients and the time from disease onset to admission was repeatedly shown to be comparable to the 10 days in our study. (2, 27, 41) Therefore, in the vast majority of patients in studies with a comparable study design the production of autologous humoral immunity against SARS-CoV-2 will have started as well. This is also supported by the notion that the generation and strength of the neutralizing antibody response correlates with disease severity. (40, 42, 43) We therefore predict that, on their own, almost all ongoing trials using a frequentist's design will be substantially underpowered to show beneficial effects from ConvP in hospitalized patients. This problem of insufficient statistical power may be partially circumvented by pooling data from ongoing trials together in real-time as suggested by others and as initiated under the COMPILE initiative in the United States. (44)

Apart from correctly identifying patients who are likely to benefit most from ConvP, choosing the optimal ConvP donor with high titers of neutralizing antibodies is likely to be equally critical. While data from formal dose finding studies of ConvP are pending, the volume of as well as the minimum antibody titer in ConvP but also the methods used to measure antibody titers vary substantially across study protocols. That this may turn out to be critical was demonstrated in a COVID-19 hamster model in which disease could be prevented with human ConvP with an exceptionally high neutralizing antibody titer of 1:2560 while ConvP with an above average antibody titer of 1:320 did not. (11) On theoretical grounds, ConvP will need to contain a certain (as of yet unknown) minimum level of neutralizing antibodies to ascertain an antiviral effect. Indeed, the antibodies in a standard 300 mL plasma unit will be ~ 1:10 diluted when given to an average human adult. With concurrent emerging data, including from our laboratory, it is likely that a neutralizing goal directed therapy to reach a minimum neutralization titer of 1:80 in vivo after transfusion should be obtained to recapitulate the 95% probability of inhibiting viral growth in vitro. This should then also take into account the approximately tenfold dilution of ConvP in human plasma during transfusion. It is therefore worrisome if ConvP compassionate use programs and trials currently lack donor screening for neutralizing antibodies or select donors on ELISA testing only. Although data show that strong ELISA signals correlate with neutralization capacity, the predictive value of any ELISA cut-off for the presence of high levels of neutralizing antibody titers seems moderate at best. (33) Without readily available

alternatives to ascertain antibody functionality, we consider the use of virus neutralization assays essential to avoid suboptimal donor selection. The use of hyperimmune Ig preparations produced from a large donor pool of ConvP (also called COVIg) as well as specific highly neutralizing monoclonal antibodies may resolve this issue in the future. (45, 46)

Our study has several limitations. First, the premature ending prevents definite conclusions regarding the lack of clinical benefit of ConvP. The COMPILE real-time meta-analysis initiative described above, should be able to solve this limitation. (44) Also, two large platform trials have opened a ConvP arm for hospitalized patients (the UK RECOVERY trial and global REMAP-CAP) (47, 48) and with their Bayesian design continue enrollment until futility or effectivity is documented. Second, the decision to end the study does not take into account that plasma could have effects unrelated to virus neutralization. We consider these effects highly unlikely because far higher doses of plasma or immunoglobulins (usually 70–150 grams) are used to reach these immunomodulatory effects than that present in a single unit of plasma (3 grams). One concern with plasma treatment is whether antibody dependent enhancement of infection could be mediated by the transferred antibodies. The findings described by Joyner et al. on over 35.000 ConvP transfusions, including many with lower antibody titers than in our study is reassuring in that perspective. Finally, we did not record the use of corticosteroids for COVID-19. During the recruitment for the trial, corticosteroids for non-ICU patients were not recommended in the Dutch COVID-19 guideline

For future directions, our data support a more prominent role for ConvP early in the disease course, potentially in the outpatient setting in particular in those with a higher risk of disease progression. It could also serve as a way to protect B-cell depleted patients or as post exposure prophylaxis after high-risk exposure. (49) Selecting hospitalized patients for ConvP treatment based on their antibody test results seems a logical way forward when plasma with high titers of neutralizing antibodies is scarce. Finally, studies in hospitalized patients will have to be sufficiently large to document a therapeutic benefit independent of dexamethasone and remdesivir therapy. In conclusion, no beneficial effects of ConvP were observed in patients recently hospitalized with COVID-19. The most likely explanation is the already high antibody titers on the day of inclusion. ConvP to treat COVID-19 should be targeted to patients as early as possible in their disease course and before a strong autologous neutralizing humoral response can be observed.

Methods

Study design and population

The ConCOVID study was a multicenter open-label randomized clinical trial including 14 secondary and academic hospitals in the Netherlands. Enrollment began on April 8, 2020. Eligible patients were at least 18 years, admitted to the hospital for COVID-19 proven by a SARS-CoV-2 genome detectable in a reverse transcriptase polymerase chain reaction (RT-PCR) test in the previous 96 hours. Patients with documented IgA-deficiency or on mechanical ventilation for >96 hours at the time of screening were excluded. Concurrent inclusion in another interventional study aimed at COVID-19 treatment was prohibited. Upon

the discretion of the research physician, eligible patients identified through screening were not included when care had entered a terminal phase or a patient had already improved significantly to a fit for discharge level.

Recovered COVID-19 patients who could potentially participate as plasma donors were informed on this option by social media notifications. Interested donors could apply by email. Eligible donors had RT-PCR confirmed SARS-CoV-2 infection and were asymptomatic for minimally 14 days. Written informed consent was obtained and a questionnaire was sent by email using Gemstracker. ConvP donors were recruited and screened by Sanquin Blood Supply (Dutch blood bank) according to existing guidelines (Appendix 2 study protocol, section 8.3 on donor eligibility criteria). Donors could voluntarily donate up to maximum of 4 times at 1-week intervals. A single serum tube for anti-SARS-CoV-2 antibody assessment was drawn on the first day of donation. Only donor ConvP with anti-SARS-CoV-2 neutralizing antibodies confirmed by ELISA and having a SARS-CoV-2 plaque reduction neutralization test (PRNT) and a PRNT50 titer of minimally 1:80 was used. (*34, 39*) For each patient, we selected the plasma with the highest PRNT50 titer from the donor pool available at the time of inclusion. Donors completed a detailed questionnaire on their medical history and COVID-19 clinical symptoms.

Study procedures and endpoints

Participants provided written informed consent, had blood group determined and were subsequently randomly assigned via a web-based system ALEA at a 1:1 ratio to the current standard of care with or without the addition of 300mL ConvP including anti-SARS-CoV-2 neutralizing antibodies with a known adequate PRNT50. The chosen volume reflects the standard volume of one plasma unit produced by Sanquin Blood Supply, and was comparable to the volume (280mL) of ConvP used in studies for SARS-CoV. (*8*) ConvP was administered intravenously on the day of inclusion. Patients without a clinical response and a persistently positive RT-PCR could receive a second unit of ConvP after five days. Off-label use of EMA-approved drugs as a treatment for COVID-19 was allowed in hospitals where this was part of the standard of care. We scored the clinical status with the ordinal 8-point WHO COVID-19 disease severity scale on days 1, 15 and 30. (*50*) Serum samples and nasopharyngeal swabs were collected at inclusion preceding treatment and day 3, 7, 14. One serum tube per participant for immunological and virological assays were collected at enrollment and on day 7 and 14. This material was used for detection of antibodies by ELISA and, with sufficient serum available, PRNT. The primary endpoint of the study was overall mortality until discharge from hospital or a maximum of 60 days after admission, whichever came first. Key secondary endpoints were the improvement on the 8-point WHO COVID-19 disease severity scale on day 15 and day 30, hospital length of stay, SARS-CoV-2 shedding from the airways, impact of ConvP on humoral immunity and inflammation. Safety of ConvP was recorded as any plasma related transfusion reaction or death.

Clinical data

All PI and the sites' study teams were trained before any study procedure through site initiation meetings. Baseline characteristics and medical history were recorded in the electronic case record (eCRF) form by a

trained research physician. 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 either type 1 or type 2. This also included diabetes with or without end organ damage. Cardiac history was defined as any chronic disorder of the cardiac function that made the subject eligible for yearly influenza vaccination according to the Dutch guidelines. (*51*) A history of pulmonary disease was defined as any chronic pulmonary condition which required inhalators or systemic medication or follow-up with a pulmonologist. A history of cancer was defined as any active cancer in the previous five years (cutaneous basal cell carcinoma was not included). A history of immunodeficiency was defined as any documented clinical relevant immunocompromised condition or active use of immunosuppressants. A history of chronic kidney disease was defined as any kidney disorder due an estimated glomerular filtration rate below 60ml/min, macroalbuminuria, peritoneal or hemo-dialysis, or prior kidney transplantation. A history of liver cirrhosis was defined as liver cirrhosis classified as Child-Pugh A or higher. Admitted participants were assessed as inpatients by the research physicians at the study timepoints on clinical endpoints. The use of experimental medication for COVID-19 was recorded in the eCRF for (hydroxy)chloroquine, lopinavir/ritonavir and remdesivir. Discharged patients were contacted at the study time-points to assess the clinical status. The most recent routinely measured serum biomarkers for COVID-19 severity were collected in the eCRF and were generally available for CRP (1 missing), LDH (3 missing), bilirubin (7 missing), lymphocyte count (8 missing), ferritin (29 missing). All sites collected and entered data into an eCRF (OpenClinica). Independent data monitors scrutinized the data quality and solved inconsistencies in the eCRF. A.G., C.J., B.R., C.R., G.P. extracted and analyzed the data.

SARS-CoV-2 Plaque reduction neutralization test

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. (*37*) 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.

Anti-SARS-CoV-2 Antibody Enzyme Linked Immunosorbent Assays

Serum was tested for the presence of anti-SARS-CoV-2 total Ig and IgM against RBD in the Wantai Enzyme Linked Immunosorbent Assay (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. (*33*) Anti-SARS-CoV-2 virus nucleocapsid protein (N-protein)-specific antibodies in serum were measured by ELISA using COVID-19 IgG ELISA (Tecan,

30177447) and COVID-19 IgM ELISA (Tecan, 30177448) according to the manufacturer's instructions. Positive cut-off for these ELISAs was 11 units.

Serum cytokine measurements

Cytokines IL-6, TNFα, IFNγ, IL-1β, IL-2, IL-4, IL-10 and IL-12p70 in serum of COVID-19 patients and plasma donors were measured using Simple Plex Cytokine Screening Panel cartridges (SPCKE-PS-003426, Bio-Techne) with the Ella Next Generation ELISA system (Bio-Techne). The lower and upper limit of quantitation was 0.21 pg/ml and 840 pg/ml for IL-1β, 0.64 pg/ml and 990 pg/ml for IL-2, 0.32 pg/ml and 1290 pg/ml for IL-4, 0.28 pg/ml and 2652 pg/ml for IL-6, 0.46 pg/ml and 5530 pg/ml for IL10, 0.46 pg/ml and 2.7 pg/ml for IL-12p70, 0.17 pg/ml and 4000 pg/ml for IFNγ, and 0.3 pg/ml and 1160 pg/ml for TNFα.

Viral culture

Vero cells, clone 118, were used for isolation of infectious SARS-CoV-2 from respiratory tract samples. Samples were cultured for seven days, and, once cytopathic effect (CPE) was visible, the presence of SARS-CoV-2 was confirmed with immunofluorescent detection of nucleocapsid proteins.

Sample size and statistical analysis plan

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. A Mann-Whitney U test, a t-test or a chi-squared test was used to describe differences in these baseline statistics. With an anticipated 50% overall mortality reduction from 20% (as the reported mortality in hospitalized patients in the Netherlands when the protocol was designed) and with a control to intervention ratio of 1:1, 426 patients were needed for the study to have 80% power with a global alpha of 0.05 and adjusted alpha for the primary endpoint of 0.0480, accounting for 1 interim analysis. Due to the premature interruption of the trial and resulting in lower event rates we present both the results of the multivariable (adjusted) logistic regression analysis as originally planned as the principle analysis as well as the unadjusted univariable analysis. 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 worse on the 8-point WHO COVID-19 disease severity scale at day 15 and day 30 after inclusion and adjusted for the seven factors mentioned above. This model was used to test 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 both with a proportional hazards model for the subdistribution of hospital discharge as proposed by Fine and Gray (1999) and by reporting the cause-specific hazards using Kaplan-Meier plots. The correlation between time and PRNT50 were assessed by Spearman correlation coefficients. The viral load (copies/ml) was analyzed using a mixed-effects model with

random-intercepts and random-slopes. A linear effect of time was used in the model. An interaction effect between time and treatment was included in the model to allow for different evolution over time between the treatment arms and to assess whether the rate of decrease is different between the two arms. The outcome was transformed using the logarithmic function to avoid deviations from the normality and homoscedasticity assumptions of the model. The value of 0.001 was added to zero values of viral load before applying the transformation to avoid minus infinity values.

Ethical considerations

The study was reviewed and approved by the institutional review board of the Erasmus University Medical Center. Written informed consent was obtained from every patient or legal representative. The DSMB consisted of a professor in biostatistics, an infectious diseases specialist and an intensivist. They reviewed the safety of the participants on a regular basis and recommended the study team regarding the further conduct of the study at predefined time points. Findings are reported according to the CONSORT statement. The study was registered as NCT04342182 at clinicaltrials.gov.

Declarations

Author contributions

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AG, CCEJ, CR, PtB, GP, MPGK and BJAR designed the study, GP is the study statistician, AG CCEJ BJAR designed the database and eCRF, JvK, NMAO, MPGK, BLH and CG were responsible for the virological

assays, RT-qPCR, PRNT and the anti-SARS-CoV-2 total Ig and IgM against RBD, YM, PK and TS were responsible for the immunological analyses, FHS organized plasma donations at the Dutch blood Bank (Sanquin Blood Supply), all other authors recruited patients and collected study data. AG, CCEJ, CR, GP and BJAR analyzed the data. BJAR and CR wrote the first draft of the paper, all authors reviewed the paper.

Potential competing interest

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Tables

Table 1
Baseline characteristics of COVID-19 patients

	SoC (n=43)	ConvP (n=43)
Male sex, n (%)	33 (77)	29 (67)
Age (years), median (IQR)	63 (55 – 77)	61 (56 – 70)
Duration of symptoms at inclusion (days), median (IQR)	11 (6 – 16)	9 (7 – 13)
Number of comorbidities, n (%)		
Diabetes Mellitus	8 (19)	13 (30)
Hypertension	11 (26)	11 (26)
Cardiac	11 (26)	9 (21)
Pulmonary	11 (26)	12 (28)
Cancer	3 (7)	5 (12)
Immunodeficiency	6 (14)	5 (12)
Chronic kidney disease	6 (14)	1 (2)
Liver cirrhosis	0	1 (2)
CRP (mg/L), median (IQR)	109 (70 – 165)	84 (50 – 133)
Ferritin (µg/L), median (IQR)	709 (525 – 1311)	702 (406 – 1060)
LDH (U/L), median (IQR)	356 (291 – 507)	336 (259 – 454)
Lymphocytes (x10^9/L), median (IQR)	0.95 (0.80 – 1.30)	1.20 (0.80 – 1.53)
Bilirubin (µmol/L), median (IQR)	8 (6 – 12)	9 (5 – 13)
WHO COVID-19 disease severity score(1), n (%)		
≤2	0	0
3	1 (2)	7 (16)
4-5	34 (79)	31 (72)
6-7	8 (19)	5 (12)

(1) WHO 8 point COVID-19 disease severity score (at study inclusion for patients and highest score ever during disease course for donors) in which **0** is no clinical or virological evidence of infection, **1** is no limitation of activities, **2** is limitation of activities, **3** is hospitalized, no oxygen, **4** is oxygen by mask or nasal prongs, **5** is non-invasive ventilation or high-flow oxygen, **6** is intubation and mechanical ventilation, **7** is ventilation and additional organ support (vasopressors, renal replacement therapy, ECMO) and **8** is death.

Figures

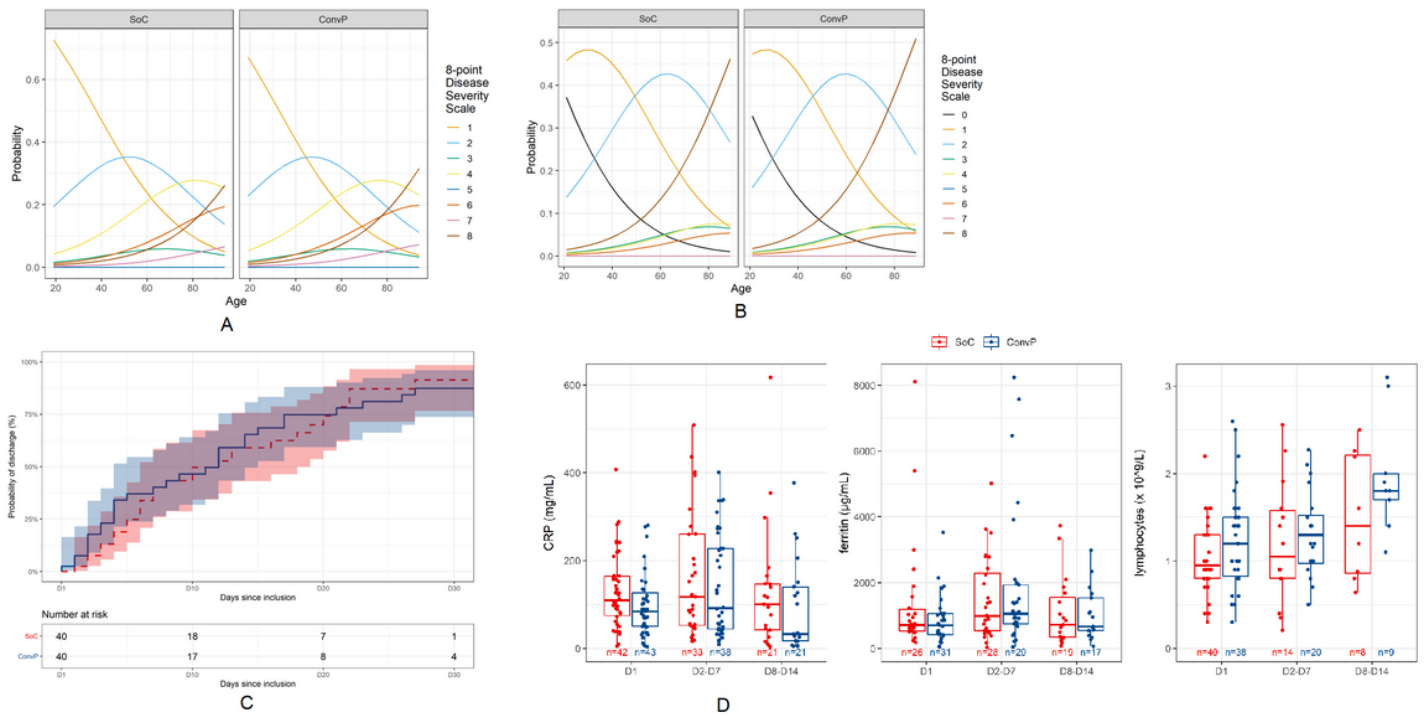
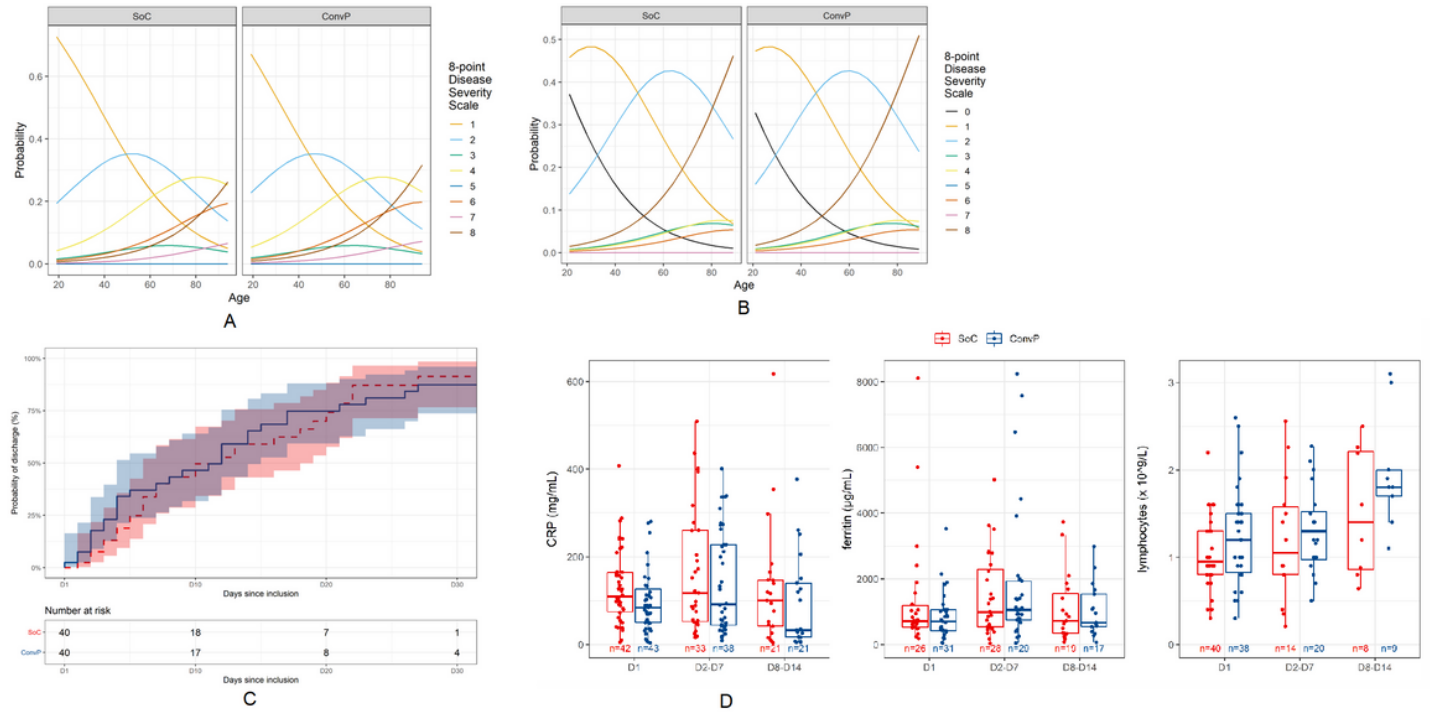


Figure 1

A. Predicted probabilities of belonging to each outcome category of the WHO COVID-19, 8-point Disease Severity Scale after 15 days over different values of age and per treatment group. The predicted probabilities are based on the proportional ordinal logistic regression model adjusted for age, sex, CRP and whether a patient was admitted to the ICU at enrollment. The values of these factors are set to the median value (if continuous) or to the most frequent value (if categorical). WHO COVID-19 8-point Disease Severity Scale: 0 = No clinical or virological evidence of infection, 1 = No limitation of activities, 2 = Limitation of activities, 3 = Hospitalized, no oxygen therapy, 4 = Oxygen by mask or nasal prongs, 5 = Non-invasive ventilation or high-flow oxygen, 6 = Intubation and mechanical ventilation, 7 = Ventilation + additional organ support = vasopressors, RRT, ECMO, 8 = Death. B. Predicted probabilities of belonging to each outcome category of the WHO COVID-19, 8-point Disease Severity Scale after 30 days over different values of age and per treatment group. The predicted probabilities are based on the proportional ordinal logistic regression model adjusted for age, sex, CRP and whether a patient was admitted to the ICU at enrollment. The values of these factors are set to the median value (if continuous) or to the most frequent value (if categorical). WHO COVID-19 8-point Disease Severity Scale: 0 = No clinical or virological evidence of infection, 1 = No limitation of activities, 2 = Limitation of activities, 3 = Hospitalized, no oxygen therapy, 4 = Oxygen by mask or nasal prongs, 5 = Non-invasive ventilation or high-flow oxygen, 6 = Intubation and mechanical ventilation, 7 = Ventilation + additional organ support = vasopressors, RRT, ECMO, 8 = Death. C. Kaplan Meier curves of the probability of discharge with 95% CI (shaded area) for the two treatment groups (SoC: red dashed line, ConvP: blue solid line) after enrollment (D=1) and table with

number of subjects at risk of discharge. Death is not accounted for as competing risk. D. CRP, ferritin and lymphocytes were measured* in the serum of COVID-19 patients (SoC: red, ConvP: blue) on day 1 of enrollment, between day 2-7 and day 8-14 after enrollment. Reported data are the highest value for CRP and ferritin and the lowest value for lymphocytes. Box indicates the median and IQR. * only measured if it was part of routine care



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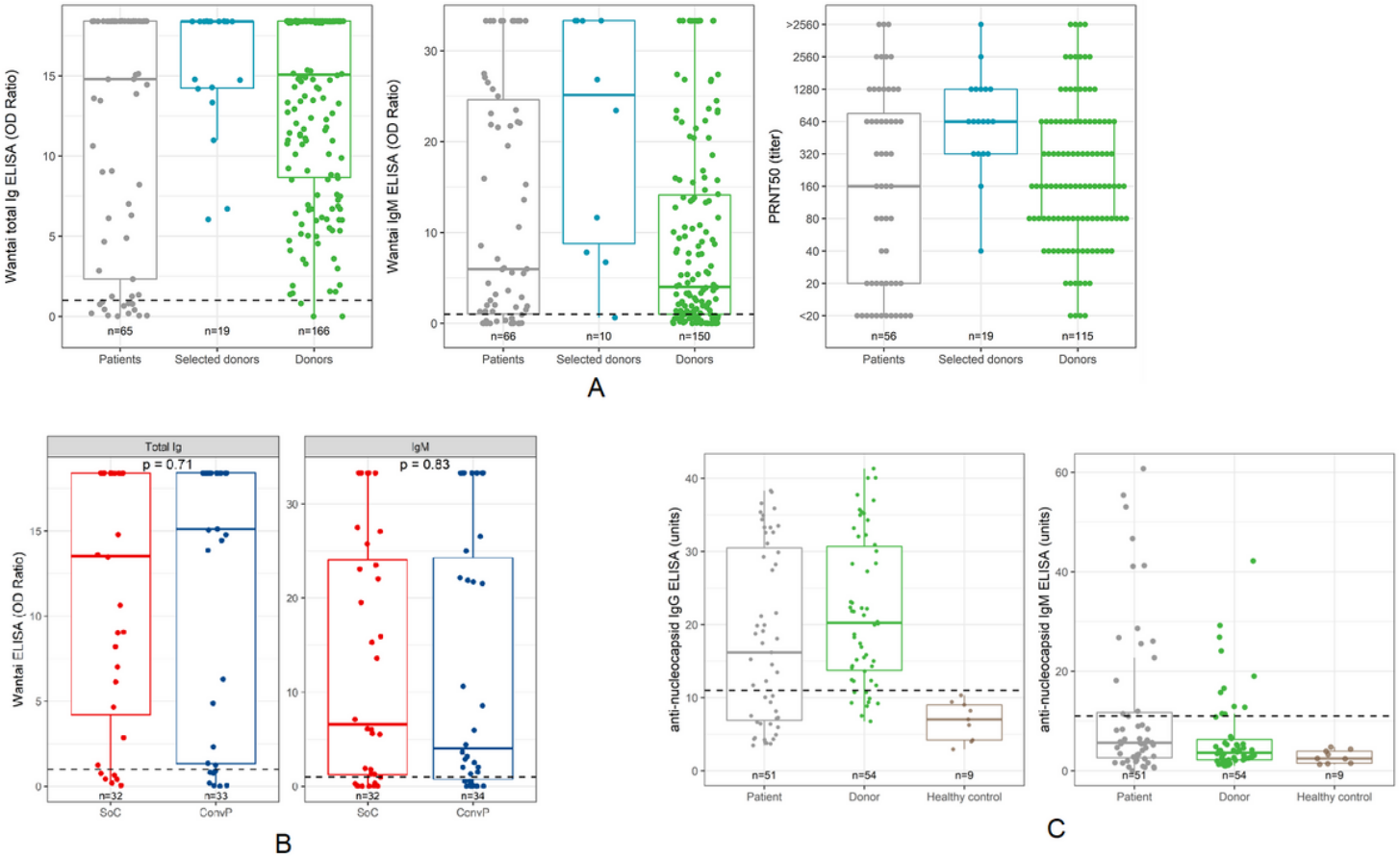


Figure 2

A. Anti-SARS-CoV-2 total Ig and IgM against SARS-CoV-2 receptor binding domain (RBD) measured by Wantai ELISA and viral neutralization capacity measured as PRNT50 titer were evaluated in the serum of COVID-19 patients (grey) at enrollment (day 1) and serum of donors at day of plasma donation (all 115 donors tested: green, donors of whom plasma was selected for use in the study: blue). Box indicates the median and IQR. Dashed line indicates positive cut-off at 1.0 OD ratio for both total Ig and IgM. B. Anti-SARS-CoV-2 total Ig and IgM against SARS-CoV-2 receptor binding domain (RBD) measured by Wantai ELISA were evaluated in the serum of COVID-19 patients (SoC: red, ConvP: blue) at enrollment (day 1). Box indicates the median and IQR. Dashed line indicates positive cut-off at 1.0 OD ratio for both total Ig and IgM. C. Anti-nucleocapsid IgM and IgG antibodies were measured in the serum of COVID-19 patients (grey) at enrollment (day 1) in serum of donors (green) at week 6 post infection and in serum of healthy

uninfected controls (brown). Box indicates the median and IQR. Dashed line indicates positive cut-off at 11 units for both IgM and IgG.

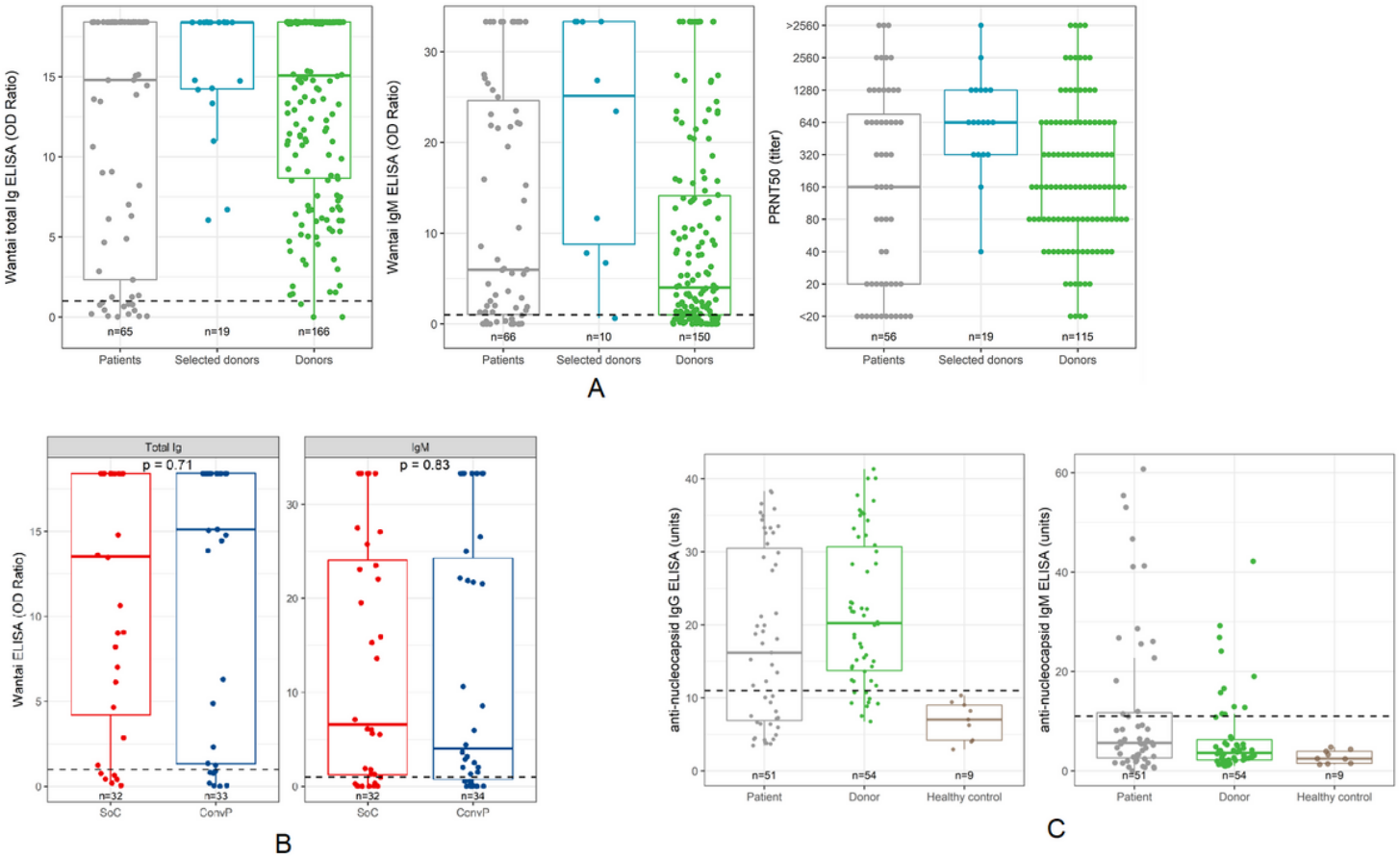


Figure 2

A. Anti-SARS-CoV-2 total Ig and IgM against SARS-CoV-2 receptor binding domain (RBD) measured by Wantai ELISA and viral neutralization capacity measured as PRNT50 titer were evaluated in the serum of COVID-19 patients (grey) at enrollment (day 1) and serum of donors at day of plasma donation (all 115 donors tested: green, donors of whom plasma was selected for use in the study: blue). Box indicates the median and IQR. Dashed line indicates positive cut-off at 1.0 OD ratio for both total Ig and IgM. B. Anti-SARS-CoV-2 total Ig and IgM against SARS-CoV-2 receptor binding domain (RBD) measured by Wantai ELISA were evaluated in the serum of COVID-19 patients (SoC: red, ConvP: blue) at enrollment (day 1). Box indicates the median and IQR. Dashed line indicates positive cut-off at 1.0 OD ratio for both total Ig and IgM. C. Anti-nucleocapsid IgM and IgG antibodies were measured in the serum of COVID-19 patients (grey) at enrollment (day 1) in serum of donors (green) at week 6 post infection and in serum of healthy uninfected controls (brown). Box indicates the median and IQR. Dashed line indicates positive cut-off at 11 units for both IgM and IgG.

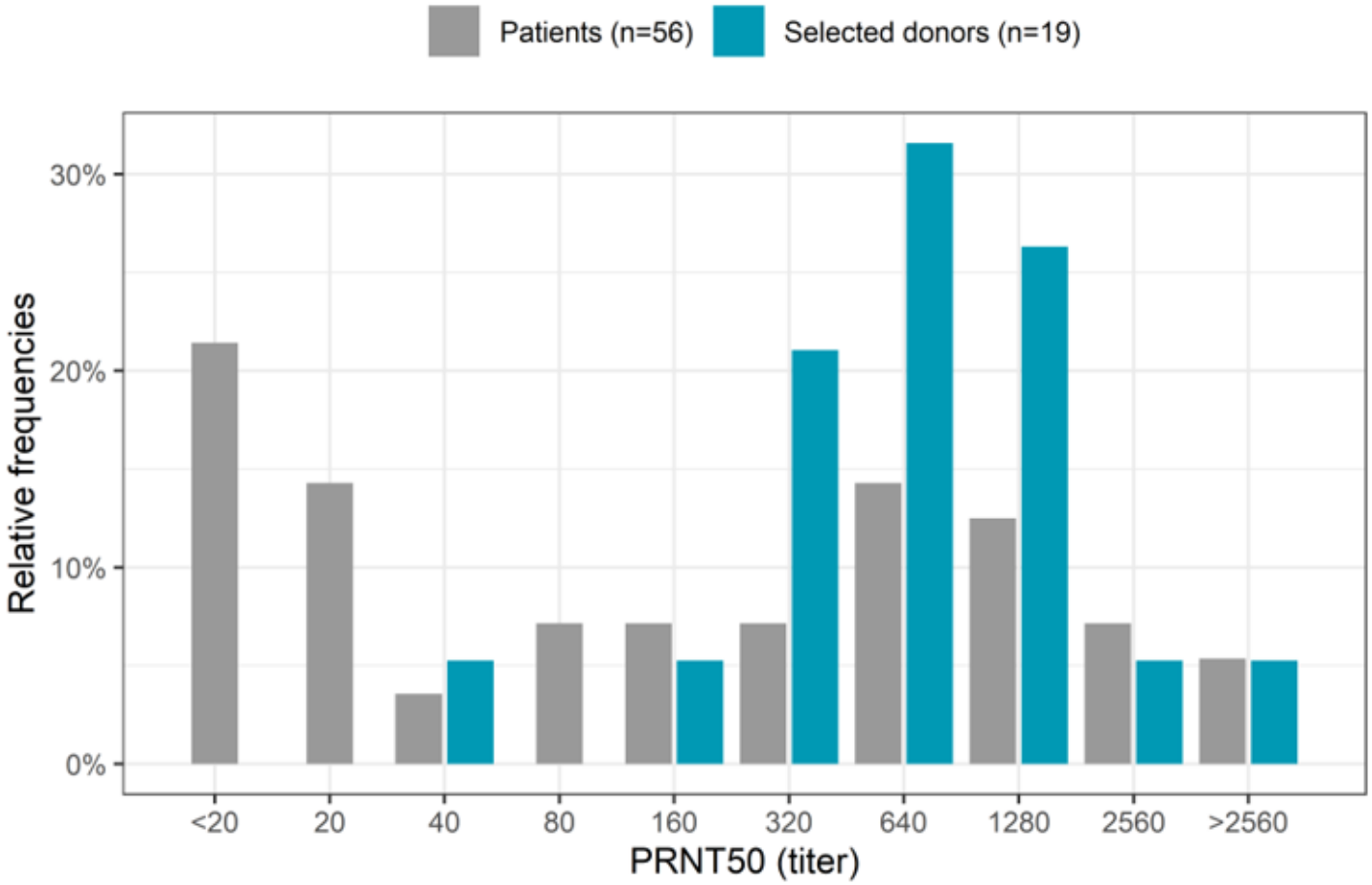
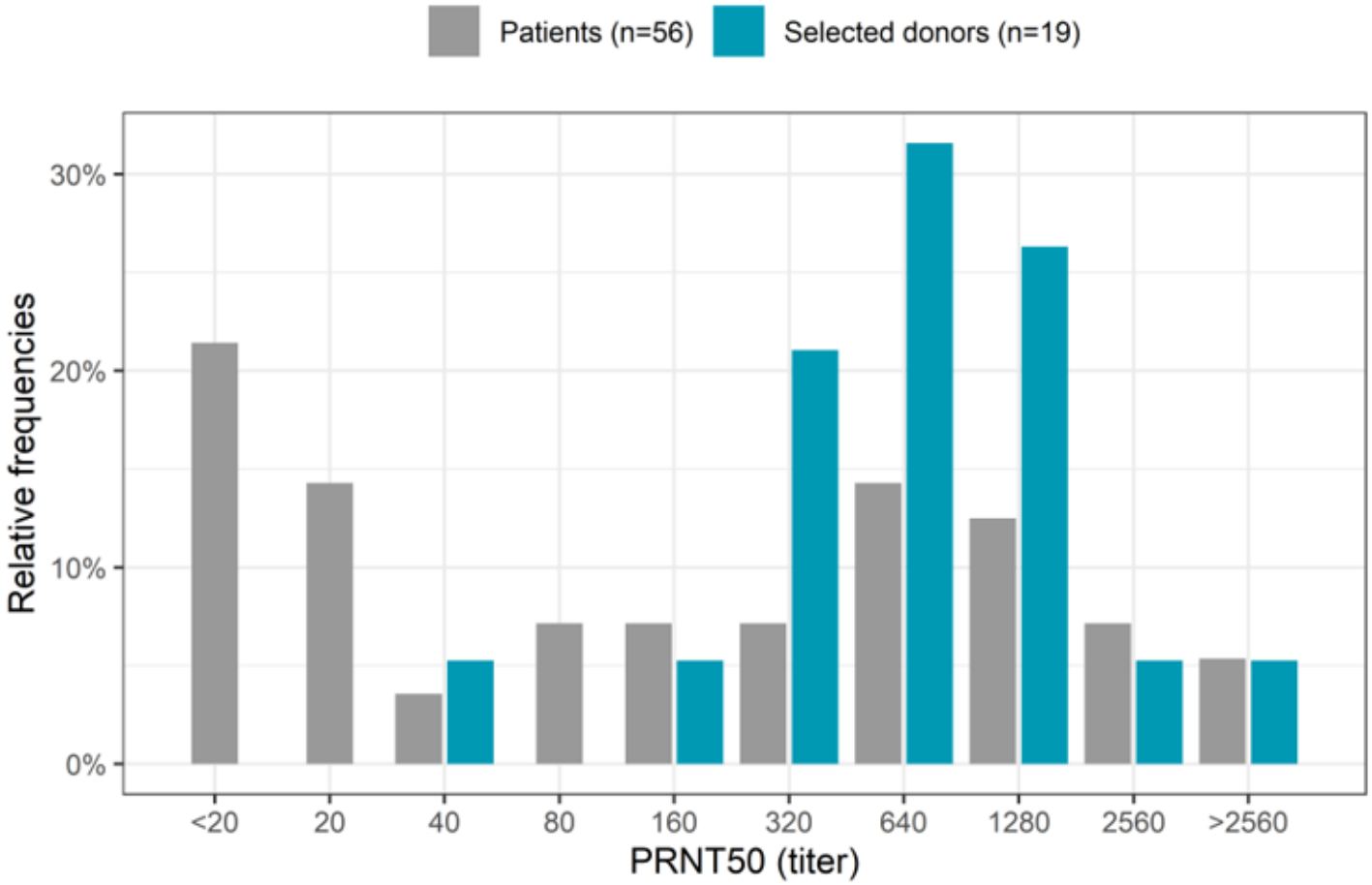


Figure 3

PRNT50 titers were measured in the serum of COVID-19 patients (grey) on day 1 of enrollment and in selected donors (blue). Height of the bars indicates the relative frequency of PRNT50 titer in that group.



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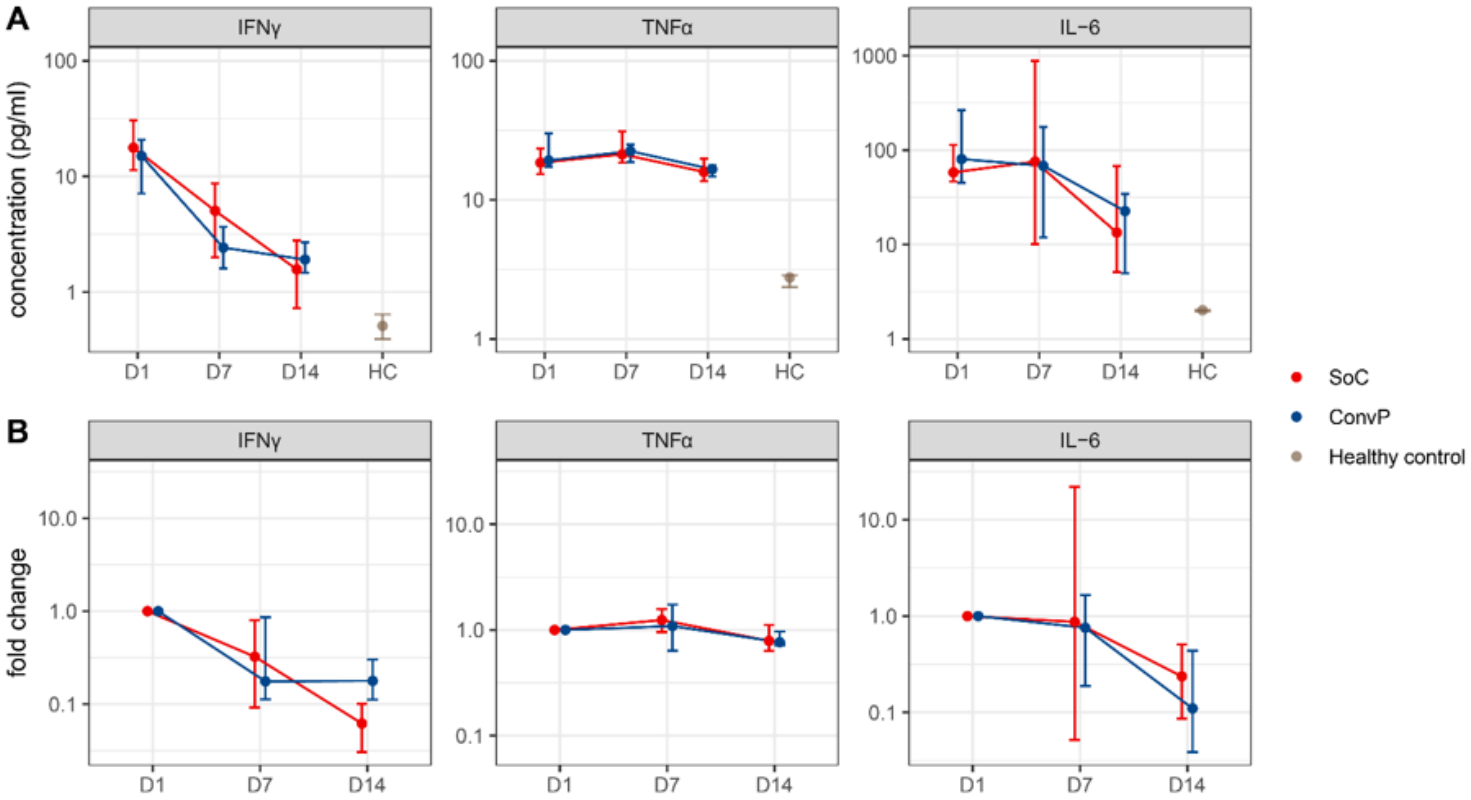
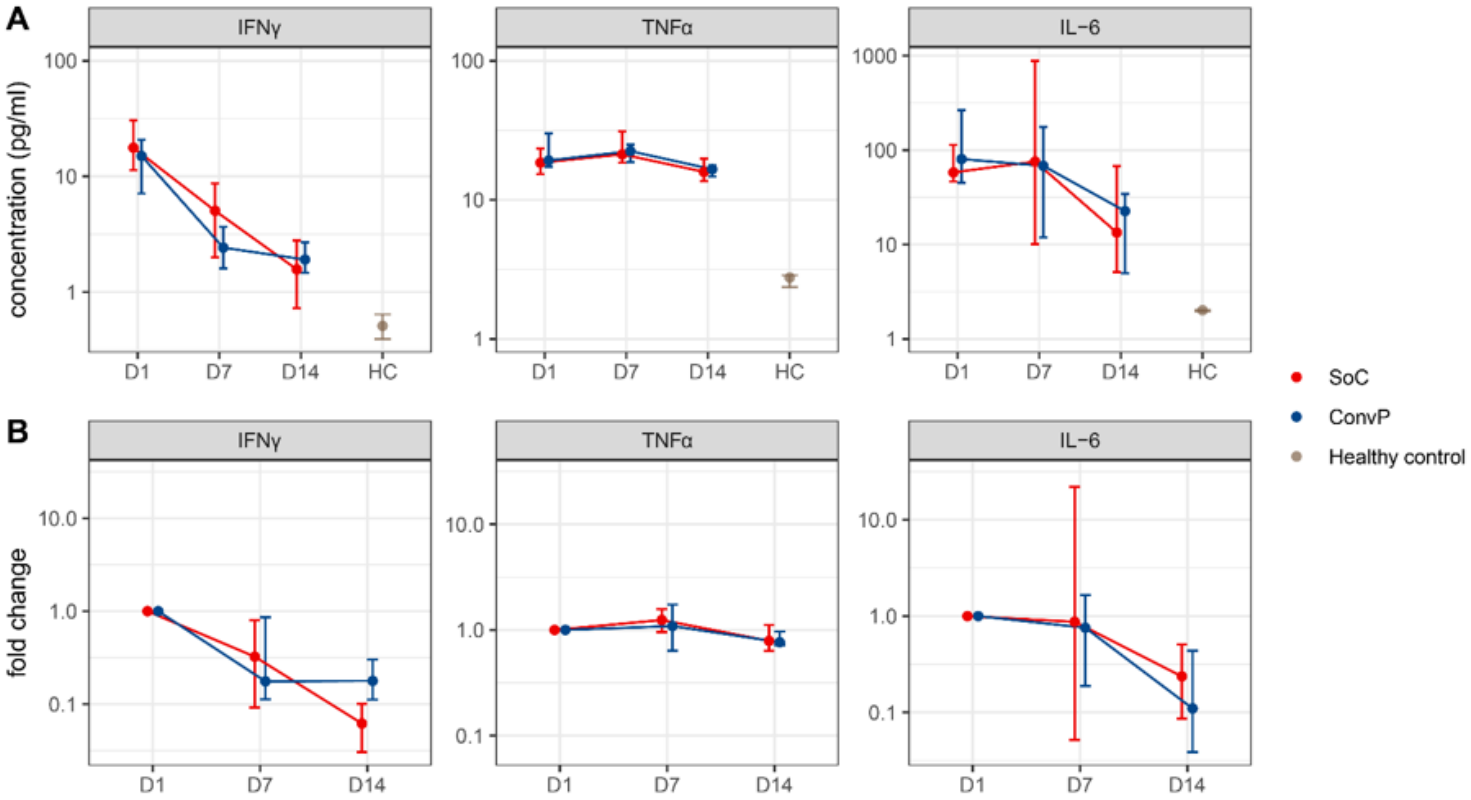


Figure 4

Cytokines IL-6, IFNγ and TNFα were measured in the serum of COVID-19 patients (SoC: red, ConvP: blue) at enrollment (day 1) and on day 7 and day 14 after enrollment and in the serum of healthy controls (brown). Shown are cytokine concentrations (A) and fold change compared to day 1 (B). Dots represent median and vertical lines represent IQR.



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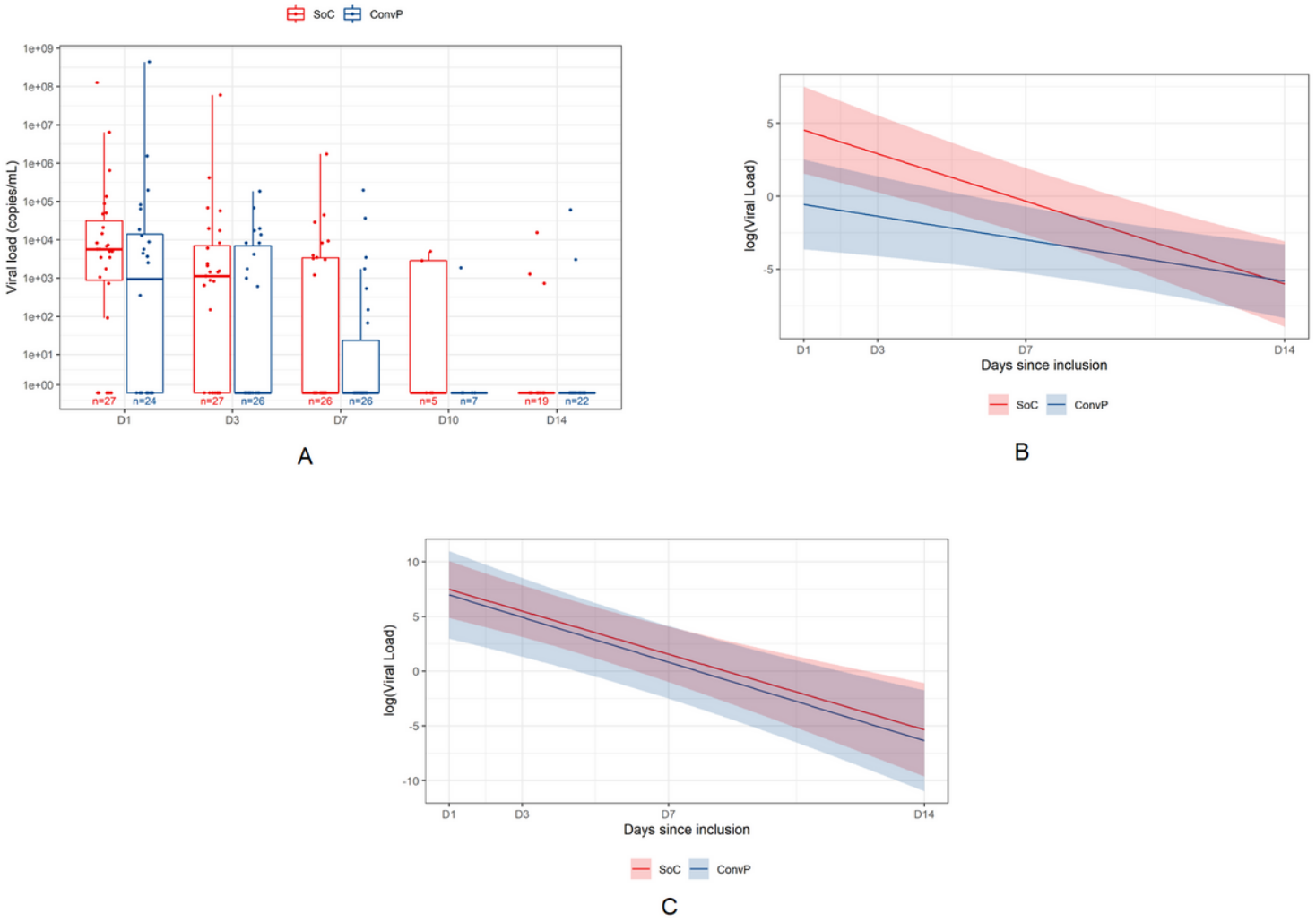
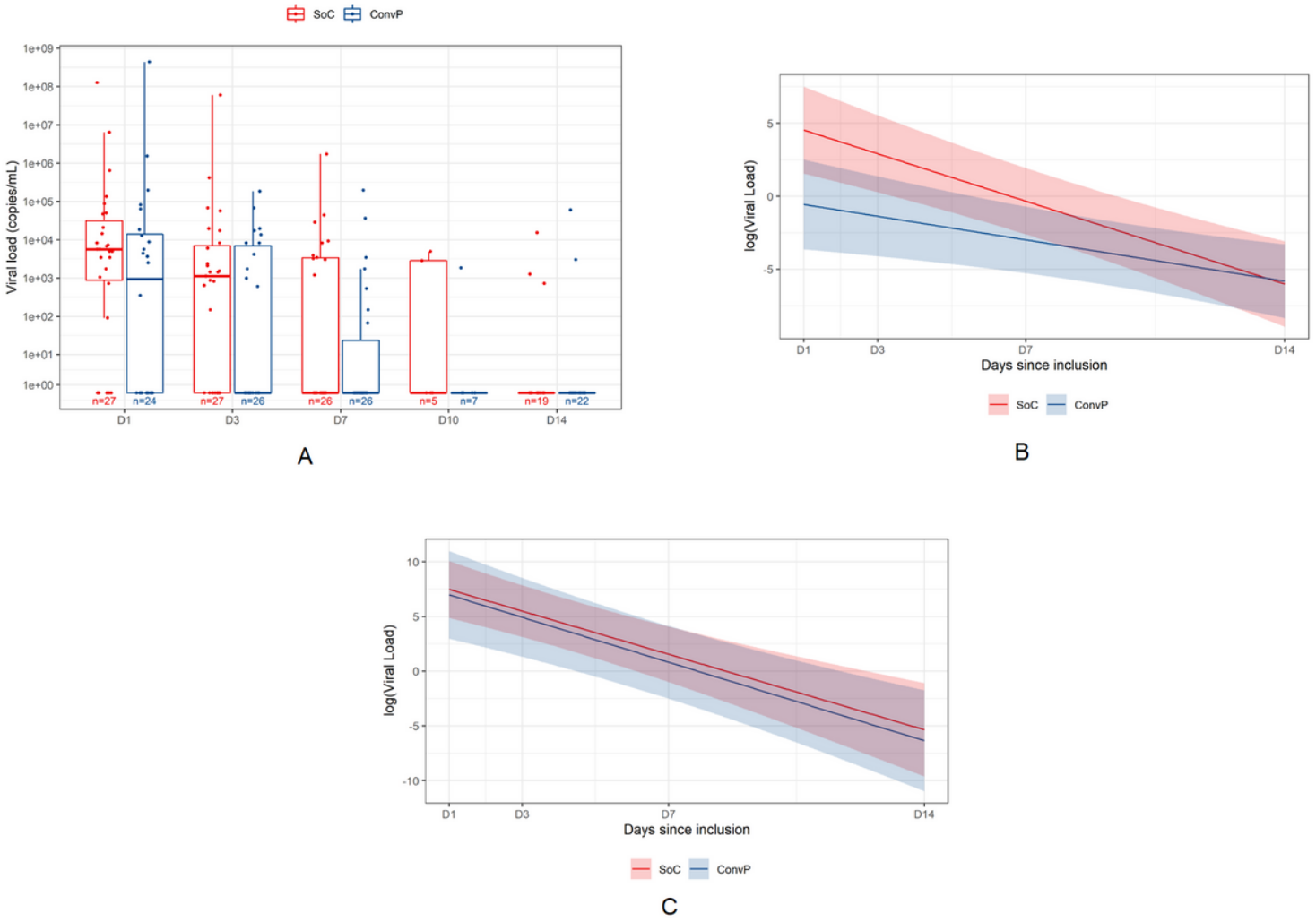


Figure 5

A. SARS-CoV-2 viral load (copies/mL) measured by RT-PCR from the nasopharyngeal swabs of COVID-19 patients (SoC: red, ConvP: blue) at enrolment (day 1) and day 3, day 7, day 10 and day 14 after enrollment. Box indicates the median and IQR. B. Predicted evolution (solid line) and 95% CI (shaded area) of SARS-CoV-2 log(Viral Load) in log(copies/ml) per day since enrollment (D=1) for COVID-19 patients (SoC: red, ConvP: blue) C. Predicted evolution (solid line) and 95% CI (shaded area) of absolute log(Viral Load) in log(copies/ml) per day since enrollment (D=1) for COVID-19 patients (SoC: red, ConvP: blue) excluding subjects who had SARS-CoV-2 viral load equal to zero at day 1 of enrollment.



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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SI.docx
- SI.docx
- C1.ProtocolCONCOVIDamendement3v3.1plain.pdf

- C1.ProtocolCONCOVIDamendement3v3.1plain.pdf