




# Characterization of pathogen-inactivated COVID-19 convalescent plasma and responses in transfused patients

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**Abbreviations and Conventions:** ACE-2 blocking nAb, angiotensin-converting enzyme 2 receptor inhibitor assay for neutralizing antibodies; ADAP S1 Ab, antibody-dependent agglutination PCR for Antibodies to S1 epitope of SARS-CoV2 spike protein; ADAP N Ab, antibody-dependent agglutination PCR for Antibody to nuclear epitope N of SARS-CoV2; Commercial ELISAs, enzyme-linked immunoassay; COVAM, coronavirus antigen microarray; dCCP, donated COVID-19 convalescent plasma; N IgG Ab ELISA, anti-Nucleocapsid IgG Antibodies Enzyme-linked immunoassay (Elecysys<sup>®</sup>; Roche Diagnostics International Ltd, Rotkreuz, Switzerland); IgG S1 Ab ELISA, anti-Spike 1 IgG Antibodies Enzyme-linked immunoassay (Euroimmun AG, Lübeck, Germany); nAb, neutralizing antibodies; PCA, principal component analysis; PRT, pathogen-reduced treatment; RVPN NT<sub>50</sub>, reporter virus particle neutralization fifty percent neutralization titers.

Maja Weisser and Nina Khanna contributed equally to this study.

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### Abstract

**Background:** Efficacy of donated COVID-19 convalescent plasma (dCCP) is uncertain and may depend on antibody titers, neutralizing capacity, timing of administration, and patient characteristics.

**Study Design and Methods:** In a single-center hypothesis-generating prospective case-control study with 1:2 matched dCCP recipients to controls according to disease severity at day 1, hospitalized adults with COVID-19 pneumonia received 2 × 200 ml pathogen-reduced treated dCCP from 2 different donors. We evaluated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in COVID-19 convalescent plasma donors and recipients using multiple antibody assays including a Coronavirus antigen microarray (COVAM), and binding and neutralizing antibody assays. Outcomes were dCCP characteristics, antibody responses, 28-day mortality, and dCCP-related adverse events in recipients.

**Results:** Eleven of 13 dCCPs (85%) contained neutralizing antibodies (nAb). PRT did not affect dCCP antibody activity. Fifteen CCP recipients and 30 controls (median age 64 and 65 years, respectively) were enrolled. dCCP recipients received 2 dCCPs from 2 different donors after a median of one hospital day and 11 days after symptom onset. One dCCP recipient (6.7%) and 6 controls (20%) died ( $p = 0.233$ ). We observed no dCCP-related adverse events. Transfusion of unselected dCCP led to heterogeneous SARS CoV-2 antibody responses. COVAM clustered dCCPs in 4 distinct groups and showed endogenous immune responses to SARS-CoV-2 antigens over 14–21 days post dCCP in all except 4 immunosuppressed recipients.

**Discussion:** PRT did not impact dCCP anti-virus neutralizing activity. Transfusion of unselected dCCP did not impact survival and had no adverse effects. Variable dCCP antibodies and post-transfusion antibody responses indicate the need for controlled trials using well-characterized dCCP with informative assays.

### KEYWORDS

COVID-19, COVID-19 convalescent plasma, neutralizing antibodies, pathogen-reduction treatment, SARS-CoV2

## 1 | INTRODUCTION

As of July 26, 2022, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections have been diagnosed in almost 572 million persons with over 6.3 million deaths worldwide.<sup>1</sup> Antiviral<sup>2–6</sup> and immunotherapeutic drugs such as dexamethasone,<sup>7</sup> tocilizumab,<sup>8–10</sup> and baricitinib<sup>11,12</sup> have demonstrated moderate clinical efficacy while early treatment with anti-spike neutralizing antibodies (nAb) has shown to prevent progression to severe disease.<sup>13–15</sup> Mutational variants have led to a global

surge in cases despite the initiation of effective vaccines.<sup>16</sup> Variant-based donated COVID-19 convalescent plasma (dCCP) may help in specific patient groups in whom nAb are less likely to be elicited.<sup>17</sup>

To date, almost 80 clinical studies reported on the use of dCCP in COVID-19.<sup>18–44</sup> Three trials—the RECOVERY trial, the Concor-1, and the SIREN-3CPO<sup>37,45,46</sup>—did not show a clinical benefit, while a recent trial on the early application of high-titer plasma in outpatients reduced hospitalization by more than 50%.<sup>38</sup> In two large meta-analyses mortality in dCCP recipients was reduced,<sup>47,48</sup>

while another showed no improvement.<sup>49</sup> All studies confirmed the safety of dCCP transfusion.<sup>47,50</sup> Mortality reduction has been shown to be associated with antibody titer.<sup>30,48,51,52</sup>

Most early studies measured total IgG antibody against SARS-CoV-2 spike (S) protein without assessing viral neutralization efficacy.<sup>53</sup> In general, total anti-S antibody correlated poorly with neutralization activity.<sup>54</sup> Mostly, patients received a single unit of dCCP (200–250 ml) with the assumption that dCCP from recovered patients contain sufficient levels of nAb. The results of these studies have continued to create equipoise about the therapeutic efficacy of dCCP for COVID-19.

In this hypothesis-generating study, initiated early in the first epidemic wave in Switzerland, we extensively characterized dCCP for antibody profile and neutralization efficacy using multiple complementary assays. We treated dCCP with amotosalen-UVA for pathogen reduction, a method that demonstrated efficacious inactivation of SARS-CoV-1 and of SARS-CoV-2 in preliminary studies.<sup>55,56</sup> The hypotheses proposed in the present study were that pathogen reduction treatment (PRT) does not affect antibody activity that characterizing antibodies in dCCP would facilitate the selection of dCCP with high neutralization capacity, and that neutralization activity would translate into therapeutic efficacy.

## 2 | METHODS

### 2.1 | Ethics and regulatory oversight

This study was conducted at the University Hospital Basel and the Regional Blood Transfusion Service, Swiss Red Cross, Basel, Switzerland from March to June 2020. The study was approved by the ethics committee of Northwestern and Central Switzerland (Req-2020-00508 and EKNZ-2020-00769) and registered at [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT04389944). Informed consent was obtained from COVID-19 convalescent plasma (CCP) donors and recipients or their surrogate decision maker if incapacitated due to critical illness.

### 2.2 | Study design

This is an exploratory single-center study including 15 cases and 30 matched controls. Cases were prospectively included from the University Hospital Basel. Controls were selected from the hospital data system among patients hospitalized with COVID-19 during the same period. The 2:1 matching for disease burden was done using a standardized clinical risk score<sup>57</sup> on the day of hospitalization and concomitant use of tocilizumab.

### 2.3 | Endpoints

The primary endpoints were the characterization of dCCP and antibody responses in dCCP recipients. Secondary endpoints were the safety and efficacy of dCCP transfusions as well as changes in biomarkers in dCCP recipients.

### 2.4 | Eligibility and selection of dCCP donors

CCP donors were males aged 18–60 years with a nasopharyngeal swab positive for SARS-CoV-2 by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR),<sup>58,59</sup> who had not been hospitalized. According to Swiss regulations, donors were eligible for plasma collection if asymptomatic for at least 14 days after the first positive swab. Donors presenting 14–28 days after symptom resolution were tested twice by nasopharyngeal swabs to confirm negativity; those symptom-free >28 days after COVID-19 diagnosis were not tested.

### 2.5 | Plasmapheresis and dCCP products

From each donor, 650 ml plasma was collected by apheresis using an Aurora device (Fresenius Kabi, Frankfurt, Germany) according to national regulations.<sup>60</sup> dCCP manufacturing was performed under good manufacturing practice conditions complying with current regulations<sup>60</sup> and standard procedures. The collected plasma underwent PRT, consisting of the addition of amotosalen followed by UVA illumination (INTERCEPT Blood System for Plasma, Cerus BV, Amersfoort, Netherlands), then distributed in 200 ml bags, frozen within 18 h after collection, and stored at  $-30^{\circ}\text{C}$ . Upon request, single dCCP units were thawed in a temperature-controlled water bath at  $37^{\circ}\text{C}$  for 18–20 min. The median time from dCCP collection to transfusion was 15.67 days (IQR 5–31).

### 2.6 | Procedures for dCCP recipients

Patients hospitalized with qRT-PCR confirmed COVID-19 were eligible for dCCP transfusion if aged  $\geq 18$  years, with respiratory symptoms, typical COVID-19 infiltrates on chest CT scan, and oxygen saturation on room air of  $< 92\%$ . Patients with IgA deficiency, previous severe allergic reactions to blood products, and pregnancy were excluded. Patients received two ABO-compatible dCCP units of 200 ml each from a different donor  $\geq 12$  h apart. Additional medications were continued independent of

dCCP administration. Standard treatment for COVID-19 consisted of hydroxychloroquine and lopinavir/ritonavir for those without a contraindication to these medications. Additionally, tocilizumab was applied in patients with hyperinflammation and remdesivir in those with oxygen desaturation.<sup>61</sup>

## 2.7 | Clinical evaluation

Laboratory analyses from blood samples taken on day 0 before and day 1, 3, 7, 14, and 28 after dCCP transfusion consisted of a full blood picture, creatinine, liver enzymes, and C-reactive protein. Data on the clinical course and adverse events of dCCP transfusion were obtained from the electronic medical record system including hemovigilance data. If discharged before day 28, patients were followed-up in the outpatient clinic.

## 2.8 | Antibody testing

In CCP donors, serum/plasma for SARS-CoV-2 antibodies was obtained on the days of donor screening and CCP donation. Additionally, dCCP samples were tested for antibodies before and after PRT. In dCCP recipients, SARS-CoV-2 antibodies were measured on day 0 before and on days 1, 3, 7, 14, and 28 after dCCP transfusion. The results of antibody tests were not available at the time of transfusion and during patient follow-up.

## 2.9 | Antibody assays

### 2.9.1 | Total immunoglobulin assays for anti-Nucleocapsid (N) activity and anti-Spike (S1) in donor and patient plasma

Anti-Nucleocapsid IgG antibodies (N IgG Ab) to SARS-CoV-2 were determined with Elecsys<sup>®</sup> Anti-SARS-CoV-2 N electrochemiluminescence immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) according to the manufacturer's instructions using the Cobas<sup>®</sup> e801 analyzer.<sup>62</sup> Results are expressed as absolute cutoff indices: a cutoff index <0.7 indicates non-reactivity/negativity and an index >1.0 reactivity/positivity. Additionally, N IgG Ab was determined by a second electrochemiluminescence immunoassay (Abbott, Abbott Ireland Diagnostics Division, Finisklin Business Park Sligo Ireland). Anti-Spike 1 IgG antibodies (S1 IgG Ab) were assessed using an ELISA assay (Euroimmun AG, Lübeck, Germany).

### 2.9.2 | Antibody-dependent agglutination PCR (ADAP) assay

Total S1 and N protein antibodies (IgG, IgM, and IgA) from dCCP and recipient plasma samples were assayed using PCR-based methods reported previously (Appendix S1)<sup>63,64</sup> including the Antibody-dependent agglutination PCR for S antibodies to S1 epitope (ADAP S1 Ab) and to nuclear epitope N of SARS-CoV2 (ADAP N Ab). For nAb, a soluble angiotensin-converting enzyme 2 receptors (ACE-2) inhibitor assay (Enable Biosciences, South San Francisco; ACE-2 blocking nAb) was used.<sup>64-66</sup> Based on plasma samples from healthy control donors prior to COVID-19, the negative cutoff ( $\Delta C_t$ ) values for S1, N, and ACE inhibitors were respectively: 1.5, 4.0, and 0.4. These assays have been used in a larger dCCP data set recently reported.<sup>67</sup>

### 2.9.3 | Coronavirus antigen microarray antibody profile

The coronavirus antigen microarray (COVAM) analyzed antibody reactivity in dCCP and recipients of dCCP to 61 antigens of respiratory viruses. In brief, the COVAM included 11 SARS-CoV-2 antigens, 5 SARS-CoV, 5 MERS-CoV, 12 common cold coronaviruses, 12 influenzas, 4 adenoviruses, 3 metapneumoviruses, 4 parainfluenzas, and 4 respiratory syncytial virus.<sup>68</sup> The antigens were printed onto nitrocellulose microarrays, probed with dCCP or recipient plasma diluted 1:100, and analyzed as previously described.<sup>69-71</sup>

### 2.9.4 | Reporter virus particle neutralization (RVPN) assay

dCCP and recipient samples were analyzed for neutralization activity with a SARS-CoV-2 Reporter Virus Particle Neutralization (RVPN NT<sub>50</sub>) assay<sup>53</sup> determining fifty percent neutralization titers by calculating the percent of no serum control and plotting non-linear regression curves (GraphPad Prism version 8.4, GraphPad Software, San Diego, CA; Appendix S1). dCCP samples were considered to lack sufficient nAb if the titer was <1:40.

## 2.10 | Statistical analysis

For analyses of the case-control study, baseline characteristics were compared by Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Conditional logistic regression was used to calculate

odds ratios for associations between dCCP therapy and different binary outcomes. Continuous outcomes were compared by applying the Mann–Whitney U test, as these were non-normally distributed. All analyses were performed using STATA version 15.0 (Stata Corp., College Station, Texas, USA). *p*-values of less than or equal to 0.05 were considered significant.

We compared antibody results using the Wilcoxon matched-pairs signed rank test, best-fit curves, and R2 (correlations) were assessed based on linear trendlines unless specified otherwise. Error bars represent SEM. Statistical analysis was performed in Microsoft Office 365 Excel (Microsoft, Seattle, WA) or GraphPadPrizm 7 (GraphPad Software, San Diego, CA).

### 3 | RESULTS

#### 3.1 | CCP donors, antibody properties of dCCP, and effects of PRT

Overall, 55 donors provided dCCP at a median of 32 days (IQR 15–39) after a SARS-CoV2-positive nasopharyngeal swab and 29 days (IQR 28–35) after symptom resolution. CCP products from 13 donors containing 650 ml each were used for transfusion to patients.

N IgG Ab and S1 IgG Ab levels in dCCP measured with commercial assays after PRT showed marked donor variation (Table S1), but were not impacted by PRT (Table S1, Figure 1). Two of the 13 dCCP products used for transfusion (dCCP 2640 and 2827) had low to undetectable nAb by RVPN NT<sub>50</sub> and ACE-2 blocking nAb (Table S1B, Figure 1). Nineteen dCCP products (including the 13 used for transfusion) were analyzed by COVAM to define antibody profiles. Principal Component Analysis (PCA) revealed four distinct clusters (Figure 2) regarding the recognition of 11 SARS-CoV-2 antigens: Cluster 1 dCCP units were non-reactive to most SARS-CoV-2 antigens; cluster 2 had broad reactivity to most SARS-CoV-2 antigens; cluster 3 had an intermediate activity with proportionally higher reactivity to the N antigen, and cluster 4 had an intermediate activity with higher reactivity to S1 antigens. Importantly, PRT did not alter the antibody reactivity COVAM profiles.

A positive correlation ( $r^2 = 0.66$ ) was observed between ADAP S1 Ab and ACE-2 blocking nAb (Figure 3A) and between the ACE-2 blocking nAb and RVPN NT<sub>50</sub> ( $r^2 = 0.75$ , Figure 3B). The correlation of S1 IgG Ab by Euroimmun assay and ADAP S1 Ab (Figure 3C) was moderate ( $r^2 = 0.46$ ) and higher ( $r^2 = 0.68$ ) with the ACE-2 blocking nAb (Figure 3D). For the 13 dCCP units transfused to the patients classified by COVAM PCA, we observed no functional

neutralizing activity in cluster 1 ( $n = 2$ ) by either the ACE-2 blocking nAb or the RVPN NT<sub>50</sub>. Cluster 1 dCCP demonstrated some anti-S1 reactivity. In contrast, dCCP neutralizing activity for SARS CoV-2 was observed in COVAM cluster 2 ( $n = 3$ ), cluster 3 ( $n = 4$ ), and cluster 4 ( $n = 4$ ) (Figure 4).

#### 3.2 | Recipients of dCCP transfusions

Of the 15 dCCP recipients (Table 1), one patient was treated at another hospital without serial plasma sampling available. Twelve of 15 dCCP recipients (80%) were male, aged 64 years (median) with a median BMI of 26.2 (IQR 24.2–29.3). Four recipients (27%) were smokers and nine (60%) had arterial hypertension. Overall, 4 of 15 recipients were on an immunosuppressive treatment: 2 of 5 recipients with a hematological malignancy had a B-cell depleting therapy, one received rituximab for vasculitis, and another fingolimod for multiple sclerosis.

Presenting symptoms were fever (93%), cough (87%), and dyspnea (53%) with a median of 8 days (IQR 3–11) from symptom onset to diagnosis (Table 1). In 8 recipients (53%) oxygen saturation was <92% on room air. All patients had typical radiological patterns on chest computer tomography. Five (30%) recipients were admitted to intensive care for mechanical ventilation directly upon admission - one in cardiogenic shock. Eleven dCCP recipients (73%) were classified as high-risk and 4 medium-risks using a standardized COVID-19 clinical severity score.<sup>57</sup>

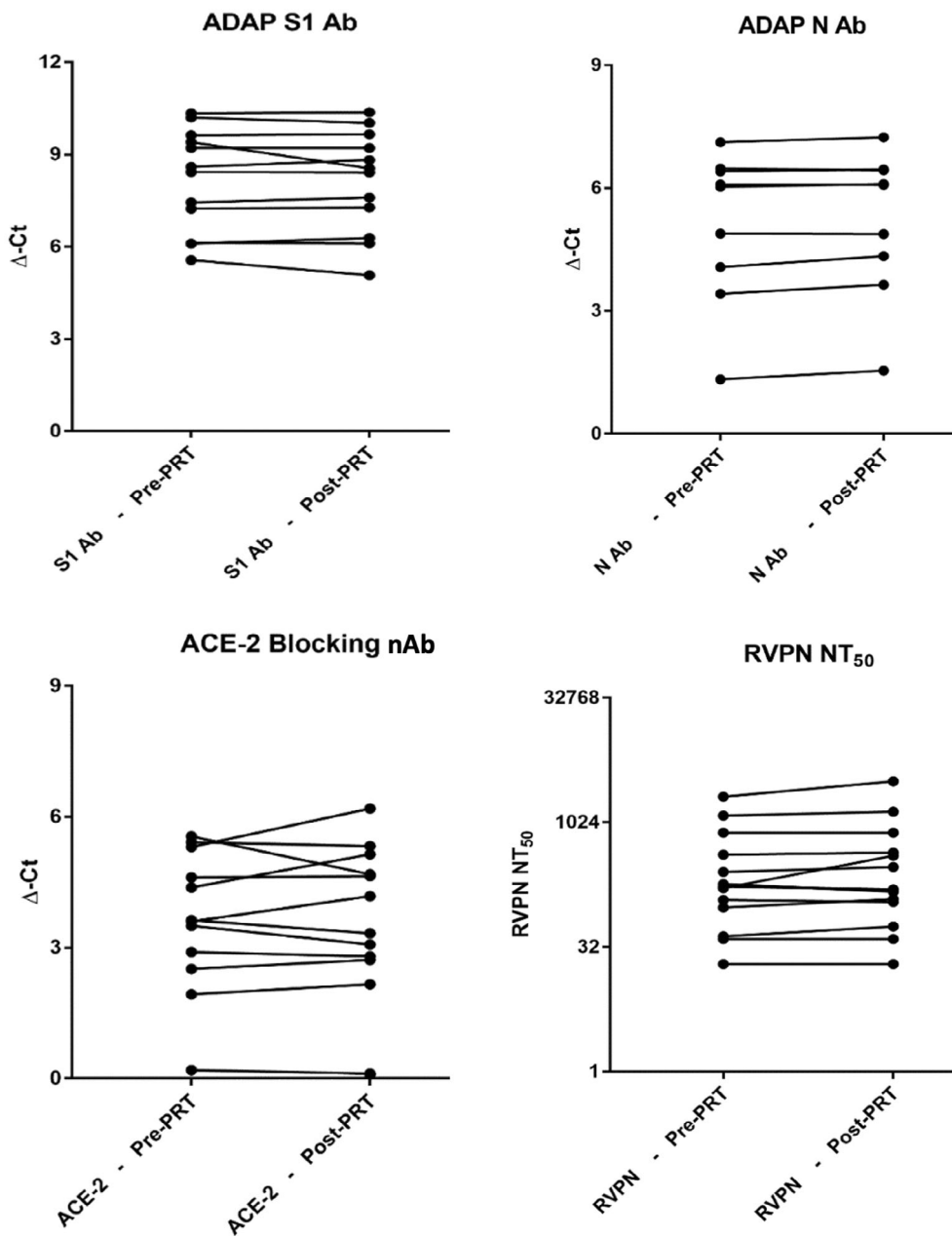
#### 3.3 | Controls

Baseline characteristics of the 30 controls were comparable to those of dCCP recipients (Tables 1 and S2)—twenty-two (73.3%) were male, mean age of 65 years (IQR 53–73), and median BMI was 27.8 (IQR 24.5–30.0). CT lung scan abnormalities, baseline oxygen saturation, and oxygen requirements were similar in dCCP recipients and controls (Table S2).

#### 3.4 | Transfusion and safety of dCCP

dCCP was transfused after a median of 1 day after hospitalization and 11 days after the onset of symptoms. Recipient plasma volumes estimated by a gender-specific, height-weight-based formula ranged from 2.25 to 4.24 L (median 3.32).<sup>72</sup>

Each patient received at least one dCCP unit with effective nAb by COVAM PCA assignment (Table S3). The patient in cardiogenic shock received 5 units of dCCP, all from COVAM PCA Cluster 2 and 3 upon the



**FIGURE 1** dCCP antibodies to SARS-CoV-2 S1 and N antigens, nAbs by ADAP, ACE-2 blocking nAb and RVPVN NT<sub>50</sub> assays before and after PRT. The upper 2 figures show Anti-S and anti-N activity in dCCP before and after PRT expressed as  $\Delta$ Ct by ADAP assay (ADAP S1 Ab and ADP N Ab). The lower 2 figures show dCCP virus neutralization before and after PRT by ACE-2 blocking Ab assay expressed as  $\Delta$ Ct PCR assay and RVPN NT<sub>50</sub> before and after PRT.

decision of the treating physician in the absence of other treatment options. No adverse effects of plasma transfusion were observed.

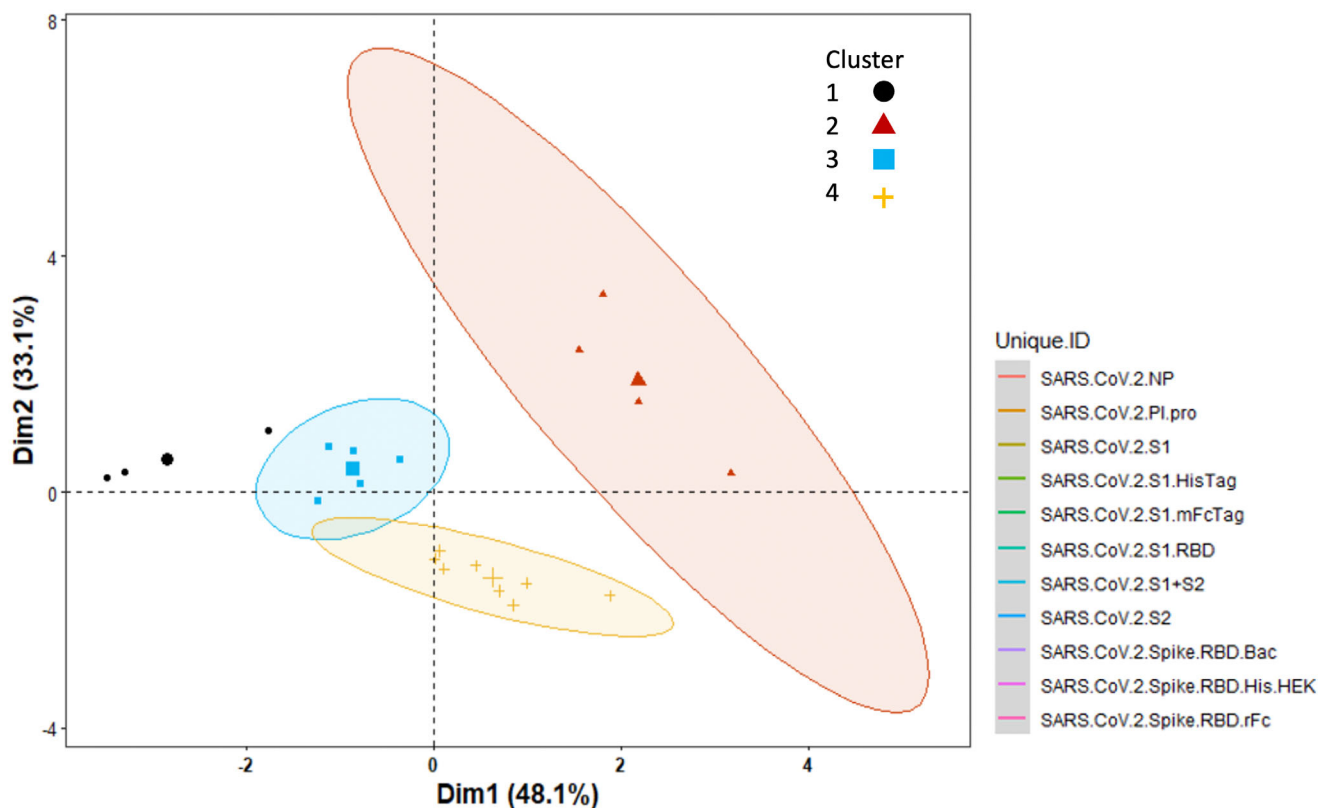
### 3.5 | Clinical outcomes

One of 15 dCCP recipients died during hospitalization (6.7%) compared to 6 (20.7%) of 30 controls (odds ratio (OR) 0.25; 95% confidence interval (CI) 0.03–2.44;  $p = 0.233$ ; Table 2). This patient (patient 7) died on day 8 after hospitalization in ICU with cardiogenic shock, multiorgan failure, and disseminated intravascular coagulopathy. He received in total 5 dCCP since no other treatment

options were available due to multiorgan failure. ICU admission and progression to intubation as well as days in hospital did not differ between the two patient groups. There was a trend ( $p 0.053$ ) toward a faster C-reactive protein normalization in the dCCP recipients compared to controls (Table 2). The low number of transfused patients and the variable clinical presentation does not allow a correlation of the quality of dCCP with clinical outcomes.

### 3.6 | Serial antibodies in recipients

Analysis of S1 IgG Ab and N IgG Ab by commercial assays showed detectable antibodies at baseline in 2 of



**FIGURE 2** Donor CCP profiles after PRT by COVAM PCA using 11 SARS-co-V-2 antigens. Principal component analysis (PCA) of plasma reactivity with 11 SARS-CoV-2 antigens (Unique ID), determined by COVAM shows the spatial distribution of the CCP along the first and second principal components and revealed four clusters: non-reactive (cluster 1 - black); broad reactivity to all antigens (cluster 2 - red); intermediate reactivity primarily S reactive with lower reactivity to N (cluster 3 - blue); and intermediate reactivity primarily to N with lower reactivity to the other antigens (cluster 4 - yellow). The large symbols within each cluster represent the mean values. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

15 recipients for S1 and 4 to N (Figure 5). On day 1, post-transfusion antibodies measured by ACE-2 blocking nAb increased in 8 recipients and by RVPN NT<sub>50</sub> in 6 (Table S4, Figure 6). Antibody increases by ADAP S1 Ab were detected in 8 patients and by S1 IgG Ab (Euroimmun assay) in 7 (Figures 5 and 6, Table S4). The 2 patients with detectable ADAP S1 Ab at baseline exhibited similar levels on day one after transfusion (Figure 6, Table S4).

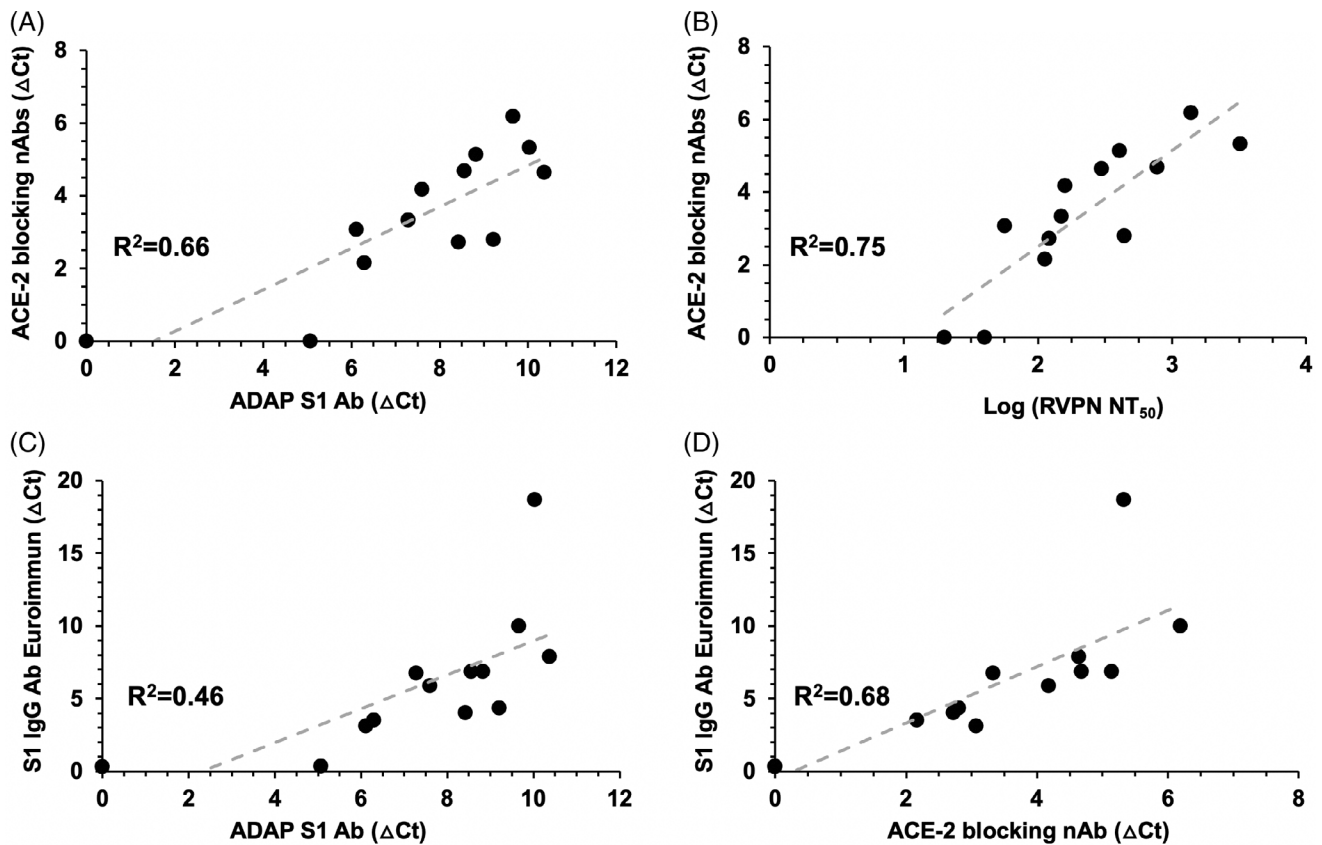
The 3 patients (patients 6, 9, and 14) on B-cell depleting therapies and the patient on fingolimod (patient 13) showed no S1 IgG Ab or N IgG Ab at baseline and post-transfusion. All four patients survived until day 28 (Figure 5, Table 2).

Immune profiles by serial COVAM IgG for 11 different SARS-CoV2 antigens pre-transfusion (day 0 or day -1) and on days 1, 3, 7, and 14 after dCCP transfusion (Figure S1 and Figure S2) exhibited increases in antibodies contained in COVAM profiles after dCCP transfusion and during the post-transfusion clinical course, however, these were not consistent in all patients. IgM

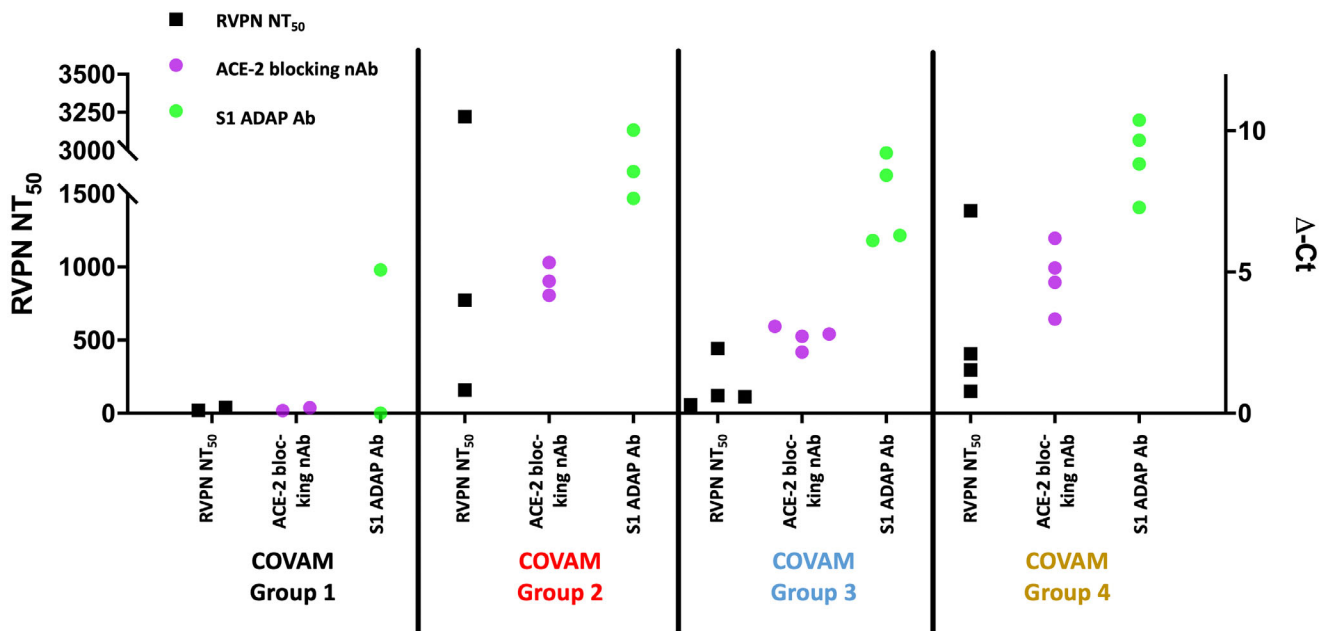
antibody profiles were not informative due to very low and sporadic levels. Five patients (patients 3, 4, 8, 10, and 12) already had detectable IgG to SARS-CoV-2 antigens at baseline (days 0 or -1) (Figure S2) by COVAM. Patient 1 (Figure S1) serves as an example of increasing endogenous antibody responses over time. Notably, dCCP transfusion did not impair later endogenous antibody responses which generally increased after post-transfusion day 7. The 4 immunosuppressed patients<sup>6,9,13,14</sup> demonstrated muted endogenous antibody responses to COVAM antigens indicative of impaired immune recovery.

## 4 | DISCUSSION

In this pilot hypothesis-generating case-control study, we used different assays to assess antibody profiles and neutralizing activity in unselected dCCP from recovered donors after mild disease during the first COVID-19 pandemic wave in Switzerland. While limited conclusions about the clinical outcomes can be made from our study



**FIGURE 3** Correlation of the different antibody assays used to assess PRT dCCP. Correlation of S1 ADAP Ab with neutralizing activity (ACE-2 blocking nAbs) expressed as  $\Delta$ Ct PCR cycle time (A). Correlation of RVPN NT<sub>50</sub> titer expressed as  $\log_{10}$  with neutralizing activity (ACE-2 blocking nAbs) expressed as  $\Delta$ Ct PCR cycle time (B). Correlation of S1 IgG Ab measured by Euroimmun with S1 ADAP Ab expressed as  $\Delta$ Ct PCR cycle time (C). Correlation of S1 IgG Ab measured by Euroimmun with ACE-2 blocking nAbs expressed as  $\Delta$ Ct PCR cycle time (D). Respective  $R^2$  values are indicated for each analysis.



**FIGURE 4** Correlation of COVAM PCA with S1 ADAP ab, ACE-2 blocking nAb, and RVPN NT<sub>50</sub> for 13 CCP transfused to recipients. dCCP ADAP anti-S, and nAb by RVPN assay and ADAP ACE-2 inhibition assay according to COVAM PCA Group. Thirteen dCCP were used for transfusion of recipients with acute COVID-19 infection. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



TABLE 1 Baseline demographics of dCCP recipients and controls

	dCCP recipients (n = 15)	Controls (n = 30)	p-value
Sex (male; %)	12 (80.0%)	22 (73.3%)	0.736
Age, years (IQR)	64 (52–73)	65 (53–73)	0.673
Body mass index, kg/m <sup>2</sup>	26.2 (24.2–29.3)	27.8 (24.5–30.0) <sup>a</sup>	0.625
Currently smoking (%)	4 (26.7%)	10 (33.3%) <sup>b</sup>	0.117
Days (IQR)			
• From symptoms to diagnosis	8 (3–11)	7 (5–10)	0.847
• From symptoms to plasma therapy	11 (8–17)	--	
Comorbidities (%)			
• Number of comorbidities	1 (1–3)	1 (0–2)	0.333
• Arterial hypertension	9 (60.0%)	14 (46.7%)	
• Cardiovascular disease	6 (40.0%)	8 (26.7%)	
• Cerebrovascular disease	0 (0.0%)	2 (6.7%)	
• Chronic obstructive lung disease	0 (0.0%)	3 (10.0%)	
• Chronic renal impairment	3 (20.0%)	5 (16.7%)	
• Diabetes mellitus	3 (20.0%)	6 (20.0%)	
• Cancer	5 (33.3%) <sup>c</sup>	4 (13.4%)	
• Autoimmune disorder	5 (33.3%)	0 (0.0%)	
• HIV-infection	0 (0.0%)	2 (6.7%)	

Abbreviations: dCCP, donated COVID-19 convalescent plasma; IQR, interquartile range.

<sup>a</sup>Missing values in 5 patients.

<sup>b</sup>Unknown smoking status in 6 patients.

<sup>c</sup>Not recorded for 1 patient.

TABLE 2 Clinical outcomes of dCCP recipients and controls

	dCCP recipients (n = 15)	Controls (n = 30)	Odds ratio	95%CI	p-value
Mortality, n (%)	1 (6.7%)	6 (20.7%)	0.25	0.03–2.44	0.233
Duration of hospitalization <sup>a</sup> , days (IQR)	13 (7–18)	12 (8–18)	-	-	0.830
Duration of O <sub>2</sub> supply	9 (4–15)	6 (1–10)	-	-	0.208 <sup>b</sup>
Duration of SARS CoV-2 shedding	15 (10–18)	10 (7–14)	-	-	0.179
Duration of intubation <sup>a</sup> , days (IQR)	21 (8–28)	14 (8–28)	-	-	0.833
Duration of intensive care unit <sup>a</sup> stay, days (IQR)	30 (10–41)	9 (3–25)	-	-	0.124
Lymphocyte count normalization <sup>c</sup>	13 (86.7%)	22 (73.3%)	2.14	0.43–10.71	0.356
C-reactive protein normalization <sup>c</sup>	13 (86.7%)	17 (56.7%)			0.053
Ferritin normalization <sup>c</sup>	8 (53.3%)	8 (26.7%)	2.15	0.50–9.16	0.301

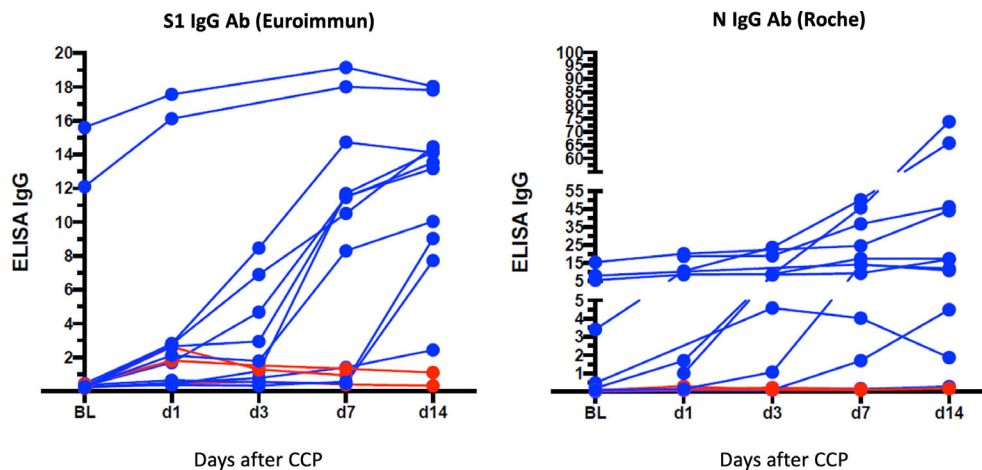
<sup>a</sup>Only for survivors.

<sup>b</sup>0.340 after exclusion of non-survivors.

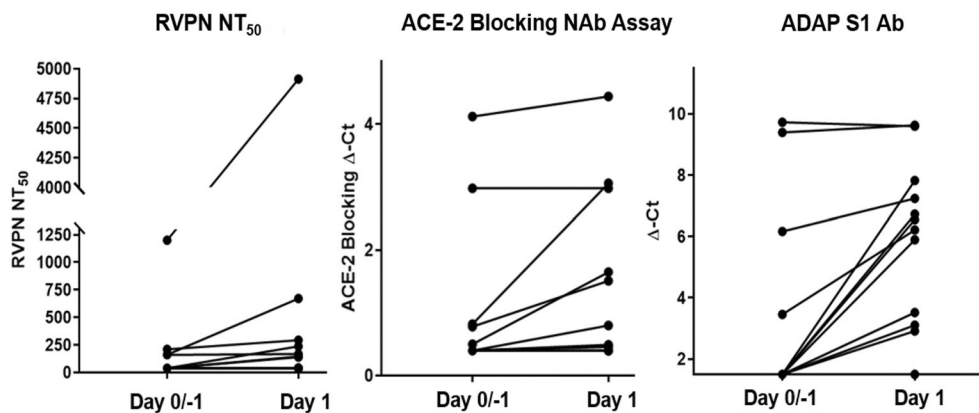
<sup>c</sup>Normalization was assessed on day 28 and was defined as follows: lymphocytes >1 G/L, C-reactive protein <10 mg/L and ferritin <300 µg/l.

due to the small number of patients, we have four important observations: (i) pathogen reduction did not affect the neutralizing capacity of dCCP, (ii) using five different antibody assays, the best correlation was established with neutralizing activity for anti-S1 measured by the RVPN and ADAP assays, (iii) COVAM profiles of serial recipient plasma samples after dCCP showed

retention of endogenous immune responses, with the exception of the four immunosuppressed patients, and finally, no adverse events or antibody-dependent disease enhancement after intravenous transfusion of dCCP were observed in recipients. The major difference from other studies was the use of dCCP products from 2 different donors to broaden the immunologic repertoire of dCCP.



**FIGURE 5** dCCP recipient baseline and serial plasma antibodies to IgG S and N proteins by commercial assay. Recipient IgG antibody to S and N were measured in recipient plasma samples at baseline (BL) and after CCP transfusion on days (D) 1, 3, 7, and 14. S1 IgG Ab was measured using Euroimmun ELSA and N IgG Ab by Roche ELISA assay. Immune suppressed recipients are noted in red. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 6** dCCP recipient antibodies to SARS-CoV-2 at baseline and one day after transfusion. NAb was measured in recipients before dCCP transfusion (Day 0 or -1) and the day after dCCP transfusion (Day 1). Antibody neutralizing activity was determined by RVPN NT<sub>50</sub> and ACE-2 blocking nAb assay and total S1 ADAP Ab expressed as  $\Delta$ Ct.

Based on previous experience with the SARS-CoV-1 epidemic showing a benefit of early administration of dCCP in shortening hospitalization,<sup>73</sup> we initiated this study in the early days of the pandemic by transfusing dCCP without knowledge of antibody content and specificity. In contrast to other studies,<sup>25</sup> we aimed to increase the diversity of antibody composition of dCCP by using two different donors for each patient, which was achieved in all but two dCCP products without detectable nAbs (COVAM cluster 1). The five applied antibody assays demonstrated some level of correlation and provided complementary information in characterizing dCCP. The highest correlation of total IgG antibody with neutralizing capacity was found for RVPN NT<sub>50</sub> and ACE-2 blocking nAbs. Correlation between responses of different antibody assays to SARS CoV-2 in other studies has been conflicting in the early stages<sup>54</sup> indicating the complexity of the immune response and different assays. In early 2021, the FDA released a document containing the definition of high-

titer dCCP using different antibody assays,<sup>74</sup> which was updated in December 2021.<sup>75</sup>

Efficacy of PRT by amotosalen-UVA in inactivating Coronaviruses including SARS-CoV-2 has been reported previously.<sup>55,56</sup> In our study, PRT did not affect levels or specificities of binding measured by ACE-2 competition assays or neutralization activity of dCCP. All five antibody assays indicated no significant impact by PRT consistent with recent observations in a larger data set of dCCP.<sup>67</sup> Specifically, reactivity against the S protein using the ADAP S1 Ab assay and virus neutralization efficacy using two different assays (RVPN NT<sub>50</sub> and ACE-2 blocking nAb) were unaffected by PRT.

All but two dCCP recipients had undetectable SARS-CoV-2 antibodies and little or no neutralizing activity before dCCP transfusion—emphasizing the importance of transfusing CCP early after infection.<sup>30</sup> On day 1 after transfusion, we observed variable recipient responses of ADAP S1 Ab and ACE-2 blocking nAb, and fewer

responses using the RVPN NT<sub>50</sub> assay. The inability to detect post-transfusion antibody levels might partly be explained by the relatively small dCCP volume transfused. While 2 × 200 ml is comparable to what other studies used, it corresponds on average to only about 12% of the recipient plasma volume.<sup>30,51</sup> We note that in the study of Hueso et al, they transfused 4 PRT dCCPs per patient.<sup>17</sup> Of the 8 patients, who received high reactive dCCP (COVAM cluster 2), two had endogenous ACE-2 inhibitor levels at baseline, five displayed ACE-2 inhibitor-increased levels after receiving dCCP, and one with low baseline levels had no detectable antibody. These data indicate that the assays used were enough sensitive to detect dCCP nAb post-transfusion in some patients.

Post-transfusion COVAM analysis showed that, except for the 4 immunosuppressed recipients, dCCP recipients demonstrated endogenous immune responses to SARS-CoV-2 antigens over 14–21 days post dCCP suggesting that endogenous immune responses were not suppressed by dCCP exposure. No patients treated with dCCP demonstrated antibody-dependent enhancement of disease, a concern for dCCP use in the early days of the pandemic.<sup>76,77</sup>

Immunosuppression—especially B-cell depletion—poses a special risk for patients with COVID-19.<sup>17,78</sup> The four immunosuppressed dCCP recipients—three treated with anti-CD20 antibodies and one with immune modulation with fingolimod—had very low immune responses. Whether the small nAb increase in two patients conferred a protective effect remains unclear. In B-cell-depleted patients, the application of dCCP has shown promising results.<sup>17,79</sup> All four of our immunosuppressed patients had a favorable initial response.

While 28-day mortality was lower in the dCCP cohort (6.7% vs. 20.0%), it did not reach significance. There was a trend toward normalization of inflammation indicated by decreasing CRP levels. Data on the mortality benefit of dCCP remains conflicted in the literature.<sup>25,38,46–49</sup> A clear reduction in progression to severe disease was demonstrated in elderly patients with early application of high-titer dCCP.<sup>30,38</sup> In our study, dCCP recipients had a median of 11 days of symptoms but mostly did not have antibodies or neutralizing activity before transfusion of CCP. Differences in impact on patient outcome in different studies might partly be due to unknown antibody content in plasma in the early studies, small doses of dCCP, as well as late application.<sup>25,39,80</sup>

Limitations of our study are the small number of dCCP recipients and the lack of randomization. Concomitant drugs might have affected the outcome despite matching for disease severity. The lack of a randomized control group did not allow a definitive assessment of

clinical effectiveness. In addition, CCP was administered to some patients at an advanced stage of the disease, albeit early in hospitalization. It appears that the administration of CCP has the greatest clinical effectiveness in the early, highly viremic early phase of infection.<sup>22,48</sup> However, within our hospitalized patient population we did administer dCCP as early as one day post hospitalization.

The limitations of our study must be considered in the light of the necessity to treat COVID-19 patients in an emergency when no other valid treatment options existed and experience with SARS-CoV-1 suggested a benefit of dCCP. With the evolution of SARS-CoV-2 variants, three of the assays used (ADAP S1 and N Ab, ACE-2 blocking nAb, N and S1 IgG Ab, and COVAM) are feasible for rapid selection of effective dCCP from an inventory. Additionally, these assays can be modified to evaluate cross-reactive dCCP in inventory and to characterize newly collected dCCP for reactivity to the antigenic variants. This flexible strategy for identifying dCCP with high antibody titers and activity can improve the therapeutic efficacy of dCCP for improved intervention as the epidemic evolves. Additionally, vaccine-boosted dCCP with hybrid/uber-antibodies might be promising to increase the neutralization efficacy of CCP.<sup>81–85</sup> Applying the novel characterizations of dCCP in the setting of early disease treatment, for example, in outpatient settings as shown by Sullivan et al<sup>38</sup> might optimize the effect of CCP transfusion. Resource-limited settings with reduced access to expensive monoclonals and antiviral agents could also profit from such a flexible way of identification of high titer dCCP products.

In conclusion, this hypothesis-generating study shows the variable immunologic composition of dCCP. Additional studies are needed to determine if a particular type of anti-viral reactivity profile in CCP affects clinical efficacy. Local production of dCCP from recovered donors with nAb against variant viruses of concern may offer the potential to mitigate the severity of variant COVID-19.<sup>86</sup> Our experience suggests that further studies with well-characterized PRT dCCP prior to transfusion are warranted, especially in view of surging variant viruses of concern that may not be responsive to current monoclonal antibody therapy.

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## CONFLICT OF INTEREST

LI collaborates with Cerus. Anil Bagri, Johannes Irsch, and Laurence Corash are employees of Cerus. Maja Weisser, Karoline Leuzinger, Hans Pargger, Nikolaus Deigenesch, Anil Bagri, and Nina Khanna have no conflict of interest. Michael Paul Busch, Graham Simmons, and Mars Stone are employees of Vitalant Research and have no conflict of interest. Philip L. Felgner, Rafael R de Assis, and Saahir Khan are employees of the University of California and the University of Southern California and have no conflict of interest. Cheng-ting Tsai, Peter V Robinson, and David Seftel are employees of Enable Biosciences—manufacturers of the ADAP technology assays which are not yet approved for commercial use.

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### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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