

## ORIGINAL PAPER

# Antibody response and intra-host viral evolution after plasma therapy in COVID-19 patients pre-exposed or not to B-cell-depleting agents

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**Summary**

Administration of plasma therapy may contribute to viral control and survival of COVID-19 patients receiving B-cell-depleting agents that impair humoral immunity. However, little is known on the impact of anti-CD20 pre-exposition on the kinetics of SARS-CoV-2-specific antibodies. Here, we evaluated the relationship between anti-spike immunoglobulin G (IgG) kinetics and the clinical status or intra-host viral evolution after plasma therapy in 36 eligible hospitalized COVID-19 patients, pre-exposed or not to B-cell-depleting treatments. The majority of anti-CD20 pre-exposed patients (14/17) showed progressive declines of anti-spike IgG titres following plasma therapy, contrasting with the 4/19 patients who had not received B-cell-depleting agents ( $p = 0.0006$ ). Patients with antibody decay also depicted prolonged clinical symptoms according to the World Health Organization (WHO) severity classification ( $p = 0.0267$ ) and SARS-CoV-2 viral loads ( $p = 0.0032$ ) before complete virus clearance. Moreover, they had higher mutation rates than patients able to mount an endogenous humoral response ( $p = 0.015$ ), including three patients with one to four spike mutations, potentially associated with immune escape. No relevant differences were observed between patients treated with plasma from convalescent and/or mRNA-vaccinated donors. Our study emphasizes the need for an individualized clinical care and follow-up in the management of COVID-19 patients with B-cell lymphopenia.

**KEYWORDS**

antibody kinetics, anti-CD20 therapy, convalescent plasma, immunocompromised patients, mRNA-based vaccine plasma, SARS-CoV-2, spike mutations, whole-genome sequencing

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## INTRODUCTION

Coronavirus disease 2019 (COVID-19) disproportionately affects immunocompromised patients, in the context of their underlying disease, high prevalence of comorbidities, and/or related treatment.<sup>1</sup> Haematological malignancies and solid tumours have been consistently associated with increased risk of COVID-19 complications and death.<sup>2-5</sup> Repeated administration of anti-CD20 monoclonal antibody (e.g. rituximab), an effective treatment for B-cell cancers or inflammatory autoimmune diseases, which leads to B-cell lymphopenia and hypogammaglobulinaemia, is also marked by a more severe COVID-19 course<sup>6-8</sup> and impaired anti-SARS-CoV-2 antibody response, elicited by infection or vaccination.<sup>9,10</sup>

Neutralizing antibodies represent an important correlate of recovery following SARS-CoV-2 infection.<sup>11</sup> Consequently, convalescent plasma therapy (CP), obtained from donors who have recovered from COVID-19 and containing anti-SARS-CoV-2 neutralizing antibodies, has been under massive investigation as reported in large randomized controlled trials.<sup>12-19</sup> In immunocompetent unvaccinated COVID-19 patients with high risk factors for severe disease progression, treatment with CP has shown clinical benefit and reduced incidence of hospitalization, when given early after the onset of symptoms and with high titres of neutralizing antibodies.<sup>12,13,19</sup> Similar observations were made when REGN-COV2, a neutralizing monoclonal antibody (mAb) cocktail, was administered early in the disease course and in seronegative individuals.<sup>20</sup> Most of these trials, however, failed to demonstrate a therapeutic benefit of CP, once COVID-19 patients were hospitalized with an already-established severe pneumonia.<sup>14-18</sup>

The usefulness of plasma therapy is more substantial in immunodeficient patients. There is growing evidence from cohort studies and case series, that CP therapy in frail immunosuppressed individuals, unable to mount effective anti-SARS-CoV-2 antibody responses, reduces viral load and improves clinical symptoms, even when given late after initial diagnosis.<sup>21-29</sup> Accordingly, these findings indicate that the administration of plasma with high neutralizing antibody titres is a safe and effective treatment for immunosuppressed patients.<sup>3,30,31</sup>

Patients with immunosuppression are also at specific risk for a protracted infection with SARS-CoV-2.<sup>32</sup> In an initial report, Aydiillo et al. showed no major changes in the consensus sequences of the original virus strain from serial sample genomes of 11 immunosuppressed patients, including patients treated with CP.<sup>33</sup> While there has been a limited number of reported case series,<sup>33</sup> accumulation of SARS-CoV-2 mutations has been documented in sporadic case reports of long-term-infected immunocompromised hosts.<sup>34-38</sup> Even if this phenomenon does not seem to be very common,<sup>30,39</sup> prolonged viral replication in the context of an inadequate immune response may facilitate the emergence of divergent escape variants.<sup>32</sup>

A key issue of CP therapy relates to the wide heterogeneity of neutralizing antibody titres found within CP units

from recovered individuals.<sup>40</sup> The rapid decay of circulating antibody titres within two to three months after viral infection<sup>41</sup> strongly limits the window of opportunity to collect high-dose anti-SARS-CoV-2 immunoglobulin G (IgG) titres from convalescent donor plasma.<sup>42</sup> For instance, the supply of CP from one centre revealed that high-titre collections, as defined by the US Food and drug Administration (FDA), accounted for only about 20% of plasma donations.<sup>43</sup> In turn, anti-spike protein (i.e. anti-S) IgG antibody responses induced after the second dose of mRNA vaccines are found to be similar to or even higher than the average values from convalescent serum samples.<sup>44,45</sup> Moreover, planning plasmapheresis from individuals who have scheduled their vaccination date is logistically easier than from donors recovered from COVID-19. Assuming that the main criterion of plasma efficacy is to provide the highest antiviral antibody titres, this argument supports the use of plasma from non-COVID-19 healthy adults who had recently received the second dose of an mRNA-based vaccine.

Here, we describe the long-term outcomes after treatment with CP or vaccinated plasma (VP) in an observational case series of hospitalized COVID-19 patients ( $n = 36$ ) with acquired immunodeficiencies or high risk factors, between November 2020 and July 2021. Among them, 17 had received (less than 12 months before) or were still under anti-CD20 therapy (e.g. rituximab, obinutuzumab). The main objective was to determine the impact of anti-CD20 pre-exposition on the kinetics of SARS-CoV-2-specific antibodies after plasma therapy, in comparison to patients without B-cell-depleting treatment. As a secondary outcome, we explored the relationship between antibody kinetics and the patient's clinical status on the one hand and on the other, the rate of intra-host viral evolution and immune escape. In addition, two different sources of plasma (CP vs VP) were evaluated. Each patient was thoroughly monitored over time by anti-S IgG quantification and whole-genome SARS-CoV-2 sequencing.

## METHODOLOGY

### Study design

All patients described in this observational case series were treated with either plasma from convalescent donors (CP) or from vaccinated donors (VP) between 27 November 2020 and 28 July 2021 under an experimental therapy protocol available as compassionate use only, according to the Swiss Federal Law on Therapeutic Products (LPT). Eligible patients (>18 years of age) had a polymerase chain reaction (PCR)-confirmed diagnosis of SARS-CoV-2 infection, a documented onco-haematological diagnostic or an autoimmune disease and/or a solid organ transplant and/or active solid tumour malignancy and were hospitalized with mild to severe COVID-19 according to the World Health Organization (WHO) classification.<sup>46</sup> We included four additional non-immunocompromised patients with high risk factors for severe COVID-19 (i.e. severe obesity, chronic

respiratory disease or older age),<sup>1</sup> and nosocomial infection less than 72h post symptoms or diagnosis, in agreement with Libster et al.<sup>12</sup>

## Plasma collection and preparation

Convalescent plasma was collected from 32 male donors (CP), who had fully recovered for at least 28 days after COVID-19 onset and presented anti-S protein-specific IgG titres ranging from 1.34 to 10.5 S/CO (signal to cut-off titre), with a median of 5 S/CO, by ELISA (Euroimmun AG, Lübeck, Germany). Due to the difficulties to obtain high-titre plasma from convalescent donors, we collected, from 1 March 2021 onwards, plasma from 24 non-COVID-19 male donors (VP) based on their clinical history, who had received their second dose of an mRNA-based vaccine (Moderna or Pfizer) and exhibited an anti-S protein IgG response >8 S/CO by ELISA (Euroimmun AG). Since June 2021, we harvested plasma from convalescent male donors, boosted with an mRNA-based vaccine (Moderna or Pfizer) after COVID-19 infection (CP/VP, >8 S/CO). All plasma donors (18–65 years old) were eligible for blood donation according to the requirements of the Blood Transfusion Services, Swiss Red Cross. After collection by apheresis, the leukocyte-depleted plasma was treated for pathogen inactivation (Intercept blood system, Cerus Corporation, Amersfoort, Netherlands) and standard testing according to the current regulations in Switzerland (Blood Transfusion Services CRS and Swiss Federal Act on Medical Products). The plasma was further separated into three units (200+/-20 ml each) within 24h and kept frozen at -25°C. The transfusion protocol of CP consisted in four units of ABO-compatible plasma (from two or more different donors), given on two consecutive days. In contrast, the transfusion protocol of VP (or CP/VP) involved only two units of ABO-compatible plasma (from two different donors whenever possible), given on the same day, as they contained higher titres of anti-S-protein IgG. Each unit was administered over a 45-min period.

## Therapeutic outcomes

The primary outcome was the evaluation of anti-S protein IgG antibody kinetics at Days 3–5 and then every 7–10 days in B-cell-depleted versus non-depleted patients. Secondary outcomes included monitoring of transfusion safety, follow-up of symptoms according to the WHO severity classification score<sup>46</sup> and SARS-CoV-2 RNA detection by nasopharyngeal swabs at Days 3–5 and then every 7–10 days combined with SARS-CoV-2 whole-genome sequencing. In addition, two different sources of plasma (CP vs VP) were evaluated. Since we investigated the durable effect of plasma transfusion on the serological and viral evolution of each treated patient, only those patients

alive at Day 7 after plasma transfusion were included in this study (i.e. three patients were censored, two CP and one VP).

## Virus detection by qRT-PCR

The SARS-CoV-2 RNA was detected in various clinical specimens by real-time (RT) PCR using the different platforms available in our diagnostic laboratory, namely a fully automated molecular diagnostic platform (MDx platform), the Xpert platform (Cepheid, Sunnyvale, CA, USA), the cobas 6800 platform and the cobas Liat platform (Roche, Basel, Switzerland), as described in Oputa et al. (2020) and Jacot et al. (2020).<sup>47,48</sup> All obtained cycle threshold (CT) values were converted to viral loads based on plasmids-positive controls, as reported in Jacot et al. (2020).<sup>48</sup>

## Anti-S-protein-specific IgG titres by Luminex assay

Sera from individuals at the time of plasma donations (CP vs VP) and sera from patients at different time points after plasma transfusion were collected and characterized for anti-spike protein (S1) IgG titres using an in-house-developed Luminex assay and performed as previously described.<sup>49</sup> MFI signal of each donor serum or plasma patient sample was divided by the mean signal for the negative control samples yielding a ratio over negative control.<sup>49</sup>

## SARS-CoV-2 whole-genome sequencing

RNA from clinical samples (nasopharyngeal or mouth swabs) were extracted using MagnaPure (Roche) and processed with the CleanPlex (Paragon Genomics, Hayward, CA, USA) SARS-CoV-2 panel as previously described.<sup>50</sup> Briefly, the CleanPlex SARS-CoV-2 protocol generates 343 amplicons ranging from 116 to 196 bp (median, 149 bp), distributed into two pools. All samples were sequenced using the 150-bp paired-end protocol on a MiSeq (Illumina, San Diego, CA, USA). Reads were processed using GENCOV (<https://github.com/metagenlab/GENCOV>), a modified version of CoVpipe ([https://gitlab.com/RKIBioinformaticsPipelines/ncov\\_minipipe](https://gitlab.com/RKIBioinformaticsPipelines/ncov_minipipe)). Variant calling was performed with Freebayes (parameters: - min-alternate-fraction 0.1 - min-coverage 10 - min-alternate-count 9).<sup>51</sup> Positions covered by less than 10 reads were set to *N* (unknown) if they were not identified as part of a short deletion by Freebayes. Only variants supported by at least 70% of mapped reads were considered to build consensus genomes. The consensus sequence was generated with bcftools,<sup>52</sup> was checked using our in-house quality control<sup>53</sup> and assigned to SARS-CoV-2 lineages with pangolin.<sup>54</sup>

## Intra-host mutation rate and phylogenetic analyses

Single-nucleotide variants and indels supported by more than 10% of the reads were compared between sequenced SARS-CoV-2 genomes of each patient. The mutation rate was calculated as the total number of variants supported by more than 10% of reads present in one or multiple sequenced genomes and absent from the other sequenced genome(s). In addition, the rate of mutations reaching fixation was calculated as the total number of variants supported by 70% or more of the reads in the last sequenced sample and absent (or supported by <70% of the reads) from the first sequenced genome divided by the time interval (in days) between the first and the last sample. Phylogenies were built using Nextstrain and the nCoV workflow (<https://github.com/nextstrain/ncov>,<sup>55</sup>) including publicly available genomes sequenced in Switzerland (GISAID database version 2022-01-21; <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2017.22.13.30494?crawler=true>). Sequencing reads were submitted to the International Nucleotide Sequence Database Collaboration (INSDC), whereas consensus genome sequences were submitted to GISAID and onto the Swiss Pathogen Surveillance Platform.

Statement: All measurements described in this study were taken from distinct samples.

## RESULTS

### Characteristics of patients treated with convalescent or vaccinated plasma

Seventeen COVID-19 patients (6 female/11 male) with acquired immunodeficiencies due to haematological malignancy ( $n = 15$ ; 88%) or autoimmune disease ( $n = 2$ ; 12%) were treated with CP from 27 November 2020 to 17 March 2021 (Table S1). Among them, 12 (71%) had received (less than 12 months before SARS-CoV-2 diagnosis) or were still under anti-CD20 therapy. As the Swiss vaccination campaign started in early 2021, it became possible to collect plasma from SARS-CoV-2-vaccinated regular blood donors without a previous history of SARS-CoV-2 infection. Consequently, VP was administered to 19 patients (9 female/10 male) with haematological cancer ( $n = 7$ ; 37%) or non-haematological disease ( $n = 8$ ; 42%; autoimmune disease, organ transplant and/or solid tumour) or with high risk factors for severe COVID-19 progression<sup>1</sup> ( $n = 4$ ; 21%), from 9 March 2021 to 22 June 2021 (Table S1). Patients treated with VP were predominantly infected by the alpha variant B.1.1.7, the variant of concern spreading rapidly in Switzerland at that time (from January to June 2021), in contrast to CP-treated patients (CP, 2/17 vs VP, 14/19;  $p = 0.0002$ , Mann–Whitney test) (Figure 1A). The majority of patients had a negative SARS-CoV-2 anti-IgG serology (29/36; 81%) and were not vaccinated (32/36; 89%) (Figure 1A). Most patients had B-cell lymphopenia and hypogammaglobulinaemia (total IgG)

at presentation, both of which were more profound in CP than in VP patients (Figure 1B). The median time from diagnosis to plasma treatment was 31 days (range 1–57 days) for patients receiving CP treatment. This was reduced to four days (range 1–170 days) in the cohort of VP (Figure 1B).

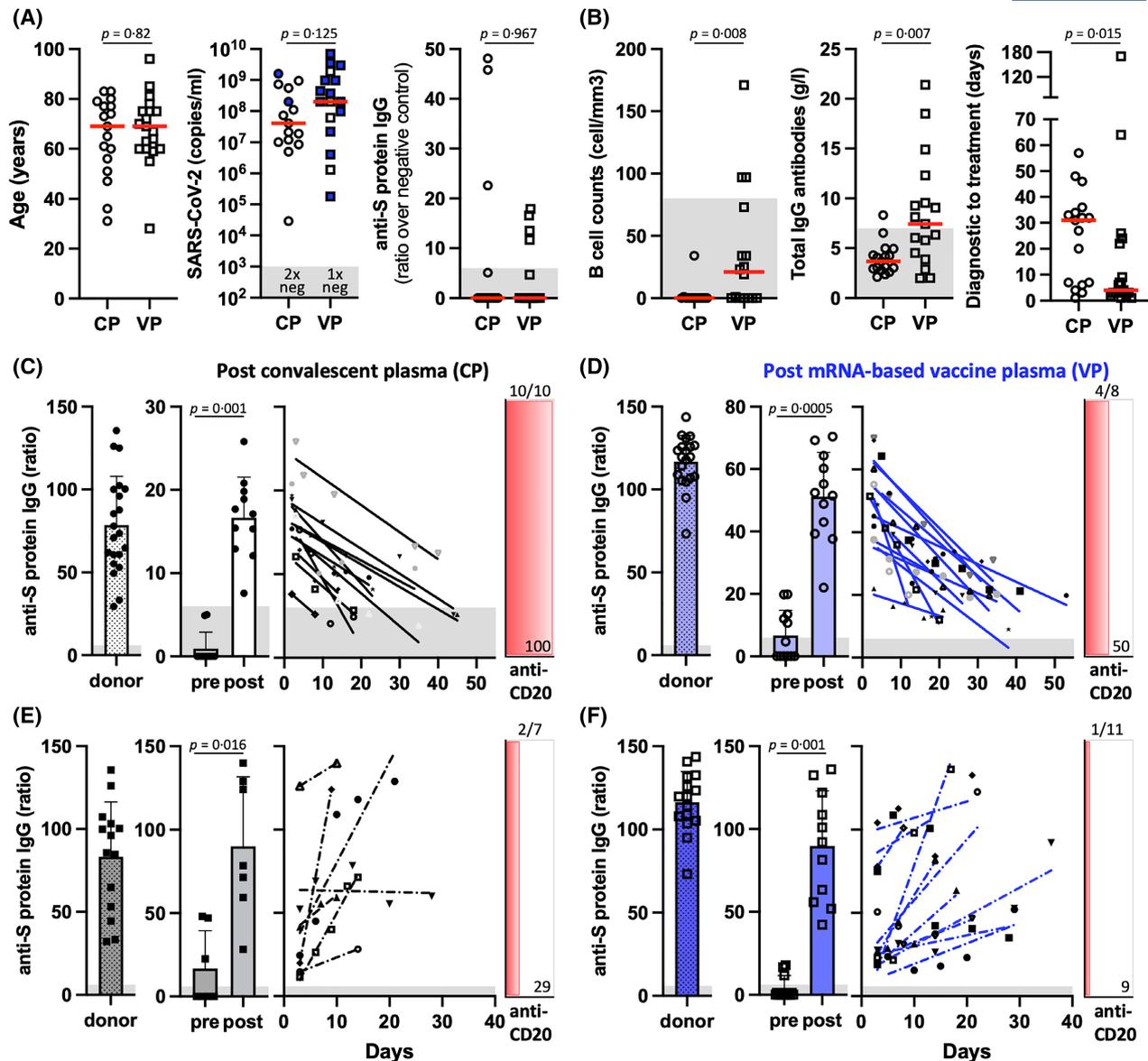
### Anti-CD20 pre-exposition is associated with anti-S IgG titre decay following plasma therapy

To address the impact of anti-CD20-targeted therapies on antibody kinetics, we assessed the anti-S IgG titres of each patient treated either with CP or VP at serial time points following plasma transfusion. Antibody levels varied greatly between unvaccinated donors of CP, whereas these titres were more homogeneous and in general higher in individuals donating plasma after the second mRNA-based vaccine injection, in line with previous reports<sup>44,45</sup> (Figure 1C–F, left panels; donor). This translated into higher levels of anti-S IgG antibodies in patients receiving VP (Figure 1D, middle panel), as compared to those transfused with CP (Figure 1C, middle panel) (CP, median, ratio of 17 vs VP, median, ratio of 52;  $p < 0.0001$ , Mann–Whitney test).

Convalescent and vaccinated plasma recipients were further classified according to their antibody kinetic pattern following transfusion. One half (18/36) of the patients presented a progressive decline in anti-S IgG levels, with a longer time to reach negative titres for VP (median 42 days, range 15–72 days) than for CP (median 25 days, range 6–58 days;  $p = 0.046$ , Mann–Whitney test) (Figure 1C,D, right panels). Six of these patients (3 CP and 3 VP), who required additional plasma transfusions due to insufficient clinical and microbiological responses, exhibited an anti-S IgG antibody decline after each treatment. Interestingly, 14 of the 18 patients showing a decline in anti-S IgG levels had been pre-exposed to an anti-CD20 therapy (10/10 CP patients and 4/8 VP patients). In contrast, the other patients (18/36) showed a progressive increase in anti-S IgG titres following plasma transfusion (Figure 1E,F), and among them only three had received an anti-CD20 treatment (CD20 pre-exposure; Ab decline, 14/18 vs Ab increase, 3/18;  $p = 0.0006$ , Mann–Whitney test). Of note, two of these patients (CP-3 and CP-10) readily presented a positive anti-S IgG serology before plasma therapy, whereas the third one (VP-19) had received rituximab (five months before SARS-CoV-2 diagnosis) in the context of an auto-immune disease (Table S2). Together, our data indicate that anti-CD20 pre-exposition is associated with a progressive decay in anti-S IgG titres following plasma therapy (CP or VP).

### Patients with progressive decline in anti-S IgG titres following plasma therapy had prolonged SARS-CoV-2 infection before complete virus clearance

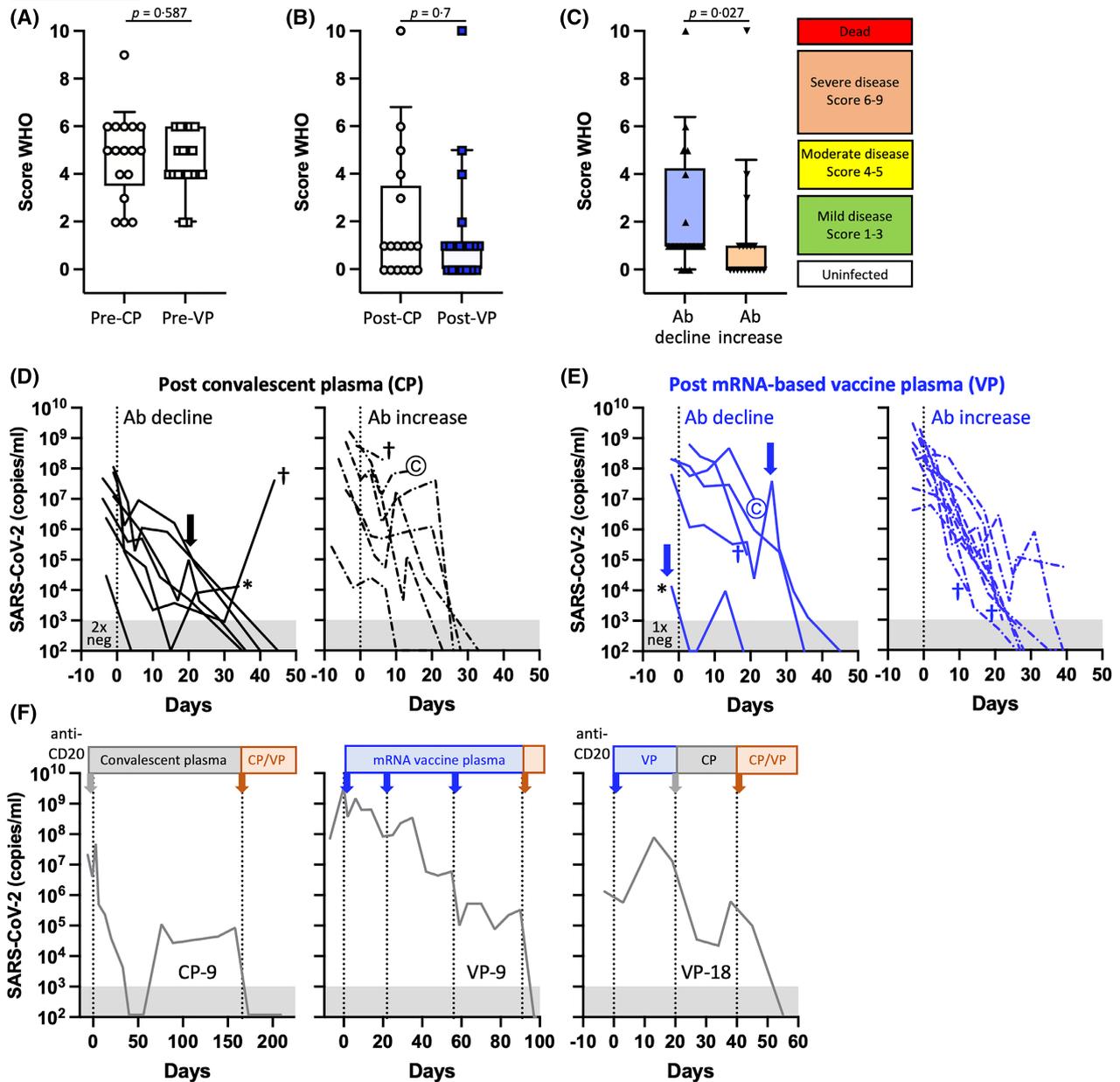
At the time of plasma treatment, 11/17 (65%) CP and 8/19 (42%) VP patients needed oxygen supplementation. Patients



**FIGURE 1** Immune status-related information and serological response follow-up. (A) Patient characteristics (age), SARS-CoV-2 viral loads (copies/ml), anti-SARS-CoV-2 S protein IgG antibody levels (ratio over negative control, negative cut-off set at 6) before treatment with convalescent plasma (CP,  $n = 17$ ) or vaccinated plasma (VP,  $n = 19$ ). Blue symbols represent patients infected by the alpha variant B.1.1.7. (B) Absolute B-cell counts (cell/mm<sup>3</sup>), total IgG antibody levels (g/l) and time lapse from diagnostic to treatment (days) before treatment with CP ( $n = 17$ ) or VP ( $n = 19$ ). (A, B) the  $p$  values are by Mann–Whitney test. (C–F, left panels) Anti-spike (anti-S) protein IgG titres for each plasma donor post COVID-19 recovery (C, E) or after the second injection of an mRNA-based vaccine (D, F). (C–F, middle panels) Comparison of anti-S IgG antibody titres in each patient before and after plasma treatment. The negative cut-off was set at six ratios over standard negative control. The maximum reached value for each patient is depicted. The  $p$  values are by Wilcoxon matched-pairs signed ranked test. (C, D, right panels) Patients presenting anti-S IgG antibody declines following plasma transfusion with CP (C) or VP (D). Of note, anti-S IgG decline kinetic is depicted after each treatment, including those patients who received serial plasma transfusion. (E, F, right panels) Patients exhibiting anti-S IgG antibody increases following plasma treatment with CP (E) or VP (F). (C–F, red panels). Proportion of patients (in percentage) pre-exposed (ongoing or <12 months) to an anti-CD20 antibody-targeted treatment.

presented a range from mild to severe COVID-19, according to the WHO classification<sup>46</sup> (CP, median score at 5, range 2 to 9, with one mechanically ventilated patient; VP, median score at 4, range 2–6) (Figure 2A). Beside one patient with a transient increase in oxygen requirement after plasma transfusion, no transfusion-related adverse events were documented (data not shown). Clinical improvement in COVID-19 symptoms within a follow-up period of

30 days (13–30 days) after plasma transfusion was reported for 34 of the 36 patients (Figure 2B). Specifically, low WHO scores, between 0 to 1, were attributed for 12/17 (71%) CP patients (versus 0/17 before CP transfusion;  $p < 0.0001$ , Mann–Whitney test) and 15/19 (79%) VP patients (versus 0/19 before VP transfusion;  $p < 0.0001$ , Mann–Whitney test). Moreover, a favourable trend was observed for patients who presented an endogenous serological response, compared to



**FIGURE 2** Clinical and viral load recovery in immunocompromised individuals after plasma therapy. (A, B) Clinical status according to the WHO classification before (A) and following CP ( $n = 17$ ) or VP ( $n = 19$ ) treatment (B). (C) WHO scores after plasma therapy in patients classified according to anti-spike (anti-S) IgG antibody decline ( $n = 18$ ) or increase ( $n = 18$ ). (D, E) Over-time follow-up of SARS-CoV-2 RNA detection in nasopharyngeal swabs (copies/ml) after treatment with CP ( $n = 16$ ; D) or VP ( $n = 17$ ; E). Patients were further classified according to their anti-S IgG antibody kinetics (decline versus increase). Three patients had undetectable viral loads at D0 of plasma transfusion (2x neg CP, 1x neg VP), but still presented clinical and/or radiological signs of active COVID-19. Arrows represent patients who received a second treatment from the same type of plasma. One patient (\*) was sequentially treated with CP and VP. Patients who died from SARS-CoV-2-related complications (©) or from their primary-evolutive malignancy (†) are depicted. (F) Over-time follow-up of SARS-CoV-2 RNA detection in three patients (1xCP, 2xVP) with prolonged SARS-CoV-2 shedding and who received serial plasma treatments (CP and/or VP and CP/VP). (A–F) CP, convalescent plasma; VP, vaccinated plasma, CP/VP, convalescent vaccine-boostered plasma. The  $p$  values are by Mann–Whitney test.

those with anti-S IgG antibody declines (Figure 2C). Two patients died from SARS-CoV-2-related complications (one CP; one VP) and five (two CP; three VP) from their primary-evolutive malignancy.

Alongside, we observed a gradual decline in SARS-CoV-2 RNA levels from nasopharyngeal swabs, with quantitative values ranging below  $10^3$  copies/ml in 11/15 (73%) CP patients and 13/18 (72%) VP patients (Figure 2D,E). The

time-to-negativity was shorter in patients treated with CP or VP, presenting an endogenous anti-SARS-CoV-2 response (median 26 days, range 13–39), compared to those with progressive anti-S IgG decline (median 38 days, range 4–49;  $p = 0.0032$ , Mann–Whitney test). Three patients (CP-9, VP-9, VP-18), who exhibited persistent SARS-CoV-2 shedding, received repeated transfusions (two to four times), including plasma from COVID-19-recovered donors boosted by an

mRNA vaccine, leading to the full undetectable SARS-CoV-2 RNA in nasopharyngeal swabs (Figure 2F). Collectively, prolonged SARS-CoV-2 infection was generally observed in the subgroup of patients displaying a progressive decline in anti-S IgG titres following plasma therapy, and included the six patients, who received serial plasma transfusions (Table S2).

### Only a minority of patients, unable to mount an endogenous humoral response, presented significant viral evolution following plasma therapy

To investigate whether persistent SARS-CoV-2 infection was associated with intra-host mutation rate following plasma treatment (Figure 3), SARS-CoV-2 genome sequencing was performed on 139 serial respiratory samples from 30 patients, pre- and post-plasma treatment, with a studied interval of up to 182 days (CP,  $n = 14$ , range 4–182 days; VP,  $n = 16$ , range 9–109 days). Twenty-six out of 30 patients showed one or more intra-host mutations in the viral subpopulations (>10% reads) at any time point (Figure 3A), some of which reached fixation (>70% reads) over time, supporting the constant within-host virus evolution. Large variations were observed in the number of mutations. Specifically, three patients (CP-9, VP-9 and VP-18), who presented a protracted SARS-CoV-2 (Figure 2F) infection and were unable to mount an endogenous humoral response, presented 26 to 65 mutations, including 20 to 50 that reached fixation for at least one sample time (Figure 3A; Table S3). Phylogenetic analyses with the most closely related published genomes from Switzerland supported the monophyletic origin of each strain documented in these three patients, hence excluding secondary infections with other circulating strains (Figure S1).

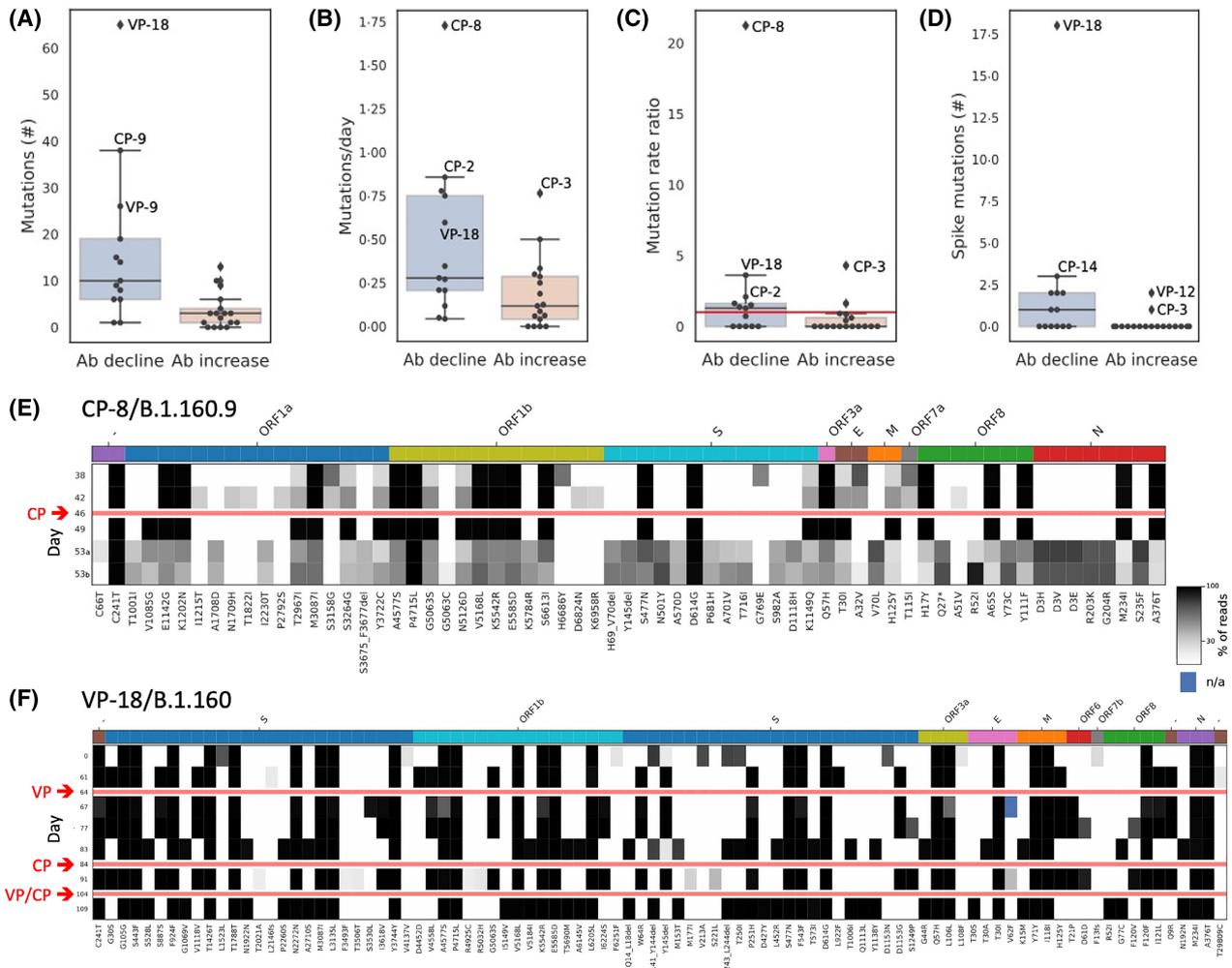
Patients who presented progressive declines in anti-S IgG titres after plasma therapy had significantly higher mutation rates than those showing an endogenous anti-SARS-CoV2 response (Figure 3B, Mann–Whitney  $U$  test;  $p = 0.015$ ). Four patients (CP-2, CP-3, CP-8 and VP-18), among which three had declining anti-S IgG titres, presented mutation rates over twice the expected approximately two mutations per month (Figure 3C).<sup>56</sup> CP-8 presented an exceptionally high number of variants supported by 10%–70% of the reads, many of whom reaching fixation in subsequent samples, suggestive of the presence of a heterogeneous viral population (Figure 3E). Viral subpopulations tend to disappear at Day 3 following CP treatment, but a very high number of mutations supported by 10%–70% of the reads were detected again at Day 7. VP-18 also presented an intriguing pattern of mutation acquisition and alternation over time (Figure 3F). In this patient, two distinct mutation profiles were observed alternatively at different time points, also supported by phylogenetic analyses (Figure S1). In spite of this viral diversification, consecutive plasma transfusion from different donors, including convalescent vaccine-boosted plasma (CP/V), led to SARS-CoV-2 infection control (Figure 2F).

Only a limited number of spike mutations were globally observed during the follow-up (Figure 3D; Figures S2 and S3) and were composed of amino acid substitutions and several recurrent deletions within the N-terminal domain (NTD) of the spike protein (i.e.  $\Delta$ L141–Y144,  $\Delta$ Y145,  $\Delta$ A243–L244) (Table S3), in line with previous studies.<sup>57</sup> Five mutations (S373L, D405N, D427Y, L452R, S494L) were included in the receptor-binding domain (RBD). Overall, our observations indicate that only few immunosuppressed patients presented an accumulation of many mutations over the course of the infection, some of which reached fixation. Patients with progressive anti-S IgG antibody declines following plasma therapy were at highest risk for enhanced viral evolution.

## DISCUSSION

This observational series of immunocompromised COVID-19 patients reports on the impact of CD20 monoclonal antibodies (i.e. rituximab, obinutuzumab) on the kinetics of SARS-CoV-2-specific antibodies. The majority of anti-CD20 pre-exposed patients showed progressive declines of anti-S protein IgG titres following plasma therapy, contrasting with the endogenous humoral response predominantly present in patients who had not been pre-exposed. Antibody decay also correlated with prolonged clinical symptoms and infection before virus clearance. Owing to an insufficient clinical and microbiological response, six patients with progressive anti-S IgG declines received additional plasma transfusions, leading to complete viral response (i.e. undetectable viral loads). Finally, 4/30 genotyped patients showed increased intra-host viral evolution and 3/30 patients, all unable to mount a humoral response, presented one to four spike mutations, potentially associated with immune escape. Collectively, our report highlights the need for an individualized clinical care and follow-up in the management of COVID-19 patients with B-cell lymphopenia, even after plasma therapy. These findings further support the key role of donor plasma selected for enhanced anti-SARS-CoV-2 antibody titres in correcting humoral deficiency and improving clinical outcomes for severely immunocompromised patients, in line with previous reports.<sup>3,28,29</sup> The strength of this study is the in-depth appraisal over time of the anti-SARS-CoV-2 antibody kinetics in 36 plasma-treated patients pre-exposed or not to B-cell-depleting agents and the ensuing evaluation of the relationship between antibody kinetics and clinical state or intra-host viral evolution.

The present report presents several limitations. First, the lack of a control group (non-plasma-treated patients) does not allow us to draw definite conclusions. It is worth noting that no randomized controlled trials have thus far been performed on immunosuppressed COVID-19 patients to evaluate plasma therapy efficacy. Second, it is likely that clinical and virological results may partially be explained by other COVID-19-related given treatments (such as remdesivir and/or steroids). Third, the cohort of patients receiving CP versus VP therapy differed substantially in terms of underlying viral variant,



**FIGURE 3** Intra-host viral evolution in immunocompromised patients before and after plasma therapy. (A) Number of mutations supported by at least 10% of the reads that differ between sequenced genomes of the same patient. (B) Mutation rate calculated as the number of mutations supported by at least 10% of the reads divided by the interval between the first and the last sequenced sample (in days). (C) The rate of mutations reaching fixation (>70% of the reads) between the first and last sequenced samples was compared to the theoretical SARS-CoV-2 mutation rate of approximately 25 mutations per year. Patients with a rate ratio larger than one (horizontal red line) present more mutations than expected. (D) Number of spike mutations supported by at least 70% of the reads. (A–D) Patients were classified according to their anti-spike (anti-S) IgG antibody kinetics (decline,  $n = 13$  versus increase,  $n = 17$ ). (E–F) overview of identified non-synonymous mutations as compared to the reference Wuhan Hu-1 reference genome, before and/or after plasma therapy for patients CP-8 (E) and VP-18 (F). Cells indicate the percentage of reads supporting each mutation (rows) in the different samples (columns). Only variants supported by at least 10% of the reads are reported. For CP-8, the sample from Day 53 was sequenced twice to rule out a contamination during the sequencing process. CP, convalescent plasma; VP, vaccinated plasma, CP-VP, convalescent vaccine-boostered plasma.

immunosuppressive treatments and nosocomial infections, which could have affected clinical outcomes. Finally, anti-S IgG antibody kinetics were monitored after plasma therapy, yet at the time of this study, we could not assess whether these antibodies were of neutralizing activity.

The effectiveness of CP is likely influenced by the quantity of neutralizing antibodies (correlating to the titres of anti-S IgG antibodies) present at the time of donation.<sup>13,58</sup> Initially, Libster and coworkers<sup>12</sup> reported a dose-dependent effect relative to the antibody titres after transfusion, with reduced COVID-19 progression. In early 2021, initiation of the Swiss vaccine campaign further enabled collecting non-COVID-19 donor plasma enriched with high and homogeneous anti-S protein IgG titres post second mRNA vaccination

(i.e. with high neutralizing titres<sup>44,45</sup>). In the meantime, two doses of mRNA vaccines were shown to remain highly effective against symptomatic SARS-CoV-2 infection and severe outcomes with different variants of concern.<sup>59,60</sup> Moreover, a single immunization can boost the neutralizing titres up to 1000-fold in donors recovered from COVID-19.<sup>61</sup> When administered to immunosuppressed patients, comprising those who received an anti-CD20 pre-treatment, VP allowed the effective transfer of anti-S IgG antibodies and led to clinical and viral load recovery comparable to that in CP therapy. In addition, three cases presenting persistent SARS-CoV-2 infection were efficiently treated with convalescent vaccine-boostered plasma. Collectively, our data show that vaccine-based plasma was at that time (i.e. March to June

2021) an alternative treatment alongside CP, in the management of COVID-19 patients with B-cell lymphopenia. However, during this period, we did not monitor the presence of anti-nucleocapsid antibody responses, and thus we cannot formally exclude an asymptomatic or undiagnosed SARS-CoV-2 infection in the population of vaccinated donors. Importantly, the use of plasma from vaccine-boostered convalescent individuals or from vaccinated ones boosted by a breakthrough infection likely broadens the spectrum of anti-SARS-CoV-2 humoral response<sup>61–63</sup> and represents currently the most convenient source of available plasma.

Only a minority of the 30 genotyped patients displayed an increased viral mutation rate, most of whom were unable to mount an intrinsic antibody response to SARS-CoV-2. Likewise, whole-genome sequencing showed the emergence of a limited number of spike mutations (e.g.  $\Delta$ L141–Y144,  $\Delta$ Y145 and L452R) potentially associated with immune escape, in different patients (CP-14, CP-5 and VP-18 respectively) following plasma therapy. Thorough follow-up allowed identifying a few cases with prolonged viral shedding who needed serial transfusion for a complete recovery. Our data are in line with a systematic review by Focosi et al.,<sup>57</sup> reporting that CP may be associated with a lower risk of emergence of resistant variants, contrasting with the documented immune escape after treatment with monoclonal antibodies, as recently shown in immunocompromised patients who received sotrovimab as monotherapy.<sup>64–66</sup> This may in part be explained by the polyclonal nature of the transfused anti-SARS-CoV-2 antibodies. Moreover, escape variants associated with plasma therapy exhibited recurrent amino acid deletions in the NTD region as well as single amino acid changes throughout the spike protein. Consistently, only five of the 31 identified spike mutations affected the RBD region. In line with these observations, escape from polyclonal plasmas likely involves larger antigenic structural changes than escape from monoclonal antibodies, targeting single epitopes.<sup>57</sup> As comparable viral evolution patterns were found following transfusion with vaccine-based plasma, this also suggests that CP and VP may share common mechanisms in antibody-mediated protection.

In summary, our case series extends on previous findings,<sup>3,29</sup> validating the concept that immunosuppressed patients, particularly those who are pre-exposed to an anti-CD20 monoclonal antibody treatment, are unable to produce a potent anti-SARS-CoV-2 humoral response and rely on the passive transfer of neutralizing antibodies. Such immunosuppressed patients with a *de novo* SARS-CoV-2 infection should quickly be identified at the daily clinical practice, since most of them require individualized clinical care and follow-up. Moreover, B-cell-depleted COVID-19 patients are at increased risk for long-term viral replication, as compared to other vulnerable individuals who are still able to develop their own endogenous antibody response. A high mutation rate was only observed in few patients with prolonged virus shedding. Yet, this observation emphasizes the need for long-term

surveillance for the emergence of new variants carrying mutations favouring escape to current population immunity by regular SARS-CoV-2 viral load and genomic monitoring. Finally, given the importance of the humoral immune response for clinical recovery,<sup>11</sup> plasma therapy from convalescent vaccine-boostered donors remains a rational option, since it is inexpensive and logistically simple to organize. Moreover, it is easily adaptable to any new variant outbreaks, and contains high titres of neutralizing antibodies against SARS-CoV-2 associated with a broad antigenic spectrum.<sup>61–63</sup>

#### AUTHOR CONTRIBUTIONS

Study design and writing of the manuscript: David Gachoud, Trestan Pillonel, Nathalie Rufer. Patient care and acquisition of data: David Gachoud, Gerasimos Tsilimidos, Dunia Battolla, Dominique Dumas, Peter Vollenweider, Oriol Manuel, Nathalie Rufer. Analysis and interpretation of data: David Gachoud, Trestan Pillonel, Gerasimos Tsilimidos, Gilbert Greub, Claire Bertelli, Nathalie Rufer. Revision and approval of the manuscript: David Gachoud, Trestan Pillonel, Gerasimos Tsilimidos, Onya Opota, Stefano Fontana, Peter Vollenweider, Oriol Manuel, Gilbert Greub, Claire Bertelli, Nathalie Rufer.

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#### CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

#### DATA AVAILABILITY STATEMENT

The datasets that support the findings of this study are available from the corresponding authors upon reasonable request.

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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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