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Characterization of CCP: Can We Use Past Convalescent Plasma from COVID-19 Patients for Treatment of New Emerging Variants?

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Highlights:

- Not all past CCP could be used to treat patients with a new SARS-CoV-2 delta and omicron infection due to the lack of specific neutralizing antibodies (Nt-Abs);
- For the moment, the neutralization test remains the gold standard to select potential CCP donors;
- We did not find a statistical difference between Nt-Abs responses in North and South Italy CCP donors.

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Abstract: Background and Objectives: New SARS-CoV-2 variants may impact the effectiveness of previously stored convalescent plasma (CCP). We defined levels of anti-delta and anti-omicron SARS-CoV-2 neutralizing antibodies (Nt-Abs) and investigated possible differences of past CCP Nt-Abs responses related to donor location in North and South Italy. Methods: Serum from 153 donors recovered from SARS-COV-2 infection (98 from northern and 55 from southern Italy) were analyzed for Nt-Abs characterization using our in house microneutralization assay. Results were compared to anti-Spike IgG measured by chemiluminescent assay (CLIA) to define a possible agreement with a more affordable test. Results: delta Nt-Abs titer in comparison to the reference strain (PV10734 D614G) showed a reduction of 82% in northern and 77% in southern Italy groups. Omicron Nt-Abs titer showed a reduction of 97%. CCP corresponding to Nt-Abs titer > 1:80 showed a median of 1365 BAU/mL for delta strain and 653 BAU/mL for reference strain. We found no statistical differences between Nt-Abs responses in North and South CCP donors. Conclusions: Not all past CCP could be used to treat patients with SARS-CoV-2 delta and omicron infections due to the lack of specific Nt-Abs. For the moment, the neutralization test remains the gold standard to select potential CCP donors. Interestingly, our study did not find NT-Abs differences between plasma collected from donors living in different areas of Italy.

Keywords: SARS-CoV-2; delta; omicron; CCP; NT-Abs

1. Introduction

The emergence of Severe acute respiratory syndrome virus 2 (SARS-CoV-2) has caused an international pandemic with more than 6 million deaths [1] and a worldwide lockdown. Vaccines were developed successfully very quickly [2], but new viral variants continue to emerge, evading humoral immunity [3]. At the beginning of the SARS-CoV-2 pandemic, in the absence of an effective vaccine or monoclonal therapeutic, treatment with convalescent plasma (CCP) corresponding to a neutralizing antibodies titer equal or greater of 1:80 was

used as a possible accessible therapy [4–6]. When a new infectious agent appears, passive immunotherapy is the only therapy available to stem a public health emergency. The CCP was recently applied to Western Africa Ebola (2013–2016) and the MERS epidemic (2014–2015) [7]. CCP has been shown to be a safe and effective treatment when high titer neutralizing antibodies are given within three days of symptom onset, resulting in a 73% reduction in the risk of coronavirus disease 2019 (COVID-19) progression [8]. However, no reduction in mortality was observed when CCP was administered later in severe COVID-19 patients [9]. Some variability in the efficacy of CCP therapy is due to the design of clinical trials specifically in the timing of patients' admission to the therapy or to antibodies neutralization levels used [10]. However, the emergence of new variants suggests that immunological differences may affect the effectiveness of both convalescent plasma and vaccines. Some studies showed that local variants may impact the effectiveness of CCP, resulting in less effective treatment against newer SARS-CoV-2 variants [11].

Thus, the aim of our study was to define the level of anti-delta and anti-omicron SARS-CoV-2 neutralizing antibodies in previously stored and characterized CCP units, collected between January and June 2021 for evaluating the effectiveness of CCP in SARS-CoV-2 delta and omicron variants infections. Moreover, SARS-CoV-2 neutralizing antibody results were compared to anti-S IgG measured by chemiluminescent assay (CLIA) in order to define a possible agreement and, thus, avoid the use of actively replicating virus that require BSL3 facility. Finally, considering the hypothesis that near-sourced convalescent plasma could reflect small structural composition variations of local viral strains [12], we compared neutralizing titer against reference strain and delta variant in CCP from two distant areas in Italy (north and south), collected in the same period, to investigate whether it is important that the CCP donor and the eligible patient to treat should be in close geographic proximity.

2. Methods

2.1. Patients

Serum samples from 153 plasma donors, 98 (64%) from northern Italy and 55 (36%) from southern Italy, recovered from SARS-COV-2 infection with no history of vaccination, were collected in the period of January/May 2021 and tested for SARS-CoV-2 NT-Abs using the European strain B.1 (PV10734 D614G) circulating in Italy in that period [12]. All the sera were retrospectively considered for SARS-CoV-2 NT-Abs using delta (B.1.617.2) and omicron (B.1.1.529) variants, since their spread worldwide. Commercial SARS-CoV-2 specific real time RT-PCRs (MGISP-NE384, MGI Tech Co., Ltd., Whuan, China) targeting RNA-dependent RNA polymerase and ORF8 genes was performed to detect the Initial infection of SARS-CoV-2 in nasal swab, according to the manufacture guidelines.

2.2. SARS-CoV-2 Variants Isolation and Characterization

SARS-CoV-2 strains PV10734 (D614G reference strain) delta (B.1.617.2) and omicron variant (B.1.1.529) were isolated from nasal swabs of hospitalized-infected symptomatic patients in our laboratory. Nasal swabs from symptomatic patients were inoculated into two wells of a confluent 24 VERO E6 (VERO C1008 (Vero 76, clone E6, Vero E6); ATCC® CRL-1586™) microplates and incubated at 33 °C 5% CO_2 with the addition of respiratory medium: EMEM plus 1% penicillin, streptomycin, glutamine, and trypsin 5 γ/m, until the appearance of CPE depending on the amount of virus present in the inoculum. After isolation all the strains were propagated in 25 cm^2 tissue culture flask till all the cells were destroyed, usually 3/5 days. Mediums were harvested from infected cells, clarified by centrifugation, titrated and frozen at −80 °C in aliquots until use. SARS-CoV-2 strains were sequenced [12] and submitted to GISAID under the following reference numbers (EPI_ISL_568579; EPI_ISL_1403609-11). Virus propagation occurred in BSL-3 laboratory. All virus concentration results are presented in median tissue culture infectious doses ($TCID_{50}$). Virus titrations were calculated using the Reed-Muench method [13].

2.3. Microneutralization Assay

SARS-CoV2 neutralizing antibodies (Nt-Abs) levels were defined as previously described [14]. Briefly 50 µL of each patients sera, starting from 1:10 to 1:640 in a serial fourfold dilution series were added in duplicate in a flat bottom tissue culture microtiter plate (COSTAR, 13 Corning Incorporated, New York, NY, USA) plus 50 µL of 100 $TCID_{50}$ of each strain and incubated for 1 h at 33 °C 5% CO_2 $\times$ 10^4 VERO E6 cells (VERO C1008 (Vero 76, clone E6, Vero E6); ATCC® CRL-1586™) in 50 µL were added. After 72 h of incubation, plates were scored for cytopathic effect (CPE), then inhibitory concentration 90% (IC90), defined as the concentration of Abs able to decrease the percentage of infected cells by 90%, were calculated as neutralizing antibodies titer [14]. Plates were then stained with Gram's crystal violet solution (Merck KGaA, 64271 Darmstadt, Germany) with the addition of 5% formaldehyde 40% *m*/*v* (Carlo ErbaSpA, Arese (MI), Italy) for 30 min and washed under running water. Blue staining of wells indicated the absence of cytopathic effect. A neutralizing antibodies titer < 1:10 was defined as negative whereas a titer greater or equal 1:10 was considered positive [14].

2.4. Quantitative SARS-CoV-2 TrimericS IgG Measurement

Serum samples were analyzed using a chemiluminescent immunoassay (CLIA) (LIAISON® SARS-CoV-2 TrimericS IgG; DiaSorin, Saluggia (VC), Italy) for the quantitative characterization of SARS-CoV-2 IgG antibodies. According to the manufacturer's instructions and consensus standards, results were given as BAU/mL and a cut-off of 33.8 BAU/mL was considered for definition of positive samples. IgG titers < 33.8 BAU/mL were given as a negative result.

3. Data Analysis

All analyses reported were performed using GraphPad Prism software (version 5; GraphPad Software Inc., La Jolla, CA, USA), descriptive statistic including mean, standard deviation and numbers of samples analyzed (N), correlation (Pearson R test), and ROC for setting up the Cut-Off.

4. Ethical Statement

The study was approved and performed according to guidelines of the Institutional Review Board of the Fondazione IRCCS Policlinico San Matteo (protocols no. P-20200035863 and P-20200027987).

5. Results

5.1. SARS-CoV-2 NT-Abs in Sera Collected from Northern Italy

Fifty-six sera from the northern Italy (57%) were considered suitable for donation since NT-Abs level against PV10734 D614G reference strain was higher than 1:80. On the other hand, only 12 (12%) sera showed NT-Abs level higher than 1:80 when tested against B.1.617.2 strain. Only 8 sera out of 98 (8.2%) showed a high level of NT-Abs (higher than 1:80) against both variants, thus suggesting that they could be suitable for transfusion in COVID-19 patients infected with PV10734 D614G or B.1.617.2.

The average Nt-Abs titer were 1:40 for delta and 1:270 for the reference strain (Figure 1). Comparison of the Nt-Abs titer of the two variants showed an 82% (Std. Dev $\pm$ 23%) reduction for the delta variant ($-$.05 Log, Std. Dev $\pm$ 0.7 Log) (Figure 2).

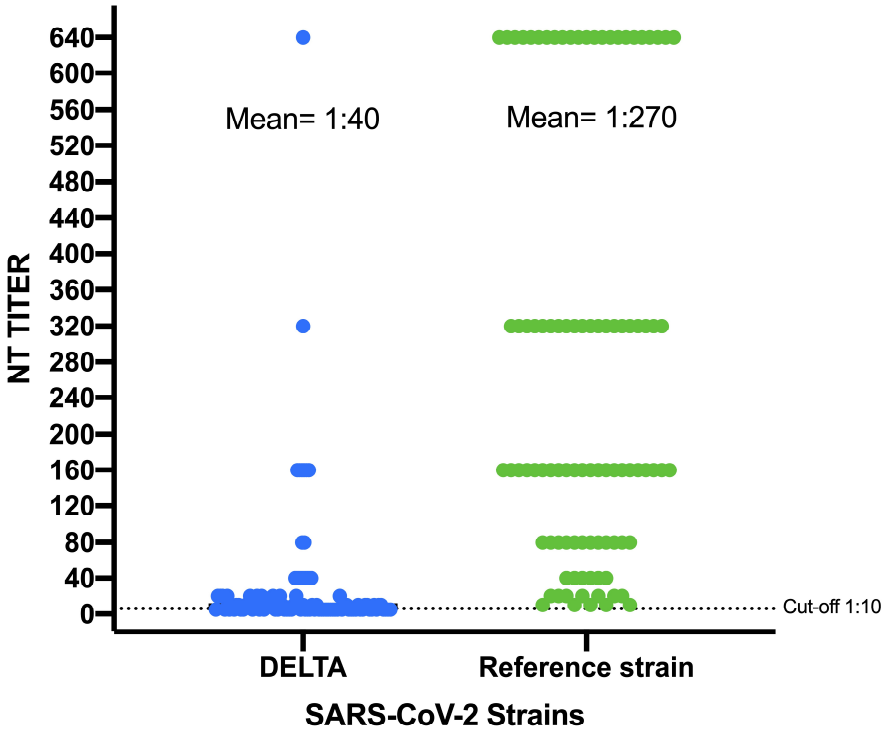

Figure 1. SARS-CoV-2 Nt-Abs characterization of 98 (n = 98) CCP serum samples from the northern area of Italy for reference strain (PV10734 D614G) and delta variants using Microtitration Assay. Positive Nt-Abs titers were considered ≥1:10, while titers ≥ 1:80 were considered suitable for CCP treatment. The average Nt-Abs titers were 1:40 for delta strain, and 1:270 for the reference strain. Twelve percent CCP were suitable for donation (NT value ≥ 1:80) for the delta strain (12/98) and 57% for the reference strain (56/98). Eight out of 98 sera (8.2%) were suitable for both variants.

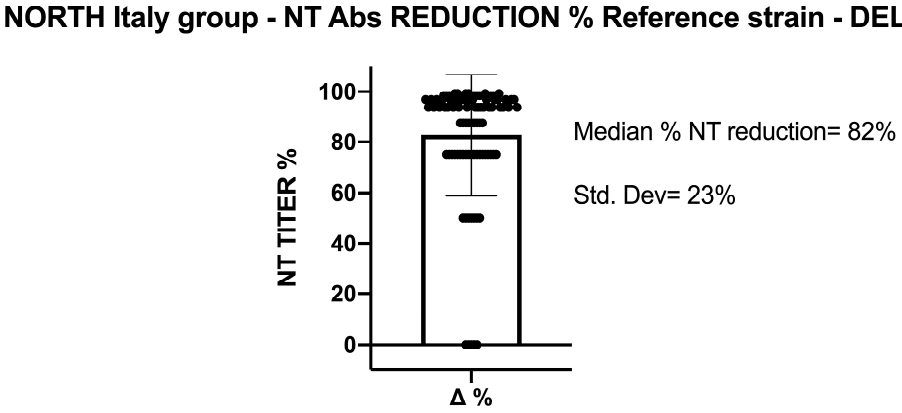

Figure 2. Percentage of Nt-Abs reduction in 98 (n = 98) CCP serum samples from the northern area of Italy against reference strain and delta variants. Delta variant, compared to the reference strain, showed 82% reduction in specific Nt-Abs (Std. Dev ± 23%) (−2.05 Log, Std. Dev ± 0.7 Log) compared to the reference strain.

5.2. SARS-CoV-2 NT-Abs in Sera Collected from Southern Italy

In the southern group only 3 sera out of 55 (5.45%) were suitable for donation (NT-Abs level ≥ 1:80) when tested against the B.1.617.2 strain, while 31 sera out of 55 (56%) showed sustained level of NT-Abs when tested against the PV10734 D614G reference strain. Only 3 sera out of 55 (5.45%) showed high SARS-CoV-2 NT- titer (NT-Abs ≥ 1:80) against both variants. The average Nt-Abs titer were 1:23 for delta strain and 1:147 for the reference strain (Figure 3). In this group, we observed a 77% decrease (Std. Dev ± 24%) in the Nt-Abs

specific for the delta variant (−1.70 Log, Std. Dev ± 0.7 Log) in comparison to the reference strain (Figure 4).

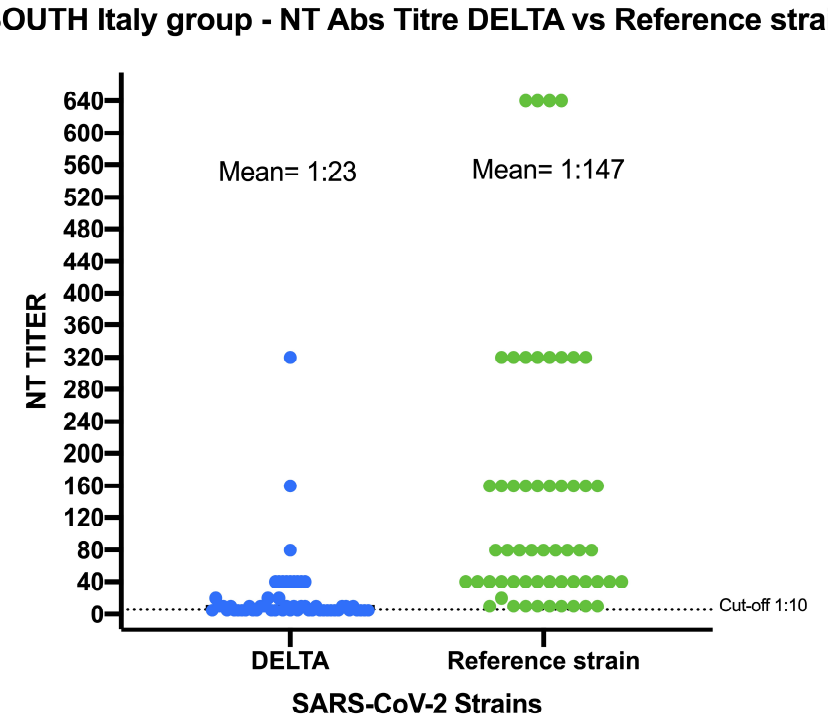

Figure 3. SARS-CoV-2 Nt-Abs characterization of Fifty-five (n = 55) CCP serum samples from the South area of Italy for reference strain and delta variants using Microtitration Assay. Positive Nt-Abs titers were considered ≥1:10, while titers ≥ 1:80 were considered suitable for CCP treatment. The average Nt-Abs titers were 1:23 for delta strain and 1:147 for the reference strain. Forty-five percent of results were suitable for donation (NT value ≥ 1:80) for the delta strain (3/55) while 56% for the reference strain (31/55). Three out of 55 sera (5.45%) were suitable for both variants.

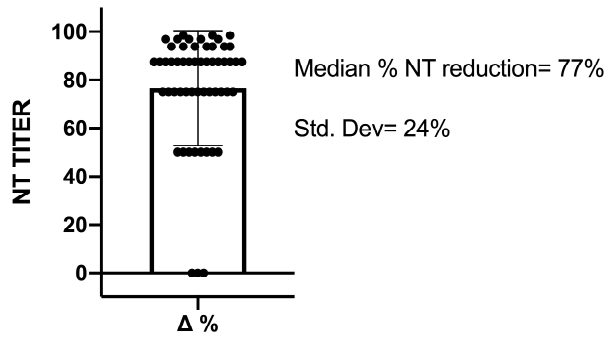

Figure 4. Fifty-five (N = 55) CCP serum samples from the South area of Italy were evaluated for the characterization of SARS-CoV-2 NT-Ab titer reduction for reference strain and delta variants using Microtitration Assay. Delta variant showed a 77% reduction (Std. Dev ± 23%) (−1.7 Log, Std. Dev ± 0.7 Log) of specific Nt-Abs compared to the reference strain.

Despite the geographical distance between the northern and southern CCP sample groups, no statistically significant difference was observed for the characterization of SARS-CoV-2 Nt-Abs response against PV10734 D614G reference strain (p = 0.1745) or delta variants (p = 0.2259).

5.3. Characterization of Total IgG Level

CCP serum samples were also tested for the quantitative evaluation of SARS-CoV-2 TrimericS IgG antibodies. We observed that sera corresponding to a Nt-Abs titer of ≥1:80 against B.1.617.2 strain showed a median of 1365 BAU/mL for Trimeric S IgG antibodies response (IQR 468.5–2080), while sera with a Nt-Abs titer ≥ 1:80 against Pv10734 D614G reference strain showed a median of 653 BAU/mL for TrimericS IgG antibodies response (IQR 219–969). (Figure 5).

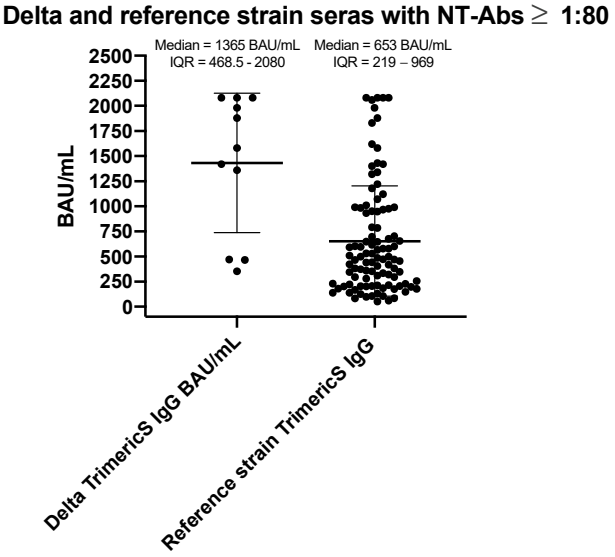

Figure 5. One hundred and fifty-three (n = 153) CCP serum samples from the northern and southern areas of Italy collected between January/June 2021 were tested for the quantitative evaluation of SARS-CoV-2 TrimericS IgG antibodies1 Sera with a Nt-Abs titer ≥ 1:80 specific for delta variant showed a median of 1365 BAU/mL (IQR 468.5–2080), while 87 sera with a Nt-Abs titer ≥ 1:80 specific for Reference strain showed a median of 653 BAU/mL (IQR 219–969).

Sorting out all sera from northern and southern Italy using a cut-off calculated using ROC curve of 1350 BAU/mL (sensitivity 69.23%, specificity 95.65%) we highlighted the presence of 66.7% (10/15) sera related to Nt-Abs titer ≥ 1:80 specific for delta variant, and 100% (15/15) sera related to Nt-Abs titer ≥ 1:80 specific for reference strain. Setting the cut-off to 650 BAU/mL (sensitivity 53.47%, specificity 75.55%) we observed the presence of 18% (7/39) sera correlated with a Nt-Abs titer ≥ 1:80 specific for delta variant, and 85% (33/39) sera correlated with a Nt-Abs titer ≥ 1:80 specific for reference strain (Figure 6).

5.4. SARS-CoV-2 NT-Abs in Sera Collected from Northern and Southern Italy against Omicron Variant and Characterization of Total IgG Level

One hundred and fifty (N = 150) CCP serum samples from both northern and southern areas of Italy collected between January/June 2021 were also tested against SARS-CoV-2 omicron variant. The average SARS-CoV-2 Nt-Abs titers were 1:30 for delta strain and 1:230 for Pv10734 D614G reference strain, whereas the omicron variant (B.1.1.529) NT-Abs average titer was negative (<1:10). Only five samples showed a titer greater or equal of 1:10 (3.3%), only one (1 sera out of 150, 0.67%) was suitable for donation (NT value ≥ 1:80) (Figure 7). The omicron variant showed a 97% of Nt-Abs reduction (Std. Dev ± 16%) compared to the reference strain Pv10734 D614G (−2.2 Log Std. Dev ± 0.6 Log) (Figure 8). CCP serum samples were also tested for the quantitative evaluation of SARS-CoV-2 TrimericS IgG antibodies. Low correlation between the SARS-CoV-2 omicron variant Nt-Abs titer and SARS-CoV-2 TrimericS IgG antibodies (R = 0.1891, p = 0.0157) was observed. It was not possible to calculate a TrimericS IgG Cut-Off for the selection of sera with omicron Nt-Abs titer ≥ 1:80 because the majority of these samples had a negative Nt-Abs titer (<1:10).

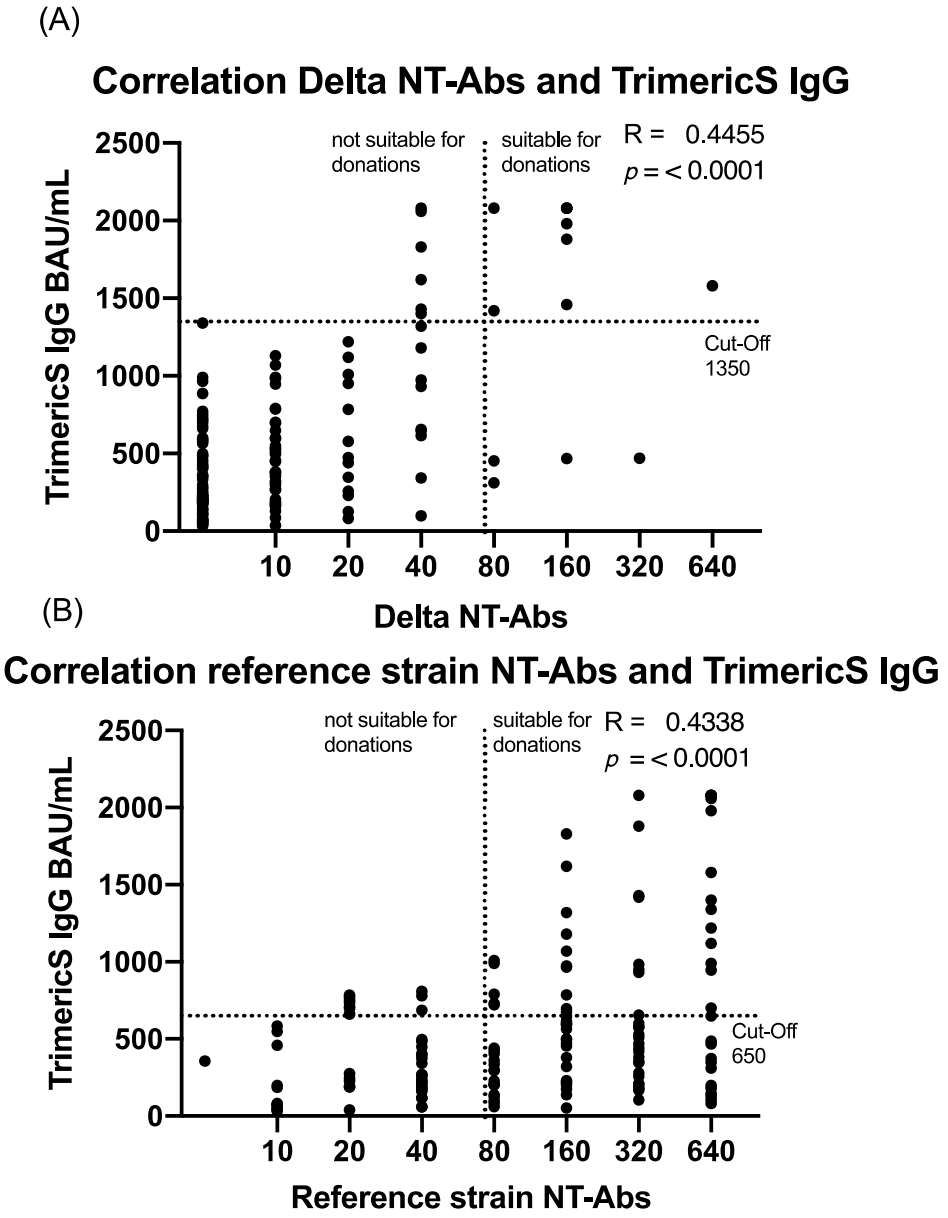

Figure 6. A total of one hundred and fifty-three (N = 153) CCP serum samples from northern and southern areas of Italy collected between January/June 2021 were analyzed. (**A**) Correlation between SARS-CoV-2 delta variant Nt-Abs titer and SARS-CoV-2 TrimericS IgG antibodies, R = 0.4455, p = <0.0001. TrimericS IgG Cut-Off set to select sera with Nt-Abs titer $\geq$ 1:80 was calculated using ROC curve (1350 BAU/mL sensitivity 69.23%, specificity 95.65%). (**B**) Correlation between SARS-CoV-2 reference strain Nt-Abs titer and SARS-CoV-2 TrimericS IgG antibodies, R = 0.4338, p = <0.0001. TrimericS IgG Cut-Off set to select sera with Nt-Abs titer $\geq$ 1:80 was calculated using ROC curve (650 BAU/mL sensitivity 53.47%, specificity 72.55%).

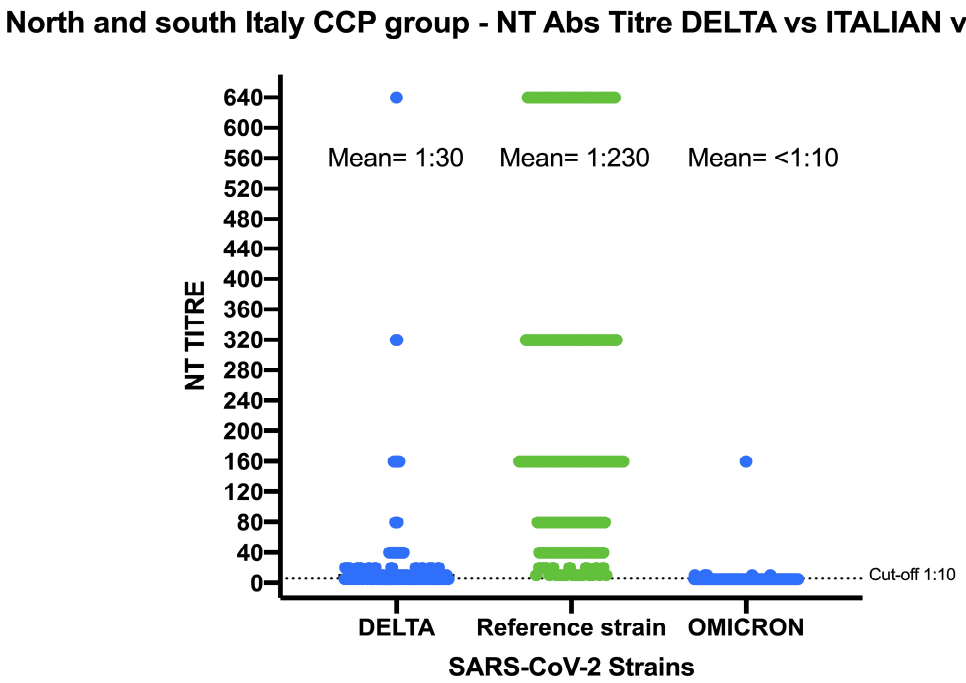

Figure 7. SARS-CoV-2 Nt-Abs characterization of 150 (N = 150) CCP serum samples from northern and southern areas of Italy for delta, reference strain, and omicron variants using microtitration Assay. Positive Nt-Abs titer were considered $\geq$ 1:10, while titers $\geq$ 1:80 were considered suitable for CCP treatment. The average Nt-Abs titer were 1:30 for delta strain, and 1:230 for the reference strain. The average omicron NT-Abs titer were negative (<1:10), only five sample shows a positive titer and only one (1/150, 0.67%) result suitable for donation (NT value $\geq$ 1:80).

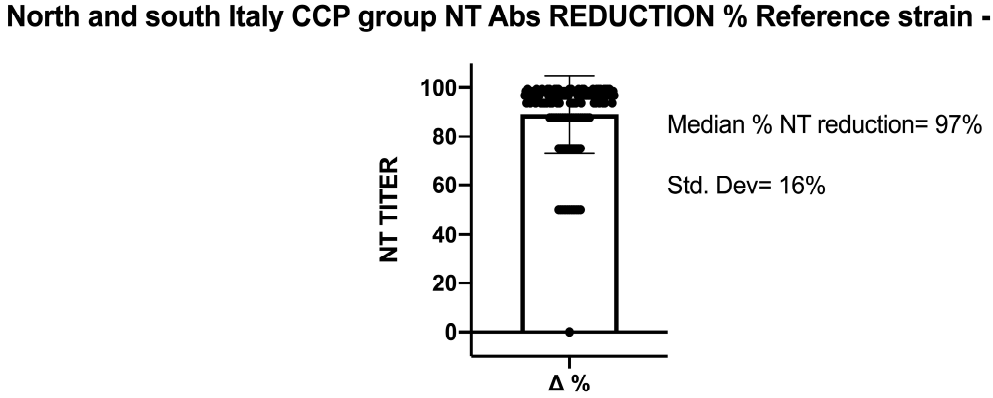

Figure 8. Percentage of Nt-Abs reduction in one hundred and fifty (N = 150) CCP serum samples from the whole area of Italy against reference strain and omicron variants. Omicron variant, compared to the reference strain, showed a 97% of specific Nt-Abs reduction (Std.Dev $\pm$ 16%) ($-$2.2 Log, Std.Dev $\pm$ 0.6 Log) compared to the reference strain.

6. Discussion

Early in the pandemic, due to the absence of specific therapies and vaccines, scientific communities evaluated the opportunity to use hyperimmune plasma transfusion for the treatment of severe COVID-19 disease [15]. However, despite positive results have been observed in some clinical trials [16], discordant data were reported regarding the real benefits of this therapy [17]. It is conceivable that the design of the trial as well as the selection of CCP product might be crucial for a successful therapy. Indeed, beyond serological tests used for the detection of SARS-CoV-2-specific IgG and NT-Abs, a different sensitivity could explain these discrepancies.

Another important issue is related to the circulation of new variants with local distribution that might affect the immune response. Over the past year, we witnessed the appearance of numerous major SARS-CoV-2 variants, some of which are much less susceptible to neutralization by antibodies elicited by previous circulating strains [18]. These SARS-CoV-2 variants tend to attract attention when they replace the previous prevalent viral strains through increased transmission, mortality and/or when they defeat vaccine immunity and antibody-based therapies through antigenic changes [19].

The antibodies present in CCP mediate their therapeutic effect through a variety of mechanisms. Those antibodies can bind to a given pathogen neutralizing its infectivity directly or they can activate antibody-mediated pathways, such as complement activation, antibody-dependent cellular cytotoxicity, and/or phagocytosis. All these mechanisms of action can contribute to CCP therapeutic effect and may also contribute to prophylaxis and/or enhance recovery [20]. We observed an in vitro Nt-Abs functionality reduction against new SARS-CoV-2 delta and omicron variants from previously stored CCP sera, while maintaining high titers against original strain. Immunological differences of emerging new variants of SARS-CoV-2 may impact the effectiveness of both convalescent plasma and vaccines developed on the previous circulating variant.

Investigating a possible difference of CCP Nt-Abs in vitro effectiveness related to donor location that could reflect small structural variations which occurs locally [12], in our study we did not find statistically relevant difference between North and South CCP donors. Consequently, we cannot confirm the hypothesis of Kunze et al. [21] on the possible efficacy of CCP within the proximity of the donation site based on our NT results. This could be due to a relatively close geographic proximity, about 1000 km: major distance needs to be further evaluated in a randomized trial.

Neutralizing antibodies were also compared with the TrimericS IgG antibodies to find an agreement with a more affordable test that does not require the use of live virus and a specific expertise. Sorting CCP with the TrimericS IgG antibodies cut-off value $\geq$ 1350 BAU/mL for delta variant and 650 BAU/mL for reference strain, we could increase the chances of obtaining a better correlation between these two tests to select suitable CCP for donation. However, selected sera through BAU/mL cut-off needs to be further confirmed by neutralization test prior donation. We observed that CCP samples related to a Nt-Abs titer $\geq$ 1:80 specific for delta variant show a higher TrimericS IgG antibodies average of response than those with a Nt-Abs titer $\geq$ 1:80 specific for reference strain. Since delta and omicron variants were not circulating when those CCP were collected, our hypothesis is that those patients could have developed specific antibodies able to bind to conserved virus structures and neutralize both SARS-CoV-2 reference strain and delta variant.

Regarding the new SARS-CoV-2 omicron variant that is widely spreading across the globe, it is characterized by 59 mutations throughout its genome with high immunoevasive potential as some of those occurring within the spike protein that are involved in host cell entry and are the main target of neutralizing antibodies [22]. Indeed, we observed in previously stored CCP in vitro a 97% Nt-Abs functionality reduction against the new SARS-CoV-2 omicron variant, confirming this escape mechanism. Furthermore, even if the BAU/mL antibodies detected with the TrimericS IgG assay remain elevated in all the CCP tested, it was not possible to correlate this test with the neutralization assay because the majority of the samples did not cross-react with omicron variant. For the moment, neutralization test remains the gold standard to select potential CCP donors due to the possibility to use new COVID-19 strains for the detection of specific Nt-Abs related variants.

In conclusion, due to the escape mechanism that involves the spike protein of new SARS-CoV-2 emerging variants, a significant reduction in neutralizing antibodies titer for delta and omicron variants put at risk the use of part of past convalescent plasma for the treatment of infected patients due to the lack of specific Nt-Abs. However, because the therapeutic effect of CCP can be explained through a variety of mechanisms and not only the NT titer, but we also cannot exclude that it could be still useful for seriously ill patients.

Interestingly, our study did not find differences between plasma collected from donors living in different areas in Italy, suggesting that is possible to send plasma in support to areas in need, even if a major distance needs to be further evaluated in randomized trials. Finally, trying to compare an NT assay with a more affordable test for the detection of anti-spike antibodies that does not require expertise, such as the TrimericS IgG assay, the NT remains the gold standard test.

Author Contributions: Conceptualization, C.P., E.P., C.D.F. and A.F.; methodology, A.F., C.D.F. and J.C.S.; software, A.F. and D.L.; formal analysis, A.S., E.P., D.L. and A.F.; resources, F.B.; data curation, A.F. and I.C.; writing original draft preparation, A.F. and E.P.; writing review and editing, E.P., I.C., C.P., C.D.F. and F.B.; visualization, F.B.; investigation, C.D.F. and E.P.; funding acquisition, F.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data will be made available on request. The data that has been used is confidential.

Conflicts of Interest: The authors declare no conflict of interest.

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