A longitudinal study of convalescent plasma (CCP) donors and correlation of ABO group, initial neutralizing antibodies (nAb), and body mass index (BMI) with nAb and anti-nucleocapsid (NP) SARS-CoV-2 antibody kinetics: Proposals for better quality of CCP collections

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Abstract

Introduction: Little is known about the neutralizing (nAb) and binding antibody kinetics in COVID-19 convalescent plasma donors, especially during the first 100 days after disease onset.

Materials and Methods: A cohort of previously RT-PCR positive (detected by nasopharyngeal swab during the acute phase), male convalescent patients, all with mild symptoms, were enrolled in serial blood sample collection for a longitudinal nAb titers and anti-nucleocapsid (NP) antibodies (IgM, IgG and IgA) evaluation. NAbs were detected by a cytopathic effect-based virus neutralization test (CPE-based VNT), carried out with SARS-CoV-2 (GenBank: MT350282).

Results: A total of 78 male volunteers provided 316 samples, spanning a total of 4820 days of study. Although only 25% of donors kept nAb titers ≥160 within 100 days after the onset of disease, there was >75% probability of sustaining nAb titers ≥160 in volunteers whose initial nAb titer was ≥1280, weight ≥90 kg or obese, according to their body mass index (BMI), as evidenced by Kaplan–Meier analysis and Cox hazard regression (all p < .02). There was no correlation between the ABO group, ABO antibody titers and persistent high nAb titers. High IgG anti-NP (S/CO ≥5.0) is a good surrogate for detecting nAb ≥ 160, defined by the ROC curve (sensitivity = 90.5%; CI95%: 84.5%–94.7%).

Conclusion: Selection of CCP donors for multiple collections based on initial high nAb titers (≥1280) or BMI ≥ 30 kg/m² provides a simple strategy to
achieve higher quality in CCP programs. High IgG anti-NP levels can also be used as surrogate markers for high nAb screening.

**KEYWORDS**

blood component preparations, blood management, donors

## 1 | INTRODUCTION

COVID-19 convalescent plasma (CCP) has been used as a passive immunotherapy in severely affected COVID-19 patients.\(^1\)–\(^7\) Several different therapeutic approaches have also been proposed for treatment; however, randomized controlled studies (RCTs) are not supportive of their current adoption,\(^8\)–\(^10\) except for dexamethasone.\(^11\),\(^12\) Thus, although there is no definitive answer about the role of CCP therapy, and despite that the procedure is considered safe, there are still many drawbacks concerning its use,\(^13\) justifying further studies on this issue. Unfortunately, the CCP source is limited and not all donor candidates fulfill the defined requirements. Until definitive medical strategies for prevention (vaccines) or treatment (e.g., monoclonal antibodies blend)\(^14\) are effectively available, it is important to define additional strategies to increase CPP collection quality.

One of the supposed pathogenic causes of severe COVID is the extreme host’s immune response (possibly related to the patient’s nAb titer production). More recently, it was found that the ABO group correlates with disease risk (O as a protector).\(^15\) Little is known about the neutralizing (nAb) and anti-nucleocapsid (NP) antibodies (IgM, IgG and IgA) in CCP donors. From our published cohort, we detected a positive correlation between donor’s weight and nAb titers.\(^16\) Thus, we studied the role of ABO type and nAb titer measured at the first sample collection, donor’s weight and body mass index (BMI), concerning the kinetics of nAb and anti-NP, defining whether there is a group of CCP donors that could sustain high and prolonged nAb levels (≥100 days after onset of disease).

## 2 | MATERIAL AND METHODS

Previously, RT PCR-positive (nasopharyngeal swab) male convalescent patients (18–60 years, body weight > 55 kgs, and full clinical recovery ≥14 days, all with mild/moderate symptoms), were enrolled in serial blood sample collection for evaluation of longitudinal nAb titers and anti-NP antibodies (IgM, IgG and IgA), as part of a previously published cohort for a CCP plasma donation program.\(^16\) Procedures were carried out according to the national legislation.\(^17\),\(^18\) Individuals were invited for a longitudinal kinetics study, with an intended 100-day period after disease onset. The initial onset of symptoms was based on descriptions by participants in their first medical interview. The participants signed an informed consent form, and underwent a series of tests, namely: (a) NAb - detected by a cytopathic effect-based virus neutralization test (CPE-based VNT), with SARS-CoV-2 (GenBank: MT350282)\(^16\),\(^19\); (b) IgM, IgG and IgA anti-NP antibodies, detected by ELISA\(^20\); (c) ABO, RhD and ABO antibody titers (anti-A, anti-B and anti-A,B), detected at room temperature (RT) and by the human antiglobulin test (AHG), using the IH-1000 platform (Biorad, Switzerland).

Variable comparisons for parametric data were made using the t-test, Fisher’s test and ANOVA. For non-parametric data, we used the Mann–Whitney U test, Spearman’s correlation, Wilcoxon or Kruskal-Wallis test. The Bonferroni adjustment was employed whenever possible. The Kaplan–Meier equality for outcome was evaluated using the log-rank test. As an outcome, we defined a total of five collections or if a given sample had a nAb titer of ≤80. Hazard ratios were evaluated using Cox hazard regression. Values with \(p<.05\) were considered significant. Tests and figures were made using STATA v15 (College Station, TX), Surfer 8.04 (Golden, CO) and JMP 15.2.1 (Cary, NC) packages.

This study was approved by IRB and the Brazilian Commission on Ethics and Research (CONEP) under request CAAE: 30259220.4.2001.5461.

## 3 | RESULTS

There were initially, 149 candidates participating in the original cohort.\(^16\) The initial data demonstrated that 15/149 (10%) cases were both classified as heavy weight (≥91 kg) and high nAb titers (≥1280) group, with 11/15 (73.3%) belonging to A or AB groups and only four from O group. We then correlated whether ABO groups, divided into A-type (A/AB) and non-A (O/B), or O and non-O types had some role in the maintenance of high nAb. The initial nAb distribution, according to weight (kg)/BMI (kg/m\(^2\)) and A-type (A vs. non-A) is shown in Figure 1.

Of these 149 individuals, only 78 (52.3%) provided at least two serial samples for the longitudinal kinetics
study. As a control group for ABO tests, we evaluated 87 first-time blood donors who donated blood before the arrival of the COVID-19 pandemic in São Paulo (A = 30, O = 30 and B = 27; no AB was included). The demographics summary is shown in Tables 1 and S1. This cohort was capable of providing 316 samples (up to five serial collections) for nAb titers and anti-NP, as shown in Table S2.

Based on initial titers from our previous paper,\textsuperscript{16} where we found that approximately 1/3 of donors would not be accepted for CCP collection (titers ≤80); we arbitrarily decided to have two additional categories based on initial acceptable nAb titers, resulting in three ranks: low (nAb ≤80; i.e., donors that would not be accepted for CCP donation), medium (640 ≤ nAb ≥ 160), and high (nAb ≥ 1280), shown in Figures 2, S1 and S2. The parallel plot shows that initial low titers (Group 1; n = 12) kept a sustained low nAb titer, not recommending plasma donation. Group 2 (medium; n = 49) had modest decline in nAb titers and most sustained qualified levels for donation. Group 3 (high; n = 17) showed the most persistent nAb titer decline, but kept levels above the necessary for donation (≥160); considered the candidates able to provide the best CCP quality concerning nAb titers. The heat map gives an overview of the nAb kinetics.

For anti-NP (IgM, IgG and IgA), we transformed the absorption (DO 450 nm) by the corresponding cut-off (0.2 - IgM/IgA; 0.3 - IgG). Because of the non-Gaussian S/CO ratio distribution, we transformed it into a natural logarithm (Ln). Figure 3 shows that except for IgM, there was
no statistical significance between the initial and subsequent collections (Wilcoxon test). We have previously demonstrated\textsuperscript{16} that IgG S/CO $\geq 5$ detected 82.4\% of samples with nAb titer $\geq 160$, which was again demonstrated, as shown in Figure 4. The adjusted $R^2$ for the five collections was stable (0.2199 to 0.3970; all $p \leq .001$). The ROC curve showed a sensitivity of 90.5\% (CI95\%: 84.5\%–94.7\%) and specificity of 41.8\% (CI95\%: 34.8\%–49.17\%).

Because of a previously published correlation between ABO groups and nAb titers,\textsuperscript{21} we checked whether the ABO group (particularly the presence of the A antigen) or weight/BMI could explain this finding. The ABO antibody titers (anti-A, anti-B and anti-A,B, by RT/AHG) had no statistically significant difference between weight or height/BMI between the cohort and control, although there was a difference in age ($p = .0212$, two-sided t-test), as shown in Table 1. The cohort ABO group did not
follow a random ABO-type population distribution, as they were not representative of the local population \( \chi^2 = 23.97, p < .001 \)). Although the control ABO group was not balanced with the cohort’s, we considered it important to have these individuals for a reasonable comparison of titer’s distribution. Figure 5 shows no difference in ABO antibodies between the cohort and controls. We stratified the cohort’s ABO antibodies based on their initial nAb rank (low, medium or high), finding no statistical significance, except for anti-B (from A individuals, by AHG) between low x medium ranks (\( p = .015 \)). Thus, ABO antibodies were not associated with the maintenance of SARS-CoV-2 nAb titers.

Because of the low number of B and AB groups, we merged the cases into two main groups: with the presence of the “A” antigen (A and AB, \( n = 47, 60.3\% \)) or without it [(non-A: O and B groups; \( n = 31 (39.7\%) \)]. We found no association between nAb titers (ranks) and the presence of A antigen (groups A/AB; \( p = .068 \)) or absence of both A and/or B antigens (O vs. non-O; \( p = .087 \), Fisher’s exact test).

Based on BMI, individuals were classified as normal (\( n = 31; 39.7\% \)), overweight (\( n = 33; 42.3\% \)), or obese (\( n = 14; 18.0\% \)). Considering weight, they were arbitrarily divided into: a) light (<75 kg), b) medium (75–90 kg), and c) heavy (>90 kg). There was a good correlation between weight and BMI (\( n = 78; \text{adj } R^2 = 0.2338, p < .001 \)), with 27/28 cases (96.4%) from the heavy group also considered overweight/obese. A positive correlation between weight and nAb titers was previously demonstrated\(^{16}\), as confirmed here in Figure 1. The heavier the donor, the higher the nAb titer, associated with a prolonged, sustainable titer level for all collection steps, as shown in Figure 6.

Since the main objective was to investigate whether any variable could be associated with persistent high nAb titers throughout the study, we used the Kaplan–Meier analysis for “non-survival” using as final event outcome nAb titers \( \leq 80 \). The 316 samples spanned 4820 days of study. The “survival” estimates of all samples, different ABO groups, initial nAb titers, and individual’s weight/BMI are shown in Figure 7. In general, nAb titers \( \geq 160 \) had a median persistence of 77 days after the onset of symptoms (CI95%: 62–92 days); however, only 25% remained at this level after 100 days. There was no statistical significance concerning A-type and O-type groups (log-rank \( \chi^2 = 0.65, p = .420; \text{HR} = 1.3; \text{CI95%: 0.7–2.5, } p = .443 \), and log-rank \( \chi^2 = 0.01, p = .992; \text{HR} = 0.9; \text{CI95%: 0.5–1.9, } p = .992 \), respectively). There was a major difference concerning initial nAb titers; according to crescent ranks, the median survival time varied from 29 to non-reachable (NR) data (\( p < .001 \)), the log-rank \( \chi^2 \)

![Image](image_url)
FIGURE 4  Correlation between Ln nAb and IgG (S/CO) from the cohort for five consecutive collections. (A) Upper left: fitted line from ordinary least square regression, showing consistent results by the corresponding adjusted R^2 (0.3970; 0.2829; 0.3249; 0.2916 and 0.2199, respectively), with p < .0001, except for collection #5, where p = .001. (B) Upper right: ROC curve for nAb samples whose titer was ≥160. (C) Lower left: contour plot showing different clusters (each line level representing 2 samples); horizontal and vertical dash lines represent the assigned cut-off for Ln nAb titer ≥160 and IgG S/CO ≥5.0, respectively. (D) Lower right: tridimensional mesh graph showing the corresponding peaks according to the different clusters from Figure 4(C).

FIGURE 5  Left: Anti-A and anti-B (anti-A,B for O group) titers according to control (n = 87) or cohort (n = 72). * There are 6 individuals from AB group that were not included, since they do not produce anti-A or anti-B antibodies Right: ABO antibody titers according to first sample nAb titer’s rank: low (nAb ≤ 80; n = 11); medium (640 ≥ nAb ≥ 160; n = 46) and high (nAb ≥ 1280; n = 15) and ABO group. There was no statistical significance for ABO antibodies (anti-A, anti-B or antiA, B) either at RT or AHG between controls and cohort (Wilcoxon test); the only statistically significant titer was for anti-B (AHG) from A individuals between ranks 1 × 2 (≤80 × 640 ≤ nAb ≥ 160; p = .015, Wilcoxon test)
ranged from 7.7 to 35.1 ($p < .001$) and the HR from 5.9 to 164.9 ($p < .001$). The same was observed for weight; median survival time ranged from 33 × 68 × 92 days after onset of symptoms (all $p < .001$), log-rank $\chi^2$ from 7.5 to 9.6 ($p < .01$), and HR from 2.6 to 11 ($p < .01$). Additional details are shown in Figure 7. Again, ABO type was not an important predictor of high nAb titer, but weight (>90 kg), BMI (overweight/obese), and initial nAb titer ($\geq 1280$) at the first collection were the best variables for selecting CCP donors, maintaining sustainable and adequately high nAb titers (at least $\geq 160$) for a considerable number of days.

4 | DISCUSSION

The scope of this work was to establish potential and simple indicators to help build a better strategy for CCP collection from a CCP cohort, providing CCP units with better quality and higher nAb titers for longer periods ($\geq 100$ days) after the onset of symptoms. There are reports presenting evidence that nAbs decline to $>50\%$ of their peak level within 90 days$^{16,22}$; thus, it is important to define a “golden-period” for CCP collection and extend it as long as possible for collections.

Our cohort comprised mild/moderate cases in male and young patients, defined as cases that had not to be hospitalized or did not have the following conditions: criteria for severe pneumonia (defined by respiratory distress: oxygen saturation of 93% or less on room air, respiratory rate $> 30$ breaths/min, and/or arterial partial pressure of oxygen (PaO$_2$)/fraction of inspired oxygen (FiO$_2$) of 300 or less). This might correlate with different nAb responses observed in severe cases, recognized as having higher nAb titers and ability to inhibit in vitro RBD-ACE2 interaction,$^{23}$ as far as S1 and S2-nAbs are concerned. Nevertheless, we observed individuals who had mounted a high and prolonged humoral response (high nAb titers). Acute patients (especially older and male) have 7–8 fold higher nAb titers than asymptomatic or previously symptomatic individuals in the convalescent phase,$^{24–30}$ perhaps because of a higher viral load and exposure period. Whether a higher level equals to better protection is under evaluation, since patients who did not develop detectable nAbs were capable of recovering from the infection (perhaps by other immunological pathways). One study in asymptomatic individuals$^{27}$ showed lower virus-specific IgG levels than severe patients and both IgG and nAb have a higher trend to become negative at early stages (up to 40%); IgG was seen in the acute phase in 81.1% and 83.8%, respectively, in asymptomatic and symptomatic patients, but dropped to 60% in asymptomatic, while remaining positive in 87.1% of symptomatic cases. All individuals from our cohort had mild/moderate symptoms and no nAbs were detected in only 8.0% (12/149).

NAbS start their ascending levels 10–15 days after onset of disease$^{31}$ with a median peak time of 33 days (IQR 24–59 days),$^{32}$ vanishing thereafter,$^{22}$ which assured that our initial sample collection fell within the expected nAb peak time, as they were collected within a mean period of 32.9 ± 10.3 days (median = 30; range: 14–78) after the disease onset.
Figure 7 Kaplan–Meier analysis for collections that kept the nAb titers ≥160 (“survivors” – upper left) or based on: A-type (presence/absence of A antigen – A or AB/B or O; upper middle); O-type (O vs. non-O group; upper right); initial rank (nAb titer – lower left), weight (lower middle) or BMI (lower right). Considerable differences are observed among individuals with initial nAb titers (rank), heavy weight (kilos) or BMI (mainly obese – BMI ≥ 30), enabling BTS to focus on special CCP donors, whose potential serial plasma collection efficiency could be more advantageous. No differences were found concerning ABO group. NR, nonreachable.
NAbs are “antibody markers of immunity against reinfection after an acute viral infection has been cleared, with capacity to reduce viral infectivity by binding to defined viral surface particles and blocking the viral replication cycle before the virally encoded transcription or synthesis in the host cell.” It is yet not quite certain the main mechanism utilized by SARS-CoV-2 nAbs (preventing virion attachment, blocking cellular receptors, interference in cellular invasion: endocytosis, fusion or direct penetration), nor on what moment it turns the virions infectious to non-infectious or how many molecules are necessary to achieve this effect, or if two or more nAbs induce additive or synergistic effects. Chen et al.23 showed that specific S1 and S2 nAbs are produced in 80.7% and 40% of cases, respectively, with 7% depending on mutual collaboration between S1 and S2-specific nAbs for viral neutralization. The best NAbs are those targeted at the spike (S) protein, against the S1 subdomains (N-terminal domain – NTD and the receptor-binding domain – RBV, responsible for viral attachment). The S2 subdomain (mediating viral-cell membrane fusion) has not been defined as an important nAb target. Whether neutralization escape mutants (naturally or passively induced) might predominate in the future is unknown, though recently demonstrated, supporting that CCP should be transfused as high-tittered NAb pooled donated units, lowering the probability of natural immune selection by a single antibody source (including monoclonal antibodies). Two groups demonstrated the isolation of potent nAbs directed against the NTD, showing immunogenicity by RBV and NTD. Epitope mapping suggests that NAbs directed against the top of RBV have a stronger capacity of block ACE2 binding than those directed at the lateral side. Possibly, the early B-cell response to S protein is polyclonal and directed outside the RBV, with no NAb activity (explaining why NAbs have a peak at the third week of infection). Another study showed a correlation between nAb titers and anti-RBD, S1 and S2 levels. Evidence demonstrates that human CCP donations provide multiple NAb specificities, acting synergistically. However, due to the low-spike amino acid sequence homology among other coronaviruses, there is a very low cross-reactivity between SARS-CoV-2 CCP and SARS-CoV-1 or MERS-CoV, suggesting specific viral-species inhibition. Nucleocapsid proteins (N/NP) are known to elicit antibodies earlier than S1 or S2, where most of the NAbs are not targeted against these proteins. Probably, anti-NP antibodies are related to antibody-dependent cellular cytotoxicity (ADCC) clearance, and are important surrogates for CCP donor selection.

This cohort shows a declining trend in nAb titers, being evident that weight/BMI has a key role in maintaining higher/sustainable titers. In addition, individuals with initial lower nAb titers (≤80) rarely had subsequent rising titers and should not be considered ideal CCP donors. NAb titer fluctuation was seen in the medium group, but most of them kept adequate levels for CCP donation. Finally, high-titer individuals (≥1280) maintained high nAb titers, considered the best CCP candidates. If this feature is combined with higher weight (>90 kilos) or BMI ≥ 25, there is a great likelihood of achieving high titer donations for longer periods and extending the ideal “golden period” CCP collections, increasing CCP program’s efficiency.

The observed waning nAb does not mean that individuals lose their immune status or are susceptible to future re-infections; only their level is below the defined cut-off for donation. Even if nAb becomes undetectable in the future, it is likely that in re-exposure, both the memory and killer T cells will be engaged in secondary immunologic response. It is possible that CCP individuals presenting low nAb titers have recovered due to additional/complementary immune mechanisms (T-cell response, cytokines) other than classical humoral response. Studies have demonstrated that SARS-CoV-2 re-infection in previously immune animals was capable of inducing a robust protective immune response, showing that immunity is maintained and protective in subsequent exposures, although human protection might last only for a period of 1–2 years.

An outbreak of COVID-19 in a confined fishing vessel demonstrated no evidence of bona fide infection in the three members with previous nAb before vessel’s departure, whereas 103/117 previously negative became infected (>85%), demonstrating the protective importance of NAbs.

Because we have not studied female volunteers, we cannot confirm whether there is a gender difference, as described by others, although studies should be adjusted for weight or BMI. Advanced age is an important comorbidity, but given that our cohort has mainly younger individuals (36.7 ± 9.1, median = 36.5 years), it could have been a protective effect against weight/BMI higher risk, as seen in older patients.

There was no significant change in IgG/IgA levels in the study (Figures 3), except for a minor IgM rise, in contrast with previous data, where IgG and IgM dropped after the early convalescent period or the time between onset of symptoms and first sample collection. Both IgG1 and IgG3 anti-S1, -NP, -RBD, and -ECD (ectodomain) were detected as early as 4–7 days after infection, with predominant IgG1 nAb, remaining stable for 90 days, with IgA anti-NP and S1 barely detectable after this period. The persistent correlation between IgG anti-NP S/CO levels and nAb titer confirms our initial
findings\textsuperscript{16} and provides additional evidence as a good surrogate for high-tittered CCP, with well-defined clusters and good sensitivity, as evidenced by the ROC analysis (Figure 4). The low specificity should not be considered a problem because volunteers were already SARS-CoV-2 convalescents. The contour plot and 3D-density clusters reveal how clustered are the high IgG S/CO samples.

Although we have found an initial association between A-type donors and higher nAb levels, unfortunately this variable has not sustained significant correlation with high nAbs after a few weeks of study. The association between ABO group and viral infections has been described,\textsuperscript{51–55} including for SARS\textsuperscript{56} and COVID-19, where higher odds for A blood group were observed.\textsuperscript{57,58} A genome wide association study (GWAS)\textsuperscript{15} evaluated >8.5 million single-nucleotide polymorphisms and identified the rs657152 polymorphism (locus 9q34.2) related to ABO blood group locus, with higher risk in blood group A than in other blood groups (OR = 1.45; \( p = .0001 \)) and a protective effect in blood group O (OR = 0.65; \( p = .000001 \)). A meta-analysis evaluating seven studies with >7500 cases and >2.9 million controls\textsuperscript{59} indicated that SARS-CoV-2 positive individuals were more likely to be group A (OR = 1.23; 95%CI: 1.09–1.40), whereas group O was more protected (OR = 0.77; 95%CI: 0.67–0.88).

Arend\textsuperscript{60} recently proposed a different model for invasion, initially independent of the ABO group, via the formation of a hybrid A-like/Tn (T-nouvelle) structure (different from the specific blood group A epitope), acting as a functional bridge between the spike protein and host mobilization (via TMPRSS2), allowing viral invasion into the host's cell (initial invasion could be present both in group O and non-O individuals, via the host’s GalNac metabolism pathway). After invasion, SARS-CoV-2 newly formed virions from group O individuals replace this initial pathway by mucin-type fucosylation and synthesis of hybrid H-type antigen, unaffected by innate anti-A/Tn or anti-B/Tn isoagglutinins, with a secondary IgG response and reduced viral binding because it happens only via the hybrid-H-type antigen since anti-A or -B/Tn isoagglutinins are preserved, explaining the protector effect on group O individuals. However, group A, B, and AB hosts replace the intermediate A-like/Tn binding site by hybrid A or B allelic mucin types, with “physiological down-regulation” of anti-A or -B/Tn activities and decreased anti-A or anti-B isoagglutinins and/or clonal selection, resulting in higher pathogen-host contact and lower protective status in A, B, or AB hosts. This explanation seems plausible for the protector effect observed on O group individuals, and a schematic model of viral neutralization capacity based on ABO groups from potential naïve individuals is shown in Table S3.

Obesity is defined by WHO when BMI is \( \geq 30 \text{ kg/m}^2 \),\textsuperscript{62} associated with visceral adipose tissue expansion, inflammation by secretion of pro-inflammatory cytokines (TNF-\( \alpha \), IL-6), adipokines (leptin), and reduction of anti-inflammatory adiponectin,\textsuperscript{63,64} leading to a pro-inflammatory status, major oxidative stress, and impaired immune response. Some comorbidities are associated with COVID-19 severity (diabetes and hypertension)\textsuperscript{65,66} The renin-angiotensin-aldosterone system (RAAS) is recognized as having two opposing counter-regulatory arms and several molecules, whose function is to regulate fluid homeostasis, blood pressure, and cardioenal function. Further details about RAAS have been described elsewhere.\textsuperscript{67–70} The most important factor between COVID-19 and RAAS is angiotensin-converting-enzyme 2 (ACE2), converting AngI to Ang (1-9) and AngII to Ang (1-7) and acting as the SARS-CoV-2 receptor (also for SARS-CoV and NL63), via the RBD, with affinity 10-20-fold of SARS-CoV; ACE2 is bound on cellular membranes (soluble form in minute amounts in the bloodstream). The cellular Ang (1–7) receptor Mas is able to reduce pro-inflammatory cytokine release. Obesity is associated with reduced ACE2/Mas axis expression and RAAS over-activation (also related to severe SARS-CoV-2 infection), prolonged viral shedding in the adipose tissue, and excessive chronic cytokine release.\textsuperscript{71} It is plausible to consider that this mechanism might explain the higher and prolonged production of nAbs, via ACE2/Mas function downregulation\textsuperscript{72,73} and higher inflammatory activity. Other genetically induced mechanisms (HLA, reduction of NK-cell cytotoxic activity, reduced ADCC and imbalance between the pro-inflammatory leptin vs. anti-inflammatory adiponectin) could have additional roles.

We recognize as a study weakness not having included women, precluding findings about their nAb kinetics. However, the normal lower weight and adiposity, combined with reduced adipocyte ACE2 response to
the RAAS, also controlled by estrogens in females, might produce a different response. In addition, the cohort was possibly subject to a selection bias, as the obese male individuals were healthy, perhaps not representing the general obese population, demanding further studies. Finally, we understand the difficulties related to adequate nAb testing using VNT with real virus, which is difficult to operationalize and cannot be adopted on a large scale.

In conclusion, despite the CCP therapeutic value remains uncertain, we have found that the initial high nAb titer ($\geq 1280$), heavy (>90 kg), or overweight/obese individuals (BMI $\geq 25$) could bear high nAb titers for a prolonged period ($\geq 100$ days), enabling BTS to optimize the CCP recruitment scheme and reduce the number of low-titer CCP units. Naturally, we cannot define what would be the nAb behavior after 100 days; however, we consider that defining strategies targeting at special donors, able to donate for approximately CCP for 100 days, bearing high nAb titers is a reasonable contribution to improve the efficiency and quality over quantity of the CCP programs.

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CONFLICT OF INTEREST
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