Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Selection of plasma donors for the production of anti-SARS-CoV-2 immunoglobulin-based therapies: Strategies for quantitative antibody measurements

Bruno Deltreggia Benites, Carolina Costa-Lima, Fernanda Batista Rosa Pinto, Vitor Antonio da Costa, Adriana da Silva Santos Duarte, Audrey Basso Zangirolami, Emerson Clayton Amaro, Fabiana Granja, José Luiz Proenca-Modena, Sara Terezinha Olalla Saad, Marcelo Addas-Carvalho

ABSTRACT

Even after two years of the pandemic, a completely effective treatment against SARS-CoV-2 has not yet been established. Considering this fact and the emergence of successive new viral variants, the development of therapies based on natural polyclonal antibodies recovered from convalescent plasma remains relevant. This study presents a comparison between different methods of screening antibodies in samples of 41 individuals previously diagnosed with COVID-19. We found a significant correlation between Abbot Architect anti-SARS-CoV-2 IgG and Abbott Allinity SARS-CoV-2 IgG II Quantitative assay intensity of reactivity and neutralizing antibody (nAb) titers. Thus, we propose an initial antibody screening with IgG anti-N Abbott Architect test, with an index of, for example, > 3.25 or SARS-CoV-2 IgG II Quantitative Abbott Allinity assay > 137.65 AU/mL as good predictors of Nab ≥ 1:80. For the quantitative method, this threshold demonstrated a 100 % sensitivity and 80 % specificity, with 97.3 % accuracy. An interesting observation was the increase in the neutralizing activity of the anti-SARS-CoV-2 antibodies with the longest interval between the end of the symptoms and the collection, demonstrating that the delay in plasma collection does not affect the achievement of adequate nAbs levels. These results demonstrate the possibility of using faster and more widely available commercial serological tests with a good correlation with viral neutralization tests in culture, allowing for optimized large-scale donor selection, which will be of utmost importance for the development of therapies such as hyperimmune immunoglobulin.

1. Introduction

Even after two years of the emergence of the new coronavirus SARS-CoV-2 (COVID-19) pandemic, there is, to date, no proven specific therapy completely effective for this disease [1,2]. Although initially promising, the use of COVID-19 convalescent plasma (CCP) has not been effective in preventing disease progression for the majority of patients, as observed in the studies published to date [3,4]. Some factors that may explain the finding of discrepant and often frustrating results between studies may be the fact that, in most protocols, patients receive CCP from only 1 or 2 donors, in addition to the differences in antibody titers and their quantification methods, and different infusion times between the study protocols [5].

Our group and others, however, have demonstrated the benefits of CCP therapy for specific patient populations such as immunocompromised individuals [6,7], which warrants the continuation of clinical trials mainly in these particular contexts. Furthermore, the knowledge accumulated in the screening and collection of CCP may now be used in the production of hyperimmune immunoglobulin. In this sense, this product would consist of fractionation and purification of plasma pooled from hundreds of different donors, leading to infusion of greater amounts of polyclonal antibodies and potentially greater coverage for the different variants. Initial studies have demonstrated production feasibility [8,9], which would be less costly than other therapies based
on monoclonal antibodies. Recently published studies have also demonstrated the viral neutralizing power of this product, in addition to the impact on increased survival and lower possibility of disease progression [10,11]. A major advantage of this therapeutic modality regarding convalescent plasma, would be the greater diversity of antigenic epitopes reached, increasing the possibility of coverage against newer variants [12].

The assays for detecting anti-SARS-CoV-2 neutralizing antibodies (nAb) are generally performed with in-house techniques that involve the incubation of the serum to be tested in cultures of infected Vero cells, observed for inhibition of the cytopathic effect, usually after 72 h. These assays require adequate protection in level 3 biosafety laboratories, in addition to manipulation by highly trained professionals which, associated with the long time elapsed until the results, prevent large-scale use [13].

There are rapid point-of-care tests that use lateral flow devices by immunochromatographic method, which have the advantage of being performed quickly, taking from a few minutes to a few hours; however, they have the disadvantage of being single-use devices. The material used in the device can be whole blood, serum, or plasma. A whole blood sample is easy to acquire by finger puncture whereas the acquisition of a serum sample requires venipuncture and processing; however, serum is more sensitive (55 % versus 96 %) [14]. Automated laboratory tests for antibody detection, in turn, are based on ELISA (Enzyme-Linked Immunosorbent Assay or enzyme-linked immunosorbent assay) and CMIA (chemiluminescent immunoassay or chemiluminescence immunoassay) that are more sensitive than rapid tests and have the benefit of carrying out several analyzes simultaneously; however, they require specific laboratories and trained teams [13,15]. Therefore, despite detecting the presence of antibodies, whether there is a specific level of detection for these commercial assays that also corresponds to the antibody functional capacity, which would be of paramount importance for screening CCP donors, remains to be established [13].

The evaluation of antibodies in the CCP units donated in our center was carried out by viral neutralization assays in culture, due to their greater precision in the functional evaluation of the neutralizing power of the antibodies that will be transfused. However, considering the aforementioned difficulties, the focus of this secondary analysis was to evaluate whether results from simpler serological tests, such as chemiluminescence immunoassay (CMIA), corresponded to some degree with the neutralizing activity detected in the functional tests. This knowledge could then reflect on greater agility in the screening of convalescent plasma donors, optimizing the release for industrial fractionation or research, including the selection of large contingents of donors for the production of hyperimmune immunoglobulin.

2. Methods

2.1. Collection and qualification of plasma from convalescent individuals

Potential donors were invited through social media, the institutional website, and through pamphlets delivered to patients who had recovered from COVID-19 at the time of hospital discharge. Individuals who fulfilled the prerequisites for donation were scheduled for collection by apheresis. Eligibility criteria included age between 18 and 60 years, SARS-CoV-2 infection documented by any diagnostic test at the time of symptoms, such as viral detection in the airways by polymerase chain reaction (PCR), rapid immunochromatographic test, serology by immunoenzymatic or chemiluminescence method, being asymptomatic for 30 days, having had no pregnancy or abortion and fulfilling the other prerequisites for blood donation in Brazil. On the day of the procedure, after agreeing with the study and signing the informed consent form, blood samples were collected for the rapid immunochromatographic test, chemiluminescence serology and tests to assess the neutralizing power of antibodies by viral neutralization in culture. The study was approved by the National Research Ethics Commission (approval number 4.021.484).

2.2. Laboratory tests

2.2.1. Rapid immunochromatographic test

Rapid tests were performed with donor serum using lateral flow chromatographic immunoassay to detect IgG and IgM anti-SARS-CoV-2 antibodies (OnSite™ COVID19 IgG / IgM, CTK Biotech, CA, USA), according to the manufacturer’s instructions. According to the manufacturer, for IgM the sensitivity is 78.03 % and the specificity is 99.39 %; for IgG the sensitivity is 96.86 % and the specificity is 100 %; for the test as a whole, sensitivity is 96.86 % and specificity is 99.39 %.

2.2.2. Chemiluminescence (CMIA)

Chemiluminescence microparticle immunoassays (CMIA) were performed in donor serum for the qualitative detection of IgM antibodies against protein S (spike) and for the qualitative detection of IgG antibodies against SARS-CoV-2 protein N (nucleocapsid) (Abbott Architect SARS-CoV-2 IgM and Abbott Architect SARS-CoV-2 IgG, Abbott Laboratories, Ireland), performed according to the manufacturer’s protocol. For IgM, the cut-off value for a positive result is Index S/CO greater than or equal to 1.00. For IgG, the cut-off value for a positive result is Index S/CO greater than or equal to 1.40. According to the manufacturer, for IgM the sensitivity is 100 % after 15 days of symptom onset and the specificity is 99.56 %; for IgG, sensitivity is 100 % after 14 days of symptoms and specificity is 99.63 %.

Subsequently, we performed automated quantitative CMIA determination of IgG antibodies using the SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, Ireland). The test measures IgG antibodies to the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2 in the Allinity system, with a positivity cutoff of ≥ 50 AU/mL as defined by the manufacturer.

2.2.3. Culture viral neutralization assays

The evaluation of the neutralizing power of specific antibodies against SARS-CoV-2 was carried out by detecting the cytopathic effect of the virus in a viral neutralization test in culture. For this test, Vero cells were incubated with a mixture of virus and donor serum with different titers. The technique was standardized by the Laboratory for Studies in Emerging Viruses of the Institute of Biology at the State University of Campinas (LEVE-IB-UNICAMP), based on the methodology described by Nurtop and colleagues [16]. By this technique, Vero CCL-81 cells were seeded in plate wells for cell culture; and were then incubated with the serum to be evaluated, in several dilutions (1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560), and with a virus sample (at 10^5 TCID50/mL concentration). After 72 h, Vero cells were evaluated by reading the plates under an inverted optical microscope. The highest dilution of serum that protected more than 80 % of the cells from the cytopathic effect was termed the neutralizing antibody titer.

3. Compilation of clinical and demographic data and statistical analysis

At the time of plasma donation by apheresis, demographic and clinical data were compiled: sex, age, blood type, weight, height, body surface, duration of symptoms, intensity of symptoms according to the WHO ordinal severity scale [17], time between end of symptoms and collection and type of test used for the diagnosis of COVID-19. The statistical analysis was performed using the software R version 4.0.3 (2020–10–10) and pROC library functions for the elaboration of ROC curves; Kruskal-Wallis and Wilcoxon tests were used to assess differences between medians, Fisher test was used to compare distributions between groups and Spearman to check correlations.
4. Results

4.1. Clinical and demographic data of convalescent plasma donors

Forty-one apheresis collections of convalescent plasma were carried out in the center, from May to September 2020, from 39 donors, and 2 donors performed 2 collections on different dates. 58.6 % were female, the median age was 33 years (18–58); most donors (92.7 %) had mild symptoms, in grades 1–2 of the WHO ordinal severity scale, while 3 donors (7.3 %) required hospitalization and oxygen support. The median duration of symptoms was 13 days, and the median of days between the end of symptoms and collection was 61 days (10–106). The ABO typing distribution of donors was 41.5 % O RhD+, 39.1 % A RhD+, 14.6 % B RhD+, 2.4 % AB RhD+ and 2.4 % AB RhD-, and recruitment and summoning of donors took into account the demand and blood type of potential recipients. These data can be observed in detail in Table 1.

4.2. Qualification of the donated product: rapid test, chemiluminescence and neutralizing antibodies in culture and correlation between different techniques

The distribution of positivity in the different tests can be observed in Table 2.

A significant correlation between qualitative IgG CMIA and neutralizing antibody titers was found (Wilcoxon rank evaluation, p = 0.034). Patients with negative IgG CMIA had a median neutralizing antibody titer of 1:20 (0–640) while donors with positive IgG CMIA had a median of neutralizing antibodies of 1:160 (0–1280). In relation to quantitative IgG CMIA, there was statistical correlation with the qualitative method (p = 0.005) and strong correlation with nAbs titers (Rho = 0.583, P < 0.001). Correlation between detected levels of neutralizing antibodies (nAbs) and CMIA results can be observed in Fig. 1.

There was also a significant correlation between IgG detection by Rapid Test with IgM CMIA reading index (Wilcoxon rank evaluation, p = 0.01) and quantitative IgG (p < 0.001) and between positivity of IgM Rapid Test with IgM CMIA reading index (Wilcoxon rank evaluation, p < 0.001), qualitative IgG CMIA (p = 0.023) and quantitative IgG (p = 0.003). Serial titrations of the samples were performed, and a significant correlation was observed between the reading index IgG CMIA versus IgG CMIA titer, with Spearman Rho rank correlation coefficient = 0.974, with p = 0, and showing that the higher the reading index with pure sample, the higher the titration in which the sample remained positive. In this cohort, a statistically significant relationship between the results of positivity for CMIA IgM and: positivity for qualitative IgG (p = 0.022) and quantitative IgG (p < 0.001) was observed.

Table 1
Clinical and demographic characteristics of convalescent plasma donors.

| Characteristics                      | Median or (minimum–maximum) |
|--------------------------------------|----------------------------|
| Age (years)                          | Median 33 y.o. (18–58)      |
| Female                               | 24 donors (58.6 %)          |
| Male                                 | 17 donors (42.4 %)          |
| Blood type                           |                            |
| A+                                   | 17 (4.5 %)                 |
| O+                                   | 16 (39.1 %)                |
| B+                                   | 6 (14.6 %)                 |
| AB+                                  | 1 (2.4 %)                  |
| AB-                                  | 1 (2.4 %)                  |
| Weight (kg)                          | Median 72 kg (52–137)       |
| Height (m)                           | Median 1.70 m (1.50 – 1.90) |
| Body surface (m²)                    | Median 1.82 m² (1.54 – 2.59) |
| Duration of symptoms (days)          | Median 13 days (0–34)       |
| Need for hospitalization or oxygen therapy | 3 donors (7.3 %) |
| Ordinal Severity Scale (8 points)    |                            |
| 1–2 (outpatient)                     | 38 (92.7 %)                |
| 3–4 (inpatient, mild)                | 3 (7.3 %)                  |
| 5–8 (inpatient, severe)              | 0                         |
| Time between end of symptoms and collection | Median 61 days (10–106) |

Table 2
Results of the different tests used to qualify convalescent plasma in this study.

| Test                      | Results                      |
|---------------------------|------------------------------|
| IgM Rapid Test positive   | 17 donors (41.5 %)           |
| IgG Rapid Test positive   | 29 donors (70.7 %)           |
| CMIA IgM positive         | 22 donors (53.7 %)           |
| CMIA IgM index (S/CO)     | 1.26 (0.05–45.86)           |
| CMIA IgG positive         | 32 donors (78.1 %)           |
| CMIA IgG index (S/CO)     | 4.45 (0.01–8.78)            |
| CMIA Quantitative IgG (AU/mL) | 892.5 (1.4–12594.3)         |
| Neutralizing Antibodies Titer | 1:160 (0–1:1280)             |

ROC curves were performed in order to define possible values for CMIA qualitative reading intensity and quantitative IgG levels that could correlate with neutralizing antibody titers equal or above 1:80. In fact, the reading intensity threshold 3.26 for qualitative IgG correlates with neutralizing antibody titers ≥ 1:80, with 71.9 % sensitivity; specificity 100 % accuracy 75.7 % positive predictive value 100 % negative predictive value 35.7 %, p = 0.005. (Fig. 2A). Regarding quantitative IgG tests, the threshold 137.65 AU/mL for detecting nAbs ≥ 1:80 demonstrated 100 % sensitivity and 80 % specificity, with 97.3 % accuracy, positive predictive value of 97.3 % and negative predictive value of 100 %, p < 0.001. (Fig. 2B).

4.3. Correlations between antibody detection and clinical and demographic characteristics of donors

There was a significant correlation between the reading index for IgM in CMIA with blood type B RhD+, according to the Wilcoxon rank test, with p = 0.023; therefore, B RhD+ donors were observed to have a higher IgM reading index by the CMIA test, compared to O RhD+ and A RhD+ donors. However, due to the low number of blood type B donors in this cohort, no conclusions could be drawn, and no ABO typing correlation was detected in the IgG quantitative or qualitative CMIA reading indexes, in the rapid immunochromatographic test or in the neutralizing activity assay.

Likewise, a significant positive correlation between the interval of days between the end of symptoms and the titer of neutralizing antibodies was identified (Spearman’s rank correlation coefficient, Rho = 0.329, with p = 0.036) as well as a significant correlation between the interval of days between the end of the symptoms and the collection with IgM positivity in CMIA (Wilcoxon, p = 0.045). Therefore, in this cohort, the interval between the end of symptoms and product collection was relatively long (median 60 days, ranging from 10 to 106), but this increase in the interval of days until collection did not impair the neutralizing power of the antibodies.

No significant correlations were found between the results of the tests and other clinical and demographic characteristics of the donors, such as sex, age, body surface, severity of symptoms and duration of symptoms.

Two donors made two donations on different occasions. Both were female; the first had mild symptoms (grade 2 of severity) for 15 days and made the first donation 30 days after the end of the symptoms, presenting rapid IgM and IgG negative test, CMIA IgM negative and IgG positive (S/CO 5.34) and neutralizing antibodies in 1:80 titer. The second donation occurred 61 days after the end of the symptoms and on that occasion, she presented a rapid immunochromatographic negative IgM and negative IgG test, negative CMIA IgM and positive IgG (S/CO 3.69) and neutralizing antibodies in title 1:320. The second donor had mild symptoms (grade 2 of severity), for 14 days and made the first donation 49 days after the end of the symptoms and presented a rapid immunochromatographic test IgM negative and IgG positive, CMIA IgM negative and IgG positive (S/CO 2.19) and neutralizing antibodies in title 1:80. The second donation took place 69 days after the end of the symptoms and presented a rapid negative IgM and positive IgG test, negative CMIA IgM and negative IgG (S/CO 1.35) and neutralizing...
antibodies in title 1:160. Therefore, despite the reduction in the index or even the abolition of IgG titer by the CMIA, there was an increase in the neutralizing antibody titer with the longest interval of time, in both donors.

Four donors tested positive at the time of symptoms, met the screening criteria, but had no antibody titer with neutralizing activity detected in the culture tests. Three of them had mild symptoms and one of them was asymptomatic and, therefore, the possible hypotheses would be a false-positive diagnostic test, or seroconversion with low antibody titers and with no detected neutralizing power.

5. Discussion

In our series, an increase in the neutralizing activity of the anti-SARS-CoV-2 antibodies was observed with the longest interval between the end of the symptoms and the collection. This data was also confirmed in the direct observation of 2 donors who underwent plasma collection on 2 occasions, with an increase in the neutralizing antibody titers. Other studies also demonstrated stable antibody levels remaining for as long as 8 months [18,19]. This data is encouraging for the serial collections of CCP and for the development of other plasma-based therapies, such as hyperimmune immunoglobulin, as the delay in the collection of plasma does not affect the achievement of a product with good nAbs levels.

Despite the maintenance of the identification of high titers of neutralizing antibodies over time, this is not necessarily the issue for commercial serological assays, where a progressive decline in their detection capacity could be expected. In our study we found a significant correlation between Abbott Architect anti-SARS-CoV-2 IgG and SARS-CoV-2 IgG II Quantitative assay intensity of reactivity and neutralizing antibody titers. Analyzing the ROC curves, the S/CO reading > 3.26 for qualitative CMIA or 137.65 AU/mL for quantitative CMIA correlated with neutralizing antibody titers ≥ 1:80. For the quantitative method, this threshold demonstrated a 100 % sensitivity and 80 % specificity, with 97.3 % accuracy. Thus, these automated tests could represent a quick and viable alternative to estimate the neutralizing activity of antibodies in culture and guide the choice of donors, optimizing workflow mainly considering the potential use in the recruitment of donors for large scale immunoglobulin production. These maintained positivity findings support the decision, for example, to screen donors despite no previous history of COVID-19.

In a study carried out in two other Brazilian hospitals, a correlation was also detected between ELISA indexes IgG, IgM and IgA (all anti-N),
Fig. 2. ROC curves for correlation between commercial serological assays and neutralizing antibody titration in cell culture. (A) Correlation of qualitative chemiluminescence assay (CMIA) read index with neutralizing antibody titers ≥ 1:80. The reading index threshold of 3.26 is correlated with neutralizing antibody titers ≥ 1:80 (p = 0.005, sensitivity = 71.9 %, specificity = 100.00 %, accuracy = 75.7 %, positive predictive value = 100 %, negative predictive value = 35.7 %). (B) Correlation of quantitative IgG CMIA test with neutralizing antibody titers ≥ 1:80. The threshold of 137.65 AU/mL is correlated with neutralizing antibody titers ≥ 1:80 with sensitivity = 100 %, specificity = 80.00 %, accuracy = 97.3 %, positive predictive value = 96.9 %, negative predictive value = 100 %, p < 0.001).
with neutralizing antibody titers; in this study, a cutoff value of the ELISA IgG test of S/CO \( \geq 5 \) was proposed to predict donors with neutralizing antibody titers \( \geq 1:160 \), and this serological test could be a substitute when the viral neutralization technique in culture is not available [20]. Anti-S antibodies are believed to be the main responsible agent for viral neutralization, however in our work, as well as in the others mentioned above, serological IgG anti-N tests were evaluated, with evidence of correlation with neutralizing activity of the antibody; therefore, we believe that the anti-N IgG test may also be useful for estimating neutralizing activity. This finding is further supported by the fact that the quantitative tests used in our study evaluate IgG anti-S, which also correlated with the IgG anti-N test and with the titers of neutralizing antibodies.

Considering the greater accuracy presented by the anti-S test, this test should be preferred whenever possible over the anti-N test, which is also in accordance with the latest FDA recommendations released in December 2021. (https://www.fda.gov/media/141477/download) Although this guide recommends a much higher antibody titer than our initial proposition (\( \geq 1280 \) AU/mL), it should be noted that this lower cutoff in our study was capable of demonstrating a reasonable titer of functionally neutralizing antibodies (above 1: 80). In the case of hyperimmune immunoglobulin, we must consider the greater condensation of antibodies in smaller volumes, and origin from a pool of donors, which would render the use of lower cut-off points in this specific case plausible, this should become more evident with the progress of further studies.

One concern is to what extent these results can be extrapolated to new variants of concern, as our analyses were performed in 2020. As mentioned earlier, a major advantage of hyperimmune immunoglobulin over convalescent plasma is its origin from a mixed pool of donors, potentially increasing their antigenic coverage. And indeed, results from more recent studies have pointed to this more reassuring conclusion, demonstrating a greater recognition of multiple antigenic sites spanning the entire spike protein, when aliquots of hyperimmune immunoglobulin were tested against ordinary convalescent plasma [21]. This coverage diversity may eventually prove even greater considering the effects of vaccination [22].

There was no significant correlation between clinical and demographic data with the antibody tests in our study, contrary to what was exposed in some studies, which revealed a correlation of neutralizing antibodies with symptom severity, duration of symptoms, hospitalization, age and male sex [18,23–26]; the Brazilian study by Wendel et al. further showed a correlation with greater body mass [20], however, none of these variables showed an impact on our sample, which may be due to the relatively small number of donors. Thus, our results reaffirm the possibility of transitioning the screening of donors of convalescent plasma from neutralization tests in cell cultures to commercial serological tests. In this sense, our proposal would be to use the CMIA IgG anti-S SARS-CoV-2 IgG II Quantitative Abbott Allinity assay with \( > 137.65 \) AU/mL as the initial screening to predict donors with \( \geq 1:80 \). This titration could then be confirmed by functional tests, with however, potentially high titers guaranteed in the product. There is also the possibility of this screening with anti-N Abbott Architect test, with a S/CO threshold \( > 3.25 \); but given the FDA recommendation for the use of anti-S tests, this alternative should be reserved only for situations where anti-S tests are not readily available. Our results further demonstrate the feasibility of a 30-to-60-day donation interval between the end of the symptoms and the donation, in addition to serial collections, when necessary. We found no clinical-demographic profile of individuals recovered from COVID-19 in our sample that could be a focus of concentration of potential fundraising efforts from convalescent plasma donors. It should also be noted that these analyzed samples correspond to a period prior to the emergence of vaccines, and therefore their interference in the tests was not evaluated in this specific study. Some limitations of this study included the small sample of cases, in addition to the analysis of tests of only one commercial brand of re-agents. However, a great differential of the study is the demonstration of the possibility of using a platform that can be used in parallel with the usual serological screening of blood donors, which allows the selection of potential donors of material for the production of plasma-based therapies, regardless of their previous history of SARS-CoV-2 infection.

Funding

This work was supported by Grant from the National Council for Scientific and Technological Development (CNPq, #401977/2020), and STOS by the University Fund for Support to Research (FAEPEX #519292).

CRediT authorship contribution statement

BDB, CCL and MAC designed the study, FBRP, VAC, ASDS, ABZ, ECA and FG participated in donors recruitment and selection, and conducted laboratory assays, BDB, JLPMS, STOS and MAC analyzed results and prepared the manuscript.

References

[1] Zou H, Yang Y, Dui H, Xiong Y, Wang J-Q, Lin L, et al. Recent updates in experimental research and clinical evaluation on drugs for COVID-19 treatment. Front Pharmacol 2021;12:732403.

[2] Sun Y, Yang H, Ji W, Wu W, Chen S, Zhang W, et al. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses 2020;12:4.

[3] Menichetti F, Popoli P, Puopolo M, Spiga Alegiani S, Tiseo G, Bartoloni A, et al. Effect of high-titer convalescent plasma on progression to severe respiratory failure or death in hospitalized patients with COVID-19 pneumonia: a randomized clinical trial. JAMA Netw Open 2021;4(13):e2136246.

[4] Axfors C, Janiaud P, Schmitt AM, Van’t Hooft J, Smith ER, Haber NA, et al. Association between convalescent plasma treatment and mortality in COVID-19: a collaborative systematic review and meta-analysis of randomized clinical trials. J Infect Dis 2021;21(1):1170.

[5] Cheng ZJ, Li B, Zhan Z, Zhao X, Xue M, Zheng P, et al. Clinical application of antibody immunity against SARS-CoV-2: comprehensive review on immunonas and immunotherapy. Clin Rev Allergy Immunol 2022.

[6] Ribeiro LC, Benites RD, Ulaf RG, Nunes TA, Costa-Lima C, Addas-Carvalho M, et al. Rapid clinical recovery of a SARS-CoV-2 infected common variable immunodeficiency patient following the infusion of COVID-19 convalescent plasma. Allergy, Asthma Clin Immunol 2021;17(1).

[7] Sankar PW, Klassen SA, Ford SK, Senese KA, Wiggins CC, Bostrom BC, et al. Use of convalescent plasma in COVID-19 patients with immunosuppression. Transfus [Internet] 2021;61(8):2503–11. https://doi.org/10.1111/trf.16525.

[8] Ali S, Uddin SM, Ali A, Anjam F, Ali R, Shalim E, et al. Production of hyperimmune anti-SARS-CoV-2 intravenous immunoglobulin from pooled COVID-19 convalescent plasma. Immunotherapy 2021;13(5):397–407.

[9] Vandeberg P, Cruz M, Díez JM, Merritt WK, Santos B, Trukawinski S, et al. Production of anti-SARS-CoV-2 hyperimmune globulin from convalescent plasma. Transfusion 2021;61(6):1705–9.

[10] Díez JM, Romero C, Cruz M, Vandeberg P, Merritt WK, Pradenas E, et al. Anti-severe acute respiratory syndrome coronavirus 2 hyperimmune immunoglobulin demonstrates potent neutralization and antibody-dependent cellular cytotoxicity and phagocytosis through N and S protein. J Infect Dis 2022;225(6):938–46.

[11] Ali S, Uddin SM, Shalim E, Sayeed MA, Anjam F, Saleem F, et al. Hyperimmune anti-COVID-19 IVIG (C-IVIG) treatment in severe and critical COVID-19 patients: A phase I/II randomized control trial. ClinicalMedicine 2021;36:109926.

[12] Zubia FT, Bellucci S, Grubbs G, Goulding H, Khurana S. Neutralisation of circulating SARS-CoV-2 delta and omicron variants by convalescent plasma and SARS-CoV-2 hyperimmune intravenous human immunoglobulins for treatment of COVID-19. Ann Rheum Dis 2022.

[13] CDC Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings.

[14] Costa SF, Buss L, Espinoza EPS, Vieira JM, de Oliveira da Silva LC, de Souza MM, et al. Performance of a qualitative rapid chromatic immunoassay to diagnose COVID-19 in patients in a middle-income country. J Clin Virol 2020;131 (August):9–12.

[15] Kohner N, Westhaus S, Rühl C, Ciesek S, Rabenau HF. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. J Clin Virol 2020;129(May): 104480.

[16] Nurtop E, Villarroel PMS, Pastorino B, Ninove L, Drexler JF, Roca Y, et al. Correction to: combination of ELISA screening and seroneutralisation tests to expedite Zika virus seropositive studies. Virilol 2019;16(1):4–9 (Virology Journal (2018) 15 (192):DOI: 10.1186/s12985-018-1105-5).

[17] World Health Organization. WHO R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis. World Heal. Organ. 2020;(February 18, 2020, Geneva, Switzerland):1–9.
[18] Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral immune response to SARS-CoV-2 in Iceland. N Engl J Med 2020; 1–11.

[19] Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021; 371(6529):eabf4063.

[20] Wendel S, Kutner JM, Machado R, Fontão-Wendel R, Bub C, Pachini R, et al. Screening for SARS-CoV-2 antibodies in convalescent plasma in Brazil: preliminary lessons from a voluntary convalescent donor program. Transfusion 2020;60(12):2938–51.

[21] Tang J, Lee Y, Ravichandran S, Grubbs G, Huang C, Staut CB, et al. Epitope diversity of SARS-CoV-2 hyperimmune intravenous human immunoglobulins and neutralization of variants of concern. iScience 2021;24(9):103006.

[22] Rojas-Jiménez G, Solano D, Segura A, Sánchez A, Chaves-Araya S, Herrera M, et al. In vitro characterization of Anti-SARS-CoV-2 intravenous immunoglobulins (IVIg) produced from plasma of donors immunized with the BNT162b2 vaccine and its comparison with a similar formulation produced from plasma of COVID-19 convalescent donors. Front Med Technol 2021;3:772275.

[23] Jungbauer C, Weseslindner L, Weidner L, Gansdorfer S, Farcket MR, Gschaider-Reichhart E, et al. Characterization of 100 sequential SARS-CoV-2 convalescent plasma donations. Transfusion 2020;August:1–5.

[24] Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature 2020;584(7821):437–42.

[25] Tan W., Lu Y., Zhang J., Wang J., Dan Y., Tan Z., et al. Viral Kinetics and Antibody Responses in Patients with COVID-19. 2020;

[26] Luchsinger LL, Ransegna B, Jin D, Muecksch F, Weisblum Y, Bao W, et al. Serological assays estimate highly variable SARS-CoV-2 neutralizing antibody activity in recovered COVID19 patients. medRxiv 2020;58(12):1–14.