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BRIEF REPORT

TRANSFUSION

Characterization of 100 sequential SARS-CoV-2 convalescent plasma donations

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Abstract

Background: Transfusion of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) convalescent plasma is a promising treatment for severe coronavirus disease 2019 (COVID-19) cases, with success of the intervention based on neutralizing antibody content. Measurement by serologic correlates without biocontainment needs as well as an understanding of donor characteristics that may allow for targeting of more potent donors would greatly facilitate effective collection.

Study Design and Methods: One hundred convalescent plasma units were characterized for functionally active SARS-CoV-2 neutralizing antibodies, as well as for SARS-CoV-2 binding antibodies, with the intention to establish a correlation between the functionally more relevant neutralization assay and the more accessible enzyme-linked immunosorbent assay (ELISA). Donor demographics such as COVID-19 severity, age, and sex were correlated with antibody titers.

Results: A mean neutralization titer 50% of 230 (range, <8-1765) was seen for the 100 convalescent plasma units, with highly significant ($P < .0001$) yet quantitatively limited ($R^2 = 0.2830$) correlation with results of the ELISA. Exclusion of units with particularly high titers (>500) from analysis improved correlation ($R^2 = 0.5386$). A tendency of higher-titer plasma units from donors with increased disease severity, of advanced age, and of male sex was seen, yet the functional relevance of this difference is questionable.

Conclusion: The ELISA-based correlation to neutralization titer enabled a threshold proposal that could be used to eliminate lower-titer units from the

Abbreviations: COVID-19, coronavirus disease 2019; CP, convalescent plasma; ELISA, enzyme-linked immunosorbent assay; MERS-CoV, Middle East respiratory syndrome coronavirus; MNT, microneutralization test; NT_{50}, microneutralization titer; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; $TCID_{50}$, tissue culture infectious dose 50%; WHO, World Health Organization.

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clinical supply for COVID-19 treatment. Disease severity may be associated with the development of higher titers of neutralizing antibodies, although larger case numbers will be needed for additional confirmation.

KEYWORDS

convalescent donor selection, COVID-19, ELISA correlation, severe acute respiratory syndrome coronavirus 2, virus neutralization

1 | INTRODUCTION

The transfusion of convalescent plasma (CP) for the treatment of infectious diseases has been in medical use for more than 100 years and was found worthy of the first Nobel Prize in Medicine, awarded to Emil von Behring in 1901. Based on some earlier successes of CP therapy with the other two zoonotic coronaviruses that have caused large numbers of severe respiratory infections in humans during the past 2 decades, that is, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV),[1,2] its use was quickly initiated after the emergence of the now pandemic SARS-CoV-2 in late 2019.[3–5]

For the identification of potential SARS-CoV-2 CP donors, different analytical approaches are possible, and some have been suggested.[6,7] These include detection of the virus by nucleic acid testing during acute infection and, after convalescence, the detection of virus-binding antibodies by enzyme-linked immunosorbent assay (ELISA), lateral flow, or Western blot assays, or the detection of functional antibodies by virus microneutralization test (MNT).[8] Only an MNT provides for a functional correlate of antiviral efficacy, and this assay is therefore still considered the "gold standard" in serologic testing, yet testing needs to be conducted under Level 3 biosafety containment restrictions, requires a few days to generate results, and is more complex to perform. It would thus greatly facilitate testing for SARS-CoV-2 antibodies in potential plasma donors and their donations if a more simple and easily scalable binding assay could be correlated against functional antibody activity as determined by MNT. In addition, CP collections might become more effective if donor characteristics were understood to correlate with higher functional antibody activity, such asdisease severity, days between disease onset and plasma collection, or age and sex of donors.

The Austrian Red Cross Blood Service for Vienna, Lower Austria, and Burgenland initiated the collection of virus-inactivated CP by plasmapheresis at the Vienna Blood Centre. Here, we report the characterization of the first 100 CP units collected for functionally active neutralizing antibodies by MNT as well as by a binding antibody assay.

2 | MATERIALS AND METHODS

2.1 | Collection and virus inactivation of CP

All CP donors had polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infections. Plasma was collected at least 28 days after the end of clinical symptoms in concordance with all applicable legal requirements and relevant recommendations. The donors signed an informed consent regarding CP and special testing.

The plasma was collected using an automated blood collection system (Trima Accel) apheresis version 7.0 devices (Terumo; Eschborn, Germany) with Trima Accel MultiPlasma sets (Ref. 82 700). The collection volumes ranged from 440 to 650 mL adapted to body weight of the donors. Samples of each donation were investigated for SARS-CoV-2 antibody titers by neutralization assay and ELISA (Anti-SARS-CoV-2 ELISA Assay [IgG]; Euroimmun, Lübeck, Germany).

CP was pooled (at least two plasmas per pool) based on the neutralization assay titer results yielding for final antibody titers of 300 or greater in the therapeutic units. Subsequently, the pooled plasma was pathogen inactivated with a processing set for plasma (Intercept; Cerus Europe, Amersfoort, Netherlands; Ref. INT3104B-1,) with the Intercept UVA-Illuminator (Ref. INT100). The final volume of the therapeutic CP units was adjusted to 200 mL. At the time of submission of this paper, 12 patients had received 27 units (ranging from 1 to 3 units per patient, median 2.0 units) in Vienna and Lower Austria.

2.2 | SARS-CoV-2 infectivity assay and testing of CP for neutralizing antibodies

SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 was kindly provided by the Charité Universitätsmedizin,

Institute of Virology, Berlin, Germany; European Virus Archive 026V-03883. Vero cells (American Type Culture Collection CCL-81), sourced from the European Collection of Authenticated Cell Cultures (84113001) were cultured in tissue culture–Vero medium supplemented with 5% fetal calf serum, L-glutamine (2 mM), nonessential amino acids (1×), sodium pyruvate (1 mM), gentamicin sulfate (100 mg/mL), and sodium bicarbonate (7.5%). For determination of SARS-CoV-2 infectivity by tissue culture infectious dose 50% ($TCID_{50}$) assay, 8-fold replicates of serial half-log sample dilutions were incubated on cells for 5 to 7 days before any cytopathic effect was assessed microscopically. Virus concentrations were calculated according to the Poisson distribution.

For virus microneutralization assays, CP samples were serially 1:2 diluted and incubated with 100 $TCID_{50}$ of SARS-CoV-2 per well. The samples were subsequently applied onto Vero cells seeded in tissue culture microplates and incubated for 5 to 7 days, when cells were evaluated for the presence of a cytopathic effect and the SARS-CoV-2 microneutralization titer (NT_{50}), that is, the reciprocal sample dilution resulting in 50% virus neutralization, was determined with use of the Spearman-Kärber formula.

2.3 | Testing of CP for binding antibodies by ELISA

Anti-SARS-CoV-2 IgG antibodies were quantified by SARS-CoV-2 IgG ELISA (Euroimmun; Lübeck, Germany) with a Euroimmun Analyzer I with the protocol recommended by the manufacturer. IgG concentration is given as a ratio, calculated with optical density and an internal calibrator provided by the manufacturer. The Euroimmun SARS-CoV-2-IgG ELISA uses the recombinant structural protein (S1 domain) of the spike protein as antigen. Serum samples were tested in serial dilutions of 2-fold steps from 1:5 to 1:1280. When the IgG ratio in a sample still exceeded 1.1 (the cutoff recommended by the manufacturer) at the dilution of 1:1280, further dilutions (1:5120 to 1:20480) were performed for an accurate interpolation.

3 | RESULTS

3.1 | CP characterization by neutralization assay

The first 100 CP units collected by the Austrian Red Cross Blood Service for Vienna, Lower Austria, and Burgenland were tested for functionally active neutralizing antibodies by MNT. While the absolute numbers generated by any such assay need to await availability of international reference standards before any meaningful comparison becomes possible, on average the CP units had a high neutralizing antibody titer, with a mean NT_{50} of approximately 230 (Figure 1A: NT_{50} histogram, median, 100; 25th percentile, 54; 75th percentile, 231; range, <8-1765). Somewhat surprisingly for individuals who had successfully recovered from PCR-confirmed SARS-CoV-2 infection, neutralizing antibodies were undetectable in 1 plasma unit, and for a total of 6 units (6%) the NT_{50} was below 23, that is, inverse $\log_2$ NT_{50} 4.5 (Figure 1A).

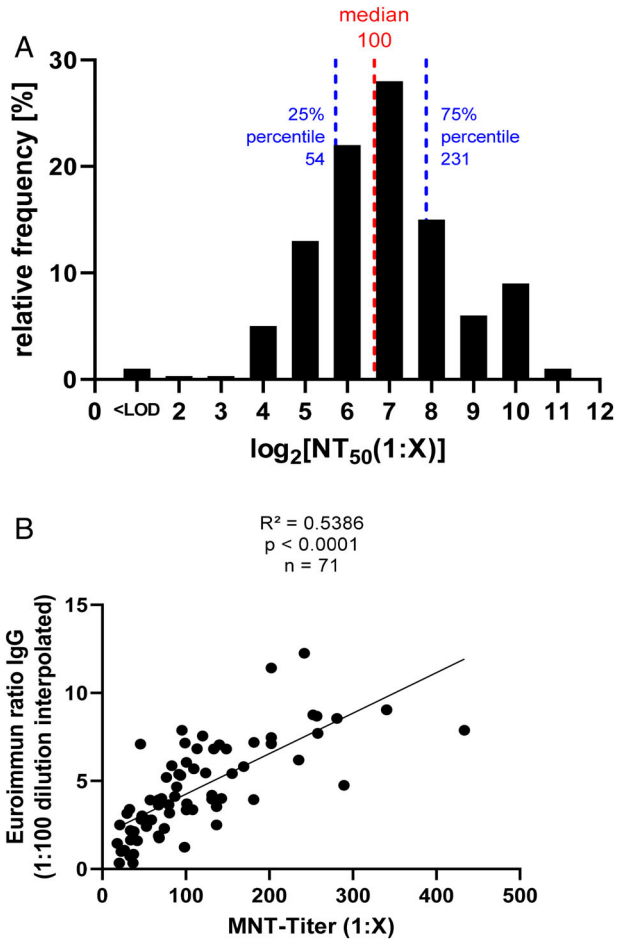

FIGURE 1 Characterization of 100 convalescent plasma donations collected at the Vienna Blood Centre for (A) SARS-CoV-2 neutralizing antibody content, reported as $\log_2$ microneutralization titers 50% (X) plotted against the relative frequency (percent) of occurrence. B, NT_{50} titers were correlated against Euroimmun IgG ELISA signal ratios obtained for the same donations [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | CP characterization by binding assay

A binding antibody assay was also performed on these CP units, with the intention to establish a correlation

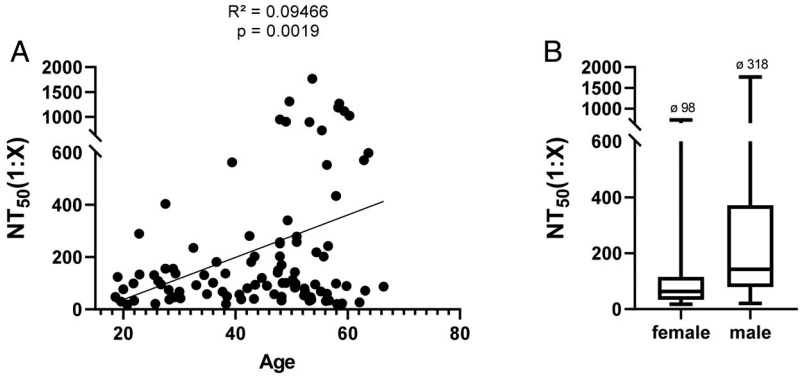

FIGURE 2 COVID-19 convalescent plasma donor demographics illustrating (A), correlation of SARS-CoV-2 neutralizing antibody titers (NT_{50}) with donor age and (B) difference in NT_{50} between female and male donors

between the functionally more relevant MNT and the more accessible ELISA, as well as an ELISA threshold to allow for the elimination of subpotent units and the qualification of CP units for transfusion. For the totality of the samples for which valid results from both assays were available (N = 83), the correlation between results from the MNT and the ELISA was highly significant ($P < .0001$), yet quantitatively limited ($R^2 = 0.2830$). With the intention of using the ELISA primarily for establishing a lower threshold for CP units to issue them for treatment of COVID-19 cases, the analysis was repeated excluding particularly high NT_{50} titers (>1:500, N = 12 / 14.5%), which improved the correlation ($R^2 = 0.5386$; Figure 1B). Using the Euroimmun ELISA 1.1 cutoff for positivity to qualify units for transfusion, 6 units (7.2%) with an average NT_{50} of 29 would have been excluded, with a corresponding increase of the mean NT_{50} of all collected plasma units from 233 (N = 83) to 249 (N = 77). A final verdict about whether this cutoff is suitable for use of CP in the treatment of COVID-19 will have to await an evaluation of clinical efficacy in correlation to these antibody measurements.

3.3 | CP characterization by donor demographic

For the targeted collection of high-antibody-titer CP units, it would be helpful to understand donor characteristics that might correlate with higher antibody potency; for example, it has been suggested that increasing disease severity may result in the development of higher antibody titers.[9] Of the 100 plasma donors, 90 were classified into World Health Organization (WHO) disease severity scores of 1 and 2,[10] with an average NT_{50} of 208, vs only six donors with disease severity scores of 3 to 6, who had a mean NT_{50} of 696. While these results may seem to support the notion of higher titers with increased disease severity, the numbers of CP donors with higher WHO scores in this study are too low to determine significance. For CP donor age (Figure 2A), the NT_{50} was significantly correlated ($P = .0019$), yet with little predictive value ($R^2 = 0.09466$), and targeting advance age donors for CP collection may therefore not be very effective. On average, the CP collected from male donors (N = 61) had a significantly higher mean NT_{50} than from female donors (N = 38), yet whether the mean titer difference of 220 has any functional relevance is questionable (Figure 2B).

4 | DISCUSSION

While functional testing for SARS-CoV-2 neutralizing antibodies would be desirable for CP units before issuing them for treatment of COVID-19, biosafety requirements and considerations around assay duration and complexity make this approach impractical. An adequate pooling strategy of CP may level out variations of antibody titers and quality in the therapeutic units. Here, we have established an ELISA-based correlate to the MNT, with a threshold proposal that could be used to eliminate lower-titer units from the clinical supply for COVID-19 treatment. Disease severity may be associated with the development of higher titers of neutralizing antibodies, although larger case numbers will be needed for a final conclusion. And while age and sex are significantly correlated with MNT antibody levels, the differences are small and thus probably not helpful for CP donor targeting.

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CONFLICT OF INTEREST

M.R.F., E.G.-R., and T.R.K. are employees of Baxter AG, Vienna, Austria, now part of the Takeda group of companies. M.R.F. and T.R.K. have Takeda stock interest. All other authors declare no conflict of interest.

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