

Running Title: SARS-CoV-2 convalescent plasma

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

Authors: Christof Jungbauer^{1*}, Lukas Weseslindtner^{2*}, Lisa Weidner¹, Simon Gänsdorfer¹,
Maria R. Farcet³, Eva Gschaider-Reichhart³ and Thomas R. Kreil³

* CJ and LW contributed equally

Affiliations:

¹ Austrian Red Cross, Blood Service for Vienna, Lower Austria and Burgenland, Vienna, Austria

² Center for Virology, Medical University of Vienna, Vienna, Austria

³ Global Pathogen Safety, Baxter AG, a Takeda Company, Vienna, Austria

Abstract

Transfusion of SARS-CoV-2 convalescent plasma is a promising treatment for severe COVID-19 cases, with success of the intervention based on neutralizing antibody content. Measurement by serological correlates without biocontainment needs, and an understanding of donor characteristics that may allow for targeting of more potent donors would greatly facilitate effective collection.

Text

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 last two decades, i.e. SARS-CoV and 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 ELISA, lateral flow or Western blot assays, or the detection of functional antibodies by virus microneutralization test (MNT)(8). Only a MNT provides for a functional correlate of antiviral efficacy and this assay is therefore still considered as ‘gold standard’ in serological testing, yet testing needs to be conducted under level 3 biosafety containment restrictions (BSL3), 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 can 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 as e.g. disease 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 and the first 100 CP units collected were tested for functionally active neutralizing antibodies by MNT (Attachment). 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 high neutralizing antibody titer, with a mean microneutralization titer 50% (NT₅₀) of approx. 1:230 (Figure 1A – NT₅₀ histogram, median 1:100, 25% percentile 1:54, 75% percentile 1:231, range < 1:8 – 1:1765). Somewhat surprisingly for individuals who had successfully recovered from NAT-confirmed SARS-CoV-2 infection, neutralizing antibodies were undetectable in one plasma unit, and for a total of six units (6%) the NT₅₀s were below 1:23, i.e. $\text{INV log}_2 \text{NT}_{50} 4.5$ (Figure 1A).

A binding antibody assay was also performed on these CP units (Attachment), with the intention to establish a correlation between the functionally more relevant MNT and the more accessible ELISA, as well as an ELISA threshold to allow for the elimination of sub-potent 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 ($p < 0.0001$), yet quantitatively limited ($R^2 = 0.2830$) (Figure 1B). 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 NT₅₀ titers ($>1:500$, $N = 12 / 14.5\%$), which improved the correlation ($R^2 = 0.5386$; Figure 1B). Using the Euroimmun ELISA 1.1 cut-off for positivity to qualify units for transfusion, 6 units (7.2%) with an average NT₅₀ of 1:29 would have been excluded, with a corresponding increase of the mean NT₅₀ of all collected plasma units from 1:233 ($N = 83$) to 1:249 ($N = 77$). A final verdict about whether this cut-off 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.

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 WHO disease severity scores of 1 and 2 (10), with an average NT₅₀ of 1:208, versus only 6 donors in disease severity scores of 3 to 6, who had a mean NT₅₀ antibody titer of 1:696. While these results may seem to support the notion of higher titers with increased disease severity, the numbers of CP donors in higher WHO scores in this study are too low to determine significance. For CP donor age (Figure 2A), the NT₅₀s were significantly correlated ($p = 0.0019$), 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 NT₅₀ than from female donors ($N=38$), yet whether the mean titer difference of 1:220 has any functional relevance is questionable (Figure 2B).

Conclusions

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 convalescent plasma 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 gender are significantly correlated with MNT antibody levels, the differences are small and thus probably not helpful for CP donor targeting.

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109 **Disclaimers**

110 MRF, EG-R and TRK are employees of Baxter AG, Vienna, Austria, now part of the Takeda
111 group of companies. MRF and TRK have Takeda stock interest.

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113 **Author Bio** (first author only, unless there are only 2 authors)

114 Dr. Jungbauer is medical doctor and specialist in immunohematology and transfusion
115 medicine. He currently is medical director of the Austrian Red Cross Blood Service for the East
116 Region, based in Vienna, Austria. His research interests are virus diseases and molecular cell and
117 tissue antigen typing.

118 **Address for correspondence:** Thomas R. Kreil, Takeda, Global Pathogen Safety,
119 Benatzkygasse 2-6, 1220 Vienna, Austria; email: thomas.kreil@takeda.com

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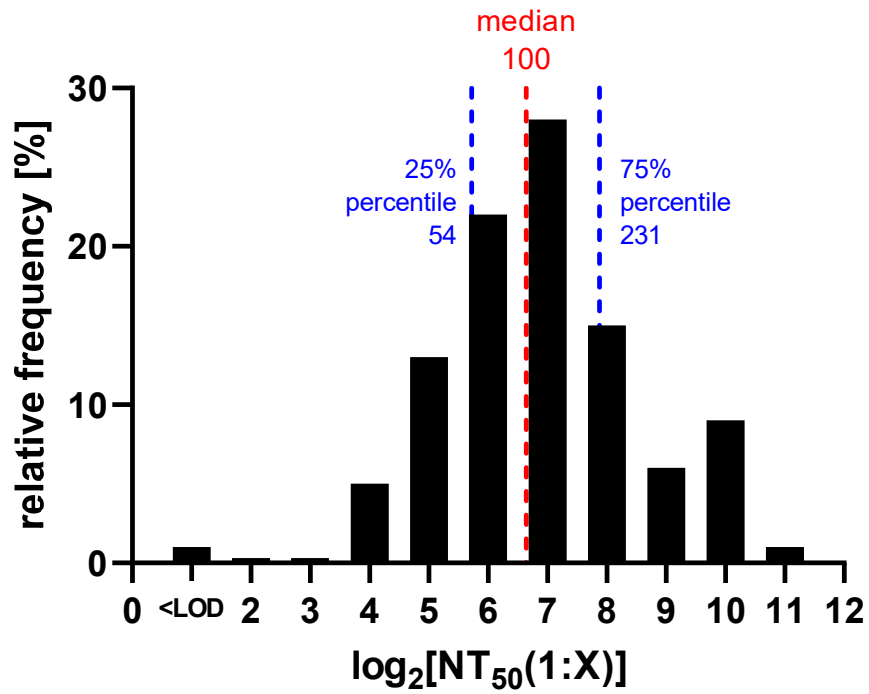
Figure Legends

Figure 1 Characterization of 100 convalescent plasma donations collected at the Vienna Blood Centre for **(1A)** SARS-CoV-2 neutralizing antibody content, reported as log₂ microneutralization titers 50% (1:X) plotted against the relative frequency (%) of occurrence. **(2A)** NT₅₀ titers were correlated against Euroimmun IgG ELISA signal ratios obtained for the same donations.

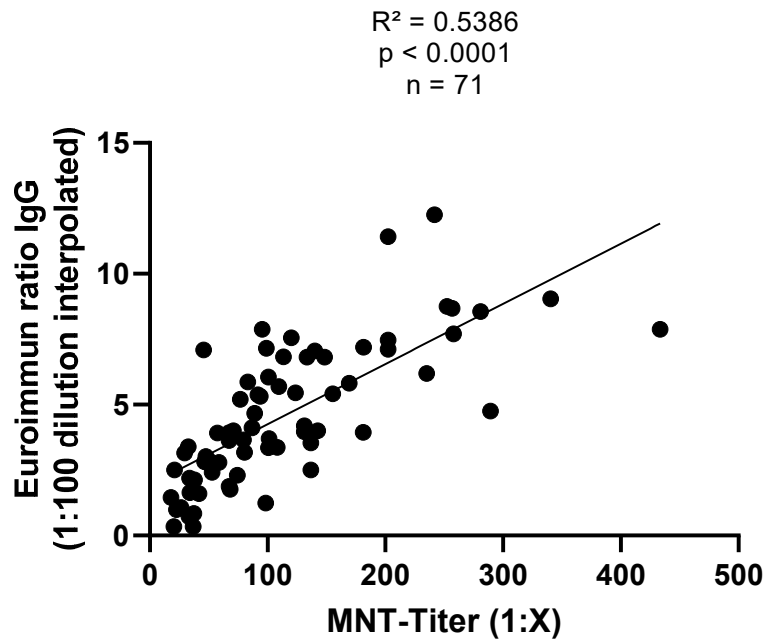
Figure 2 COVID-19 convalescent plasma donor demographics illustrating **(2A)** correlation of SARS-CoV-2 neutralizing antibody titers (NT₅₀) with donor age and **(2B)** difference in NT₅₀ between female and male donors.

130 **Figure 1**

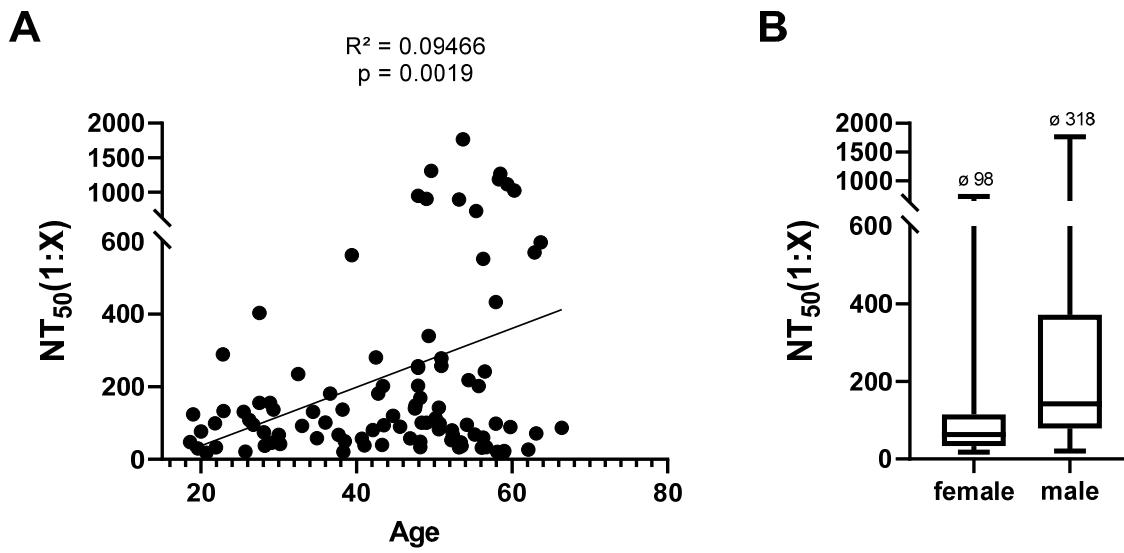
A



B



132 **Figure 2**



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Materials and Methods

Collection and virus-inactivation of CP

All CP donors had 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 Trima Accel® apheresis version 7.0 devices (Terumo, Eschborn, Germany) with Trima Accel® MultiPlasma sets (ref. 82700). The collection volumes ranged from 440 to 650 ml adapted to bodyweight of the donors. Samples of each donation were investigated for SARS CoV-2 antibody titers by neutralization assay and Euroimmun Anti-SARS-CoV-2-ELISA (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 $\geq 1:300$ in the therapeutic units. Subsequently the pooled plasma was pathogen inactivated using Intercept® Processing Sets for Plasma (Ref. INT3104B-1, Cerus Europe, Amersfoort, Netherlands) with the Intercept® UVA-Illuminator (Ref. INT100). The final volume of the therapeutic CP units was adjusted to 200 ml. At time of submission of this manuscript twelve patients had received 27 units (ranging from 1 to 3 units per patient, median 2.0 units) in Vienna and Lower Austria.

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; EVAg 026V-03883. Vero cells (ATCC CCL-81), sourced from the ECACC (84113001) were cultured in TC-Vero medium supplemented with 5% FCS, L-glutamine (2mM), nonessential amino acids (1x), sodium pyruvate (1mM), Gentamycin sulfate (100 mg/ml) and sodium bicarbonate (7.5%). For determination of SARS-CoV-2 infectivity by tissue culture infectious dose 50% (TCID₅₀) assay, eightfold replicates of serial half-log sample dilutions were incubated on cells for 5-7 days before any cytopathic effect (CPE) 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 TCID₅₀ of SARS-CoV-2. The samples were subsequently applied onto Vero cells seeded in tissue culture microplates and incubated for 5-7 days, when cells were evaluated for the presence of a CPE and the SARS-CoV-2 microneutralization titer (NT₅₀), i.e. the reciprocal sample dilution resulting in 50% virus neutralization, was determined using the Spearman-Kärber formula.

Testing of CP for binding antibodies by ELISA

Anti-SARs-CoV-2-IgG antibodies were quantified by the Euroimmun SARS-CoV-2 IgG ELISA (Euroimmun, Lübeck, Germany) using an Euroimmun Analyzer I with the protocol recommended by the manufacturer. IgG concentration is given as Ratio, calculated using 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

44 were tested in serial dilutions of twofold steps from 1:5 to 1:1280. When the IgG Ratio in a sample
45 still exceeded 1.1 (the cut-off recommended by the manufacturer) at the dilution of 1:1280, further
46 dilutions (1:5120 to 1: 20480) were performed for an accurate interpolation.