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Narrative review

Evidence-based dosing of convalescent plasma for COVID-19 in future trials

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ABSTRACT

Background: Two years into the pandemic, convincing evidence in favour of convalescent plasma (ConvP) as a treatment for coronavirus disease 2019 (COVID-19) is still lacking. This contrasts sharply with the efficacy of potent virus-neutralizing monoclonal antibodies. However, resistance of the Omicron variant against almost all licensed monoclonals turns back the clock, and we can expect that ConvP will regain interest. Indeed, the efficacy of virus-neutralizing monoclonal antibodies supports the premise that ConvP will work when used at the right time, at the right dose, and containing antibodies with the right affinity.

Objectives: This study aimed to review available evidence on dosing of ConvP for COVID-19 and provide guidance for future trials or patient care.

Sources: Because no dose-finding human trials were ever performed, we reviewed COVID-19 animal model studies and human trials that provide (in)direct data on the pharmacokinetics and pharmacodynamics of ConvP. We also discuss the identification of appropriate ConvP donors in the context of emerging severe acute respiratory syndrome coronavirus 2 variants.

Content: Compared with dosing in animal studies, almost all human trials used substantially lower doses. Identifying donors with sufficiently high virus-neutralizing antibody titres is challenging, in particular when new variants escape immunity induced by ancestral variants. Ways to avoid underdosing are (a) use of ConvP from two different donors, (b) use only ConvP known to neutralize the variant with which the patient is infected, (c) use two ConvP units with a neutralizing antibody titre $\geq 1/1250$ (when only one plasma unit is available, neutralizing antibody titre of $\geq 1/2500$ is recommended), (d) use an antibody test that correlates well with virus neutralization (use of international units per ml (IU/ml) for virus neutralization is strongly encouraged), and (e) use of donors shortly after a third mRNA vaccination may simplify the donor selection process.

Implications: In future trials on ConvP for COVID-19, more stringent donor selection criteria and/or higher volume transfusions should be used. **Bart J.A. Rijnders, Clin Microbiol Infect 2022;28:667**

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Introduction

Two years into the coronavirus disease 2019 (COVID-19) pandemic, convincing evidence in favour of convalescent plasma (ConvP) as a treatment for COVID-19 is still lacking. Any future role of ConvP for COVID-19 was therefore considered limited, and the

most recent WHO guideline formally recommends against the use of ConvP for hospitalized patients, unless in the context of a clinical trial. Several highly potent virus-neutralizing monoclonal antibodies have become a valuable part of our COVID-19 armamentarium, and this seemed to limit any role of ConvP even further.

Until recently, newly emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOCs) had a limited impact on the therapeutic value of monoclonal antibodies. Unfortunately, high-level resistance of the Omicron VOC (BA.1) against casirivimab/imdevimab has recently been described by

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several independent laboratories [1,2]. Almost all other licensed monoclonals seem to be impacted by Omicron as well, with sotrovimab as an exception [2]. Furthermore, the BA.2 subvariant of Omicron which is becoming the dominant strain in many countries was recently shown to have a 27-fold lower susceptibility to sotrovimab in one study and was not inhibited at all by this drug in another study [19,20].

What nobody had foreseen is that ConvP may become relevant again as a treatment for COVID-19. The effectiveness of virus-neutralizing monoclonal antibodies supports the premise that passive immunotherapy alters the viral pathogenesis; therefore, ConvP will very likely work as long as it is used at the right dose, with the right affinity, in the right patient, and at the right time. Specifically, ConvP has hypothesized advantages, including that a polyvalent antibody titre approach may provide broader antiviral activity. Before we start studying ConvP again, however, we must learn from our recent mistakes. We learned that the risk of underdosing ConvP is high and that a discrepancy between the VOC that infected the donor and recipient can affect efficacy. We also learned that antibody-based therapy works best in patients who are not yet producing virus-neutralizing antibodies. This implies that the window of opportunity is small. In this paper, we focus on the former and try to provide guidance on appropriate dosing of ConvP in future trials or when ConvP is considered for the treatment of immunocompromised patients unable to clear SARS-CoV-2.

Pharmacokinetics and dynamics in animal studies

In a study in rhesus macaques, purified Ig obtained from ConvP with a SARS-CoV-2 50% plaque-reduction neutralizing antibody titre (NAb) of 1/1581 was used to treat COVID-19. Immediately after administration of the Ig at a dose of 250 mg/kg or 25 mg/kg, the NAb titres in the treated animals were 1/511 to 571 and 1/42 to 49, respectively. Both the 250 mg/kg and 25 mg/kg doses were effective at preventing disease, but it became apparent that only the higher dose was effective for treatment. The peak viral load in bronchoalveolar lavage fluid and in the nasopharynx was reduced by 1.84 and 1.26 log, respectively, with the 250 mg/kg dose, but the viral load reduction after the 25 mg/kg dose was much more limited (0.64 log) in the bronchoalveolar lavage fluid and completely absent in the nasopharynx [3].

The study in macaques may provide a framework for an initial extrapolation of the successful 250 mg/kg dose in the animal-to-human dose. With an average total Ig in plasma of ± 13 g/L, a macaque weighing 3 kg would need ± 60 mL (= 750 mg/(13 000 mg/1000 mL)) of ConvP to get the 250 mg/kg dose of Ig. Compared with a total plasma volume of ± 160 mL, this means a transfusion of 37.5% of the plasma volume of the animal. To achieve a human–macaque equivalent systemic exposure, we could adjust for bodyweight dosing and total plasma volume. Hence, an adult weighing 70 kg and receiving ConvP with the 1/1280 titre would require 17.5 g of Ig, which equals approximately 1350 mL of ConvP, to reach a 250 mg/kg dose. Even when using plasma from donors with Nab titres of 1/2560, 600 to 800 mL of ConvP would still be required.

In our experience with 115 human ConvP donors, we found a median Nab titre of 1/160, with only 17% (20 of 115) having Nab titres of $\geq 1/1280$. Thus, a stringent donor selection process is crucial to identify the donors with the highest SARS-CoV-2 antibody.

A different approach was taken in a pre-exposure prophylaxis study in a COVID-19 Syrian hamster model in which inoculation with SARS-CoV-2 resulted in viral pneumonitis and temporary weight loss. Preceding the viral challenge, the animals received 0.5 mL of human ConvP, which is 10% of the plasma volume of hamsters

and therefore comparable to a 300 mL ConvP transfusion in humans. Although ConvP with a Nab titre of 1/320 did not prevent disease, ConvP with a Nab titre of 1/2560 fully prevented weight loss and limited viral pneumonitis [4]. This study therefore confirmed that ConvP with extremely high Nab titres of 1/2560 would be required if only 300 mL of ConvP is used. Takamatsu et al. took this experiment one step further and also showed protection from disease when the ConvP was administered 24 hours after virus inoculation. However, they used 2.0 mL rather than 0.5 mL and showed efficacy with Nab titres of 1400, 1100, and 400, but not with ≤ 200 titres. Extrapolating this dose to humans would mean that at least 300 mL with a Nab titre of 1/1600 or 600 mL with a titre of 1/800 would be required [5].

Human pharmacokinetic and pharmacodynamic data

The animal data described suggest that the administration of ConvP that results in Nab titres of $\pm 1/500$ in the recipient peripheral's blood will result in a positive treatment effect. We measured Nab titres in 11 consecutive, immunocompromised, seronegative patients treated with ConvP for COVID-19 and observed that after a median of 600 mL of ConvP with a Nab titre of 1/640 to 1/1280, the post-transfusion Nab titre increased from $<1/20$ to 1/40 or 1/80 [6]. In an ongoing phase 1/2 study [21], we evaluated the pharmacokinetics of Nab in five immunocompromised but otherwise healthy patients who had remained SARS-CoV-2 antibody negative despite vaccination. Twenty-four hours after the infusion of 600 mL ($n = 6$) of ConvP with a NAb titre of 900 IU/mL, the NAb titres in the patient's serum increased from <20 to 125 IU/mL (interquartile range, 86–165), respectively (Fig. 1). This illustrates that doses as high as 600 mL ConvP with a Nab titre of 1/2560 (and preferable 1/5120) would be required to achieve a Nab titre that approaches the 1/500 titre that was the most effective in the rhesus macaque model.

In a recent randomized trial that we completed in the Netherlands and Spain, ConvP was compared with regular non-ConvP in 792 outpatients age ≥ 50 years with <8 days of symptoms from COVID-19. The median Nab titre was 386 IU/mL, and patients received one unit (200–300 mL) of ConvP. No significant reduction in hospital admission or impact on any other clinical endpoint was observed. In this population, monoclonal antibodies have clearly proven their benefit and reduced hospital admission by 50% to 82%. The most likely explanation of the limited benefit observed in this trial is the low dose of NAb titres given [7].

Antibody titres and functionality in plasma donors

Early into the pandemic, we measured the NAb titres of 115 convalescent patients infected with the original Wuhan SARS-CoV-2 variant. NAb titres were measured a median of 34 days after symptom resolution for COVID-19 infection. Although all donors tested positive with an anti-S total Ig antibody test, the NAb titres varied enormously and ranged from $<1/20$ to $>1/2560$ [8]. This illustrates that appropriate donor selection is a crucial first step and cannot be based on a qualitative antibody test only. However, direct NAb titre measurement may not be feasible on a large scale in real time, and easier-to-use quantitative anti-spike antibody tests that correlate reasonably well with NAb titres are often used [9]. It is also important to acknowledge the substantial interlaboratory variation of NAb titres. The recently introduced international unit to standardize NAb titre results across laboratories should therefore be used whenever possible [10]. Unfortunately, as soon as a new variant circulates and partially or fully escapes humoral immunity against the ancestral virus, laboratories will have to perform additional standardization steps to be able to express NAb titres in international units in relation to the variant that is circulating. The

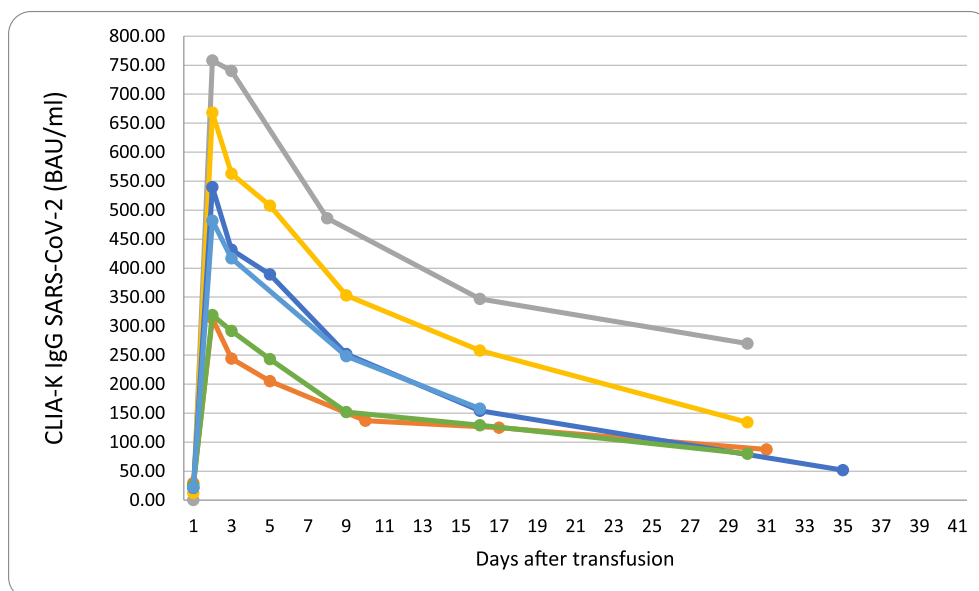


Fig. 1. Evolution of anti-SARS-CoV-2 IgG titer after convalescent plasma (ConvP) administration. BAU = Binding antibody units measured with the Diasorin Liaison Trimeric S antibody test. Neutralizing antibody titres (Nab) are expressed in international units. Patients received 600 mL of ConvP with a NAb titer of 900 IU/ml. BAU can be approximately converted to NAb when it is divided by 3.4.

European Union-funded E-support initiative is helping laboratories with this and providing calibrants [11]. Even when international standards are used, we suggest using ConvP from two different donors whenever possible to reduce the chance that a patient will unintentionally receive ConvP with insufficient NAb.

On December 28, 2021, the US Food and Drug Administration (FDA) revised its emergency use authorization of ConvP and now allows the use of ConvP for immunocompromised patients with COVID-19 [12]. The letter concludes that, based on the totality of the scientific evidence available, it is reasonable to believe that the known and potential benefits of COVID-19 ConvP with high titres of anti-SARS-CoV-2 antibodies outweigh its known and potential risks for the treatment of COVID-19 in immunocompromised patients. Unfortunately, the list of antibody tests and the cut-offs provided in table 1 of that document will in no way guarantee that plasma with high titres of NAb is selected. The provided cut-offs are merely a way to ensure that the plasma contains any detectable level of NAb. For instance, according to the label of the Diasorin TrimericS antibody test, plasma with a titre of ± 100 BAU/mL has a median NAb titre of 1/40 [13]. In an ongoing phase 1/2 study, we tested plasma from eight plasma donors with a NAb titre of 900 IU/ml with this TrimericS test. The median titre was 3070 BAU/ml (interquartile range, 2263–3607). Therefore, the cut-off of 87 BAU/mL, as well as the other cut-offs in the FDA letter, cannot be used to assure that plasma contains high-enough Nab titres. Furthermore, now that the Omicron variant is dominant, these cut-offs will not even guarantee any level of neutralizing activity against Omicron.

For the aforementioned reasons, the accurate identification and collection of ConvP from convalescent patients with very high Nab is challenging. On the upside, now that vaccines are available that predictably induce Nab in almost all immunocompetent persons, the selection of donors should become somewhat easier. Furthermore, several studies have shown that Nab titres increase further

after a third vaccination with an mRNA vaccine and, in particular, mRNA1273 [14]. This seems to be the case against Omicron as well, because antibodies induced by a third BNT162b2 vaccination or a BNT162b2 vaccination after infection with the original Wuhan-01 virus neutralized Omicron efficiently, with titres comparable to or even higher than against Wuhan-01 [15]. Therefore, probably the best ConvP donors with the highest Nab titres are young healthy persons 2 to 4 weeks after they received a third vaccination (or after vaccination after a COVID-19 infection). On the downside, Nab assays will have to be updated and validated for the Omicron variant, and it will take some time to learn how well ConvP from patients who recovered from an infection with Omicron will neutralize Omicron.

Finally, some methods for pathogen inactivation may alter Ig function. Although neither the FDA nor the European Center for Disease Control recommend pathogen-reduction technologies for ConvP, several national authorities consider that, under emergency settings, donor screening and conventional viral nucleic acid testing would not be enough to ensure safety. The two major approaches to pathogen inactivation involve methods that inactivate lipids (e.g. solvent/detergent treatment) and methods that damage nucleic acids (e.g. amotosalen + ultraviolet-A light, riboflavin + ultraviolet light, methylene blue + visible light). Data on the effect of these methods on antibody activity have shown mixed results. For instance, photoinactivation methods do not seem to reduce neutralizing antibody content or reduce the cell receptor binding capacity of the Fc-region. Nonetheless, current evidence does not rule out an effect on Fc-dependent functions, including phagocytosis, complement activation, and antibody-dependent cellular toxicity.

In Table 1, we summarize our recommendations. Although some antiviral activity was documented with lower doses, extrapolation from animal studies should be done cautiously because the timing of treatment in these studies is optimal (24 hours after inoculation).

Table 1
Recommendations on selection and use of ConvP for coronavirus disease 2019

Use two ConvP units from two different donors
Use ConvP of which the Nab titre against the variant of concern the patient is infected is known ^a
Use two ConvP units of 250–300 mL each, with Nab titre of at least 1/1250 and whenever available 1/2500
When only one unit of 250–300 mL is available, Nab titre of approximately 1/2500 and if possible 1/5000 should be used
Only use Nab test that is well validated, and interlaboratory comparison should be performed
Preferably use ConvP that has not undergone photoinactivation

ConvP, convalescent plasma; Nab = neutralizing antibody titres.

^a Relates to the *in vitro* neutralization of the variant that the patient who will receive the ConvP is infected with. Ideally, this should be plasma from a donor who was previously infected with the same variant of concern. Alternatively, plasma from donors who received a third mRNA booster or those previously infected with a different variant can be used if shown that its neutralizing activity is unaltered against the variant infecting the recipient.

Discussion

With the reality check we face as Omicron spreads across the globe, the use of ConvP for the treatment of COVID-19 will probably regain interest. Indeed, Omicron demonstrates *in vitro* resistance against all currently licensed monoclonal antibodies, with the exception of sotrovimab. We reviewed *in vitro*, human, and animal data and propose a ConvP dose that should be considered in future trials or treatments. Our recommendations certainly have their limitations. First, a multitude of methods is being used to measure Nab titres. This makes the comparison of titres between studies difficult, and interlaboratory comparisons and the use of the recently introduced international unit for Nab tests is strongly encouraged and will help to evaluate ConvP across clinical trials [10,11,16]. Another limitation of our recommendations is the lack of phase 2 dose-finding trials. The only trial so far that suggests a potential benefit of ConvP was limited by its small sample size, and a beneficial effect was only observed in this study in patients receiving ConvP with much higher titres than the cut-off of 1000 anti-S IgG used to select donors [17]. We acknowledge that, on one hand, doses below what we recommend in Table 1 may still be effective; after so many negative trials, however, we should do everything to avoid underdosing in future trials and we chose to keep the lower limit of our recommendations relatively high. On the other hand, we cannot exclude that higher doses than those recommended may still be more effective. Finally, at least outside of the context of a clinical trial, ConvP is typically used to treat immunocompromized patients with COVID-19 in whom the autologous antibody response can be limited, delayed, or even completely absent. As far as we know, none of the animal models have evaluated ConvP in immunocompromized animals. Also, several case reports have shown viral evolution and development of resistance during the treatment of severely immunocompromized patients with ConvP [18]. Therefore, it is certainly possible that higher doses will be required in immunocompromized patients, which is another reason to stay on the safe side of dosing.

The dose indications provided herein always relate to ConvP from donors who had been infected with the same virus that was used for the Nab measurement. This has become a crucial aspect of trials on ConvP since the occurrence of the Delta and, very recently, the Omicron VOC. Indeed, Nab produced by a patient after infection with the original Wuhan SARS-CoV-2 strain will neutralize other variants less efficiently or not at all. This means that as a rule, treatment with ConvP of a donor who was infected with another

VOC should be avoided unless ConvP from vaccinated donors can be used and cross-variant neutralization is well-established. Thus, new trials on ConvP for the treatment of COVID-19 caused by Omicron can only start when appropriate plasma donors become available.

In conclusion, we hope to provide guidance regarding the use of ConvP for the treatment of COVID-19 in the context of newly emerging variants of concern.

Transparency declaration

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Author contribution

All authors declare equal contribution to this paper. Conceptualization: BJAR and OM; investigation: BJAR, OM, SH; writing—original draft: BJAR, OM, SH; writing—review and editing: BJAR, OM, SH.

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