Impact of pathogen reduction methods on immunological properties of the COVID-19 convalescent plasma

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Authors contribution

Design of the study – A. Kostin; convalescent plasma donor recruiting – V. Ganchin, E. Dombrovskiy; collection of data – E. Ladygina, K. Chirkova, A. Kamalova; development of test systems for NtAbs and anti-RBD - A. Gintsburg, D. Shcheblyakov, I. Dolzhikova, D. Logunov; experimental work M. Godkov, A. Bazhenov, A. Bogdanova, V. Shustov; organization of the working processes for collection of convalescent plasma - S.Petrikov, A. Bulanov, N. Drozdova, N. Borovkova; statistical analysis and interpretation of the data and drafting of the manuscript – A. Kostin, M. Lundgren, S.Volkov. All co-authors critically reviewed and approved the manuscript.

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ABSTRACT

Background and Objectives: COVID-19 convalescent plasma has become an experimental treatment option against SARS-CoV2. The aim of this study is to assess the impact of different pathogen reduction methods on the immunological properties of COVID-19 convalescent plasma.

Materials and Methods: A total of 140 plasma doses collected by plasmapheresis from COVID-19 convalescent donors were subjected to pathogen reduction by three different methods: methylene blue (M), riboflavin (R), and amotosalen (A). To conduct a paired two-sample comparison, individual plasma doses were divided into 2 samples that were subjected to one of these methods. The titres of SARS-CoV2 neutralizing antibodies (NtAbs) and levels of specific immunoglobulins to RBD, S- and N- proteins of SARS-CoV-2 were measured before and after pathogen reduction.

Results: All methods reduced NtAbs titres significantly but not equally: among units with the initial titre 80 or above, 81% of units had unchanged titres while 19% decreased by 1 step after methylene blue; 60% were unchanged and 40% decreased by 1 step after amotosalen; whereas after riboflavin 43% were unchanged, 50% had a one-step decrease and 7% a two-step decrease. Paired two-sample comparisons (M vs A, M vs R and A vs R) revealed that the most prominent and statistically significant decrease in all studied parameters (except anti-RBD) resulted from the riboflavin treatment.

Conclusion: Pathogen reduction with methylene blue and amotosalen provides the greater likelihood of preserving the immunological properties of the COVID-19 convalescent plasma compared to riboflavin.

Keywords: COVID-19 convalescent plasma, NtAbs, pathogen reduction, amotosalen, riboflavin, methylene blue

Introduction

The new coronavirus infection (COVID-19) caused by the SARS-CoV-2 virus continues its march around the world, causing a global crisis as the number of new cases and deaths continues to rise. The treatment is supportive care mostly aimed at relieving symptoms. Candidate vaccines are still going through different stages of clinical trials, and different classes of drugs are being tested to inhibit virus replication and reduce inflammation [1, 2, 3] .

Passive immunotherapy with polyclonal antibodies from the blood plasma of convalescents was tested earlier in the outbreaks of SARS-CoV, influenza and other dangerous infections of the twentieth century [4, 5, 6]. Experts from different countries came to consider the possibility of using COVID-19 convalescent plasma (CCP) for therapeutic purposes in patients with COVID-19 [7, 8, 9]. In many countries, national campaigns have been launched to collect CCP.

Various mechanisms have been suggested as responsible for the therapeutic effect of CCP such as virus neutralization and immunomodulation [10]. Virus neutralizing antibodies (NAbs) of IgG, IgM and IgA classes bind to different parts of glycoprotein S, including the region of the receptor-binding domain (RBD), spatially blocking its interaction with the membrane protein ACE2 of host cells, which limits the penetration of the virus into the cell, thereby limiting viral replication [11, 12, 13].

Every plasma transfusion is associated, however, with risks of virus transferal such as HIV, HBV, HCV, etc. [14]. At the moment, there are no scientific publications reporting on the transmission of SARS-CoV-2 through the transfusion of blood components [15]. The Working Party on Global Blood Safety of the International Society of Blood Transfusion (ISBT) recommended the use of pathogen inactivation of convalescent plasma to minimize the residual risk of blood-borne infections and to address the problem of possible superinfection with the SARS-CoV-2 virus [8].

To date, there is no data on how pathogen reduction affects the immunological properties of CCP and what methods of pathogen reduction are preferable to use to maintain its quality and effectiveness.

The objective of this study is to assess the effect of various methods for pathogen reduction on the immunological characteristics of CCP.

Materials and methods

The COVID-19 convalescent plasma procurement program in Russia was launched on April 2, 2020 at the Department of Transfusion Medicine of the Sklifosovsky Research Institute of Emergency Medicine, Moscow. At present, this program involves many hospitals in several regions and has more than 3000 donations and about 1500 transfusions of CCP in Moscow alone. According to the adopted regulations, donors of convalescent plasma were recruited among the individuals with prior diagnosis of COVID-19 infection documented by a positive RT-PCR-test who received treatment either in a hospital setting or on an outpatient basis. Donors fulfilled the standard blood donor selection criteria. Plasma was collected at least 2 weeks after the complete disappearance of clinical symptoms.

Plasmapheresis procedures were performed using Auto-C, Aurora and PCS2 machines. Plasmapheresis was carried out in accordance with standard protocols collecting an amount of 650 ml plasma. Pathogen reduction procedures were carried out immediately after the end of plasmapheresis. For comparison, three systems for pathogen reduction were selected: Intercept – (amotosalen plus UVA light), Mirasol (riboflavin plus UVB light) and Macotronic (methylene blue plus visible light). Subsequently, the plasma was frozen and became available for clinical use after receiving negative results of all serology/virology tests for transfusion-transmitted diseases.

This study included 140 doses of plasma obtained by plasmapheresis from 140 COVID-19 convalescent donors at our Department of Transfusion Medicine. From each plasma unit, samples were collected before and after pathogen reduction for the determination of the titres of SARS-CoV2 neutralizing antibodies (NtAbs), as well as quantitative determination of specific IgG to the receptor-binding domain (RBD) of the glycoprotein S of the SARS-CoV-2 virus, and specific IgM and IgG to S- and N- proteins of this virus.

To conduct a paired two-sample comparison to assess the effect of each of these methods on the immunological parameters of CCP, the plasma dose from one donor was divided into 2 parts and each part was simultaneously subjected to a pathogen reduction procedure by one of the two methods according to the following scheme:

pair 1: methylene blue (M) vs. riboflavin - 48 pairs;

pair 2: amotosalen (A) vs. riboflavin (R) - 36 pairs;

pair 3: methylene blue (M) vs. amotosalen (A) - 56 pairs;

Since we had 140 samples *before* treatment, in total 420 (=48*2+36*2+56*2+140) samples were analyzed.

The titres of SARS-CoV2 neutralizing antibodies were measured by a microneutralization assay on a Vero E6 cell culture [16] modified at the Gamaleya National Research Center of Epidemiology and Microbiology, Moscow, with an assessment of the virus' cytopathic effect after 96 hours of incubation. The virus neutralization titre of the studied plasma was taken as its highest dilution, at which the cytopathic effect is suppressed in 2 out of 3 wells.

Determination of specific anti-RBD IgG was carried out at the same institution by the enzyme-linked immunosorbent assay (ELISA) using their own SARS-CoV-2-RBD-IFA-Gamaleya test system (registered in Russia, RZN 2020/10393).

In parallel, quantitative determination of IgM and IgG antibodies to a mixture of recombinant S- and N-proteins of SARS-CoV-2 was carried out by the immunochemiluminescent method (IHLA) using reagent kits for Shenzhen Mindray Bio-Medical Electronics Co., Ltd (China).

Since the data for NtAbs are presented in the format 10 multiplied by an integer power of two (i.e., 20, 40, 80, 160 etc.) we log-transformed the data: $y=\log_2(x/10)$, where x is the reported value of NtAbs

In order to identify the methods of pathogen reduction which have the least negative effect on NtAbs levels, we applied the two-sample paired T-tests (M vs A, M vs R, and A vs R) to the difference in reduction in titres of NtAbs, anti-RBD IgG, and anti-S + N IgG and IgM titres respectively, after pathogen reduction by different methods. The p-values < 0.05 were considered to be significant. No corrections were made for multi-significance. Confidence intervals were obtained using the standard methods for estimation of proportions. The software used for the analysis was Maple™.

Results

The assessment of the impact of various methods of pathogen reduction on the titres of SARS-CoV2 neutralizing antibodies (NtAbs) showed a statistically significant decrease in antibody titres after all pathogen reduction processes (Figure 1, Table 1).

If all plasma units, regardless of the initial titre were included in the analysis, it was shown that in 88% (n=104; confidence interval 81% - 94%) of units NtAbs titres did not decrease after pathogen reduction with methylene blue whereas a one step titre reduction was observed in remaining 12%. In 70% (n=88; 95% confidence interval 61% - 80%) of units treated with amotosalen the NtAbs titre did not change, and in 30% it decreased by 1 step. Pathogen reduction with riboflavin left NtAbs titres unchanged in 61% (n=83; confidence interval 51% - 72%) of the units, in 35% decreased by one step and in 4% by two steps.

To compare the impact of different methods of pathogen reduction, we used the data collected on paired data: the plasma units from the same donor were treated using e.g. method A and M, and then the resulting NtAbs were noted for both methods. We had three different datasets: one compared A vs. R, another dataset M vs. R and the third M vs. A. The results are the following: M is better than R (p-value=0.00002, n=48), A is better than R (p-value=0.0002, n=36), M is better than A (p-value=0.0012, n=56).

When only units with the initial NtAbs titre 80 or above were chosen (this is the level that is generally considered to be suitable for therapeutic purposes) the distribution was similar: after treatment with methylene blue, 81% of plasma samples had unchanged NtAbs titres (n=53; confidence interval 71% - 92%), while in the remaining 19% of samples the titres decreased by 1 step. Pathogen reduction with amotosalen gave worse results: 60% of samples had the same NtAbs after the reduction (n=55; confidence interval 47% - 73%), while in the remaining 40% samples the titres decreased by 1 step. Finally, after treatment with riboflavin, only 43% of the samples preserved the level of NTABs titres (n=30; confidence interval 26% - 61%), whereas a one-step decrease was observed in 50% samples, and a two-step decrease in 7% of samples.

The decrease in anti-RBD IgG in paired comparison with baseline values was more pronounced after pathogen reduction with riboflavin whereas after amotosalen or methylene blue there were no significant differences (Table 1).

Plasma pathogen inactivation with methylene blue did not lead to a significant decrease in anti-S + N IgG and IgM, whereas the use of amotosalen significantly reduced the level of anti-S + N IgG (Table 1). In the study of 83 pairs of samples before and after pathogen reduction with riboflavin, the differences were significant in the anti-S + N levels of both IgG and IgM (Table 1).

Paired two-sample comparisons (M vs A, M vs R and A vs R) revealed the most prominent and statistically significant decrease in titres of NtAbs, anti-S + N IgG and IgM, but not anti-RBD IgG titres, after pathogen reduction by riboflavin (Table 2).

Discussion

The key safety issue of using convalescent plasma is played by the choice of a pathogen-inactivation method that minimizes the residual transfusion risk of transmissible viruses in the final product, while maintaining a high titre of antibodies to the SARS-Cov-2 virus. A number of different pathogen reduction methods are available today [17]. Ultraviolet (UV) A [18, 19] and UVB radiation [20], in combination with amotosalen and riboflavin, respectively, makes it possible to inactivate nucleic acids of pathogenic organisms. These systems can reduce the activity of SARS and MERS viruses in plasma or platelet concentrates to varying degrees.

Methylene blue is a phenothiazive compound that, in combination with visible light, is also capable of inactivating coronaviruses in plasma [21, 22]. The photoactive agents used in these methods have different chemical structures and are activated at different wavelengths of radiation (visible light about 590 nm, UVA from 400 nm - 315 nm and UVB from 315 nm - 280 nm). Consequently, various mechanisms are involved in ensuring the reduction of pathogens through the binding of nucleic acids.

The current study is the first to examine the impact of pathogen reduction methods on SARS-CoV-2 antibody levels in convalescent plasma.

The hypothesis tested in this study is that different types of photo-chemical reactions used in the methods of pathogen reduction can have a different effect on the amount and functional activity of SARS-CoV2 specific IgG and IgM antibodies in the final product - convalescent plasma. The results obtained indicate a lesser effect on the immunological quality of CCP of pathogen reduction with methylene blue and amotosalen.

Based on the study, we can recommend using inactivation methods with methylene blue and amotosalen to ensure the safety and quality of CCP, due to the greater likelihood of preserving the immunological properties of the final product. Since even these methods of pathogen reduction are associated with a risk of reducing the quantity and quality of antibodies against SARS-CoV-2, it is recommended to transfuse at least 2 units of convalescent plasma (200-300 ml) from different donors to

one patient, especially in those medical institutions where it is impossible to routinely reproduce techniques for determining NtAbs titres.

In those blood establishments where pathogen reduction with riboflavin is traditionally used, it may be worth to consider increasing the dose of transfused convalescent plasma in order to compensate for the decrease in the baseline neutralizing antibody titres after this method of pathogen reduction.

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Table 1: Individual methods of pathogen reduction, comparison of the initial values *vs.* post treatment values using two sample paired t-test. The numbers show differences in means and respective p-values in parentheses below. Statistically significant differences are in **bold font**.

Method (sample size)	NtAbs*	Anti-RBD IgG (AU)	Anti S+N IgG (U/L)	Anti-S+N IgM (COI)
Methylene blue (n=104)	**0.10** (0.01)	**0.03** (0.03)	1.1 (0.23)	0.007 (0.59)
Amotosalen (n=88)	**0.23** (0.0003)	0.0 (0.99)	**1.7** (0.008)	0.01 (0.53)
Riboflavin (n=83)	**0.40** (<0.0001)	**0.07** (0.001)	**8.9** (<0.0001)	**0.19** (<0.0001)

* The shown values are $\log_2$-transformed and divided by 10.

Table 2: Comparison of different methods of pathogen reduction using two sample paired t-test. The numbers show differences in means and respective p-values in parentheses below. Statistically significant differences are in **bold font**.

Method (sample size)	NtAbs*	Anti-RBD IgG (AU)	Anti S+N IgG (U/L)	Anti-S+N IgM (COI)
Methylene blue vs Amotosalen (n=56)	**0.27** (0.001)	-0.02 (0.60)	3.1 (0.21)	0.045 (0.29)
Amotosalen vs Riboflavin (n=36)	**0.33** (0.0002)	0.06** (0.27)	**6.7** (<0.0001)	**0.20** (<0.0001)
Methylene blue vs Riboflavin (n=48)	**0.42** (<0.0001)	0.007 (0.82)	**6.6** (<0.0001)	**0.16** (<0.0001)

* The shown values are $\log_2$-transformed and divided by 10.

** one data point is missing, so n=35 here

Figure 1. Proportion of COVID-19 convalescent plasma units that had no decrease in NtAbs titre (), one-step titre decrease () or two steps titre decrease () after pathogen reduction with methylene blue (A, D), amotosalen (B, E) and riboflavin (C, F) among all plasma units (A, B, C) or only units with initial NtAbs titre 80 or above (D, E, F).