SAFETY AND EFFICACY OF CONVALESCENT PLASMA FOR COVID-19: THE PRELIMINARY RESULTS OF A CLINICAL TRIAL

V.P. Baklaushev[1, 2], A.V. Averyanov[1, 2], A.G. Sotnikiva[1, 2], A.S. Perkina[1, 2], A.V. Ivanov[3], G.M. Yusubalieva[1] O.N. Novikova[1], V.E. Shikina[1], N.V. Dupik[1], A.G. Kedrova[1], A.E. Sanjarov[1], E.V. Shirshova[1], O.I. Balionis[1], V.T. Valuev-Elliston[3], N.F. Zakirova[1,3], Ya.N. Glazov[4], I.A. Panukhina[1], N.A. Solov'ev[1], A.G. Vinokurov[1], Yu.V. Ivanov[1], V.N. Vasiliev[1], T.V. Klypa[1], A.V. Troitskiy[1]

[1] Federal Scientific and Clinical Center of Specialized Types of Medical Care and Medical Technologies of the Federal Medical and Biological Agency of Russia, Moscow, Russian Federation

[2] Research Institute of Pulmonology of the Federal Medical and Biological Agency of Russia, Moscow, Russian Federation

[3] Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences, Moscow, Russian Federation

[4] Blood Center of the Federal Medical and Biological Agency of Russia, Moscow, Russian Federation

Background. *The lack of effective etiotropic therapy for COVID-19 has prompted researchers around the globe to seekr various methods of SARS-CoV-2 elimination, including the use of convalescent plasma.* ***Aim.*** *The aim of this work was to study the safety and efficacy of the convalescence plasma treatment of severe COVID-19 using the plasma containing specific antibodies to the receptor binding domain (RBD) of SARS-CoV-2 S protein in a titer of at least 1:1000.* ***Methods.*** *A single-center, randomized, prospective clinical study was performed at the FRCC FMBA of Russia with the participation of 86 patients who were stratified in two groups. The first group included 20 critically ill patients who were on mechanical ventilation the second group included 66 patients with moderate to severe COVID-19 and with spontaneous respiration. The patients in the second group were randomized into two cohorts in a ratio of 2:1. In the first cohort (46 patients), pathogen-reduced convalescent plasma was transfused (twice, 320 ml each), in the second cohort (20 patients) a similar amount of non-immune freshly frozen plasma was transfused to the patients.* ***Results.*** *The use of plasma of convalescents in patients with severe COVID-19 being on mechanical ventilation does not affect the disease outcome in these patients. The mortality rate in this group was 60%, which corresponds to the average mortality of COVID patients on mechanical ventilation in our hospital. In the second group, clinical improvement was detected in 75% and 51%, for convalescent and non-immune plasma, respectively. Of the 46 people who received convalescent plasma, three patients (6.5%) were transferred to mechanical ventilation, two of them died. In the group receiving non-immune plasma, the need for mechanical ventilation also arose in three patients (15%), of which two died. The hospital mortality in the group of convalescent plasma was 4.3%, which is significantly lower than the average COVID-19 hospital mortality at our Center (6.73%) and more than two times lower than the hospital mortality in the control group (n=150), matched by age and by the disease severity.* ***Conclusions.*** *Thus, we demonstrated a relative safety of convalescent plasma transfusion and the effectiveness of such therapy for COVID-19 at least in terms of the survival of hospitalized patients with severe respiratory failure without mechanical ventilation. In the absence of bioengineered neutralizing antibodies and effective etiotropic therapy, the use of hyperimmune convalescent plasma is the simplest and most effective method of specific etiopathogenetic therapy of severe forms of COVID-19.*

Keywords: *SARS-CoV-2, COVID-19, plasma of convalescents, antibodies to RBD S-protein, ARDS, mechanical ventilation.*

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List of abbreviations

APV — artificial pulmonary ventilation
CT — computed tomography
CT_0 – CT_4 — classification of radiological manifestations of COVID-19
ICU — resuscitation and intensive care unit
ARDS — acute respiratory distress syndrome
PCR — polymerase chain reaction
RNA — ribonucleic acid
CRP — C-reactive protein
ACE2 — angiotensin-converting enzyme 2
COVID-19 (Coronavirus Disease 2019) — a new coronavirus infection caused by SARS-CoV-2
Ig — immunoglobulin
IL — interleukin
INFγ — interferon gamma
MERS — Middle East Respiratory Syndrome
$SatO_2$ (oxygen bound to hemoglobin) — oxygen saturation of arterial blood
SARS — severe acute respiratory syndrome
SARS-CoV-2 (Severe Acute Respiratory Syndrome, Coronavirus-2) — a new strain of coronavirus, from the genus Betacoronavirus
RBD — receptor-binding domain
TNFα — tumor necrosis factor alpha
TRALI — transfusion-related acute lung injury

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journal of clinical practice 2020 Том 11 №2

BACKGROUND

The COVID-19 pandemic, which spread from China through Europe to the West, namely to the USA, Brazil, and to the East, to Russia and the post-Soviet countries, became a challenge for the entire medical community. In the absence of etiotropic therapy, the search for effective treatment methods was performed, in fact, under conditions of a global experiment and was sometimes based on reports from countries that were the first to be affected by the epidemic and had no adequate evidence base [1]. The above fully applies to the hyperimmune plasma of convalescents, which has been used for more than a hundred of years with varying degrees of success in influenza pandemic (Spanish flu, H1N1 [2, 3]), measles, poliomyelitis and a number of other diseases, including SARS and MERS coronavirus infections [4, 5], but as of April 2020 has no proven efficacy against COVID-19.

Studies of efficiency of convalescent plasma in the treatment of MERS, which is characterized by a significantly higher mortality rate than COVID-19, revealed that the titer of virus-neutralizing antibodies, disrupting the normal cycle of virus penetration into target cells, is of fundamental importance [5]. In the new coronavirus SARS-CoV-2, cell penetration is performed through the interaction of the spike S-protein with the receptor to angiotensin-converting enzyme (ACE2); therefore, neutralizing antibodies must firmly bind to the receptor-binding domain of the S-protein, displacing it from the connection with ACE2 [6].

The presence of virus-neutralizing antibodies in the plasma used is also important from the standpoint of preventing potential adverse events. The experience of studies of the immune response to various serotypes of the causative agent of Dengue fever and the results of some preclinical studies on MERS indicated that the presence of antibodies that do not have neutralizing activity may not only not contribute to protection against an infectious agent, but, on the contrary, cause the phenomenon of increased infectivity and aggravate the course of the disease [7, 8].

A high titer of virus-neutralizing antibodies is not registered in all patients with the history of COVID-19 [6; own data], although in general, the immune response to SARS-CoV-2 is quite active, as by the day 15 of illness, the vast majority of patients have IgM and IgG (94.3% and 79.8% of the diseased, respectively) [9].

The first reports on the efficacy of anti-COVID plasma for COVID-19 were received from China and looked promising, although they were performed in a format of clinical case series. It has been demonstrated that convalescent plasma leads to clinical improvement, normalization of O_2 saturation, and regression of acute respiratory distress syndrome (ARDS), including in critically ill patients and on artificial pulmonary ventilation (APV) [10–12]. In all of these studies, no serious side effects were recorded, however, given the sample size, it was not possible to draw significant conclusions about the safety and efficacy of hyperimmune plasma transfusion in COVID-19. Moreover, there were isolated reports in which, along with potential efficacy, the possibility of life-threatening complications from plasma transfusion was also noted, especially in critically ill patients [13]. At the same time, given the lack of effective etiotropic therapy against SARS-CoV-2, phase I/II clinical studies of convalescent plasma are currently performed worldwide [14–17], and their preliminary results enabled to include plasma therapy in the latest version of temporary guidelines of the Ministry of Health of the Russian Federation on COVID-19 [18].

This work aimed to analyze the safety and efficacy of transfusion of single-group hyperimmune plasma from convalescents to patients with COVID-19 in the form of polysegmental pneumonia with respiratory failure and ARDS.

METHODS

Study design

An open, prospective, randomized, single-center, comparative study of the safety and efficacy of pathogen-reduced immune plasma from convalescents with the history of COVID-19 and fresh frozen plasma from healthy donors.

Compliance criteria

Inclusion criteria:

- signed informed consent of the patient or his legal representative, in case the patient is unconscious;
- men or women over the age of 18 (women of fertile age can be included in the study regardless of pregnancy);
- the presence of COVID-19 confirmed by a smear test for SARS-CoV-2 RNA from the upper respiratory tract by the polymerase chain reaction (PCR) method, and/or clinical and radiological method (the presence of a typical clinical presentation and characteristic signs of polysegmental viral pneumonia COVID-19 with computed tomography (CT) of the chest);
- the presence of at least three of the following indicators:
  — SpO_2 level lower than 93% in ambient air;

— fever of 38.5°C or higher during the last 3 days;

— lymphopenia lower than 0.85 × 10^9/l;

— the concentration of C-reactive protein (CRP) higher than 50 mg/ml;

— ferritin concentration higher than 600 μg/ml;

— increase in the concentration of one of the indicators (IL6, IL2, TNFα, INFγ) during the last 3 days by 3 times or higher;

— the presence of adverse factors in the severe course of COVID-19 (age 65+, diabetes mellitus, hypertensive disease, obesity, chronic diseases with impaired function of vital organs, other comorbid conditions that worsen the prognosis for recovery);

— respiratory index of 300 or lower;

— the current intake of immunosuppressive drugs or within the last 2 weeks.

Non-inclusion criteria:

- lack of informed consent;
- contraindications to the transfusion of donor immune plasma;
- duration of APV for more than 48 h;
- chronic lung diseases with chronic respiratory failure;
- the need for constant oxygen therapy at home before the onset of the present disease;
- serum creatinine level higher than 150 μmol/l;
- the disease with a prognosis of survival less than 1 year;
- treatment with monoclonal antibodies to IL6, IL2, IL1, TNFα.

Exclusion criteria

The criteria for early termination of patient participation in the study were the following:

- withdrawal of informed consent by the patient;
- newly diagnosed conditions and/or diseases listed in the exclusion criteria;
- serious adverse events;
- adverse events that do not meet the criteria for severity, the development of which, in the opinion of the researcher, could be detrimental to the patient's health or well-being in further participation in the study;
- administrative reasons (termination of the study by the sponsor or regulatory authorities), as well as gross violations of the protocol that could affect the results of the study;
- the patient is receiving/requires additional treatment that may affect the outcome of the study or the safety of the patient;
- individual intolerance to the study drugs.

Study conditions

The study was performed in the Federal Clinical Research Centre of the Federal Medical-Biological Agency of Russia in the period from 28.04.2020 to 30.06.2020, during the work of the infectious hospital for the treatment of COVID-19.

Description of the medical intervention

Harvesting hyperimmune plasma

695 convalescents who with the history of COVID-19 took part in the study voluntarily and free of charge; and 146 potential donors of hyperimmune anti-COVID plasma with a high titer of virus-neutralizing antibodies to the receptor binding domain (RBD) of the SARS-CoV-2 S protein were selected from them. The antibody titer was determined by enzyme-linked immunosorbent assay using a test system developed at the National Medical Research Center of Hematology of the Ministry of Health of Russia. Plasma was harvested from donors without blood-borne diseases, with normal biochemical parameters and coagulogram, and with an anti-RBD antibody titer of at least 1:1000. The collection was performed by the plasmapheresis method, the volume of the harvested plasma was 640 ml per donation; donations were repeated every 2 weeks, up to three times, with the obligatory repeated determination of the antibody titer.

Therapy scheme

The administration of freshly thawed single-group pathogen-reduced anti-COVID plasma was performed twice with an interval of 24 hours according to the following scheme:

- Day I: 320 ml, intravenously, slowly, after a biocompatibility test;
- Day II: 320 ml, intravenously, slowly.

In order to prevent possible allergic and hyperthermic reactions, 8 mg of dexamethasone was injected intravenously each time before plasma administration.

Study outcomes

Main endpoints:

- for patients hospitalized for a severe course of COVID-19 and with spontaneous breathing: the frequency of transfer to APV within 7 days after the first injection of plasma and mortality within 30 days;
- for patients with an extremely severe course, who use APV, mortality within 30 days.

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journal of clinical practice 2020 Том 11 №2

Secondary endpoints:

- incidence of acute respiratory distress syndrome;
- duration of stay in the resuscitation and intensive care unit;
- total duration of hospital stay;
- clinical status assessed in accordance with the World Health Organization recommendations;
- duration of use of APV;
- duration of the need for oxygen therapy;
- increase in the titer of antibodies to SARS-CoV-2 in blood plasma;
- changes over time of oxygen saturation level (arterial blood gases);
- changes over time of cytokines IL6, IL10, and TNFα;
- changes over time of the CRP level;
- changes over time of the level of fever;
- the frequency of indications for the administration of monoclonal antibodies to the IL6 receptor.

This report presents the results for achievement of the primary endpoints, as well as the secondary endpoints in the form of the changes over time of the levels of fever and CRP.

Ethical considerations

The study protocol was approved by the Local Ethics Committee of the Federal Clinical Research Centre (meeting minutes No. 4 dated 28.04.2020) and posted in the international clinical trials registry Clinicaltrials.gov (NCT04392414). The study included 85 COVID-19 patients and 146 anti-COVID plasma donors. All study participants who were conscious at the time of enrollment signed an informed consent. Patients in an extremely severe, unconscious state were included in the study by the decision of the case conference and the medical board in accordance with the temporary recommendations of the Ministry of Health of Russia for the purpose of transfusion of hyperimmune plasma for vital indications. The randomization was not performed in the latter case.

Statistical analysis

Statistical data processing was performed for all patients who received at least one plasma infusion and who have at least one post-baseline assessment in terms of safety and efficacy.

Data processing was performed in an electronic database (Excel) using the IBM SPSS Statistics 23.0 program. The changes over time of parametric data were analyzed using a two-way ANOVA for parametric indicators, as well as using nonparametric methods and comparison of results based on contingency tables (Pearson's χ^2 chi-squared test).

RESULTS

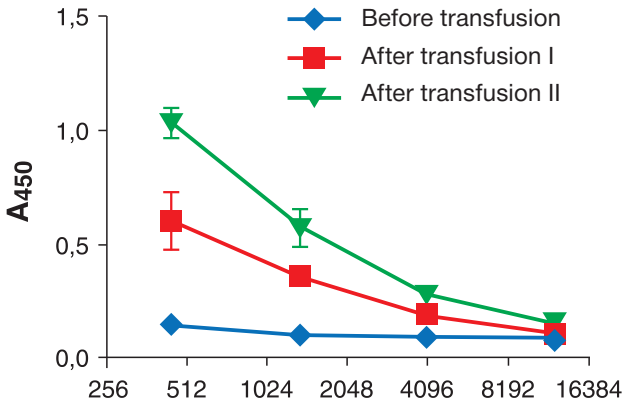
Subjects (participants) of the study

The study included 85 patients with COVID-19. Patients were enrolled according to the inclusion criteria from among those newly admitted to the infectious diseases hospital with a clinical presentation of viral pneumonia, confirmed by the presence of SARS-CoV-2 RNA and/or a characteristic X-ray image on chest CT. The patients enrolled who signed informed consent to participate in the clinical study were stratified into two groups. The group I (n = 20) included patients with severe and extremely severe course, who used APV; and the group II (n = 65) included patients with moderate (n = 28) and severe (n = 37) COVID-19 course, having spontaneous breathing. The group II was randomized into 2 cohorts with the ratio 2:1. The cohort 1 (odd randomization numbers) received hyperimmune pathogen-reduced anti-COVID plasma as therapy (n = 45), and the cohort 2 (n = 20) received non-immune fresh frozen plasma according to the same scheme. The study also analyzed two additional historical control groups (HC1 and HC2) of COVID-19 patients who were treated at the Federal Clinical Research Centre, receiving the same standard therapy, but without plasma. The HC1 group consisted of 70 ICU patients with severe and extremely severe forms of COVID-19, who used APV. The HC2 group consisted of 150 patients in inpatient departments, with registered moderate (45%) and severe (55%) conditions at the time of hospitalization, with an average age comparable to that of group II.

The titer of neutralizing antibodies was determined in all patients, starting from the day 1 after transfusion of immune plasma. This study has not yet been completed, however preliminary results showed that after transfusion, there is a significant increase in the titer of anti-RBD antibodies (up to 1:1000 and higher), detected for at least 3 days after the last transfusion (Fig. 1). Before the administration of immune plasma, neutralizing antibodies were not detected in the blood serum of the enrolled patients.

Group I patients received convalescent plasma transfusion while on APV for no more than 48 hours. One of the group I patients with a full-scaled presentation of ARDS received plasma while breathing spontaneously, however, already having indications for high-flow oxygen therapy ($SatO_2$ 90% at a flow of 15 L O_2/min in prone position). Within 12 hours, he was intubated and transferred to APV therefore it was decided to classify him as a group I patient whose ARDS had already developed. The average age of the group I patients was 62.9 ± 14.6 years (Table 1); 12 out of 20

Fig. 1. Determination of the titer of virus neutralizing antibodies to RBD S-protein of SARS-CoV-2 in a patient after transfusion of two doses of plasma.



patients had concomitant pathology in the form of hypertension, diabetes mellitus, angina pectoris, or cerebrovascular diseases. At the same time, in the group with an extremely severe course of the disease, there were 6 young men (30%) with a normal body mass index, who did not have any clinically significant comorbidities. All patients of the group I, having acute respiratory distress syndrome and severe respiratory failure, had laboratory signs of a cytokine storm, and showed severe lymphopenia (0.85 ± 0.21), very high CRP level (166 ± 44 mg/ml), significantly increased IL6 (102 ± 18 pg/ml) (Table 1). The vast majority of patients in this group (15 out of 20) at the time of initiation of plasma therapy had subtotal lung lesions (CT3 or CT4).

Group II patients were younger to some extent (mean age 55.3 ± 10.6 and 57.4 ± 12.3 years in the cohorts 1 and 2, respectively); more than 80% had comorbid pathology (Table 2). All patients of the group II had signs of an acute inflammatory reaction (lymphopenia, increased CRP, ferritin, moderate increase in IL6) and polysegmental viral pneumonia confirmed by CT with a characteristic X-ray presentation (areas of ground glass opacity and consolidation). 58% and

Table 1

General characteristics and main clinical and laboratory parameters of the group I patients who received convalescent plasma therapy (*n* = 20)

No.	Indicator	Value, M ± STD
1	Age, M ±STD (min; max), years	62.9 ± 14.6 (35; 89)
2	Gender, m/f	13/7
3	Body mass index, kg/m^2	28.5 ± 4.2 (26; 31)
4	Time from the onset of the disease, days	7 (5; 11)
5	Condition at the time of plasma administration	Severe — 6 (30%) Extremely severe — 14 (70%)
6	SpO_2 at the time of admission to the ICU, Me (min; max)	87 (83; 89)
7	Respiratory support	HFOT — 6 (30%) APV — 14 (70%)
8	Duration of APV before the start of plasma therapy, days, *n* (%)	1 day — 7 (35) 2 days — 8 (40)
9	Duration of APV, M ±STD (min; max), days	14.9 ± 7 (6; 28)
10	PCR-confirmed presence of SARS-CoV-2, *n* (%)	16 (80)
11	Stage by computed tomography, *n* (%)	CT2 — 5 (25) CT3 — 11 (55) CT4 — 4 (20)
12	CRP, mg/l	166 ± 44
13	Lymphocyte count, 1 × 10^9/l	0.85 ± 0.21
14	Ferritin, mg/l	874 ± 214
15	Glucose, mmol/l	7.14 ± 1.2
16	IL6 level, pg/ml	102 ± 18
17	Concomitant pathology worsening the disease prognosis, *n* (%)	12 (60)
18	Distribution by blood groups, *n* (%)	O (I) — 6 (30) A (II) — 10 (50) B (III) — 3 (15) AB (IV) — 1 (5)

Note. ICU — resuscitation and intensive care unit, HFOT — high-flow oxygen therapy, APV — artificial pulmonary ventilation, CRP — C-reactive protein.

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journal of clinical practice 2020 Том 11 №2

Table 2

General characteristics and main clinical and laboratory parameters of the group II patients.

No.	Indicator	Immune plasma *n* = 46	Non-immune plasma *n* = 21
1	Age, M ±STD (min; max), years	55.3 ± 10.6 (28; 89)	57.4 ± 12.3 (25; 78)
2	Gender, m/f	28/17	12/8
3	Body mass index, kg/m^2	26.5 (20; 31)	25.0 (20; 28)
4	Duration of the disease, days	9 (5; 14)	8 (5; 11)
5	Condition severity at the time of inclusion in the study, *n* (%)	Moderate — 19 (42) Severe — 26 (58)	Moderate — 8 (40) Severe — 12 (60)
6	SpO_2 at the atm. air at the time of inclusion in the study, %	91.5 ± 4.7	93.0 ± 3.0
7	SpO_2 during oxygen therapy, %	94.0 ± 1.7	94.1 ± 2.3
8	Body temperature at the time of inclusion in the study, °C	38.2 ± 0.6	38.1 ± 0.8
9	Duration of fever ≥ 38.0°C, days, Me (min; max)	5 (4; 8)	5 (3; 7)
10	Positive PCR test for SARS-CoV-2 RNA before plasma therapy, *n* (%)	38 (83)	17 (81)
11	Stage by computed tomography, *n* (%)	CT1 — 5 (11) CT2 — 17 (37) CT3 — 20 (44) CT4 — 3 (6)	CT1 — 2 (10) CT2 — 13 (65) CT3 — 5 (25) CT4 — 0
12	CRP, M ± STD, mg/L	89.4 ± 12.4	80.6 ± 19.9
13	Lymphocyte count, 1 × 10^9/l	1.01 ± 0.36	1.06 ± 0.60
14	Ferritin, mg/l	863.4 ± 678.1	823.0 ±429.9
15	Glucose, mmol/l	7.11 ± 2.96	6.44 ± 1.95
16	IL6 level, pg/ml	44 ± 11	38 ± 11
17	Distribution by blood groups, *n* (%)	O (I) — 14 (31) A (II) — 22 (49) B (III) — 5 (11) AB (IV) — 4 (9)	O (I) — 5 (25) A (II) — 9 (45) B (III) — 4 (20) AB (IV) — 2 (20)
18	Concomitant pathology worsening the disease prognosis, *n* (%)	40 (88)	16 (80)

Note. PCR — polymerase chain reaction, CRP — C-reactive protein.

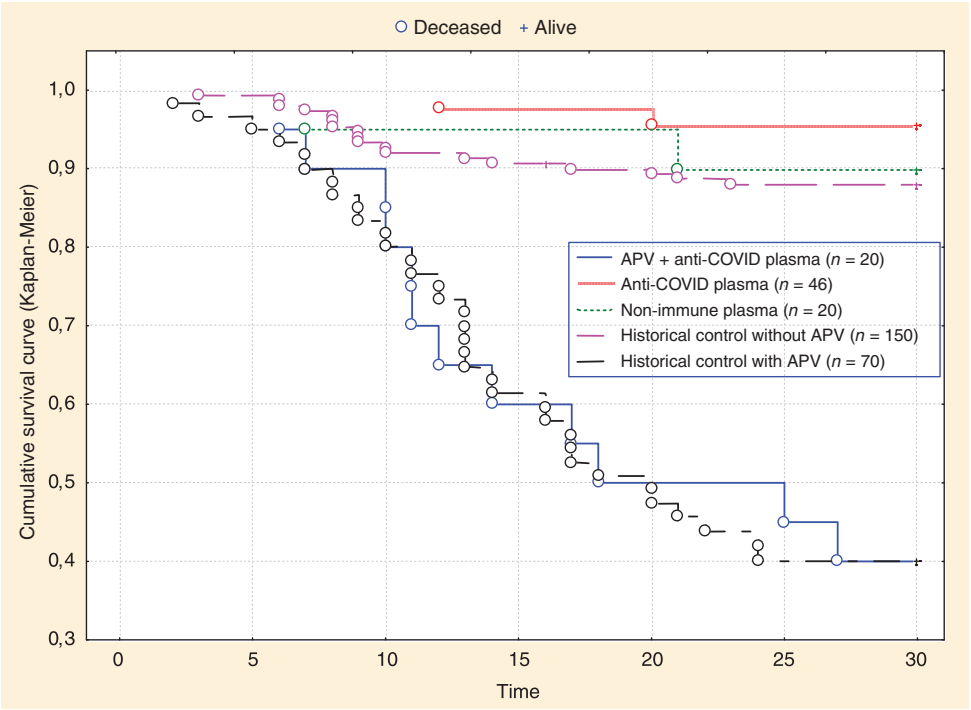
60% of patients in the cohorts 1 and 2, respectively, had signs of respiratory failure, which enabled to classify their condition as severe.

Key research results

In patients of the group I, who used APV, no serious adverse events were registered. No clinically significant reactions were also noted during transfusion of fresh frozen plasma in patients of the group II (for more details, see our previous work focused on the analysis of safety of therapy with convalescent plasma [19]). The most common side effects in all patients who received both immune and non-immune plasma were urticaria-type rash (7 (10.8%) and 1 (5%) cases), as well as febrile non-hemolytic reactions (5 (7.6 %) and 2 (10%) patients in the immune and non-immune plasma groups, respectively). One patient of the group II with a severe course of COVID-19, who received anti-COVID plasma, developed noncardiogenic pulmonary edema one hour after transfusion, which was regarded as a manifestation of the transfusion-related acute lung injury (TRALI). In total, adverse events in the anti-COVID plasma group (in total in the resuscitation and intensive care unit and linear departments) were registered in 14 patients, which amounted to 21.5% of the total number of patients in the group, while in those who received fresh frozen plasma, these were only in 3 (15 %).

In most patients of the group I, after administration of anti-COVID plasma, despite the detectable titer of neutralizing antibodies, there was a further increase in the serum concentration of CRP and ferritin, and the

Fig. 2. Cumulative survival curves of patients receiving immune and non-immune plasma



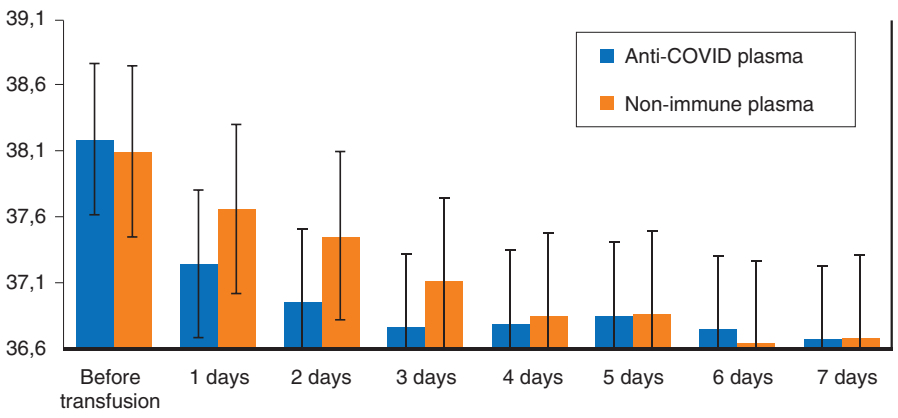
Note. APV — artificial pulmonary ventilation.

progression of lymphopenia. We did not reveal any other clinical and laboratory signs of the efficiency of plasma therapy in the first three days after administration in patients on APV. The thirty-day mortality rate of patients in the group I who received anti-COVID plasma was 60%, which almost completely corresponds to the average mortality rate of patients on APV in group HC1 (57.9%), determined from 70 completed cases in our hospital [20] (Fig. 2).

In the overwhelming majority of the group II patients (75%), after transfusion of convalescent plasma within 1–5 days, clinical improvement occurred, characterized by regression of fever, a decrease in respiratory failure, and normalization of laboratory parameters. In the subgroup of patients who received non-immune plasma, clinical improvement during the first 5 days was registered in 51% of cases.

The majority of patients in the group II who received transfusion of immune and non-immune plasma showed a significant antipyretic effect. At the same time, in the group of immune plasma, the temperature returned to normal somewhat faster, but the difference with the group of fresh frozen plasma did not reach a statistically significant level (Fig. 3). A similar tendency of slightly higher efficiency of hyperimmune plasma was noted when analyzing the changes over time of CRP concentration (Fig. 4), as on the day 2 after transfusion of immune and non-immune plasma, the differences almost reached the level of statistical significance (p = 0.1)

Fig. 3. Changes over time of body temperature in patients after plasma transfusion.



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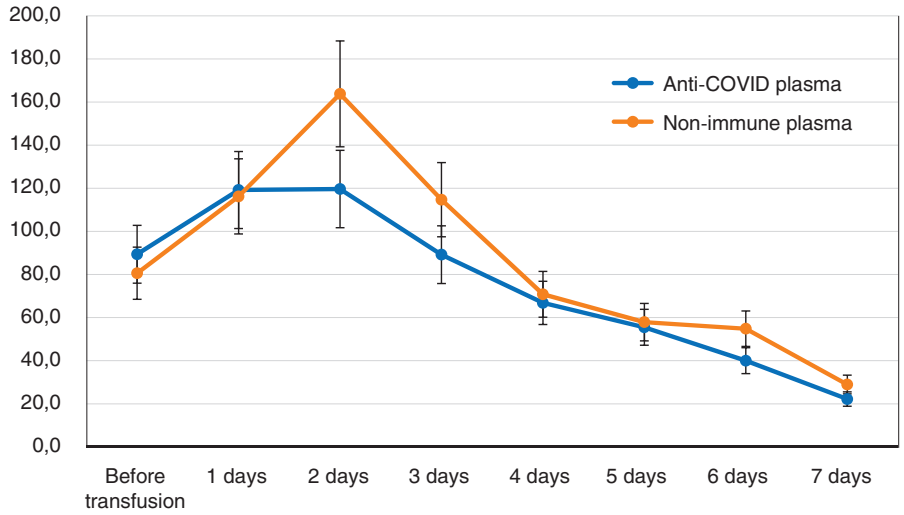
journal of clinical practice 2020 Том 11 №2

Fig. 4. Changes over time in the blood serum concentration of C-reactive protein in patients after plasma transfusion.



Out of the 46 patients of the subgroup 1 of group II who received anti-COVID plasma during the first 2 days after transfusion, 3 (6.5%) patients were transferred to APV, and two of them died. The cause of death of one patient was coronavirus pneumonia in presence of decompensated myeloblastic leukemia, while in another patient, who had severe course of COVID-19 after plasma administration, noncardiogenic pulmonary edema occurred.

In the group that received fresh frozen plasma, the need for APV also arose in 3 (15%) patients, and two of them died. Thus, hospital mortality in the group that received convalescent plasma was 4.3%, which is significantly lower than the total hospital mortality rate determined for all completed cases in our hospital (6.73%). In the retrospectively assessed control group HC2, corresponding to the anti-COVID plasma group in terms of the condition severity, age, and concomitant pathology, hospital mortality rate was 12.0% (18 patients out of 150) (Fig. 2). Thus, convalescent plasma therapy enabled to reduce the hospital mortality rate in moderate and severe course patients with spontaneous breathing by more than 2 times.

DISCUSSION

We initiated this clinical study at the peak of the increase in the incidence of COVID-19 in the city of Moscow due to the lack of effective etiotropic therapy and the urgent need to develop an emergency aid for patients with a severe course of the disease. At the same time, we were aware that the transfusion of such a biologically active substance as donor plasma, especially plasma of convalescents, can be accompanied by serious adverse events, and the decision to conduct a transfusion should be made each time only in cases where the risk of progression and lethal outcome of the disease outweighs significantly the possible risks of treatment complications. Given the lack of an evidence base for the efficiency of plasma therapy at the start of our studies, the FDA-recommended format of an open randomized clinical trial was chosen [20], which was preceded by a population-based study on the selection of hyperimmune plasma donors. Our experience has shown that among potential donors with the history of COVID-19, only 21% of the patients examined had a high titer of antibodies to S-protein RBD (1:1000 and higher). The proportion of hyperimmune donors can be increased only by selecting those who have had severe pneumonia (CT2, CT3) no more than 2 months ago, since the titer of neutralizing antibodies may decrease significantly (but not completely) after a specified time.

The first work to assess the safety and efficacy of convalescent plasma in the treatment of COVID-19 has already been completed, and the results have been published, while several dozen studies, including the one presented, are ongoing or are at the stage of analyzing the results obtained. It is worth noting that one of the first reports on the successful treatment of 5 critically ill COVID-19 patients, who used APV and received 2 plasma infusions with a volume of 200–250 ml, inspired many researchers and doctors [10]. Another study from China involving 10 critically ill patients, three of whom used APV, showed clinical and laboratory improvement after transfusion within 3 days of the follow-up in all patients, as well as a rapid reduction of CT symptoms [11]. However, further more representative and carefully planned studies did not confirm the optimistic conclusions about the efficiency of convalescent plasma in

critically ill patients. More recently, after the completion of enrollment of patients in our study, the results of one of the first open randomized multicenter studies of the safety and efficacy of convalescent plasma, conducted by L. Li et al., involving 103 patients in seven medical centers in Wuhan, were published [22]. According to these data of comparison of patients who received plasma (n = 52) or standard therapy (n = 51), there were no significant differences in the incidence of clinical improvement and in 28-day mortality, although the viral load after plasma therapy decreased significantly faster than in the control group. At the same time, after analyzing separately the subgroups of patients with severe and extremely severe course, with the use of APV, it turned out that the outcomes in patients who received plasma before the development of a critical condition and the initiation of APV were significantly better when compared with the corresponding control, while the use of plasma in a critical condition does not influence the outcome in any way.

Almost simultaneously, the results of researchers from the USA were published, where several medical centers in New York also conducted a randomized study of the safety and efficacy of convalescent plasma [23]. T. Sean et al. monitored the effect of plasma only in those patients who had not yet been intubated, although many of them received high-flow oxygen therapy [23]. Thus, the data of our study, in which we did not reveal a significant effect of plasma therapy on the disease outcome in patients used APV, are fully consistent with the results of multicenter studies from Wuhan and New York. It should be added that not only convalescent plasma, but also monoclonal antibodies against IL6 receptors and other immunosuppressive drugs are no longer effective in this category of patients with full-scaled acute respiratory distress syndrome and subtotal lung damage. The insidiousness of COVID-19 consists in the fact that this stage of the disease can develop very quickly, within a matter of hours, which requires an immediate response from an attending physician at the first signs of a cytokine storm.

The key result we have achieved was a more than twofold increase in the survival rate of patients breathing spontaneously after plasma therapy, compared with the control group. At the same time, in our study, it was shown that the decrease in mortality in patients after plasma administration is not associated with the nonspecific action of plasma immunoglobulins, since a significant decrease in mortality rate is not registered after transfusion of non-immune fresh frozen plasma. These results indicate that the decrease in mortality rate after hyperimmune plasma transfusion is most possibly due to the action of virus-neutralizing antibodies that inactivate the pathogen and cause turning in the course of the disease. At the same time, the antipyretic and anti-inflammatory effects of transfusion of immune and non-immune plasma did not differ significantly and, most probably, were caused by the action of nonspecific immunoglobulins and other factors present in normal plasma.

It should be noted that almost all studies noted good tolerance of convalescent plasma therapy, with rare and mild side effects; TRALI was not recorded in any case. Unfortunately, our experience has shown that adverse events occur more frequently with hyperimmune plasma transfusion than with transfusion of non-immune fresh frozen plasma. Given the occurrence of TRALI in one of the patients with a severe course of COVID-19, it is not necessary to conclude that hyperimmune plasma transfusion is not a completely safe method.

CONCLUSION

Thus, to date, conclusion can be made on the limited safety of using plasma from patients with the history of COVID-19 and the efficiency of this therapy in terms of at least a twofold increase in the survival rate of hospitalized patients with severe respiratory failure, who do not use APV. In the absence of bioengineered virus-neutralizing antibodies and effective etiotropic therapy, the use of hyperimmune plasma of convalescents is the simplest and most effective means of specific etiopathogenetic therapy for severe forms of COVID-19.

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journal of clinical practice 2020 Том 11 №2

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AUTHOR CONTRIBUTIONS

V.P. Baklaushev and A.V. Averyanov made an equal contribution to the planning and implementation of this study (V.P. Baklaushev performed analysis of the level of neutralizing antibodies, prepared plasma, and wrote the article; A.V. Averyanov enrolled the patients by inclusion criteria, monitored the clinical study implementation, and proofread the article). A.V. Perkina, O.I. Balionis were responsible for the patient management, and filling in the case report forms. A.V. Ivanov, V.T. Valuev-Elliston performed an enzyme immunoassay. A.G. Sotnikova, O.N. Novikova, N.V. Dupik, A.G. Kedrova, A.E. Sanzharov, N.A. Soloviev, A.G. Vinokurov, Yu.V. Ivanov, and V.N. Vasiliev performed the patient management in linear departments. G.M. Yusubalieva prepared the samples, and performed immunochemical studies; Ya.N. Glazov supervised the purveyance of blood plasma; T.V. Klypa managed the patients in the ICU. A.V. Troitsky was responsible for the overall project management. All authors took an active part in the execution of the work, read, made corrections and approved the final version of the article.

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AUTHORS INFO

The author responsible for the correspondence:

Vladimir P. Baklaushev, MD, PhD; **address:** 28, Orechovy boulevard, Moscow 115682, Russia];
e-mail: baklaushev.vp@fnkc-fmba.ru, **SPIN-код:** 3968-2971, **ORCID:** https://orcid.org/0000-0003-1039-4245

Co-authors:

Alexander V. Averyanov, MD, PhD, Professor; **e-mail:** dr.averyanov@gmail.com, **SPIN-код:** 2229-7100, **ORCID:** https://orcid.org/0000-0003-1031-6933

Anna G. Sotnikova, MD, PhD; **e-mail:** sotnikoffaa@gmail.com, **ORCID:** https://orcid.org/0000-0003-1237-8134

Anastasia S. Perkina, MD; **e-mail:** anastasyaperkina@gmail.com;
ORCID: https://orcid.org/0000-0003-0351-1802

Alexander V. Ivanov, PhD; **e-mail:** aivanov@yandex.ru, **SPIN-код:** 5776-5496,
ORCID: https://orcid.org/0000-0002-5659-9679

Gaukhar M. Yusubalieva, MD, PhD; **e-mail:** gaukhar@gaukhar.org, **SPIN-код:** 1559-5866,
ORCID: https://orcid.org/0000-0003-3056-4889

Oksana N. Novikova, MD, PhD; **e-mail:** Novikova_oksana_@mail.ru,
ORCID: https://orcid.org/0000-0003-2779-0383

Valentina E. Shikina, MD, PhD; **e-mail:** shik-val@mail.ru, **SPIN-код:** 8371-5054,
ORCID: https://orcid.org/0000-0002-6672-4269

Nikolay V. Dupik, MD; **e-mail:** dnv-74@yandex.ru

Anna G. Kedrova, MD, PhD; **e-mail:** kedrova.anna@gmail.com, **SPIN-код:** 3184-9760

Andrey E. Sanzharov, MD; **e-mail:** sanzh@mail.ru, **SPIN-код:** 5713-5791,
ORCID: https://orcid.org/0000-0003-1056-3053

Elena V. Shirshova, MD, PhD, **e-mail:** Shirshova60@rambler.ru, **ORCID:** https://orcid.org//0000-0001-9193-0534

Olga I. Balionis, MD; **e-mail:** balionis.oi@gmail.com, **SPIN-код:** 7208-4442,
ORCID: https://orcid.org/0000-0002-8251-4050

Vladimir T. Valuev-Elliston, PhD; **e-mail:** gansfaust@mail.ru, **SPIN-код:** 3492-4501,
ORCID: https://orcid.org/0000-0003-0365-570X

Natalia F. Zakirova, PhD; **e-mail:** nat_zakirova@mail.ru, **SPIN-код:** 8050-2592,
ORCID: https://orcid.org/0000-0001-9704-6977

Yaroslav N. Glazov, MD; **e-mail:** yglazov@bloodfmba.ru, **SPIN-код:** 3344-8644,
ORCID: https://orcid.org/ 0000-0001-7761-8093

Irina A. Panukhina, MD; **e-mail:** panyuirina@yandex.ru, **ORCID:** http://orcid.org/0000-0002-8662-3457

Nikolay A. Soloviev, MD, PhD; **e-mail:** my_docs@mail.ru, **SPIN-код:** 8024-7220,
ORCID: https://orcid.org/0000-0001-9760-289X

Alexei G. Vinokurov, MD, PhD; **e-mail:** avinok@yandex.ru

Yuri V. Ivanov, MD, PhD; **e-mail:** ivanovkb83@yandex.ru, **SPIN-код:** 3240-4335,
ORCID: http://orcid.org/0000-0001-6209-4194

Valentin N. Vasilev, MD; **e-mail:** vasilievvn@list.ru, **ORCID:** https://orcid.org/0000-0002-2853-4528

Tatyana V. Klypa, MD, PhD; **e-mail:** tvklypa@gmail.com, **ORCID:** https://orcid.org/0000-0002-2732-967X

Alexander V. Troitsky, MD, PhD, Professor; **e-mail:** dr.troitskiy@gmail.com,
ORCID: https://orcid.org/0000-0003-2411-6043

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