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We are reporting on a phase IIa study which aimed to determine the intubation rate, survival, viral clearance, and the development of endogenous antibodies in patients with COVID-19 pneumonia treated with convalescent plasma (CCP) containing high levels of neutralizing anti-SARS-CoV-2 antibodies. All 51 treated patients had COVID-19 pneumonia by radiographic and laboratory evaluation. Fresh or frozen CCP from donors with high titers of neutralizing antibodies was administered. The non-mechanically ventilated patients (n=36) had an intubation rate of 13.9% and a day-30 survival of 88.9%. The overall survival for a comparative group based on network data was 72.5% (1625/2241). Patients had rates of negative nasopharyngeal swab on day +10 and +30 of 43.8% and 73% respectively. Patients mechanically ventilated had a day-30 mortality of 46.7%; the mortality for a comparative group based on network data was 71% (369/520). All evaluable patients were found to have neutralizing antibodies on day +3 (n=47), and all but 1 had antibodies on day +30 and +60. The only adverse event was a mild rash. We are concluding that in this study of patients with COVID-19 pneumonia, CCP was safe and conferred transfer of antibodies while preserving endogenous immune response.

Clinical and laboratory evaluation of patients with SARS-CoV-2 pneumonia treated with high-titer convalescent plasma

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Authors contribution: The trial design and implementation was done by MLD, AI, SG, SR, KC, EB, JZ, KB, AU, LL, AR, MK, MV, RF, HS, DS, MG, TF, AG, AP, NB, LL, SS, SK, DSP. Management of the patients was done by BB, CC, RS, AAK, SS, SD, DA, RG, ET, KR, SS, AG, ST, AM, MB, PMcK. The statistical design and analysis were done by XG, MT. The laboratory and basic science were done by SP, SF, RK, DSP.

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SUMMARY: Patients with COVID-19 pneumonia receiving high-titers convalescent plasma showed antibody transfer with preservation of endogenous production. Database survival comparison is promising for the early group.

ABSTRACT
We are reporting on a phase IIa study which aimed to determine the intubation rate, survival, viral clearance, and the development of endogenous antibodies in patients with COVID-19 pneumonia treated with convalescent plasma (CCP) containing high levels of neutralizing anti-SARS-CoV-2 antibodies. All 51 treated patients had COVID-19 pneumonia by radiographic and laboratory evaluation. Fresh or frozen CCP from donors with high titers of neutralizing antibodies was administered. The non-mechanically ventilated patients (n=36) had an intubation rate of 13.9% and a day-30 survival of 88.9%. The overall survival for a comparative group based on network data was 72.5% (1625/2241). Patients had rates of negative nasopharyngeal swab on day +10 and +30 of 43.8% and 73% respectively. Patients mechanically ventilated had a day-30 mortality of 46.7%; the mortality for a comparative group based on network data was 71% (369/520). All evaluable patients were found to have neutralizing antibodies on day +3 (n=47), and all but 1 had antibodies on day +30 and +60. The only adverse event was a mild rash. We are concluding that in this study of patients with COVID-19 pneumonia, CCP was safe and conferred transfer of antibodies while preserving endogenous immune response.

Introduction

As of December 28, 2020, over seventy-nine million people around the world have been infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and over 1.7 million have died (1). The human and economic impact, unprecedented in our generation, has mobilized the medical community in search of effective treatment strategies. The angiotensin-converting enzyme 2 (ACE2) is necessary for SARS-CoV-2 to enter human cells (2). The initial phase of the disease occurs when SARS-CoV-2 infects the respiratory epithelial cells, but in addition to lung tissue, expression of ACE2 in found broadly, including in renal, intestinal, and adipose cells, leading to a wide viral impact on the host (3). Moreover, ACE2 has been shown to be upregulated by SARS-CoV-2 infections (4). The innate immune response to the viral infection leads to the release of cytokines, and the ensuing cytokine storm results in acute respiratory distress syndrome and multiorgan failure (5). The natural response to viral infections including coronaviruses, is the production of high affinity immunoglobulin G (IgG) during the adaptive immune response (6). SARS-CoV-2 has been associated with the suppression of this T cell-mediated immune response, which bring into question the quality of the adaptive immunity in severely ill patients (7). A therapeutic intervention focused on viral neutralization is therefore a priority.

Convalescent plasma as a method of passive immunity transfer has a long history dating to the Spanish flu of 1918 (8). More recently, convalescent plasma was deployed in the management of SARS (9) and MERS (10), with evidence of viral neutralization. Convalescent plasma therapy in the setting of SARS-CoV-2 infection is currently an active field of investigation (11-23), but information on immune transfer, the subsequent endogenous response, and the clinical course of patients at different stages of the disease remains incomplete. Furthermore, since the development of neutralizing antibody titers varies among COVID-19 recovered patients, convalescent plasma is a heterogeneous product of varying potency. In this study, we investigated both the clinical and laboratory parameters characterizing patients treated with high-titer anti-SARS-CoV-2 neutralizing convalescent plasma.

Results

Between April 15 and June 16, 2020, 52 patients were enrolled, one had a negative SARS-CoV-2 nasopharyngeal swab RT-PCR and was ineligible. For research purposes across studies, patients with COVID-19 at our institution were divided in three tracks based on acuity, track 1 being attributed to outpatients, track 2 for patients hospitalized but not requiring positive pressure mechanical ventilation, and track 3 for patients receiving positive pressure mechanical ventilation. Fifty-one patients were treated, 36 met criteria for track 2, and 15 patients met criteria for track 3. All 51 patients had radiographic evidence of pneumonia. A significant proportion of patients in track 2 were either immunocompromised (22%) or had active cancer (19%), as our hospital harbors a cancer center and stem cell transplant program. Demographic and baseline characteristics of patients in track 2 and 3, along with patients in our network COVID-19 database are summarized in table 1.

Among the 36 patients in track 2, 24 (66.7%) were infused with 500 mL of liquid fresh irradiated plasma and 12 (33.3%) received 400 mL of fresh frozen plasma. Distribution of fresh or frozen plasma was strictly based on availability. The median dose of plasma IgG_{1-4} infused was 27,537 ug/kg (IQR 21,550-61,408; n=23); 12/36 (33.3%) received plasma with viral neutralizing anti-spike protein titers >1:10,000 and 22/36 (61.1%) with titers 1:1000-10,000 and 2/36 (5.6%) with neutralizing titers 1:500-1000. The primary endpoint analysis for track 2 showed that patients had an intubation rate of 13.9% (95% CI: 4.7%-29.5%), enough evidence to reject our null hypothesis. Univariate analysis of numerous parameters was performed and is described in table 2. Older age was associated with an increased risk of intubation. The univariate analysis significance of tocilizumab cannot be ascertained as it was administered to patients with more severe disease. The rates of negative nasopharyngeal swab by RT-PCR on day +10 and +30 post treatment were 43.8% (95% CI: 26.4%-62.3%) and 73% (95% CI: 52.2%-88.4%) respectively. There was only one COVID-19-related readmission and the patient was subsequently discharged.

Secondary endpoints analysis for track 2 demonstrated a day-30 survival rate of 88.9% (32/36; 95% CI:73.9%-96.9%) (figure 1). Survival was compared with our network database for hospitalized patients with COVID-19 pneumonia for the months of March to May 2020. Data fields were selected for age $\geq$ 18, a positive SARS-CoV-2 PCR and an abnormal chest x-ray or CT scan, and excluded for positive pressure mechanical ventilation. 2241 patients met these criteria with a survival rate of 72.5% (1625/2241; p=0.036). Compared with the database,

the track 2 study group was younger, had more patients with active cancer or pregnancy, and had greater oxygen requirements. There were also more females and more patients receiving remdesivir (table 1).

Among the 15 patients in track 3, 12 (80%) were infused with 500 mL of liquid fresh irradiated plasma, 3 patients received fresh frozen plasma either 200 mL (1 patient) or 400 mL; volume and frozen status was strictly based on availability. The median dose of infused plasma IgG_{1-4} ug/kg was 38,260 (IQR 33,3076-50,426; n=12); 5/15 (33.3%) received plasma with neutralizing anti-spike protein titers >1:10,000 and 9/15 (60%) with titers 1:1000-10,000. The primary endpoint analysis for track 3 showed that patients had a day-30 mortality of 46.7% (7/15; 95% CI:21.3%-73.4%). Based on our study statistical plan, track 3 was closed after 15 patients as the null hypothesis could not be rejected. In a post-hoc analysis, the track 3 mortality rate was compared with our network database for hospitalized patients with COVID-19 pneumonia during the months of March to May 2020. Data fields were selected for a positive SARS-CoV-2 PCR and an abnormal chest x-ray or CT scan, and positive pressure mechanical ventilation. 520 patients met these criteria with a mortality rate of 71% (369/520; p=0.08). Compared with track 3 study patients, database patients were older, and more were receiving invasive mechanical ventilation (table 1). Secondary endpoints analysis for track 3 study patients demonstrated rates of negative nasopharyngeal swab or endotracheal secretion analysis by RT-PCR at day +10 and +30 of 85.7% (95% CI: 42-100%; n=7) and 100% (95% CI: 63-100%; n=8) respectively, with a median time from symptom onset to treatment of 15 days (IQR 9-19). There were no readmissions.

There was a single adverse event for all 51 patients, one patient developed a grade 2 rash (CTCAE v4.0) for which hydrocortisone 100 mg IV was administered once with resolution. Univariate analysis of numerous parameters was performed and is described in tables 2 and 3. For either tracks, there was no statistically significant difference in survival, duration of hospitalization, post infusion antiviral titers, and post infusion inflammatory markers (CRP, ferritin, IL-6 and D-dimers) between fresh and frozen plasma, infused plasma immune globulin subtype (IgA, IgM, IgG_{1-4}) content, or concomitant medications (listed in table 1). There was also no difference in these endpoints within the ranges of donor IgG antiviral titers used, which were all above >1:500 (2 donors) and predominantly >1:1000. The overall survival plots for each track are shown in figures 1 and 2. Track 2 and 3 survival comparison with network data is summarized in table 4.

Transfer of immune titers was evaluated by measuring the recipients' anti-SARS-CoV-2 neutralizing anti-spike protein RBD titer levels immediately pre-infusion and again on day +3. Eight patients (22.2%), all in track 2, had no pre-infusion titers, and subsequently all 8 were found to have anti-SARS-CoV-2 neutralizing titers on day +3. One transplant patient on immunosuppression was found to have undetectable titers on day +10. Patients in track 3 all had anti-SARS-CoV-2 titers pre-infusion, 4/15 (27%) >1:10,000, 10/15 (67%) 1:1000-10,000, and 1/15 (7%) 1:500-1000. However, we observed an increase on day +3 with 12/15 (80%) >1:10,000 and 3/15 (20%) 1:1000-10,000. All but one evaluated patient on study were found to have neutralizing titers on day +30 (n= 33) and all patients on day+60 (n=31) (figure 3).

Discussion

In this prospective study investigating the therapeutic use of convalescent plasma in patients with COVID-19 disease, we showed that the administration of high-titer donor plasma is safe and effectively transfers antiviral titers, while preserving the endogenous development of immunity. The study was conducted at the height of the epidemic in New Jersey, when most patients were hospitalized only if requiring oxygen supplementation. In congruence with this fact, all patients treated had pneumonia. Only 17% of patients concomitantly received remdesivir, allowing for the evaluation of convalescent plasma as the sole antiviral agent administered for most patients. Our results showed an intubation rate of 13.9% and for the ventilated patients a day-30 mortality of 46.7%. In a post-hoc analysis, the overall survival of the treated non-mechanically ventilated patients compares favorably with our network database, within the limitations of non-matched controls. Within the ranges of plasma antiviral titers above 1:1000, we were not able to see a difference in outcome based on titer levels. Frozen plasma was not inferior to fresh plasma. Plasma was infused without adverse events, except for one mild rash, to a wide spectrum of recipients including those who were ventilated, elderly, pregnant and immunocompromised.

In the search for antiviral therapy, our findings clearly demonstrate the safety of convalescent plasma and the passive transfer of antiviral titers. As the original data from China used fresh liquid plasma (11) and most centers in the United States make use of fresh frozen plasma, the lack of a significant difference between these products is important information. Frozen plasma allows the flexibility of use, as it can be accumulated and rapidly deployed

during a viral surge. Since most of the plasma were from donors with titers above 1:1000, we cannot determine a lowest level acceptable. However, we can ascertain within the statistical limits of this study that we need not limit our donor pool to those with the highest titers >1:10,000, and a cut-off of 1:1000 will be used for our subsequent studies.

Early viral neutralization, with the ensuing prevention of the catastrophic immune response to viral damage, forms the basis for the infusion of high-titer convalescent plasma. Our expectation at protocol inception was to have access to patients early in the course of their disease. The reality, however, of conducting a clinical trial in the setting of an overwhelming influx of cases meant that most patients were not hospitalized until later in their course, during the inflammatory phase. We therefore conducted an analysis of the non-immune patients which included patients early in their course and patients unable to mount an immunity, such as immunocompromised patients. Understanding the kinetics of immune response to the virus is important and has been recently elegantly described. In a series of 23 patients with mild or severe disease (24), IgG antibodies emerged at 10-15 days post onset of symptoms, were sustained for at least 6 weeks and with a similar IgG response for both the mild and severe groups. Based on these kinetic descriptions, we can confirm that the presence of antibodies on day +3 was from passive transfer and not time related. Interestingly, the same authors reported that most patients with severe disease still had viral shedding 30-40 days post onset of disease, bringing into question the neutralizing capability of those endogenous antibodies (25). In our study, recipients demonstrated a high level of viral clearance at post infusion day +10 and +30.

Track 3 represents a group of severely ill patients, either non-invasively or invasively mechanically ventilated, all with endogenous immune titers. Our management of patients with COVID-19 from mid-April 2020 on, reserved invasive ventilation almost exclusively for patients failing non-invasive positive pressure ventilation measures. The clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia has been previously reported (26, 27). In a series of 52 patients similar to our track 3 patients, receiving either invasive or non-invasive mechanical ventilation, 32 (61.5%) had died by day 28, and of the remaining 20 patients, only 8 (15.4%) were discharged (26). In our current study, track 3 patients had a day-30 discharge alive rate of 46.7% and a viral clearance of 86.7% at day +10 post treatment. This may support the position that passive transfer of antiviral titers may be of benefit even in patients with immunity.

The focus of most antiviral therapy has been early in the course of the disease. The track 2 patients day-30 discharge alive rate was 88.9%, even though 22% of patients were immunocompromised either from cancer or transplantation, 100% had pneumonia, and 89% required oxygen supplementation. A recent randomized study evaluated the effect of convalescent plasma on the time to symptom improvement in severe COVID-19 disease (13). Patients were excluded if they had high titers of S-protein-RBD-specific IgG antibodies ($\geq$ 1:640), leaving a similar patient population to our non-immune or minimally immune patients ($\leq$1:100-500). The median volume infused was 200 mL compared to 400-500 mL in our study. In this randomized study the day-28 mortality was 15.7% for the patients in the plasma group, with a discharge rate of 51%. Details of the plasma content or immunity transfer was not provided. There was a statistically significant increase in the rate of viral negativity by PCR in the plasma group, but no difference in the primary outcome of time to clinical improvement. This study was unfortunately limited by the small sample size.

Our study was limited by the lack of randomization to a control group, and the access to patients early in the disease course, where antiviral interventions is presumed to be of greatest impact. Our study was also not powered or designed to evaluate the optimal donor antiviral titer level, or the optimal dose of IgM and IgG to be infused. We are conducting a randomized study of convalescent plasma in high-risk patients with early onset disease with the aim of reducing hospitalizations.

In conclusion, we aimed at gaining a better understanding of the clinical and laboratory effects of high-titer convalescent plasma in hospitalized patients with severe COVID-19 pneumonia. We found that the infusion of convalescent plasma is safe, effectively transfers of antiviral titers, leads to a high incidence of viral clearance, and does not preclude the development of endogenous immunity. The low rate of intubation and the survival at day 30 are encouraging and warrant further evaluation within the context of a randomized study.

Methods

Study design

We conducted a single institution prospective phase IIa clinical trial. The study was performed at Hackensack University Medical Center. Patients were included if they were aged 18 years or older and were hospitalized for the management of symptoms associated with a documented infection with SARS-CoV-2. Patients were excluded for a history of severe transfusion reactions, infusion of immunoglobulins with 30 days, AST or ALT greater than 10 times the upper limit of normal, requirement for vasopressors and dialysis. Patients requiring intermittent vasopressors for sedation management were treated. The patient referral process was done by requests to a central research team. Any treating clinician could refer their patients to a central COVID-19 research basket requesting participation in this study and others. A research nurse would perform an initial screen, and patients who appeared eligible for this convalescent plasma study were then approached by the study's research nurses for final confirmation of eligibility and consenting purposes.

Prospective plasma donors were included if they were aged 18 to 60 years, had a history of a positive nasopharyngeal swab for COVID-19 or a positive antibody test, were at least 14 days from resolution of symptoms, had one subsequent negative swab, were found to have high titers of neutralizing antibodies against SARS-CoV-2 (>1:500), and met institutional and FDA regulations for donation of blood products.

Procedures

Volunteer donors were recruited through advertising in the local community. Individuals who agreed to participate and gave informed consent were evaluated at the John Theurer Cancer Center where they underwent a physical examination, completed a donor health questionnaire, had a nasopharyngeal swab for SARS-CoV-2 and blood drawn for complete blood count and chemistry, infectious disease markers, and HLA antibodies for female donors. These donors were then collected either at our facility or referred to our affiliated blood center. Plasma collected on site was distributed fresh in 500 ml bags, plasma collected through our affiliated blood center was frozen in 200 ml bags. Collection at either site was based solely on availability.

The presence of SARS-CoV-2 neutralizing antibodies was evaluated using the previously described COVID-19 ELISA protocol with recombinant spike receptor binding domain (RBD) as capture antigen, using Goat anti-Human IgG (H+L) Secondary Antibody, HRP from Thermo Fisher Scientific, catalog #31410 (26). High-titer sera was evaluated for virus neutralization in a viral cytopathic assay performed with Vero E6 cells at 100 x the TCID50 value. The assay using SARS-CoV-2 in Vero E6 cells was established under biosafety level 3 (BSL-3) containment to assess intracellular inhibitory potencies of small molecules. Final assay conditions were 30,000 Vero E6 cells per well and virus at a MOI of 0.01-0.05 in 200 ul. The plates were incubated for 48 or 72 hours at 37°C and 5% CO_2. Viral ToxGlo™ Luminescent Cell Viability Kit (Promega Corp, Madison, WI, USA) was used to provide a semi-quantitative measure of virus infected cell viability. We also assessed the levels of IgM using the RBD antigen as per the IgG ELISA, with Goat anti-Human IgM Secondary Antibody, HRP Thermo Fisher Scientific, catalog#31415. Donors found to have neutralizing IgG Spike RBD greater than 1:500 were selected for plasma donation, with a preference for titers 1:1000-10,000 and >1:10,000. Donors underwent plasmapheresis using the Trima Accel® system for either a planned fresh infusion of 500 mL or for cryopreservation in aliquots of 200 mL.

Recipients were referred by the clinical teams through the institutional COVID-19 research request process and were treated if eligible. A single infusion of convalescent plasma was administered at a rate less than 250 mL per hour. Premedication with diphenhydramine 25 mg IV and hydrocortisone 100 mg IV with or without acetaminophen was given. The use of fresh versus frozen plasma was based solely on the availability of product at the time of request. Exploratory blood work including serology for anti-SARS CoV-2 titers was performed immediately pre-infusion and on day +3, +10, +30 and +60 post treatment. SARS CoV-2 testing by RT-PCR from nasopharyngeal or endotracheal tube secretions was done on day +10 and if positive again on day +30. A 10 mL sample of plasma was collected at the bedside from the donor plasma bag immediately pre-infusion for analysis.

For comparison, we evaluated the outcomes of patients treated for COVID-19 within our hospital network system. Data was collected from the electronic health records of patients hospitalized. Patients in the database were selected if SARS-CoV-2 PCR tests were positive. The data was manually abstracted by nurses and physicians from the John Theurer Cancer Center as part of an unrelated Cancer Center Outcomes Division Covid-19 project. We selected all patients from this database with characteristics closest to the patients in our two treatment cohorts.

Statistical analysis

It is important to note that at the time of the study's statistical design in March 2020, the availability of outcomes data was more limited. Our statistical plan for this study included only patients ascribed to tracks 2 and 3. The primary endpoint for patients in track 2 was to evaluate the efficacy of convalescent plasma in reducing the rate of intubation. The primary objective for patients in track 3 was to evaluate the efficacy of convalescent plasma in reducing the mortality rate at day +30. The safety of convalescent plasma was also a primary objective. Secondary objectives for both groups included duration of hospitalization, overall survival, rate of virologic clearance by nasopharyngeal swab RT-PCR at day +10 and +30, impact of donor neutralizing antibody titer levels on the primary objectives, and recipient anti-SARS-CoV-2 titer levels pre-infusion and on days +3, +10, +30 and +60. Comparison with patients in the COVID-19 data base was not planned at inception and was done post-hoc.

We used a multistage design based on the sequential conditional probability ratio test, which is more efficient than the Simon's 2-stage design and has the flexibility of unplanned analysis (29). The design for each track had a type I error rate of 0.1 with statistical power of at least 0.8. The statistical design was based on the following hypothesis: for track 2 the null hypothesis assumed an intubation rate of 30% and the alternative hypothesis was an intubation rate $\leq$ 15%. The first stage analysis was after 12 patients. If 6 or more of the first 12 patients required mechanical ventilation to the therapy, there was < 0.059% chance that the mechanical ventilation rate would be less than 30% should the study continue to enroll all 36 patients. On the other hand, if 0 of the first 12 patients required mechanical ventilation, it was certain that the trial would meet its goal even if we enrolled all 36 patients. For track 3, the null hypothesis assumed a mortality rate of at least 49% with an alternative hypothesis of $\leq$ 25%. The first stage analysis was after 6 patients, if 5 or more of the first 6 patients died, there would be a less than 0.091% chance that the mortality rate would be less than 49% should the study continue to enroll all 19 patients. On the other hand, if 0 of the first 6 patients died, it is certain that the trial would meet its goal even if we enroll all 19 patients. In accordance with this statistical plan, track 3 enrolled a total of 15 patients before closing. The decision to accept or reject the null hypothesis was made based on interim data analysis in a three-stage process. Descriptive statistics were used to characterize the baseline profile of the subjects and exploratory outcomes. Frequency and percentages were used for categorical variables; mean (SD) and median (IQR) were used for the continuous variables. Confidence intervals for the intubation and mortality rates, and virologic clearance at day+10 and +30 was calculated using exact binomial. Kaplan-Meier method was used for overall survival (OS). Log-rank statistics was used to compare the OS between product types, donor titers, and pre-treatment immunity. Cox proportional hazards model was utilized to assess the effect of infused plasma neutralizing titers on OS. Univariate test was performed to explore associations between exploratory outcomes and interested groups. Fisher's exact test was used for categorical variables, and t-test/ANOVA, or its non-parametric version, for the continuous variables based on the normalized of the data. P-value less than 0.05 was considered significant. Statistical analyses were performed using SAS (Version 9.4) and RStudio (Version 0.99.902).

Study approval

The study is registered with ClinicalTrials.gov NCT04343755, FDA IND approval obtained 4/4/2020 and approved by our Institutional Review Board.

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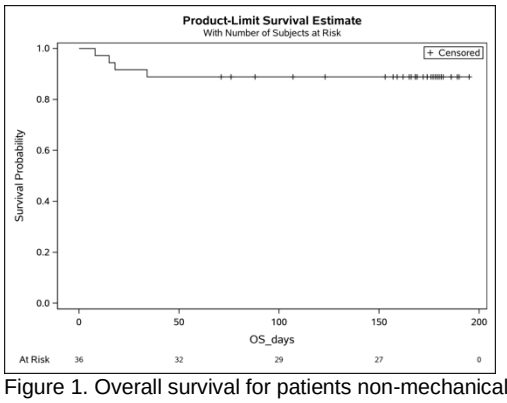

Figure 1. Overall survival for patients non-mechanically ventilated (track 2)

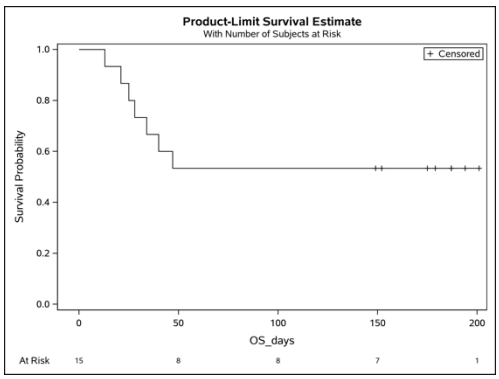

Figure 2. Survival of patients on positive pressure mechanical ventilation (track 3)

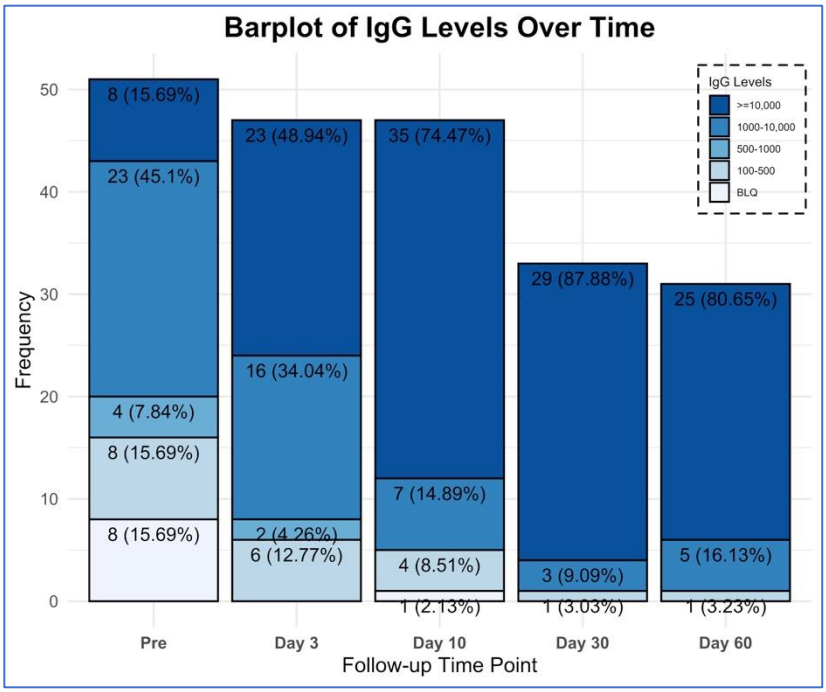

Figure 3. Recipients neutralizing antibody titers percentage and frequency over time for all patients

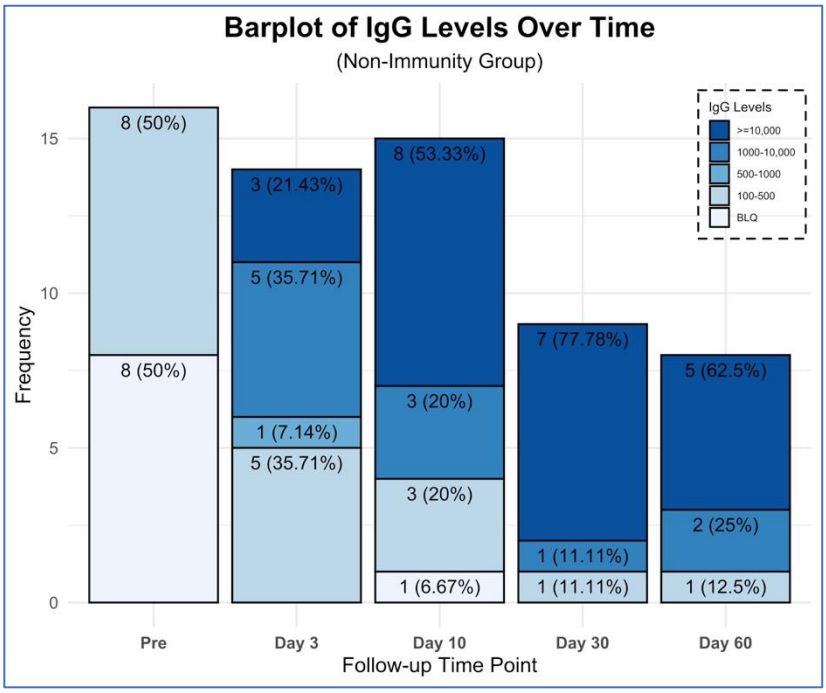

Figure 4. Neutralizing antibody titers percentage and frequency over time for non-immune or minimally immune patients (≤1:100)

	Track 2 n=36	database n=2241	p	Track 3 n=15		database n=520	p
Demographics							
Male sex	15 (41.7%)	1408 (63%)	0.014	10 (67%)		355 (68%)	1
Female sex	21 (58.3%)	833 (37%)	0.014	5 (33%)		165 (32%)	1
Median age, years	58 (IQR 49-68)	64 (53-76)	0.03	53 (IQR 45-58)		66.5 (IQR 56-76)	0.0002
Race							
Hispanic	22 (61%)			10 (67%)			
Caucasian	7 (19%)			3 (20%)			
Black	1 (3%)			1 (7%)			
Asian	5 (14%)			1 (7%)			
other	1 (3%)						
Clinical characteristics							
BMI, median	29 (24-34)	29 (IQR 25-33) n=2189	1	29 (25-32)		30.9 (IQR 26-34) n=513	1
Obese/morbidly obese	17 (47%)	917/2189 (42%)	0.5	4 (27%)			
Pregnant	3 (8%)	24 (1.1%)	0.008	0		4 (0.8%)	1
Hypertension	16 (44%)	1219 (54%)	0.24	6 (40%)		338 (65%)	0.06
Diabetes	9 (25%)	709 (32%)	0.47	7 (47%)		205 (39%)	0.6
Smoking	5 (14%)	71/1978 (3.6%)	0.01	2 (13%)		13/434 (3%)	0.09

COPD or asthma	8 (22%)			1 (7%)			
Immunocompromised	8 (22%)			1 (7%)			
Active cancer	7 (19%)	17 (0.8%)	<0.0001	1 (7%)		12 (2.3%)	0.31
Disease status on treatment day							
Pneumonia by CXR	34 (94%)	2241 (100%)	0.0002	15 (100%)		520 (100%)	1
Oxygen supplementation	32 (89%)	1022 (46%)	<0.0001	15 (100%)		520 (100%)	1
100% non-rebreather mask	8 (22%)	130 (13%)	0.001	n/a		n/a	
Positive pressure non-invasive mechanical ventilation	n/a			11 (73%)		118 (23%)	<0.0001
Invasive mechanical ventilation	n/a			4 (27%)		402/520 (77%)	<0.0001
Median days symptom to treatment	7 (IQR 4-10)	≥7; 987 (44%), <7;1067 (48%), unk;187 (8%)				≥7; 186 (36%), <7;259 (50%), unk;75 (14%)	
Concomitant medications							
Hydroxychloroquine	19 (53%)			12 (80%)			
Steroids	21 (58%)			13 (87%)			
Tocilizumab	5 (14%)			8 (53%)			
Remdesivir	7 (19%)	32/2015 (2%)	<0.0001	2 (13%)		26/434 (3%)	0.2
Inflammatory markers							
Median ferritin ng/mL	542 (IQR 200-1160) n=29			1520 (IQR 1100-2097) n=13			
Median CRP mg/L	10.6 (IQR 4.3-16.7) n=31			3.8 (IQR 1.8-9)			
Median IL-6 pg/mL	5 (IQR 5-10) n=15			10.5 (IQR 5.5-21.5)			

Table 1. Baseline characteristics of study patients and COVID-19 database patients

		No intubation	Intubation	p-value
N		31	5	
Age, median		56 (IQR 47, 64)	69 (IQR 66, 74)	0.007
Gender	Female	17 (55%)	4 (80%)	0.38
	male	14 (45%)	1 (20%)	
BMI median (Q1-Q3)		29 (25-34)	24 (23-32)	0.583
Hypertension	No	17 (55%)	3 (60%)	1
	Yes	14 (45%)	2 (40%)	
Diabetes	No	25 (81%)	2 (40%)	0.088
	Yes	6 (19%)	3 (60%)	
Heart disease	No	30 (97%)	5 (100%)	1

	Yes	1 (3%)	0	
Smoking	No	26 (84%)	4 (80%)	0.622
	Yes	4 (13%); 1 unknown	1 (20%)	
COPD or asthma	No	25 (81%)	3 (60%)	0.305
	Yes	6 (19%)	2 (40%)	
Active Cancer	No	25 (81%)	4 (80%)	1
	Yes	6 (19%)	1 (20%)	
Non-rebreather mask O_2, day 0	No	25 (81%)	3 (60%)	0.305
	Yes	6 (19%)	2 (40%)	
Steroids	No	13 (42%)	2 (40%)	1
	Yes	18 (58%)	3 (60%)	
Tocilizumab	No	29 (94%)	2 (40%)	0.013
	Yes	2 (6.5%)	3 (60%)	
Remdesivir	No	24 (77%)	5 (100%)	0.559
	Yes	7 (23%)	0	
Product: fresh/frozen	Fresh	21 (68%)	3 (60%)	1
	Frozen	10 (32%)	2 (40%)	
Infusion volume, mean		471 (SD 46)	440.00 (SD 54.77)	0.188
Donor titers	> 1:10,000	8 (26%)	4 (80%)	0.075
	1:1000-10,000	21 (68%)	1 (20%)	
	1:500-1000	2 (7%)	0	
IgM ug/kg infused, median		4678 (IQR 3600, 6775)	5805(IQR 4743, 9295)	0.598
IgG1 ug/kg infused, median		12275 (IQR 9514, 16289)	12700.30 (IQR 9218.29, 17697.79)	0.891
IgG2 ug/kg infused, median		9753 (IQR 5367, 14754)	8301 (IQR 6306, 10614)	0.441
IgG3 ug/kg infused, median		2428 (IQR 1180, 4659)	3449 (IQR 2754, 4301)	0.441
Ig G4 ug/kg infused,median		2415 (IQR 891, 19129)	18831 (IQR 744, 41669)	0.968
IgA ug/kg infused, median		5704 (IQR 3446, 8619)	7800 (IQR 533, 10360)	0.303
Total IgG ug/kg infused, median		27537 (IQR 21550, 47961)	47390 (IQR 22675, 74738)	0.839
Recipient IgG titers Pre	>1:10,000	4 (13%)	0	0.947
	1:1000-10,000	10 (32%)	3 (60%)	
	1:500-1000	3 (10%)	0	
	1:100-500	7 (23%)	1 (20%)	
	BLQ	7 (23%)	1 (20%)	
Recipient IgG titers Day3	>1:10,000	11 (39%)	0	0.168
	1:1000-10,000	11 (39%)	2 (50%)	
	1:500-1000	1 (4%)	1 (25%)	
	1:100-500	5 (18%)	1 (25%)	
Recipient IgG titers Day10	> 1:10,000	21 (68%)	3 (75%)	0.553
	1:1000-10,000	5 (16%)	0	
	1:500-1000	1 (3%)	0	
	1:100-500	3 (10%)	1 (25%)	

	BLQ	1 (3%)	0	
Recipient IgG titers Day 30	> 1:10,000	21 (91%)	2 (100%)	1
	1:1000-10,000	1 (4%)		
	1:100-500	1 (4%)		
Recipient IgG titers Day 60	> 1:10,000	18 (78%)		1
	1:1000-10,000	4 (17%)		
	1:100-500	1 (4%)		
Recipient titers IgM Pre	Negative	14 (45%)	2 (40%)	1
	Positive	17 (55%)	3 (60%)	
Recipient titers IgM Day 3	Negative	3 (11%)	1 (25%)	0.43
	Positive	25 (89%)	3 (75%)	
Recipient titers IgM Day 10	Negative	3 (10%)	1 (25%)	0.41
	Positive	27 (90%)	3 (75%)	
Ferritin Day 0, ng/mL median		525.15 (IQR 198.05, 1235.59) n=28	792.89 (IQR 763.50, 1972.29) n=5	0.353
Ferritin Day 3, median		522.60 (IQR 223.25, 752.97) n=24	1496.84 (IQR 1440.53, 1936.41) n=4	0.033
CRP Day 0, mg/L median		9.12 (IQR 4.05, 16.01) n=29	15.63 (IQR 15.00, 15.98) n=5	0.145
CRP Day 3, median		2.79 (IQR 1.21, 9.92) n=25	34.10 (IQR 25.35, 35.77) n=4	0.013
IL-6 Day 0, pg/mL median		5.00 (IQR 5.00, 11.70) n=15	5.00 n=1	0.056
IL-6 Day 3, median		5.00 (IQR 5.00, 5.00) n=9	203.00 (IQR 107.50, 298.50) n=2	0.064
D-dimer Day 0, mg/L median		1.07 (IQR 0.87, 1.48) n=28	1.77 (IQR 1.55, 2.12) n=4	0.040
D-dimer Day 3, median		0.94 (IQR 0.55, 1.61) n=25	2.76 (IQR 1.78, 7.72))n=4	0.029

Table 2. Distribution of variables and univariate analysis for patients not mechanically ventilated (track 2).

Page Break

		Alive	Dead	p-value
N		8	7	
Age, median		55.5 (IQR 50.00, 59)	49 (IQR 41, 56)	0.267
Steroids	No	2 (25%)		0.467
	Yes	6 (75%)	7 (100%)	
Tociluzumab	No	5 (63%)	3 (43%)	0.619
	Yes	3 (37%)	4 (57%)	
Remdesivir	No	6 (75%)	7 (100%)	0.467
	Yes	2 (25%)	0	
Product: fresh/frozen	Fresh	6 (75%)	6 (85.7%)	1
	Frozen	2 (25%)	1 (14.3%)	
Infusion volume, mean		450 (SD 106.9)	486 (SD 37.8)	0.619
Donor IgG titers	>1:10,000	2 (25%)	3 (44%)	1
	1:1000-10,000	5 (62.5%)	4 (57%)	
	1:500-1000	1 (12.5%)	0	
IgM ug/kg infused, median		6638.97 (IQR 5439.39, 8279.42)	4475.55 (IQR 3920.08, 6727.85)	0.194

IgG1 ug/kg infused, median		16535.29 (IQR 12813.00, 22444.04)	12009.06 (IQR 10317.84, 15954.94)	0.256
IgG2 ug/kg infused, median		15094.85 (IQR 8622.97, 18774.82)	7389.73 (IQR 6058.87, 12827.95)	0.104
IgG3 ug/kg infused, median		3150.47 (IQR 3031.66, 3281.62)	4169.17 (IQR 2343.29, 9080.77)	0.745
IgG4 ug/kg infused, median		4931.74 (IQR 1312.22, 13930.51)	1513.87 (IQR 657.88, 5799.86)	0.626
IgA ug/kg infused, median		6379.23 (IQR 6377.62, 10133.75)	6564.96 (IQR 5763.48, 6774.62)	0.871
Total IgG ug/kg infused, median		46536.05 (IQR 35410.90, 73192.06)	33628.45 (IQR 29786.79, 43242.17)	0.104
Recipient IgG titers Pre	>1:10,000	2 (25%)	2 (28.6%)	0.765
	1:1000-10,000	6 (75%)	4 (57.1%)	
	1:500-1000	0	1 (14.3%)	
Recipient IgG titers Day 3	>1:10,000	6 (75%)	6 (85.7%)	1
	1:1000-10,000	2 (25%)	1 (14.3%)	
Recipient IgG titers Day 10	>1:10,000	7 (87%)	4 (80%)	1
	1:1000-10,000	1 (12.5%)	1 (20%)	
Ferritin Day 0, ng/mL median		1778.07 (IQR 1057.64, 2109.22) n=8	1288.90 (IQR 1186.70, 1635.94) n=5	0.942
Ferritin Day 3, median		1046.03 (IQR 745.52, 2187.83) n=7	1448.58 (IQR 999.67, 2370.05] n=6	0.830
Ferritin Day 10, median		1102.88 (IQR 956.82, 1248.94) n=2	1479.57 (IQR 1098.72, 1642.57] n=5	0.561
CRP Day 0, mg/L median		2.34 (IQR 1.73, 10.82) n=8	6.86 (IQR 3.17, 8.86) n=7	0.524
CRP Day 3, median		6.1 (IQR .28, 14.2) n=7	7.19 (IQR 2.62, 16.89) n=7	0.443
CRP Day 10, median		2.27 (IQR 1.57, 3.96) n=3	7.12 (IQR 3.57, 14.98) n=4	0.596
IL-6 Day 0, pg/mL median		13.00 (IQR 11.25, 25.50) n=4	6.00 (IQR 5.00, 19.75) n=6	0.331
IL-6 Day 3, median		9.00 (IQR 4.75, 29.50) n=3	9.00 (IQR 5.00, 75.25) n=6	0.694
IL-6 Day 10, median		5.00 n=1	79.00 (IQR 51.00, 212.50) n=3	0.371
D-dimer Day 0, mg/L median		1.84 (IQR 1.59, 11.15) n=7	2.65 (IQR 1.58, 6.29) n=7	0.848
D-dimer Day 3, median		2.17 (IQR 1.35, 12.50) n=7	5.89 (IQR 3.56, 11.64) n=7	0.522
D-dimer Day 10, median		1.92 (IQR 1.39, 5.75) n=3	2.35 (IQR 1.43, 5.76) n=5	0.766

Table. 3 Distribution of variables and univariate analysis for patients on positive pressure mechanical ventilation (track 3).

		Track 2	Track 2 database controls	p-value
	n	36	2241	
Survival status (%)	alive	32 (88.9%)	1625 (72.5%)	0.036
	dead	4 (11.1%)	616 (27.5%)	
		Track 3	Track 3 database controls	p-value
	n	15	520	
Survival status (%)	alive	8 (53.3%)	151 (29%)	0.08
	dead	7 (46.7%)	369 (71%)	

Table 4. Survival comparison of track 2 and 3 with database control.