Original Research Article

CONVALESCENT PLASMA INFUSIONI IS NON EFFECTVE IN REDUCTION OF MORTALITY LATEIN ITALIAN HOSPITALIZED PATIENTS WITH SEVERE COVID-19 PNEUMONIA: A CASE-CONTROL PROSPECTIVE STUDY

Abstract

Background: This case-control prospective study aimed to describe the effects of COVID convalescent plasma (CCP) therapy in patients diagnosed with coronavirus disease 2019 (COVID-19) pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, during the fourth trimester of 2020 in Venice Prefecture (North East Italy).
Methods: 214 consecutive patients admitted to COVID-19 Hospitals of our district were enrolled. The confirmation of SARS-CoV-2 infection was made through the reverse transcription-polymerase chain reaction test in nasopharyngeal swabs. The severity of each patient's clinical condition was determined following the Berlin score. The clinical data, laboratory test reports and imaging diagnosis of all patients were analysed at hospitalization. All subjects were showing symptoms of severe COVID-19 pneumonia and were treated with conventional therapy; 108 patients received standard therapy and 106 received standard therapy in combination with CCP. The endpoint was mortality at 30 days.
Results: The two patient series were comparable for gender, age, risk factors, Berlin score, PaO2/FiO2. Both groups received standard therapy: NSAIDs, ventilator support, LMWH, steroids, antibiotics and antivirals; 106 subjects were additionally administered CCP. The mortality rate at 30 days was 30.6% among subjects treated with standard therapy versus 30.2% among subjects treated with standard therapy plus CCP. Considering the radiological findings and ventilatory support data, it would appear that the disease was more severe in patients treated with ST plus CCP.
Discussion: This case-control prospective study showed that in a cohort of hospitalized patients suffering from severe 19 pneumonia, the addition of CCP to the standard therapy did not impact the mortality rate at 30 days. However, we believe that further studies are needed to evaluate the effectiveness of convalescent plasma therapy.

Keywords: COVID-19, Convalescent Plasma, Mortality, Pneumonia, Risk factors.

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Introduction

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At the end of 2019, a new coronavirus strain was reported in the Chinese province of Wuhan and was named SARS-CoV-2, its subsequent disease was named COVID-19. The rapid spread of this infection in Italy led to a high number of hospitalizations and deaths, and exceeded the response capacity of our National Health System [1-3]. Moreover, all available antiviral treatments proved to be only partially effective [4-6].Previous data on the use of convalescent plasma during the recent Ebola, SARS and MEV outbreaks suggested that patient prognosis can be improved by administering neutralizing antibodies from convalescent subjects [7-9].Effectiveness of CCP therapy in COVID-19 patients is currently being discussed, with some authors reporting a satisfactory therapeutic impact when treating COVID-19 patients with CCP [10-13], while analysis of two large open-label randomized trial: CONCOR-1 [14] and RECOVERY [15] demonstrated tha convalescent plasma did not reduce the risk of intubation or death at 30 days in hospitalizated patients with COVID-19.

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Interpretation of published studies is made more complex by the variability of the volume of CCP administered. Furthermore, the definition of CCP appears to be ambiguous. From a methodological perspective, neutralization is considered the reference method for antibody quantification [16-18]. Recommendations regarding antibody titer differ, with the IstitutoSuperiore di Sanità (ISS) strictly recommending a titer > 1/160 [16], the FDA allowing for a titer >1/80 [17] and the European guidelines prescribing a titer > 1/320 [18].

The aim of this case-control prospective study was to report some considerations about our experience treating hospitalized COVID-19 patients with pneumonia and moderately – severely compromised respiratory function. The study was performed in the Venice area between October - December 2020, during the second epidemic wave in Italy.

Materials and Methods

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Study Design: This was a single center case-control prospective study on the long-term effectiveness and safety of the infusion of CCP in hospitalized patients with COVID-19 pneumonia with moderately to severely compromised respiratory function, according to the

Berlin score [19]. Moreover the radiological findings were classified as follow 1) bilateral pneumonia, 2) presence of ground-glass opacities,3) evidence of pulmonary consolidations. The primary endpoint of the study was 30-day mortality. Data were recorded at hospital admission as reported in clinical documentation.

Setting and population: The study was conducted in two COVID-19 hospitals in Venice Prefecture (North East Italy) and was approved by the local ethical committee. Patients were enrolled between October 01, 2020 and December 31, 2020. Follow-up concluded on January 31, 2021. All of the 214 patients admitted to Hospital with COVID-19 pneumonia were enrolled in this study and received standard treatment with steroids (ST), low molecular weight heparin (LMWH), non-steroidal anti-inflammatory drugs (NSAID), antibiotics (AB) and antiviral drugs (AV); of these, 106 received CCP infusion as well.Patientsreceived standard therapy alone or in combination with CCP, according to the indications of the clinicians who were treating them, meaning no randomization was involved. The enrollment protocol for the administration of CCP envisaged a maximum time interval of 12 days from the onset of symptoms.

Convalescent donors and plasma: Males and females aged from 18 to 60 years and with no history of blood transfusions or pregnancies who had recovered after a symptomatic and microbiologically confirmed (positive molecular nasopharyngeal swabs) SARS-CoV-2 infection were recruited. Each donor gave specific written consent to the procedure after receiving adequate information in a confidential interview with a doctor. Their suitability for plasma donation was assessed according to current Italian guidelines and transfusion law [20]. Further enrollment criteria were: complete clinical recovery, two consecutive negative nasopharyngeal molecular swabs performed between 7 - 30 days before potential recruitment as donors and a neutralizing titer of anti SARS-CoV-2 antibody $\geq$80 [21]. In addition to the tests required by law, CCP donors were tested for SARS-CoV-2-RNA, HAV-RNA, HEV-RNA, Erytrovirus-DNA, Anti-SARS-CoV-2 antibodies.Plasma (650-700 mL) was collected using latest-generation cell separators (Aurora – Fresenius Kabi), and, after each procedure, was immediately equally divided into three bags (about 200 mL) using a sterile tubing welder. Plasma pathogen reduction was performed with the INTERCEPT processing system (Cerus Europe BV) [22]. The units collected were stored at a controlled temperature ranging from - 40°C to - 30°C.

Plasma infusion: Plasma was delivered ready-for-use by the Transfusion Medicine Service to Clinical Units and was administered to patients over 30 to 60 minutes, on three

consecutive days, under supervision of the treating physician. Each patient gave specific written consent to the procedure after receiving adequate information in a confidential interview with a doctor.

SARS-CoV-2 micro neutralization assay: The titerof neutralizing antibodies against SARS-CoV-2 was determined as previously described [23]. In summary, 50 mL of a serum sample from each donor, starting from 1:10 in a serial 4 - fold dilution series, were added to two wells of a flat-bottomed tissue culture microtiter plate mixed with an equal volume of 50, 50% tissue culture infectious dose ($TCID^{50}$) of a previously titrated, SARS-CoV-2 strain. Plates were incubated at 33°C in 5% CO^2. After 1 h of incubation, VERO E6 cells were added to each well. After 48 additional hours of incubation the wells were scored to evaluate the degree of cytopathic effect compared to that of the virus control. Blue staining of the wells denoted the presence of neutralizing antibodies. The neutralizing titer was the maximum dilution that presented a 90% reduction of the cytopathic effect. A positive titer was defined as equal or greater than 1/10. Positive and negative controls were included in all test runs [24,25].

*Statistical Analysis:*Data were analyzed using MedCalc Ver.8.0.0 (Medcalc SW Bvda, Ostend, Belgium). Categorical data are presented as numbers (percent) and continuous data as median (I and III quartiles). Value distribution was evaluated using the Skewness (CS) and Kurtosis (CK) coefficients, normality was estimated using the D'Agostino-Pearson (DP) test. Proportions comparison was performed using a Chi square test; median comparison was performed using a Mann-Whitney test. A $p<0.05$ was considered statistically significant.The primary outcome was analyzed using a two-sided Wald test of the null hypothesis that probability of death at 30 days is the same among patients receiving standard therapy or standard therapy plus CCP.

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Results

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We recruited 214 patients, 106 were treated with standard therapy (ST) and also received CCP, 108 received only standard therapy. As reported in table I,at hospital admission,the two series were comparable for age and gender distribution; comorbidity and risk factors such as tobacco smoke, hypertension, hyperlipemia, diabetes, obesity, cardiovascular disease, respiratory disease (RD), neoplastic disease, chronic kidney impairment (CKI).

As reported in table II, at hospital admission the two series of were comparable for PaO2/FiO2 and severity of pulmonary function impairment. No significant difference could be detected. As a matter of fact, we observed a moderate ARDS (Acute Respiratory Distress Syndrome) in 57 (53.7%) patients treated with CCP infusion versus 59 (54.6%)

patients treated without CCP, and a severe ARDS in 49 (46.3%) patients treated with CCP infusion versus 51 (45.4%) patients treated without CCP.
Regarding chest imaging we observed a higher (p=0.02) prevalence of evidence of pulmonary consolidations. in patients treated with CCP.

In this patient series all subjects required O2 support. Among patients treated with CCP, we observed 14 patients (13.2%) that needed low flow O2 with a simple mask, 22 (20.7%) needed high flow O2 with a reservoir or a Venturi mask, 70 (66.1%) needed C-Pap and/or intubation. Among patients treated with ST, we observed 23 patients (21.3%) that needed low flow O2 with a simple mask, 21 (19.4) needed high flow O2 with a reservoir or a Venturi mask, 65 (60.3%) needed C-Pap and intubation. Among patients treated with CCP we observed a statistically significant higher (p=0.01) prevalence of subjects needing intubation.
As reported in Table III all patients received AB and NSAIDs, no significant difference was observed between the two patient series for ST, LMWH and Tozicilizumab. A higher percentage of subjects treated with ST alone was treated with Remdesivir, compared to patients that received CCP therapy (88.9% versus 76.4%, p=0.01).
Among 106 patients treated with CP,collateral effects were observed in 4 (3,7%): 3 urticaria and 1dyspnoea.
With regards to mortality within 30 days from diagnosis, we recorded 32 deaths (30.2%) among patients treated with CCP infusion versus 33 (30.6%) among patients treated without CCP plasma infusion (a two-sided Wald test did not allow to exclude the null hypothesis).

Discussion

Based on experience gained during the SARS-CoV epidemic in 2002 [9] and Ebola virus outbreak in 2015 [8], the use of convalescent plasma has been considered a possible therapeutic solution for COVID-19 patients. Obviously, CCP must meet the safety requirements established by Italian National transfusion regulations as well as the specific product standard agreed upon by the National Blood Centre [20,26]; it must also adhere to specific characteristics and quality requirements (e.g. treatments or presence / concentration of anti-SARS-CoV-2 antibodies) defined when used as part of the clinical protocol for which it is requested [26,27].
At present, despite the numerous clinical studies and meta-analysis conducted so far, while the safety of therapy with CCP has been confirmed, conclusive results with respect

to its effectiveness have not yet been provided [29-31]. A recent WHO document recommended against administering CCP except in the context of clinical trial [32].

During the second epidemic wave in Italy, therapeutic protocols for the treatment of patients with COVID-19 were not fully defined and they were based on: ventilatory support, NSAIDs [33], low molecular weight heparin [34], steroids [35], antibiotics [36] and antivirals (mainly Remdesivir) [37]. In Italy, CCP has never been considered a standard therapy for COVID-19 patients, meaning its use largely depended on the clinician's "beliefs" on its effectiveness [16,21,22]. Therefore, patients were not randomized and CCP was assigned as required by the clinical departments.We chose to study patients with COVID pneumonia at hospitalization [37]. Our case-control prospective study was performed to compare the outcome of patients with COVID-19 pneumonia and moderate to severe ARDS. 214 patients admitted to the two COVID-19 hospitals (Dolo and Jesolo) set up in the prefecture of Venice in the fourth quarter of 2020 were enrolled; 108 subjects received ST alone and 106 ST plus CCP. At hospital admission the two patient groups were comparable for age, gender distribution, comorbidity such as hypertension, diabetes, hyperlipemia, cardiovascular disease, CKI, RD and risk factors such as tobacco smoke and obesity. Moreover, at first evaluation the two groups were also similar for PaO2/FiO2 ratio and disease severity classification using the Berlin score [19,39-41].

Taking into account radiological findings, we found a lower prevalence of subjects with evidence of pulmonary consolidations among patients treated with standard therapy than in patients who additionally received CCP infusion. Moreover, patients treated with standard therapy alone showed a higher need of low flow O2 support than patients who received CCP infusion. These data would seem to suggest that patients treated exclusively with ST presented a milder disease than patients treated with ST plus CCP.

In the authors' experience, considering 30-day mortality as the endpoint, no effectiveness in the use of convalescent plasma could be highlighted.In fact, in the 108 patients treated with standard therapy the mortality at 30 days was 30.6% while in patients treated with standard therapy plus convalescent plasma the mortality at 30 days was 30.2%. As a matter of fact, a two-sided Wald test did not allow to exclude the null hypothesis. The results relating to the ineffectiveness of CCP treatment in reducing mortality in long-term (30 days) in hospitalized patients are confirmed by the CONCUR-1 and RECOVERY studies [14,15].

In our patient series we detected a mortality ratio higher than data reported by other authors [42,43]. We believe that this observation can be traced back to the high

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percentage of critically ill patients enrolled in this study. It should be noted that, in the group of 108 subjects treated only with standard therapy, 38 (35.2%) patients were admitted to Intensive Care Units (ICU); while, among 106 subjects treated with standard therapy plus CP, 36 (36.8%) were admitted to ICU. A mortality of around 30% is in keeping with what is expected for patients classified with moderate - severe ARDS according to the Berlin score [16,21].In subjects treated only with standard therapy, we observed 23 deaths out of 38 patients admitted to ICU (60.5%), versus 10/70 (14.3%) patients who did not need intensive care, whereas in subjects treated with CCP plus standard therapy, we observed 23 deaths out of 39 patients admitted to ICU (59.1%), against 9/67 (13.4%) among patients who did not need to be admitted to ICU.

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In this patient series plasma infusion was generally well tolerated, in fact only four (3.7%) mild adverse reactions were observed, none of which required the suspension of therapy [44,45].

The authors are aware that the study has some limitations.First of all, it is not a polycentric prospective randomized study but a single center case-control prospective study.Secondly, only one primary endpoint (30-day survival) was defined.Thirdly, a high percentage of critically ill patients, hospitalized in intensive care units, was enrolled in both groups, which may explain the high mortality rates observed. Moreover during the second wave of COVID-19 pandemic in Italy, due to the lack of evidence-based treatment for COVID-19, pre-hospitalization treatment was not well-defined and probably heterogeneous, although ministerial guidelines recommended "watchful waiting". On that background, patients might have been treated differently before being hospitalized for severe disease, with unknown effect on the clinical outcome. Unfortunately we do not have any data about the treatments carried out at home before admission to the hospital.

In our opinion the most interesting aspect of this report study, is that we used an age-gender, comorbidity, and other parameters COVID-19 matched group for comparison, as in most of the previous studies, control groups used for comparison were not composed of age-gender, comorbidity, and other COVID-19 treatments matched patients [38-43]. One further aspect to consider is the level of expertise of staff. It is obvious that the expertise of clinical staff and available technology could not but influence the outcome [38-43]. In our study, all the patients were hospitalized and treated in the same two COVID-19 hospitals in our Prefecture. We therefore believe that any influence linked to the level of expertise of the clinical staff and available technology was minimal.Furthermore, we also tried to reduce, as much as possible, the variables related to the hyper immune plasma, and that

is why, for the group also treated with CCP the dose was standardized: one 200 mL unit per day for three consecutive days, the transfusion was carried out centrally using hyper immune plasma with a neutralizing antibody titer≥ 80 [10,11,16,33].

In conclusion, SARS-CoV-2 has spread rapidly across the globe and preparedness of National Health System in the Western World proved to be inadequate. In the absence of a specific and effective treatment during the current pandemic, thousands of patients died, thousands more needed intensive care and the Hospital system nearly crashed. In these critical conditions it seems CCP therapy should be effective for a better outcome of COVID-19 infection in severe and critically ill patients. Our data do not confirm this hypothesis as it was not possible to observe a decrease in the mortality rate at 30 days.However, we believe that further studies are needed to evaluate the usefulness of convalescent plasma therapy, the selection criteria for patient selection needed to evaluate the effectiveness of convalescent plasma therapy, the criteria for patient selection, assessment of CCP titre, administration schedule warrants further consideration.

Ethical Statement: The study was authorized by the Ethics Committee for Clinical Research of the province of Venice with decree N ° 196828 of 15/05/2020.
All the procedures described in the study, and which involved human beings were implemented in accordance with the ethical standards established by the Helsinki Declaration of 1964 and subsequent amendments.
Each patient / donor has issued written informed consent for enrolment in the convalescent plasma collection project.
Each patient / recipient gave written informed consent for enrolment in the arm that involved the administration of convalescent plasma.
No animal studies were performed in the conduct of the study.

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Table I: Patient series profile at hospital admission

	Plasma Infusion YES	Plasma Infusion NO	Statistical Significance
Patients	106	108	p>0.05
Male	83 (78.3%)	79 (73.1%)	p>0.05
Age (median)	63 (from 32 to 86)	64 (from 35 to 91)	p>0.05
Smoke	19 (17.9%)	20 (18.5%)	p>0.05
Hypertension	65 (61.3%)	63 (58.3%)	p>0.05
Hyperlipemia	20 (18.9%)	17 (15.7%)	p>0.05
Diabetes	23 (21.7%)	21 (19.4%)	p>0.05
Obesity (BMI>30)	21 (19.8%)	25 (23.1%)	p>0.05
Cardiovascular diseases	8 (7.5%)	9 (8.3%)	p>0.05
Respiratory diseases	7 (6.6%)	4 (3.7%)	p>0.05
Tumor	7 (6.6%)	9 (8.3%)	p>0.05
Chronic kidney impairment	9 (8.5%)	11 (10.2%)	p>0.05

No statistically significant difference was observed comparing the two groups.

Table II: Patients' clinical characteristics at hospital admission

	Plasma Infusion YES	Plasma Infusion NO	p
Patients	106	108	
PaO2/FiO2	166 (IQ113 – IIIQ220)	171 (IQ125 - IIIQ234)	p>0.05
Berlin score			
Moderate	57 (53.7%)	59 (54.6%)	p>0.05
Severe	49 (46.3%)	51 (45.4%)	p>0.05
Chest Imaging			
Bilateral Pneumonia	18 (17.1%)	23 (21.3%)	p>0.05
Ground Glass Opacities	31 (29.2%)	36 (33.3%)	p>0.05
Pulmonary Consolidation	57 (53.7%)	49 (45.4%)	p=0.02
Ventilatory support			
Low flow	14 (13.2%)	23 (21.3%)	p=0.01
High flow	22 (20.7%)	21 (19.4%)	p>0.05
Intubation	70 (66.1%)	65 (60.3%)	p=0.01

Table III: Comparison of therapeutic approach in the two patient groups

	Plasma Infusion YES	Plasma Infusion NO	p
Patients	106	108	--
Ventilatory support	106 (100%)	108 (100%)	p>0.05
NSAIDs	106 (100%)	108 (100%)	p>0.05
LMWH	104 (98.1%)	104 (96.3%)	p>0.05
Steroids	102 (96.2%)	106 (98.1%)	p>0.05
Antibiotics	106 (100%)	108 (100%)	p>0.05
Remdesivir	81 (76.4%)	96 (88.9%)	P=0.01
Tocilizumab	10 (9.4%)	12 (11.1%)	p>0.05
Colchicine	1 (0.9%)	0 (0.0%)	p>0.05
Plasma Infusion	106 (100%)	0 (0.0%)	--

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