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IMMUNOTHERAPY WITH NEUTRALISING F(ab')2 ANTI-SARS-CoV-2 NEUTRALISING ANTI-SARS-CoV-2 ANTICUPERPOSES FROM EQUINE SERUM IN THE TREATMENT OF AMBULATORY PATIENTS WITH BILATERAL COVID-19 PNEUMONIA

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ON BEHALF OF THE EQUINE SERUM RESEARCH GROUP OF THE BOLIVAR HOSPITAL

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Summary Introduction: Passive immunotherapy is a therapeutic alternative for patients with COVID-19. **Methods:** **A decision was made** to conduct a prospective database of patients with COVID-19.
with a diagnosis of non-hypoxemic SARS-CoV-2 pneumonia, treated on an outpatient basis at the Hospital de Bolívar, Dr. Miguel Capredoni, province of Buenos Aires, Argentina, with the aim of evaluating the efficacy of treatment with hyperimmune equine serum in the reduction of severe cases and hospitalisations. We conducted a retrospective analysis of the period from 26/05/2021 to 28/08/2021, 151 patients were included. The options were meprednisone and colchicine associated with two infusions of horse serum (n=92) or meprednisone and colchicine for 10 days orally (n=59). **Results:** No differences in population characteristics and comorbidities were observed between the two groups. Forty-six per cent (69) of patients had received at least one dose of COVID-19 vaccine. During follow-up 23% (35) required hospitalisation, with no difference between the equine serum group and the control group (p = 0.89). There was a non-significant trend in the risk of prolonged hospitalisation of 15.7% (Equine serum group 38.1% vs. control group 53.8%, *Fisher Exact test* p = 0.41). Mortality in the equine serum group was 3.97% (4), with no differences between the two groups. Differences were observed between vaccinated and unvaccinated patients on hard points such as need for MRA (0% vs. 8% p = 0.001) and death (0% vs. 8% p = 0.001). **Discussion:** Although hospitalisation and death rates were lower than expected, the use of hyperimmune horse serum in the outpatient setting appears to be of no clinical benefit.

Keywords: COVID-19 pneumonia, immunotherapy, hyperimmune equine serum

Abstract ***Immunotherapy with anti-SARS-COV-2 neutralyzing F(ab')2 antibodies from equine serum in the treatment of out-patients with bilateral COVID-19 pneumonia***

Introduction: Passive immunotherapy is a therapeutic alternative for patients with COVID-19. **Methods**: The decision was made to create a prospective database of patients diagnosed with SARS-CoV-2 pneumonia, non-hypoxemic, treated on an outpatient basis at the *Hospital de Bolívar, Dr. Miguel Capredoni*, province of Buenos Aires, Argentina, with the aim of evaluating the efficacy in reducing severe cases and hospitalizations of treatment with hyperimmune equine serum in this subgroup of patients. We performed a retrospective analysis of the period from 05/26/2021 to 08/28/2021, where a total of 151 patients were included. The options were meprednisone plus colchicine associated with two equine serum infusions (n = 92) or oral meprednisone and colchicine for 10 days (59). **Results**: No differences were observed between the population characteristics and comorbidities between both groups. A 46% (69) of the patients had received at least one dose of vaccine against COVID-19. During follow-up, 23% (35) required hospitalization, with no differences between the equine serum group and the control group (p = 0.89). A non-significant trend of 15.7% was observed for the risk of prolonged hospitalization (Equine serum group 38.1% vs. control group 53.8%, Fisher Exact test p = 0.41). Mortality between the equine serum group was 3.97% (4), with no differences between the two groups. Differences were observed between vaccinated and unvaccinated patients in hard points such as the need for MRA (0% vs. 8% p = 0.001) and death (0% vs. 8% p = 0.001). **Discussion**: Although the rate of hospitalization and death were lower than expected, the use of hyperimmune equine serum in the outpatient setting impresses as not providing clinical benefit.

Key words: pneumonia COVID-19, immunotherapy, equine hyperimmune serum

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KEY POINTS
Current knowledge

- The use of passive immunotherapy with plasma from convalescent patients for COVID-19 pneumonia has mixed results. The use of hyperimmune horse serum in severe COVID-19 pneumonia does not provide reduction in clinical hard spots.

Contribution of the article to knowledge

- Hyperimmune equine serum infusion provides no clinical benefit in non-hypoxaemic bilateral COVID 19 pneumonias.

Since the beginning of the circulation of the disease now known as COVID-19 and its declaration as a pandemic in March 2020, there have been multiple pharmacological interventions that have sought to obtain a benefit in the treatment of this disease1 .

One of the most paradigmatic cases is the use of dexamethasone, which has been shown to improve survival in patients with oxygen requirements2 . Simultaneously and subsequently, there have been other studies that have attempted to meet the same objective. Hyperimmune equine serum as part of the treatment then emerged as a possible therapeutic option (CoviFab® Hyperimmune Equine Serum).

Different human anti-receptor-binding domain (RBD) monoclonal antibodies (mAb) have been evaluated in the treatment of COVID-193. Although some degree of activity was observed in patients with mild disease, no consistent effect has been demonstrated in patients with moderate and severe disease4. It is known from previous data that RBD of the viral glycoprotein elicits high mAb titres against SARS-CoV-2 when used as an immunogen in horses. In this regard, equine polyclonal antibodies (EpAbs) may represent a practical and effective source of mAb.

In view of this pathophysiology, since March 2020, several studies have emerged that attempt to answer the question of the benefit of passive immunity and in which group of patients it could be of most benefit. There are a myriad of studies with discordant results in this regard, in which no benefit was observed in patients with moderate to severe disease3, 4. It is believed that patient selection, the evolution in compression and treatment of the pathology, as well as the standardisation of care (universal use of dexamethasone, for example) have contributed to a lack of clarity about the efficacy of this therapy or about the exact population and time in which the treatment provides the greatest benefit5 .

At the beginning of the pandemic, due to the scarce evidence on the management of these patients, it was decided to carry out a prospective registry of the treatments, toxicities and results of patients treated with COVID-19 at the Hospital de Bo- Ivar, Dr. Miguel Capredoni, in the province of Buenos Aires, Argentina.

The usual practice in our centre was the co-administration of colchicine and meprednisone.

Based on initial studies showing benefit from the use of hyperimmune equine serum, from May 2021 to August 2021, it was decided to offer this treatment to patients with bilateral, non-hypoxaemic pneumonia treated on an outpatient basis, according to their acceptance and the availability of the drug at the time of diagnosis.

The aim of this study was to compare the rate of hospitalisation between the group of patients treated with the centre's standard (colchicine and meprednisone) associated with horse serum vs. those who received only colchicine and meprednisone.

A retrospective analysis of prospectively collected data from patients diagnosed with COVID 19 pneumonia, non-hypoxaemic, treated on an outpatient basis was performed.

Hospitalisation requirement was assessed as the primary outcome variable, other variables analysed were mortality, mechanical ventilation requirement, prolonged hospitalisation and adverse effect rate.

Materials and methods

Design

A retrospective analysis was performed on a prospective basis in adult patients with a tomographic diagnosis of bilateral COVID-19 pneumonia (confirmed by specific method) without hypoxaemia and initially managed on an outpatient basis. The population receiving hyperimmune equine serum and the current standard of care (meprednisone and colchicine) was compared to the population receiving only the centre's standard of care (meprednisone and colchicine). All patients were followed up in a structured manner until 28 days from inclusion.

Equine serum group**:** **they** received treatment with hyperimmune equine serum, doses of 0.13 ml/kg, with an interval of 48 hours in between (according to the laboratory's leaflet), intravenously, in infusion chairs located in the respiratory triage, associated with anti-inflammatory treatment with meprednisone 40 mg and colchicine 0.5mg every 12 hours for 10 days.

Control group**:** Patients treated with corticosteroids and colchicine alone, equal dose.

The study period was between 26/05/2021 and 28/08/2021. This period was chosen because it was a time when there were no substantive differences in the diagnosis, risk categorisation, supportive measures or treatment of adult patients with a confirmed diagnosis of COVID-19 pneumonia, and is therefore a valid period for comparison.

Data collection was performed through a prospective database created in the hospital of Bolívar, Dr. Miguel Capredoni, province of Buenos Aires, Argentina. Data were subsequently anonymised, respecting patient confidentiality.

The study was conducted in the same hospital. Since the beginning of the pandemic, several of the hospital wards, both general inpatient and intensive care, have been completely dedicated to the care of COVID-19 patients.

Population

For inclusion in this study, patients with a diagnosis of COVID-19 with bilateral, non-hypoxaemic pneumonia, confirmed by tomography, treated at this hospital in Bolivar, who met the following criteria, were selected.

Inclusion criteria

1. Patients over 18 years of age
2. Confirmed diagnosis of bilateral CO-IDV-19 pneumonia, non-hypoxemic, evidenced by CT scan.
3. The start of treatment has been treated on an outpatient basis.
4. Confirmed diagnosis of COVID-19 by SARS-CoV-2 antigen test or qualitative reverse transcriptase-polymerase chain reaction (qRT-PCR -GeneoDX Co, Ltd or similar).
5. At diagnosis, oxygen saturation greater than or equal to 94%, and less than 10 days from the onset of symptoms.

Exclusion criteria

1. Asymptomatic disease (individuals with a positive test for SARS-CoV-2, but no symptoms), or mild (individuals with compatible symptoms, but no dyspnoea and a normal chest imaging study), moderate (individuals with evidence of lower respiratory tract disease on clinical or imaging assessment, and oxygen saturation of the respiratory tract), or mild (individuals with evidence of lower respiratory tract disease on clinical or imaging assessment, and a normal chest imaging study).
≥ 94% room air) without bilateral pneumonia.
2. Patients with any type of immunosuppression (HIV transplant recipients, with use of immunosuppressive medication). Clinical suspicion or confirmation of another bacterial, viral or fungal infection concomitant with the diagnosis of COVID-19 infection.

Study variables

Inpatient outcome: Patients were monitored by telephone on a daily basis by a team of doctors assigned to follow up COVID-19 positive cases. Body temperature, oxygen saturation and dyspnoea progression were monitored.

Those who evolved with persistent febrile registers, with oxygen saturation records of less than 94% and/or progression of their dyspnoea, were re-evaluated in the COVID-19 respiratory hall where clinical and laboratory evaluation was carried out with arterial blood gases and chest CT, and hospitalisation was decided if the O_2 saturation was less than 94% by blood gases and/or tomographic progression of pulmonary infiltrates.

For the purposes of the study, demographic variables (age, sex, BMI), presence of comorbidities (hypertension, diabetes, obesity, cancer, pulmonary disease, lung disease, heart disease, diabetes, diabetes, obesity) and other comorbidities (hypertension, diabetes, obesity) were included.

The following variables are related to the patient's diagnosis and baseline status at the time of infusion (diagnostic method, date of symptom onset, O saturation$_2$, oxygen requirement, type of device indicated, fever) and related to the evolution of the patient. Operationalisation of variables.

Sampling and sample calculation

All patients treated during the study period were included in a 2:1 ratio of patients not exposed to hyperimmune horse serum (2 exposed patients to 1 unexposed patient).

Because all patients exposed in the study period were included and because they are a small and fixed number, a power estimation was made with the achieved sample size.

Descriptive analysis

Categorical variables were presented as absolute frequency and relative frequency as a percentage. Continuous variables were presented as mean and standard deviation or median and interquartile range according to observed distribution.

Characteristics of both groups of patients were compared to detect potential confounders according to imbalance between the exposed and unexposed arms. Categorical characteristics were compared using the Chi-square or Fisher's exact test according to assumptions. Quantitative characteristics were compared using the t-test or Mann Whitney U-test according to assumptions.

Primary objective of effectiveness and secondary objectives

Considering hospitalisation and death events as right-censored events, without competing events corresponding to the primary objective of effectiveness, the cumulative incidence was estimated using the Kaplan Meier method, with its 95% confidence intervals (95%CI). The survival curves between the 2 study arms were compared using the Cox-Mantel hypothesis test, with the null hypothesis corresponding to equality of the survival curves between the exposed and unexposed.

A univariate Cox proportional hazards regression model was used to estimate the *Hazard Ratio* (HR) of the exposed relative to the unexposed, using hospitalisation or death as the outcome variable.

The same analysis was repeated for the secondary objectives.

The p's were considered statistically significant.
values less than 0.05

Statistical analysis was performed with STATA statistical software version 15.1 MP - Parallel Edition (Copyright 1985-2017 StataCorp LLC - StataCorp. 4905 Lakeway Drive, College Station, Texas 77845 USA).

Ethical considerations

The study was approved by the central ethics committee of the Ministry of Health of the Government of the Province of Buenos Aires according to Act-2021-31086070. The conduct of this research was carried out in compliance with ethical principles in accordance with the regulatory standards for human health research at national and international level, in accordance with the Resolution of the Ministry of the Nation Number 1480/2011, ANMAT Provision 6677/10, the Helsinki Declaration of the World Medical Association and all its amendments, and respecting the ICH E6 Good Clinical Practice Guidelines.

The patients, in all cases, have voluntarily signed the informed consent form recommended by the person responsible for the medicinal product. All study data were treated with maximum confidentiality in an anonymous manner, with restricted access only for authorised personnel for the purposes of the study in accordance with the current legal regulations Ley Nacional de Protección de Datos Personales 25.326/00 (Ley de Habeas data) and Ley 26. 529 /09.

The autonomy and confidentiality of the participant was respected. The identity of the personal data was kept under absolute confidentiality and anonymity, and only the researchers involved had access to it.

Results

Between 26/05/2021 and 28/08/2021, 151 patients were registered with a tomographic diagnosis of bilateral, non-hypoxaemic pneumonia, with etiological confirmation of COVID-19, who were initially treated ambulatory. Ninety-two patients were treated with meprednisone, colchicine and two infusions of horse serum (immunotherapy group) and 59 patients were treated with meprednisone and colchicine (control group).

The median age was 48 years (RIC 39-58), 54% (82) were male, all had bilateral infiltrates on CT, 11% (16) with bilateral infiltrates in more than 50% of the lung parenchyma. No statistically significant differences were observed between study groups in terms of comorbidities. Forty-six percent (69) of the patients had received at least one dose of COVID-19 vaccine. Further data are presented in table 1. In the subgroup analysis between vaccinated vs. non-vaccinated population, a statistically significant difference was found in progression to MRA 0% (0) vs. 8%, p = 0.001 and death 0% vs. 8%, p = 0.001. The information in Table 2.

TABLE 1.- *Clinical, demographic and tomographic features*

Features	Equine serum N:92 N (%)	Colchicine N:59 N (%)
Age (years)	50.8 (Average)	50.1 (Average)
Sex		
Male	47 (51.1)	38 (64.4)
Risk factors		
HTA	31 (33.7)	14 (23.7)
DBT	8 (8.7)	10 (16.9)
DLP	7 (7.6)	3 (5.1)
COPD	2 (2.2)	1 (1.7)
Asthma	4 (4.4)	1 (1.7)
IAM	0	3 (5.1)
Autoimmune	1 (1.1)	0
None	51 (55.4%)	38 (64.5)
Weight		
Normal	32 (34.8)	20 (33.9)
Overweight	32 (34.8)	21 (35.6)
Obesity	28 (30.4)	18 (30.5)
Vaccine		
None	53 (57.6)	32 (54.2)
1 dose	26 (28.3)	22 (37.3)
2 doses	13 (14.1)	5 (8.5)
CT infiltrates		
Mild CT < 25%.	53 (57.6)	36 (61)
Moderate CT 25-50	29 (31.5)	19 (32.2)
Severe CT > 50%.	10 (10.9)	4 (6.8)

COPD, chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease; DBT, diabetes; BPD, dyslipidaemia; HT, hypertension;
AMI: acute myocardial infarction; CT: computed axial tomography; CT: computed tomography.

TABLE 2.- *Results: vaccinated vs. not vaccinated*

Features	Vaccinated % (N)	Unvaccinated % (N)	p
Median age RIC	57 (47-70)	44 (36-50)	0.001
Sex Male	51 (23)	49 (22)	0.14
Equine serum	23 (9)	77 (31)	0.28
Internment	22 (9)	23 (12)	0.94
Prolonged hospitalisation	10 (4)	10 (5)	0.95
ARM	0	8 (4)	0.001
Death	0	8 (4)	0.001

MRA: mechanical ventilation

Primary objective

During the 28-day follow-up, 23% (35) required hospitalisation or died, with no statistically significant differences between immunotherapy and control groups (Cox proportional hazards model p = 0.89). Exploratory analyses also showed no differences between the subgroups analysed.

Secondary objective

Of the 35 patients who required hospitalisation, 18 had a prolonged hospitalisation, exceeding 7 days of hospitalisation. There was a 15.7% numerical reduction in the risk of prolonged hospitalisation in favour of the equine serum treatment group (equine serum group 38.1% vs. control group 53.8%) although this was not statistically significant (Fisher Exact test p = 0.41). Mortality during follow-up was 3.97%, with no differences between the two groups[4].

In this subgroup of patients, infusion of hyperimmune equine serum did not change the rate of admission to mechanical ventilation.

Discussion

Equine polyclonal antibodies have been used for decades in the management of clinical emergencies, such as snake and scorpion poisoning, tetanus toxin, botulinum poisoning and severe avian influenza infections[6-9]. With the advent of the COVID-19 pandemic, its usefulness for this pathology was raised, with the aim of preventing the progression of severe forms of SARS-CoV-2 pneumonia.

In our work, which included patients with bilateral, non-hypoxaemic pneumonia treated on an outpatient basis, the addition of hyperimmune equine serum to standard treatment did not demonstrate benefit in the objective primary reduction in the rate of hospitalisation. In both groups 23% of cases required hospitalisation, with no statistically significant differences (Cox proportional hazards model p = 0.89).

These results are similar to those presented by the PLASMAR study, which compared passive immunisation with convalescent hyperimmune serum vs. placebo in patients with severe bilateral pneumonia. This study found no decrease in mortality with the passive immunotherapy strategy, 10.96% in the intervention group vs. 11.43% in the placebo group (95% CI -7.8 to 6.8). It also showed no benefit in other efficacy parameters[10]. In another clinical study that also included patients diagnosed with moderate to severe pneumonia requiring hospitalisation, and compared the use of hyperimmune horse serum vs. placebo, there was no evidence of benefit with immunotherapy in its primary endpoint, which considered improvement in eight pre-defined clinical parameters at 28 days[11].

Given the lack of efficacy of passive immunotherapy in severe cases, its use, when the clinical picture is already established, is not considered to be an appropriate strategy.

In the INFANT-COVID-19 study, which used early convalescent plasma in patients with milder forms of COVID and at risk of a torpid course, with the aim of preventing severe forms of the disease, a significant reduction in the proportion of severe respiratory disease was observed (relative risk, 0.52; 95% confidence interval [CI], 0.29 to 0.94; P = 0.03)[12]. This study is the only one in which treatment was instituted early, before the development of bilateral pneumonia, and it may be considered that, if passive immunotherapy is used, it must be done early for it to be effective.

On the other hand, in our study, both groups received double anti-inflammatory treatment with mepred- nisone and colchicine[13] , which was considered the recommended treatment practice in our centre at that time.

moment. We cannot rule out that this may have played a role
in the results.

In our study, we identified a non-statistically significant benefit in the secondary endpoint of hospitalisation rate greater than 7 days, being 38.1% in the hyperimmune immunoglobulin group vs. 53.8% in the control group; with a relative risk reduction of 15.7% (Fisher Exact p = 0.23). This result is in line with the REMDESIVIR study which demonstrated a statistically significant reduction in hospital stay in patients with moderate to severe pneumonia, with a median of 10 days in the intervention group vs 15 days in the placebo group (rank frequency for recovery, 1.29; 95% CI, 1.12 to 1.49; P < 0.001, by a log-rank test)[14]. In view of the above, we consider that the use of hyperimmune serum in critically ill patients does not provide benefits, so that in the treatment approach for this subgroup of patients, the use of hyperimmune serum in critically ill patients is not recommended.
other options should be considered.

Based on the evidence that vaccines prevent severe forms and mortality, we believe that health systems should aim for widespread use in the population.

In conclusion, although the rate of hospitalisation and death were lower than expected, the use of hyperimmune horse serum in the outpatient setting did not appear to be of clinical benefit in patients with non-hypoxaemic COVID-19 pneumonia.

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Conflicts of interest: None to declare

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