British Journal of Healthcare and Medical Research - Vol. 11, No. 06
Publication Date: December 25, 2024
DOI:10.14738/bjhr.1106.18040.
Caimmi, P. P. & Mascaro, G. (2024). Convalecent - Hyperimmune Plasma Use and Immunological Charachteristics in the Covid-19 Pandemic: A Preliminary Experience of a Single Center in Italy as Fast Available Therapy Model in New Virus Pandemic. British Journal of Healthcare and Medical Research, Vol - 11(06). 310-317.

Convalecent - Hyperimmune Plasma Use and Immunological Charachteristics in the Covid-19 Pandemic: A Preliminary Experience of a Single Center in Italy as Fast Available Therapy Model in New Virus Pandemic

Philippe P. Caimmi
Medical Directorate, University Hospital of Novara,
Corso Mazzini 18, Novara, Italy and General Medical Practitioner,
Local trust of Biella (ASL Biella, Italian NHS), Italy

Gennaro Mascaro
Transfusion Service, University Hospital of Novara,
Corso Mazzini 18, Novara, Italy

ABSTRACT

Objectives: Hyperimmune - convalescent plasma transfusion appears to be a great resource in the current SARS-CoV-2 coronavirus pandemic and seems to be one of the few effective treatment modality available. However, its efficacy is variable and dependent on antibody/plasma volume concentration. Methods & Results: This single center analysis from a forefront COVID-19 Italian hospital in 43 convalescent plasma donors demonstrated that this variability in neutralizing antibody (NAB) levels is related to the harvesting period after SARS-CoV-2 recovery and interestingly, on blood group type. Specifically, the optimal plasmaferesis time point was approximately two weeks post test negativation with a significant drop in NAB plasma levels (112±102 vs 60±26.9, p=0.003) observed at 30 days. In contrast, no correlation between NAB and IgG plasma levels was observed over time (r=0.48.). Subgroup analyses demonstrated that NAB levels where higher in male (115.1±110.7, p=0.0001) and in blood group AB (200±138.6, p=0.036) donors while group A patients (81.2±82.7, p=0.062) had the lower levels of NAB concentration. Conclusion: This analysis demonstrated that male patient with a AB blood group plasma which was donated within three weeks of convalescence contains the highest therapeutic levels of antibodies and these patients should be actively recruited to become donors.

Keywords: Convalescent - Hyperimmune Plasma, Blood Transfusion, COVID-19, Pandemic, SARS-CoV-2 Corona Virus.

INTRODUCTION

In the recent literature, the data in favor of the use of COVID-19 convalescent - hyperimmune plasma for the management of a SARS-CoV-2 infection are growing (1). However, it is becoming increasingly clear that the efficacy of this therapeutic modality, which seems currently at the same of monoclonal antibodies the only potentially effective one available, is possibly variable and dependent on the concentration of virus specific neutralizing antibodies in the donated plasma. It is obvious that during the subsequent pandemic peaks which seems will be of similar

magnitude to the initial one we will need a way to assess the immunological effectiveness of this valuable resource possibly through a rudimentary screening process.

The purpose of this study was to analyze the epidemiological, immunological and demographic data that have been acquired during the initial months of COVID-19 convalescent plasma banking and usage at a tertiary university affiliated care facility which is in the forefront of COVID-19 management in the north of Italy, and from these derive indications for the best use and applicability of this important resource.

METHODS

A retrospective analysis of collected data on the characteristics of convalescent plasma at our hospital over a period of three months (from March 2020 to May 2020), was performed. This review was conducted according to the guidelines of the hospital's institutional review board. All individuals had consented to become plasma donors and to have routine data collected. These data were held securely in a centralized hospital computer database

Selection of Convalescent Donors

During the pandemic a designated care protocol for convalescent plasma harvesting, from patients who had contracted and recovered from SARS-CoV-2 infection thus developing immunity, was developed in our hospital for clinical use. Eligible donors' names which had a negative SARS-CoV-2 polymerase chain reaction (PCR) test following a quarantine period of at least two weeks were provided to the transfusion service of our hospital by the Local Health Authority and they were approached so as to become plasma donors. Following their consent for donation, a peripheral blood screen for transmittable diseases (Hepatitis B & C, human immunodeficiency virus - HIV) and for quantification of neutralizing antibody (NAB) titers was performed.

Recovered patients with NAB titers ≥ 1:40 who were medically suitable and consented to donation were subjected to plasmapheresis. At the time of plasma donation each donor had to have two negative nasopharyngeal swab PCR tests for SARS-CoV-2 and a negative SARS-CoV-2 PCR test in blood. Donors were recalled again after one month to undergo repeat blood sampling for immunological and serological testing. All donors' suitability was assessed according to current Italian guidelines and laws regulating blood product transfusion practice (1). Plasma collection was performed at the University Hospital Blood Transfusion Service.

Collection Technique and Convalescent Plasma Analysis

The collection technique was as previously described by Perotti and colleagues (1). Plasma collection was performed with the latest generation cell separation and apheresis (Trima Accel®, Terumo BCT, Lakewood, Colorado, USA and Amicus™, Fresenius Kabi, Bad Homburg, Germany) devices. A plasma volume of approximately 600mL was collected during each procedure and immediately divided into three bags of 200mL each using a sterile tubing welder. Plasma collection was performed as previously mentioned at 15 days post convalescence, while serological measurements for IgG and NAB levels was performed at the 15 and 30 day time-points.

Plasma pathogen reduction was performed with the INTERCEPT® processing system (Cerus Corporation, Concord, California, USA) and the Mirasol® pathogen reduction technology (PRT)

URL: http://dx.doi.org/10.14738/bjhr.1106.18040.

System (Terumo BCT, Lakewood, Colorado, USA). The collected units were stored at a controlled temperature ranging from -40 to -25 °C (1).

Serological and immunological profiling was performed on the plasma from each donor. NAB titers were obtained utilizing an in-house microneutralisation assay adapted to SARS-CoV-2 according to a previously reported method (1). In addition each volunteer's blood was also tested for IgG titers utilizing the LIAISON® SARS-CoV-2 S1/S2 IgG quantification test (DiaSorin, Saluggia, Piedmont, Italy). This is an indirect chemiluminescence immunoassay (CLIA) which automatically calculates SARS-CoV-2 S1/S2 IgG antibody concentrations expressed as arbitrary units (AU/mL)

Statistical Analysis

Comparison between two groups was performed using the Student's t-test while multiple comparisons were done with one-way analysis of variance (ANOVA) assuming normal distributions while Tukey's post hoc test was used to identify specific group differences. Continuous data are reported as mean ± standard deviation (SD). A p-value of 0.05 or less was considered statistically significant. Statistical analysis was carried out with the SPSS v23.0 software (SPSS Inc., Chicago, IL).

RESULTS

A total of 53 recovering patients were screened for possible plasma donation of which ten (19%) had NAB titers less than 1:40 and therefore were classified as non-responders. Therefore, 43 convalescent plasma donors participated in the study. There were 38 male and five female participants with a mean age of 40.5±9.7 years. Other demographic characteristics and inclusion/exclusion criteria were determined by current guidelines and Italian law regulating blood donation practice.

There was no statistical difference between the initial (day 15) average levels of NAB and IgG (122.8±115.3 vs. 123±89, p=0.419). In truth, no relation seems to exist between NAB and IgG levels with Pearson's correlation coefficient r been 0.48. When a subgroup analysis of initial NAB titers was performed according to blood group and gender, levels were significantly higher in group AB (200±138.6, p=0.036) and in male (115.1±110.7, p=0.0001) donors (Table. 1). A marginally significant, lower level of NAB was observed in blood group A donors (81.2±82.7, p=0.062). No statistically significant differences were seen between the other blood or rhesus groups (Table. 1).

An analysis of NAB titers by individual volunteer demonstrated 14 donors with titers of 1:40 for a total of 42 units of plasma, 13 donors had titers at 1:80 producing 39 units of plasma, 11 donors had NAB titers of 1:160 producing 33 units of convalescent plasma, four donors with titers of 1:320 for a total of 12 units and one donor with NAB titer of 1:640 producing three units of convalescent plasma.

When a comparison of NAB levels over time was performed a significant drop (112±102 vs. 60±26.9, p=0.003) was observed over a period of 30 days (from initial sampling to the 30 day re-sampling), (Table 2). In contrast, IgG levels seemed to remain constant and did not exhibit any statistically significant change thru time (Table 2).

DISCUSSION

Based on the findings of this analysis we were able to improve the organization of the plasma banking service in our institution and we thus hope to be able to offer some suggestions and improvements on the use of convalescent - hyperimmune plasma during the subsequent waves of the COVID-19 pandemic.

This analysis demonstrated that the optimal period for plasma donation is during the end of the SARS-CoV-2 patients' two week quarantine and recovery period where NAB titers are at the maximum level. Therefore, based on this, subsequent donors in our hospital were scheduled for plasmapheresis on day 15 so as to obtain the highest quality of convalescent plasma with the highest concentration of NAB per unit volume.

The subgroup analysis performed demonstrated that male donors had a significant higher level of NAB compared to female one and that groups AB and A donors had the highest and lowest plasma NAB concentrations respectively. These findings are comparable to those in recently published literature (2-4).

Although there is currently limited knowledge on the risk factors for susceptibility to SARSCoV-2, several studies have suggested that blood group type could be implicated in the susceptibility to the virus and the severity of COVID-19 infection, with a particular focus on the role of the ABO gene. Zhao et al. compared ABO blood group distribution in a cohort of 2,173 COVID-19 patients to that of comparable healthy controls and found that blood group A was associated with an increased risk of infection, whereas group O was associated with a decreased risk. (2). A similar study demonstrated an identical association (3), and similar results have been previously described for other viruses (3), including SARS-CoV-1 (4).

Recently, a private genomics and biotechnology company in California has corroborated this association (5). Their preliminary results suggest that blood type O appears to be protective against SARS-CoV-2 when compared to all other blood types. Expanding further on this concept, a number of hematologists have recently suggested that serum anti-A antibody (more specifically IgG anti-A in blood type O) is a more significant biomarker of SARS-CoV-2 protection than the blood group itself (6-8). This finding suggests the possible existence of unknown receptor sensitiveness to the SARS-CoV-2 virus in blood group A patients which contributes to the increased susceptibility.

One of the finding of this study suggested that quantitative IgG levels are not correlated to NAB titers either initially or later on in time. This is well explained in the literature as the IgG test of peripheral blood is sensitive to the anti-spike IgG antibody which is common amongst the coronaviruses family. A positive IgG test is indicative of an immune memory against an indeterminate corona virus (SARS-CoV-2 or other). In fact the plasma of IgG positive patients but with negative NAB is not immunologically active against the SARS-CoV-2 virus in vitro (9). Therefore only in NAB positive patients a SARS-CoV-2 infection is certain (9). This different specificity between serum IgG and NAB represents an important bias in most clinical series on Covid 19 convalescent plasma reported in literature up to day.

Despite the small sample size in this series it was clearly demonstrable that NAB levels decrease significantly after about 6 weeks post convalescence. This finding, despite its clinical

URL: http://dx.doi.org/10.14738/bjhr.1106.18040.

significance, is not yet clearly documented in literature. It has been shown that the substantial increase of NAB levels is related to a proportional increase of viral RNA wash-out and a corresponding decrease in viral load (9). In fact, in our protocol, potential donors were evaluated only when two nasopharyngeal swab PCR for SARS-Cov-2 tests were negative following the end of the 14 day quarantine period, an indirect undeniably marker of infectivity and viral load but one of the few reliable and easily available ones.

Future Suggestions for Convalescent Plasma Usage

One of the problems encountered with the use of convalescent - hyperimmune plasma is in its rational use and allocation; especially considering the scarcity of the resource. For example, there is no standardized transfusion dose consistent with proven benefit. In a number of reported studies the administration volume of convalescent plasma ranges between 200mL and 500mL in either a single or in double dosages (10, 11). This is quite a significant variance and could possibly lead to overtreatment and wastage. Currently, the recommendation in Europe is to administrate 3 mL/kg per dose over two days (10, 11). This dosing strategy facilitates the distribution of plasma by means of standardized units (250mL per unit) and allows the utilization of established collection and handling techniques by transfusion services throughout Europe.

In the literature effective convalescent plasma treatment contains NAB titers ranging from 80 to 480 (1, 10, 11). However, because donors have inherently different NAB titers, a protocol needs to be developed and adopted in order to administer equally efficacious convalescent plasma containing consistent and similar concentrations of NAB per plasma volume by selecting and matching plasma units with different titers to produce a standardized NAB dose. We wished to produce a constant effectiveness and provide an ethical distribution of treatment amongst our patients. Consequently, a protocol which was developed by our service based on the results of this analysis and has being utilized in our hospital is presented in Table 3.

Each of the convalescent plasma donors provided on average a total of 657.9±22.9 mL of plasma which was equally divided among three units of approximately 219.3 mL. Assuming that the observed mean NAB titer of 122.8 is a satisfactory therapeutic level for each patient to receive we aimed to calculate the required combination of units according to NAB titers, total volume of plasma, patient weight and their preload to provide that therapeutic dose without detrimental effects to the patient (Table 3). Utilizing this protocol a total of 82 patients were treated with plasma from 43 donors receiving equivalent therapeutic NAB levels comparable to that of convalescent patients' average. In addition to patients' body weight, preload level was used to decide the number and combination of units to allocate so that patient volume overload was minimized. Using this protocol, equivalency, reproducibility and safety of treatment is ensured and convalescent plasma can be potentially compared with the more expensive monoclonal antibodies. Furthermore, as the problem on vaccines availabilities in the beginning of all pandemia, it can suggest to improve in-hospital know-how to produce it-self pipelines for therapy when it is safe.

Crucial to the optimal recruitment of plasma donors from the convalescent pool is the effective and continuous communication between the local transfusion service and the local health authority for the prompt identification of potential donors who have negative nasopharyngeal swab PCR tests for SARS-CoV-2. Early donation allows for plasma with higher therapeutic NAB

titers per volume. Naturally, not all transfusion services have available assays for measuring NAB titers however based on the findings of this analysis; plasma of male patients with an AB blood group which was donated within three weeks of convalescence should contain the highest therapeutic levels of antibodies and this can be used as a surrogate assumption.

CONCLUSION

As the use of convalescent plasma therapy to treat severe COVID-19 infection becomes more widespread the establishing of guidelines based on analysis such as this one, for the optimal therapeutic administration according to neutralizing antibody levels is needed. The preliminary analysis presented herewith demonstrated a significant higher level of neutralizing antibodies in male patients belonging to the AB blood group. These antibody levels seem to deteriorate rapidly within the first 50 days following convalescence. Correspondingly, IgG titers do not seem to correlate with these neutralizing antibody levels and for this reason they cannot be utilized to evaluate immunization against SARS-CoV-2 for hyperimmune plasma donors selection. The finding of this analysis adds to the better understanding of the immunological response to this new virus and to the improvement of convalescent - hyperimmune plasma therapy against Covid 19 but also also a fast available therapy protocol at the beginning of diffusion against other new virus. Naturally, it is limited by its small sample size and retrospective nature and therefore larger, prospective studies are needed to confirm its findings.

Conflict of Interest Statement

All authors declare there are no potential sources of conflict of interests in regards to this article.

Statement of Funding

This analysis did not receive any specific grant or sponsorship from funding agencies in the public, commercial or not-for-profit sectors. The investigators had full access to the data of the study and had final responsibility for the analysis and the interpretation of the result.

Authorship Contribution Statement

All authors participated and contributed to a degree meriting authorship in the key parts of conception and design of the analysis, data acquisition, conducting the analysis and drafting and revising of the manuscript. All authors have reviewed the final version of the manuscript, have approved it and agree to be accountable for this work.

References

[1]. Perotti C, Baldanti F, Bruno R, Del Fante C, Seminari E, Casari S, Percivalle E, Glingani C, Musella V, Belliato M, Garuti M, Meloni F, Frigato M, Di Sabatino A, Klersy C, De Donno G, Franchini M, COVID-19 Plasma Task Force. Mortality reduction in 46 severe COVID-19 patients treated with hyperimmune plasma. A proof of concept single arm multicenter trial. *Haematologica*. 2020; 105(12): 2834-40. https://doi: 10.3324/haematol.2020.261784.

[2]. Zhao J, Yang Y, Huang H, Li D, Gu D, Lu X, Zhang Z, Liu L, Liu T, Liu Y, He Y, Sun B, Wei M, Yang G, Wang X, Zhang L, Zhou X, Xing M, Wang PG, Relationship between the ABO blood group and the coronavirus disease 2019 (COVID-19) susceptibility, *Clinical Infectious Diseases*, 2020; ciaa1150. https://doi: 10.1093/cid/ciaa1150.

[3]. Zietz M, Zucker J, Tatonetti NP. Associations between blood type and COVID-19 infection, intubation, and death. *Nat Commun.* 2020; 11: 5761. https://doi: 10.1038/s41467-020-19623-x.

[4]. Cheng Y, Cheng G, Chui CH, Lau FY, Chan PK, Ng MH, Sung JJ, Wong RS, ABO blood group and susceptibility to severe acute respiratory syndrome. *JAMA.* 2005; 293: 1450-51. https://doi: 10.1001/jama.293.12.1450-c.

[5]. 23andMe. (2020, September 4th) *23andMe Finds Evidence that Blood Type Plays a Role in COVID-19.* 23andMe Blog. Available at: https://blog.23andme.com/23andmeresearch/blood-type-and-COVID-19/ (Accessed on: 11 November 2020)

[6]. Severe Covid-19 GWAS Group, Genome wide association study of severe COVID-19 with respiratory failure. *N Engl J Med*. 2020; 383(16): 1522-34. https://doi: 10.1056/NEJMoa2020283.

[7]. Li J, Wang X, Chen J, Cai Y, Deng A, Yang M. Association between ABO blood groups and risk of SARS-CoV-2 pneumonia. *Br J Haematol*. 2020; 190(1): 24-27. https://doi: 10.1111/bjh.16797

[8]. Gérard C, Maggipinto G, Minon JM. COVID-19 and ABO blood group: another viewpoint. *Br J Haematol.* 2020; 190(2): e93-e94. https://doi: 10.1111/bjh.16884

[9]. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Sinai Immunology Review Project. Immunology of COVID-19: Current State of the Science. *Immunity*. 2020; 52(6): 910-941. https://doi: 10.1016/j.immuni.2020.05.002.

[10]. Joyner MJ, Bruno KA, Klassen SA, Kunze KL, Johnson PW, Lesser ER, et al. Safety Update: COVID-19 Convalescent Plasma in 20,000 Hospitalized Patients. *Mayo Clin Proc*. 2020; 95(9):1888-97. https://doi: 10.1016/j.mayocp.2020.06.028.

[11]. European Commission, Directorate-General for Health and Food Safety, Directorate B - Health systems, medical products and innovation. (2020). *An EU programme of COVID-19 convalescent plasma collection and transfusion. Guidance on collection, testing, processing, storage, distribution and monitored use.* Available at: https://ec.europa.eu/ health/sites/health/files/blood_tissues_organs/docs/guidance_plasma_covid19_en.pdf (Accessed on: 6 January 2021]

TABLES

Table 1: Subgroup analysis of initial NAB levels according to blood grouping and gender.

Groups (n)	NAB (Mean ± Standard Deviation)	p-value
Blood group A (21)	81.2 ± 82.7	†0.062
Blood group B (5)	104 ± 53.7	†0.48
Blood group O (13)	119.6 ± 65.7	†0.26
Blood group AB (4)	200 ± 138.6	†0.036
Rh positive (38)	114.6 ± 101.3	‡ 0.49
Rh negative (7)	108.7 ± 101.4	
Male (38)	115.1 ± 110.7	‡0.0001
Female (5)	43.3 ± 19.7‡	

† Analysis of Variance (ANOVA) & Tukey's, ‡ Student's T-test, NAB: Neutralizing Antibodies

Table 2: Comparison of NAB and IgG levels in donor plasma initially and at 30 days post initial convalescence (negative PCR test).

	Initial Convalescence	30 Days Post Initial Convalescence	p-value
NAB (Mean ± SD)	122.8 ± 115.3	60 ± 26.9	†0.003
IgG (Mean ± SD)	123 ± 89	155.1 ± 105.1	†0.41

†Student's T-test, NAB: Neutralizing Antibodies, PCR: Polymerase Chain Reaction, SD: Standard Deviation

Table 3: Proposed protocol for the most efficacious administration of convalescent - hyperimmune plasma based on the results of this analysis.

NAB Titer per	Patient Weight (kgr) &			Number of Units to Administer	Plasma Volume Given (mL)	Treated Patients
	Low NAB (<8 mmHg)	Normal NAB (8-18 mmHg)	High NAB (>18 mmHg)			
40	>60 kgr	>60 kgr	-	3	600	14
80	>60 kgr	>60 kgr	-	2	400	19
160	-	<60 kgr	<60 kgr	1	200	33
320	-	<120 kgr	<120 kgr	1	200	12
640	-	-	>120 kgr	1	200	3
Total Patients		82				

NAB: Neutralizing Antibodies

URL: http://dx.doi.org/10.14738/bjhr.1106.18040.