Letter to the Editor

Massimo Franchini*, Paola Moi, Marzia Cortellazzi, Nadia Danese, Stefania Caruso, Paola Pasolini, Sabina Ferrazzo, Stefania Piccinini, Anna Dall'Oglio, Paola Zovetti, Nadia Negri, Donatella Braga, Cristina Pasquali, Elisa Zuliani and Claudia Glingani

Lack of cross-reactivity between anti-A IgG isoagglutinins and anti-SARS-CoV-2 IgG antibodies

https://doi.org/10.1515/cclm-2021-0025
Received January 7, 2021; accepted January 25, 2021;
published online February 10, 2021

Keywords: anti-A isoagglutinins; COVID-19; SARS-CoV-2.

To the Editor,

The ABO blood group is the most important among human blood group systems and consists of complex carbohydrate moieties at the extracellular surface of red blood cell (RBC) membrane [1]. Along with their expression on RBCs, ABO antigens (namely, A, B, AB, and O) are also highly expressed on the surface of a variety of human cells and tissues [2]. Although the physiologic role of ABO antigens and their related anti-A and anti-B natural isoagglutinins is still largely unknown, they play a prominent role in blood transfusion and cell, tissue, and organ transplantation [2]. In addition, several studies have documented over the last 50 years a close link between ABO blood groups and a wide array of diseases, including cancers and cardiovascular disorders [3]. The latter association is particularly relevant, considering the profound influence of ABO antigens on hemostasis, particularly in modulating von Willebrand factor (WVF) and factor VIII (FVIII) circulating levels [4]. Also the ABO blood group-related susceptibility to various types of viral infections, including HIV, hepatitis B, dengue and influenza viruses, has been consistently reported by several investigators over the last 20 years [5]. This issue has recently gained a renewed interest thanks to the first observations on the association between ABO blood type and Coronavirus Disease 2019 (COVID-19) from China, where the infection began and quickly spread around the world [6]. In particular, it has been hypothesized that individuals belonging to O blood type are less susceptible to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection than those belonging to non-O blood groups or that have a milder disease [7]. The hypothesized reason for this phenomenon lies in the presence in O blood group subjects of anti-A isoagglutinins, predominantly of IgG class, which would prevent the binding of SARS-CoV-2 to its angiotensin converting enzyme (ACE)-2 receptor thereby inhibiting the virus entry into the targeted human cells [8]. Given such association, in this study we wanted to check if there is a cross-reactivity between anti-A IgG isoagglutinins and anti-SARS-CoV-2 IgG antibodies in patients recovered from COVID-19 selected for convalescent plasma donation.

Serum samples from 12 consecutive convalescent plasma donors belonging to blood group O (group I-cases) were collected and anti-A IgG antibodies were titrated according to standard technique (dilution phosphate-buffered saline-treated control serum and dithiothreitol-treated serum) using a micro-column technology (Ortho Clinical Diagnostics, Raritan, NJ, USA) [9]. All these samples were also titrated for anti-SARS-CoV-2 neutralizing antibodies using the plaque reduction neutralization test (PRNT), as previously described [10]. In addition, the chemiluminescent immunoassay (CLIA)-based technology (LIAISON SARS-CoV-2 S1/S2 IgG, DiaSorin, Vercelli, Italy) was used on these serum samples for the quantitative determination of anti-SARS-CoV-2 IgG antibodies, which were measured at basal and post-adsorption (incubation for 60 min at 37 °C) on blood group A RBCs, to remove from convalescent plasma donors' serum anti-A IgG antibodies [10]. All these tests were performed also in a

***Corresponding author: Massimo Franchini**, MD, Department of Hematology and Transfusion Medicine, Carlo Poma Hospital, Mantova, Italy, Phone: +39 0376 201234, Fax: +39 0376 220144, E-mail: massimo.franchini@asst-mantova.it
Paola Moi, Marzia Cortellazzi, Nadia Danese, Stefania Caruso, Paola Pasolini, Sabina Ferrazzo, Stefania Piccinini, Anna Dall'Oglio, Paola Zovetti, Nadia Negri, Donatella Braga, Cristina Pasquali, Elisa Zuliani and Claudia Glingani, Department of Hematology and Transfusion Medicine, Carlo Poma Hospital, Mantova, Italy

control group (group II) of 12 consecutive O blood type convalescent plasma donors, with the only difference that their serum was adsorbed on O blood type RBCs for the measurement of post-RBC adsorption anti-SARS-CoV-2 IgG antibodies.

The two groups (group I-cases and group II-controls) of convalescent plasma donors were comparable in terms of male-female ratio (5.0 vs. 3.3) and median age (46.5 vs. 46.0 years). The median anti-A IgG isoagglutinin titer and anti-SARS-CoV-2 neutralizing titer in the study group (group I) were 96 (range 32–256) and 80 (range 20–320), respectively. The median anti-A IgG isoagglutinin titer and anti-SARS-CoV-2 neutralizing titer in the control group (group II) were 80 (range 32–256) and 120 (range 20–320), respectively. No significant differences were observed in these parameters (i.e., anti-A IgG isoagglutinin titer and anti-SARS-CoV-2 neutralizing titer) between the two groups. Regarding the anti-SARS-CoV-2 IgG CLIA-based assay, the median basal and post-RBC (blood group A) serum adsorption levels in group I were 82 UA/mL (range 8–249 UA/mL) and 73 UA/mL (range 7–240 UA/mL), respectively. In group II (control group), the median basal and post-RBC (blood group O) anti-SARS-CoV-2 IgG levels were 95 UA/mL (range 33–265 UA/mL), and 81 UA/mL (range 22–254 UA/mL), respectively (Table 1). Using Student's t test for statistical analysis, no statistically significant difference between basal and post-RBC adsorption anti-SARS-CoV-2 IgG levels were detected in both group I and group II convalescent plasma donors. No difference was also observed comparing the same parameters between the two groups. A similar difference between median anti-SARS-CoV-2 IgG levels pre- and post-RBC adsorption studies was observed in group I and group II (11 and 15%, respectively). Thus, considering that in group I the donors' serum was adsorbed on A blood type RBCs and in group II it was adsorbed on O blood type RBCs, this slight difference was certainly not due to the presence of anti-A IgG isoagglutinins but rather to the adsorption procedure itself.

In conclusion, in this study we demonstrated the lack of cross-reactivity between anti-A IgG isoagglutinins and anti-SARS-CoV-2 IgG antibodies measured using an immunoassay, therefore excluding a possible interference of isoagglutinins on seroneutralizing tests.

Table 1: Relationship between anti-A IgG isoagglutinins and anti-SARS-CoV-2 IgG antibodies in cases and controls.

Convalescent plasma donors	Progressive no.	Sex	Age, years	Anti-A IgG isoagglutinin titer	Anti-SARS-CoV-2 neutralizing titer	Anti-SARS-CoV-2 IgG antibody titer		
						Basal	Post-RBC adsorption[a]	p-Value[b]
O blood type (Group I, cases)	1	Male	60	32	320	221	201	NS
	2	Female	44	32	160	81	73	
	3	Male	30	32	20	40	34	
	4	Male	47	32	160	83	73	
	5	Male	29	128	80	20	16	
	6	Male	46	128	40	130	123	
	7	Male	56	256	80	171	152	
	8	Male	44	64	160	249	240	
	9	Male	64	128	160	132	117	
	10	Male	37	64	20	8	7	
	11	Female	57	256	40	63	54	
	12	Male	50	128	20	30	26	
O blood type (Group II, controls)	1	Male	30	32	160	74	50	NS
	2	Male	45	64	20	33	22	
	3	Female	44	128	20	69	54	
	4	Male	54	64	80	110	90	
	5	Male	57	64	160	80	72	
	6	Female	30	256	160	240	220	
	7	Female	32	32	320	265	254	
	8	Male	61	256	160	223	201	
	9	Male	63	128	80	174	152	
	10	Male	47	128	160	228	221	
	11	Male	54	128	40	65	55	
	12	Male	28	128	80	35	23	

SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus-2; RBC, red blood cell, NS, not significant. [a]Post-adsorption of donors' serum on A blood type RBCs (group I-cases) or O blood type RBCs (group II-controls). [b]Student's t test.

Research funding: None declared.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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