

Minimal variation in anti-A and -B titers among healthy volunteers over time: Implications for the use of out-of-group blood components

Ulrik Sprogøe, MD, PhD, Mark H. Yazer, MD, Mads Hvidkjær Rasmussen, Berit Antonsen, Claus Bistrup, MD, PhD, and Kristian Assing, MD, PhD, Odense, Denmark

BACKGROUND:	Using potentially out-of-group blood components, like low titer A plasma and O whole blood, in the resuscitation of trauma patients is becoming increasingly popular. However, very little is known whether the donors' anti-A and/or anti-B titers change over time and whether repeated titer measurements on the same donor are required to ensure that each donation produces a low titer product.
METHODS:	The anti-A and/or anti-B titers were measured on 56 healthy adult volunteers (47 blood donors; nine blood center personnel) every 3 months for 12 consecutive months using an automated solid phase analyzer. The results were expressed as \log_2 titer steps (e.g., titer 32 = 5 titer steps).
RESULTS:	Minor variations in the average anti-A and/or anti-B titers were seen over time; the maximum individual SD in each group was 1.50 (IgG anti-A) or 1.00 (IgM anti-A, IgM, and IgG anti-B). When the SDs for the four titer measurements from all 56 volunteers were combined as appropriate, the highest overall combined SD was 0.47 titer steps for IgG anti-A. This value corresponds to a 95% confidence interval for intraindividual variation in this antibody's titer over 12 months of 0.96 titer steps. Thus, based on one measurement, an IgG anti-A with a titer step of, for example, 6 would be expected to be in the range of titer step 5 to titer step 7 over the course of 1 year with 95% probability.
CONCLUSION:	The titers of anti-A and/or anti-B among healthy adults are stable over at least 1 year. This suggests that repeated titer measurements within a year on the same donor are not necessary if donations are made at 3 months or longer intervals. (<i>J Trauma Acute Care Surg.</i> 2017;82: S87–S90. Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.)
LEVEL OF EVIDENCE:	Diagnostic study, level V.
KEY WORDS:	Anti-A; anti-B; titer; plasma; whole blood.

The use of potentially out-of-group blood components in the resuscitation of trauma patients is becoming increasingly popular. As the universal plasma donor group, AB, is a scarce resource, many hospital-based transfusion services have introduced group A plasma into their trauma resuscitation regimens. In fact, a recent survey of Level 1 trauma centers in the United States revealed that 34 (69%) of 49 centers use group A plasma for trauma recipients of unknown ABO group, and many of them have only recently begun the practice.¹ Thus, its use is likely to grow in the future. Of these 34 centers, 7 (21%) perform a titer on the anti-B in the plasma and the acceptable titers ranged from less than 25 to less than 100 using the immediate spin test for IgM antibodies.

Similarly, the use of group O whole blood (WB) is also becoming more widely adopted in the United States for use in the initial resuscitation of trauma patients. Because the plasma in group O WB contains both anti-A and anti-B, the titers of both antibodies should be considered. At two large, academic medical centers in the United States that use WB in the resuscitation of trauma patients, one center uses an anti-A and anti-B titer threshold less than 50, whereas the second uses an antibody titer threshold less than 200, highlighting the fact that there is currently no standard definition of a “low titer” WB unit.² Transfusing predominantly one to two units of group O WB with anti-A and anti-B titers less than 50 to non-group O trauma recipients did not lead to changes suggestive of hemolysis in a variety of biochemical markers including the lactate dehydrogenase, total bilirubin, and haptoglobin, nor did the WB transfusion lead to changes in the biochemical markers of renal function that were suggestive of dysfunction.³ However, what ultimately is a safe titer for use in massively bleeding non-group O recipient of group A plasma and group O WB has not yet been determined.⁴

In addition to determining a safe titer for anti-A and/or anti-B, another important question to answer is: if low titer products by any definition are to be used in the initial resuscitation of trauma patients, how often does a titer have to be performed on the donors of group A plasma and group O WB? The literature on the biological variations of anti-A and anti-B over time is scarce. One study in which an enzyme-linked immune sorbent

Submitted: November 21, 2016, Revised: December 27, 2016, Accepted: January 15, 2017, Published online: March 22, 2017.

From the Department of Clinical Immunology (U.S., M.Y., M.H.R., B.A., K.A.), Odense University Hospital, Odense, Denmark; Institute for Transfusion Medicine, and the Department of Pathology (M.Y.), University of Pittsburgh, Pittsburgh; and Department of Nephrology (C.B.), Odense University Hospital, Odense, Denmark.

Prior presentation of data: Part of this manuscript and data therein was presented as a poster at the AABB 2016 annual meeting, Orlando, FL.

Address for reprints: Kristian Assing, MD, PhD, Department of Clinical Immunology, Odense University Hospital, J.B. Winslows Vej 4, 5000 Odense C, Denmark; email: kristian.assing@rsyd.dk.

DOI: 10.1097/TA.0000000000001432

J Trauma Acute Care Surg
Volume 82, Number 6, Supplement 1

assay assay was used to measure anti-A in two group O individuals with high levels of anti-A, showed IgM and IgG levels to be stable (maximum variation 17%) over 7 measurements within the 82- to 167-day observation period.⁵ Because transfusion,^{6,7} pregnancy,⁸ and certain disease states,⁹ diet¹⁰ and some,^{11–14} but not all,¹⁵ vaccines have been shown to increase the titer of these antibodies, it is perhaps, given the scarce evidence on anti-A and-B stability, important to measure each donor's antibody titer every time they donate to ensure that their plasma does not contain a high titer of these antibodies. Checking donor titers at every donation has a cost in terms of reagents, but also in the time required for the technologists to perform the testing, and in managing an inventory that would contain both high and low titer products. Ensuring differential labeling of the high and low titer products and having to adapt the blood bank's computer system to accommodate these different types of products to prevent the accidental issuing of a high titer product to a non-ABO identical recipient can also pose an organizational challenge, as well as the logistical issue of having to determine if a unit to be titered came from a donor whose titer had been previously measured, if the antibody titer testing is not performed at the collection facility. Thus, it would be ideal if a donor's anti-A and/or -B could be checked less frequently than after each donation, although many of the aforementioned logistical challenges would still need to be resolved even with a lower frequency of titer testing. This project serially investigated anti-A and anti-B titers amongst healthy volunteers over the course of approximately one year to determine the variability of these titers over time.

METHODS

This was a prospective, longitudinal study to investigate the intra-personal variability of anti-A and/or anti-B titers in healthy volunteers who live in southern Denmark over the course of approximately 12 months. These volunteers consisted of blood donors and blood collection center employees. The volunteers were all white, and all were 18 years or older. Exclusion criteria accorded to the regional blood donor health criteria, and included pregnancy, the presence of symptomatic autoimmune disease, type 1 diabetes, previous or current malignancy, blood transmissible viral or parasitic disease, symptomatic infection, within 2 weeks of sampling and/or the ongoing use of prescription medications if taken orally or by injection. All participants gave written informed consent before they were enrolled. This protocol was reviewed and approved by the Regional Committees on Health Research Ethics for Southern Denmark (protocol-identification: S-20110085).

The study protocol involved measuring the titers of IgG and IgM anti-A and/or anti-B approximately every 3 months for the duration of 1 year (in total, four samples were drawn per volunteer). The blood samples were collected into ethylenediaminetetraacetic acid-containing tubes, and the plasma was immediately separated by centrifugation, divided into four aliquots, and frozen at -70°C until the titers could be assayed in batches. Antibody titrations were performed as follows: an aliquot of each participant's plasma was thawed at room temperature and then loaded onto the NEO solid phase analyzer (Immucor, Norcross, GA). The NEO analyzer made serial dilutions of the plasma using phosphate-buffered saline and then

dispensed samples onto microtiter plates. The entire process, from sample dilution to agglutination strength reading, was fully automated and guided by the Immucor titration assay software. The default software settings were used although a slight modification in the IgM antibody titration protocol was required for the automated titer results to correlate to those obtained by manual titration (the shake mode was set at medium; shake time lasted for 30 seconds, and the plates rested for 60 seconds before the agglutination strength was read). The maximum dilution was 1:2,048. Test cells were in-house glycerol frozen-thawed A₁ and B red cells suspended in phosphate-buffered saline.

The titer was defined as the inverse of the highest dilution that produced at least 1+ agglutination as read by the Immucor titration assay software. For the purpose of statistical analysis, titer values were converted to \log_2 titer steps (e.g., titer 2 = 1 titer step, titer 32 = 5 titer steps; a titer limit of <50 would correspond to <5.6 titer steps). A titer of zero was coded as -1 titer step. Recording of titer values and calculation of titer steps were performed in a custom-made Excel 2010 spreadsheet (Microsoft, Seattle, WA). The mean anti-A and/or anti-B IgM and IgG titer step and the standard deviation (SD) for all 4 titer measurements were calculated for each volunteer. The average titer step of each volunteer's four measurements was then averaged across all volunteers of the same ABO group to produce an overall average titer step value for that ABO group. The individual SDs were pooled across all volunteers to yield an overall SD of the repeated titer measurements. The overall SD was used in the calculation of the t-distribution (28 degrees of freedom for anti-A and 36 for anti-B) 95% confidence intervals of intrapersonal titer variation over time.

RESULTS

In total, 59 healthy volunteers entered the study. One man was excluded due to health reasons, one woman withdrew of personal reasons; additionally, one man was excluded from data analysis because only three samples were available for titer measurement. Thus, there were 56 people (36 men, 20 women; median age, 55 years; range, 24–66 years), of which 47 were blood donors, and nine were blood center employees who completed the study by having samples drawn for titer measurement four times over an approximately 12-month period. The median period over which the four samples were drawn was 43 weeks (range, 38–56 weeks). The average titer values and ranges stratified by the volunteers' ABO group are presented in Table 1, and the average titer step values with pooled SDs stratified by the volunteers' ABO group are presented in Table 2. As expected, the group O volunteers had significantly higher titer step values

TABLE 1. Anti-A and Anti-B Titers

ABO Group	No. Volunteers	Anti-A, IgM	Anti-A, IgG	Anti-B, IgM	Anti-B, IgG
O	19	50 (4–512)	66 (0–512)	44 (1–256)	31 (1–256)
A	27			14 (2–64)	3 (0–32)
B	10	39 (2–256)	2 (0–8)		

Data are presented as mean (range).

TABLE 2. The Overall Average and Pooled SD of Anti-A and Anti-B Titer Steps

ABO Group	No. Volunteers	Anti-A, IgM	Anti-A, IgG	Anti-B, IgM	Anti-B, IgG
O	19	4.7 (0.45)	4.9 (0.39)	4.3 (0.50)	3.5 (0.30)
A	27			3.5 (0.41)	0.1 (0.31)
B	10	4.0 (0.37)	0.3 (0.58)		
Overall	56	0.42 (n = 29, 0.87)	0.47 (n = 29, 0.96)	0.45 (n = 46, 0.91)	0.30 (n = 46, 0.62)

The data are presented as average (SD), except for the overall category where the data are presented as pooled SD (number of volunteers pooled together, 95% confidence interval). For reference, a titer step of 4 equals a titer of 16. For statistical analysis, see text.

of IgG anti-A and IgG anti-B compared with the group A and group B volunteers ($p < 0.0001$, two-sided t test).

The pooled SDs for the four repeated titer step measurements were small and ranged from 0.30 for the IgG anti-B in the group O volunteers to 0.58 for the IgG anti-A in the group B volunteers (Table 2). The maximum individual SD in each group was 1.50 (IgG anti-A) or 1.00 (IgM anti-A, IgM and IgG anti-B). Each maximum SD occurred only once in each antibody group and they occurred in 4 different individuals. When the SDs for the four titer measurements from all 56 volunteers were combined as appropriate (e.g., the SDs from the IgG and IgM anti-B titer step measurements in the group O and group A volunteers were combined), the highest overall combined SD was 0.47 titer steps for the IgG anti-A (Table 2). This SD value corresponded to a 95% confidence interval for intraindividual variation in this antibody's titer over 12 months of 0.96 titer steps. This means that based on one measurement, an IgG anti-A with a titer step of 6 (titer 64) would be expected to be in the range of titer step 5 (titer 32) to titer step 7 (titer 128) over the course of 1 year with 95% probability. The other 95% confidence intervals for the other antibody specificities and isotypes were lower than 0.96, indicating an even narrower range of variability over time (Table 2).

DISCUSSION

In this study of healthy adult volunteers, the IgG and IgM anti-A and/or anti-B titers demonstrated minimal intra-personal variation over time. This finding, which is agreement with the limited literature on the subject, is valuable because it suggests that if low titer plasma and WB is to be used for resuscitating patients of unknown ABO group, the donor's anti-A and/or -B titer needs to be measured only once a year.

The finding that IgG anti-A and -B are significantly higher in blood group O than in blood group A or group B volunteers is consistent with what has been previously reported.¹⁶

Although the 24 to 66 years age range of the volunteers indicates that the low level of anti-A and anti-B variability persists with increasing age, our study was not designed to address the more longitudinal, age related changes in anti-A and -B. Therefore, a study specifically addressing this aspect is needed before conclusions can be drawn regarding the true long-term (if any) validity of anti-A and anti-B measurements in blood donors.

To our knowledge, no serious immunizing events (e.g., pregnancy, transfusion, serious infection, or immunization, except for potentially the influenza vaccine), which are known potentially to increase the titers of anti-A and -B, occurred in the

volunteers during the study period. Our results therefore have to be interpreted and generalized with this in mind.

However, even with these limitations the main strength of this study was its pragmatic, longitudinal design. The lifestyle of the healthy volunteers was not intentionally altered as a result of participating in this study. Thus, they were free to engage in their usual activities of daily living, which might have included events that could have led to an increase in their anti-A and/or anti-B titers. It should be noted that an estimated 30% (n = 3) of the blood center employees, and likely some of the blood donors, received their annual influenza vaccination while they participated in this study. Despite this, there was minimal variation in the overall anti-A and/or anti-B titer step values, which is consistent with other reports on the stability of these titers after the administration of earlier versions of the influenza vaccine.¹⁵

It is possible that transient elevations in the antibody titer (s) could have occurred during the interval between antibody titer testing, that is, the antibody titer could have peaked sometime before the next 3-month measurement and returned to baseline sometime before the next sample for titer measurement was drawn. If this phenomenon occurs, it would predominantly affect plasmapheresis donors who can donate every 2 weeks rather than WB donors who can only donate five to six times per year. Thus, given that the kinetics of possible changes in anti-A and/or anti-B titers are unknown, perhaps some caution is warranted if group A plasma from frequent repeat plasmapheresis donors is to be administered to trauma patients of unknown ABO group. Furthermore, because the stimuli that incite increases in anti-A and anti-B antibodies have not been entirely elucidated, and because uniform responses to the same stimulus in different people has not been demonstrated, a conservative strategy to performing titers might depend on whether the donor endorses having experienced a potentially stimulating event, such as pregnancy or a vaccination since their last titer measurement, as has been espoused previously.⁴

This study also demonstrated that performing antibody titers using an automated instrument is feasible. Given that a common problem with manually performing antibody titer testing is the subjectivity of interpreting the results,^{17,18} and the potential to obtain different results when plasma samples are tested using different manual methods,¹⁹⁻²³ using an automated instrument is an attractive means for reducing the subjectivity of this assay compared with manual methods.

This study revealed stable titers of anti-A and/or anti-B among healthy volunteers over time. These findings suggest that, at least in the white population and in absence of potentially immunizing events, such as pregnancy or transfusion, repeat

titer measurements on the same donor are not necessary if donations are made at 3 months or longer intervals. Because the antibody titer measurements were not made at more frequent intervals in this study, caution is advised when using plasma from donors who donate multiple times in a 3-month period and also with regard to the long-term, that is, longer than 12 months, validity of the titer results.

AUTHORSHIP

U.S. participated in the data analysis, data interpretation, writing, critical revision. M.Y. participated in the literature search, data interpretation, writing, critical revision. M.H.R. participated in the data collection, data analysis, critical revision. B.A. participated in the method development, data collection, data analysis, critical revision. C.B. participated in the study design, data collection, data analysis, critical revision. K.A. participated in the literature search, data collection, data analysis, data interpretation, writing, critical revision.

DISCLOSURE

The authors declare no conflicts of interest.
No funding was obtained to perform this study.

REFERENCES

- Dunbar NM, Yazer MH; Biomedical Excellence for Safer Transfusion Collaborative. A possible new paradigm? A survey-based assessment of the use of thawed group A plasma for trauma resuscitation in the United States. *Transfusion*. 2016;56:125–129.
- Yazer MH, Jackson B, Sperry JL, Alarcon L, Triulzi DJ, Murdock AD. Initial safety and feasibility of cold-stored uncrossmatched whole blood transfusion in civilian trauma patients. *J Trauma Acute Care Surg*. 2016;81:21–26.
- Seheult JN, Triulzi DJ, Alarcon LH, Sperry JL, Murdock A, Yazer MH. Measurement of haemolysis markers following transfusion of uncrossmatched, low-titre, group O+ whole blood in civilian trauma patients: initial experience at a level 1 trauma centre. *Transfus Med*. 2016;27:30–35.
- Berséus O, Boman K, Nessen SC, Westerberg LA. Risks of hemolysis due to anti-A and anti-B caused by the transfusion of blood or blood components containing ABO-incompatible plasma. *Transfusion*. 2013;53(Suppl 1):114S–123S.
- Rieben R, Frauenfelder A, Nydegger UE. Naturally occurring ABO antibodies: long-term stable, individually distinct anti-A IgG spectrotypes. *Eur J Immunol*. 1992;22:2129–2133.
- Wiener AS, Samwick AA, Morrison H, Cohen L. Studies on immunization in man. I. The blood group substances A and B. *Exp Med Surg*. 1953;11:267–275.
- McShine RL, Kunst VA. The stimulation of immune antibodies anti-A and anti-B in patients after treatment with cryprecipitate and factor IX concentrate (P.P.S.B. according to Soulier). *Vox Sang*. 1970;18:435–440.
- Boorman KE, Dodd BE, Mollison PL. Iso-immunisation to the blood-group factors A, B and Rh. *J Pathol Bacteriol*. 1945;157.
- Miler JJ, Novotny P, Walker PD, Harris JR, MacLennan IP. *Neisseria gonorrhoeae* and ABO isohemagglutinins. *Infect Immun*. 1977;15:713–719.
- Daniel-Johnson J, Leitman S, Klein H, et al. Probiotic-associated high-titer anti-B in a group A platelet donor as a cause of severe hemolytic transfusion reactions. *Transfusion*. 2009;49:1845–1849.
- Crawford H, Falconer H, Cutbush M, Mollison PL. Formation of immune A iso-antibodies, with special reference to heterogenetic stimuli. *Lancet*. 1952;2:219–223.
- Gupte SC, Bhatia HM. Anti-A and anti-B titre response after tetanus toxoid injections in normal adults and pregnant women. *Indian J Med Res*. 1979;70:221–228.
- Springer GF. Influenza virus vaccine and blood group A-like substances. *Transfusion*. 1963;3:233–236.
- Siber GR, Ambrosino DM, Gorgone BC. Blood-group-A-like substance in a preparation of pneumococcal vaccine. *Ann Intern Med*. 1982;96:580–586.
- Delaney M, Warner P, Nelson K, Gleckler C, Price T, Madeleine M. Humoral immunomodulatory effect of influenza vaccine in potential blood donors: implications for transfusion safety. *Transfus Med*. 2011;21:378–384.
- Karafin MS, Blagg L, Tobian AA, King KE, Ness PM, Savage WJ. ABO antibody titers are not predictive of hemolytic reactions due to plasma-incompatible platelet transfusions. *Transfusion*. 2012;52:2087–93.
- Tanabe K. Interinstitutional variation in the measurement of anti-A/B antibodies: the Japanese ABO-Incompatible Transplantation Committee survey. *Transplantation*. 2007;84:S13–S16.
- Kumlien G, Wilpert J, Säfwenbergh J, Tydén G. Comparing the tube and gel techniques for ABO antibody titration, as performed in three European centers. *Transplantation*. 2007;84:S17–S19.
- Cooling LL, Downs TA, Butch SH, Davenport RD. Anti-A and anti-B titers in pooled group O platelets are comparable to apheresis platelets. *Transfusion*. 2008;48:2106–2113.
- Duez A, Flourie F, Garraud O. Antibodies titration for immunized pregnant women: conventional tube test or gel microcolumn assay. *Transfusion*. 2014;54:1200.
- Quillen K, Medrano C, Adams S, et al. Screening plateletpheresis donors for HLA antibodies on two high-throughput platforms and correlation with recipient outcome. *Transfusion*. 2011;51:504–510.
- Novaretti MC, Jens E, Pagliarini T, Bonifacio SL, Dorlhiac-Llacer PE, Chamone DA. Comparison of conventional tube test with diamed gel microcolumn assay for anti-D titration. *Clin Lab Haematol*. 2003;25:311–315.
- Josephson CD, Mullis NC, Van Demark C, Hillyer CD. Significant numbers of apheresis-derived group O platelet units have “high-titer” anti-A/A,B: implications for transfusion policy. *Transfusion*. 2004;44:805–808.