Changes in donor antibody titer levels over time in a military group O low-titer whole blood program

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BACKGROUND: The ability to rapidly administer whole blood (WB) at the point of injury is an important intervention to save lives. This can be accomplished using low titer group O WB donors. Titers of immunoglobulin M anti-A and anti-B might change over time. This study describes titer testing in a large series of donors.

STUDY DESIGN AND METHODS: Data were collected retrospectively from the Armed Services Blood Program and the Theater Medical Data Store. Soldiers assigned to the 75th Ranger Regiment were screened and titered upon completion of training or before deployment or during periodic unit readiness activities. A Ranger group O low-titer (ROLO) donor was defined as having titers of both anti-A and -B of less than 256 by immediate spin testing.

RESULTS: Between May 2015 and January 2017, of a total of 2237 participating soldiers, 1892 (84.5%) soldiers underwent antibody titering once, while 266 (11.9%) were titered twice, 62 (2.8%) were titered three times, and 17 (0.8%) were titered at least four times. The mean age was 26.5 $\pm$ 6.5, and 2197 (98.2%) were male. A total of 69.5% of donors met ROLO donor criteria on the first test. The percentage of donors meeting universal-donor criteria increased to 83.5% on the second test, 91.1% on the third test, and 100% on the fourth and fifth tests.

CONCLUSIONS: With successive titer testing, it appears that individuals display a tendency toward lower titers. This may indicate that titer testing may not be required after the second test if donors have been identified initially as low titer.

As hemorrhagic shock remains the leading cause of preventable mortality on the battlefield, efforts are being made to combat mortality from blood loss in novels ways. In 2014, as the US military's Joint Trauma System Committee on Tactical Combat Casualty Care updated its guidelines on hemorrhagic shock to promote the use of whole blood (WB) at the point of injury, the 75th Ranger Regiment began implementing its Ranger group O low-titer (ROLO) program to identify group O donors with a low titer of both anti-A and -B from within the fighting force to serve as a walking blood bank.[1,2] The ability to obtain group O WB from preidentified low-titer donors at the point of injury in the field significantly

ABBREVIATIONS: AOR(s) = adjusted odds ratio(s); ASBP = Armed Services Blood Program; GEE = generalized estimating equation; ROLO = Ranger group O low titer; TMDS = Theater Medical Data Store; TTD(s) = transfusion-transmissible disease(s); WB = whole blood.

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changes the approach to the management of hemorrhagic shock in austere environments by providing a rapid and safe source of WB. The Armed Services Blood Program (ASBP) has defined a low-titer donor to be one whose titers of both anti-A and anti-B are less than 256 by saline tube testing. Recent data suggest that the mean percentage of low-titer donors in the US military is between 70% and 75% of the soldiers tested,[3] which compares favorably with the percentage in the general population.[4,5]

While anti-A and -B titer testing mitigates the risk of a hemolytic transfusion reaction due to the incompatible plasma in the WB, many questions about the titers of these antibodies remain to be answered. Although a small study in Denmark showed stable antibody titers among blood donors and other healthy volunteers,[6] and also among patients undergoing dialysis,[7] it is currently unknown how often antibody titers change in potentially highly traveled and vaccinated military personnel. The stimulus underlying antibody titer fluctuations and the extent to which titers change are also unknown. As donor antibody titers change, so does the size and composition of the walking donor population. Thus, knowing how many eligible low-titer donors are currently available is critical for mission planning. Currently, all potential group O donors in the 75th Ranger Regiment are tested for transfusion-transmissible diseases (TTDs), and their anti-A and B titers are determined, before deployment. However, titer testing on a recurring basis presents a significant cost for a combat unit and may disrupt staffing plans if the number of low-titer donors changes within smaller troop formations. The current cost for screening one donor for TTDs and performing the anti-A and -B titers is approximately $77, and the screening process takes approximately 30 minutes per donor. If retesting did not need to occur as often, this would be beneficial to the US military and those units that may deploy on very short notice. This study investigated the antibody titers and their trends from the inception of the ROLO program in May 2015 until January 2017.

MATERIALS AND METHODS

Data and measures

This was a retrospective performance improvement study of titers in active military members. Military members consisted of Active Duty and National Guard personnel assigned to US Special Operations Command (USSOCOM) or military units in support of USSOCOM operations. A Ranger regiment typically consists of 4000 soldiers who are usually between 20 and 40 years old. These soldiers are typically deployed overseas for 4 months of the year. Military personnel undergo antibody titer and other pre–blood donation testing by ASBP staff members. The ASBP has 20 donor centers worldwide that collect and manufacture blood products in accordance with US regulatory requirements. Soldiers are screened and titered upon completion of training, before deployment, or during periodic unit readiness activities.

Volunteer donors were screened using an Food and Drug Administration (FDA)-approved standard ASBP donor screening form that consists of standard donor qualifying questions. If determined to be eligible after being interviewed by ASBP personnel, the donor was issued a unique donor identification number, and blood samples for the required TTD testing and antibody titer testing were collected, processed, and shipped to the designated reference laboratories for testing. All donor information including results from the TTD and titer testing was entered into the Theater Medical Data Store (TMDS), which is a Department of Defense Web-based information system used for donor management. Data for this study were extracted from the TMDS database for military members tested at ASBP donor centers or mobile screening locations.

Anti-A and -B titers were performed as follows: serial twofold dilutions of donor plasma were prepared using 0.9% saline and a calibrated pipette. Plasma dilutions were mixed with FDA-approved, commercially available reagent A_1 and B RBCs, incubated at room temperature for 15 minutes, and then centrifuged. Direct agglutination was measured immediately after centrifugation. The titer was recorded as the inverse of the highest dilution that produced macroscopic agglutination. For analysis of the changes in titers within an individual over time, the discrete levels were converted to numeric values of 1 through 11; for example, a value of 2 would equal a titer or 4, and a value of 5 would equal a titer of 32. To qualify as a low-titer donor, the titers of both anti-A and -B had to be less than 256. For analytical purposes, any test results that were not interpretable were grouped as high titer, since the donors would not be eligible to donate as ROLO donors until retested and shown to have low antibody titers.

In addition to titer data, each donor's age, sex, and the geographic location and the date when the testing was performed were also collected from the TMDS. Sex was extracted as male or female, and male was the reference in multivariate analyses. The geographic location of tests was recorded as the state in which testing occurred. All soldiers would have had their samples for antibody titer analysis collected within the United States. For multivariate analyses, the states/territories that performed fewer than 50 valid titer tests were grouped into a separate category called "all other." These states/territories included Florida, Hawaii, Illinois, North Carolina, Oklahoma, Guam, and Virginia. To account for potential seasonal variation in titer levels the month in which each titer test was performed was grouped into four seasonal categories, including 1) winter (December, January, February); 2) spring (March, April, May); 3) summer (June, July, August); and 4) fall (September, October, November). Winter was used as the reference group in multivariate analyses.

TABLE 1. Descriptive statistics of the titer tests performed in this study*

Variables	Total	Has the donor ever been a low-titer donor? Yes	No
Unique donors	2237	1625 (72.6)	612 (27.4)
Blood group			
A	67 (3.0)	1 (0.1)	66 (10.8)
AB	17 (0.8)	0 (0.0)	17 (2.8)
B	33 (1.5)	0 (0.0)	33 (5.4)
O	2120 (94.8)	1624 (99.9)	496 (81.1)
Age (years)	26.5 (±6.5)	26.5 (±6.1)	26.4 (±7.5)
Sex			
Female	40 (1.8)	17 (1.1)	23 (3.8)
Male	2197 (98.2)	1608 (98.9)	589 (96.2)
State of first test			
CA	69 (3.1)	42 (2.6)	27 (4.4)
CO	133 (6.0)	103 (6.3)	30 (4.9)
FL	2 (0.1)	2 (0.1)	0 (0.0)
GA	1283 (57.4)	967 (59.5)	316 (51.6)
HI	88 (3.9)	15 (0.9)	73 (11.9)
IL	1 (0.0)	0 (0.0)	1 (0.2)
KY	138 (6.2)	99 (6.1)	39 (6.4)
NC	6 (0.3)	2 (0.1)	4 (0.7)
OK	2 (0.1)	0 (0.0)	2 (0.3)
OT (Guam)	18 (0.8)	9 (0.6)	9 (1.5)
TX	66 (3.0)	50 (3.1)	16 (2.6)
VA	2 (0.1)	0 (0.0)	2 (0.3)
WA	429 (19.2)	336 (20.7)	93 (15.2)
Timing of titer test			
Winter (Dec, Jan, Feb)	507 (22.7)	351 (21.6)	156 (25.5)
Spring (Mar, Apr, May)	345 (15.4)	240 (14.8)	105 (17.2)
Summer (Jun, Jul, Aug)	631 (28.2)	458 (28.2)	173 (28.3)
Fall (Sep, Oct, Nov)	754 (33.7)	576 (35.5)	178 (29.1)
Number of titer tests performed per soldier			
One only	1892 (84.5)	1306 (80.4)	586 (95.8)
Two	266 (11.9)	241 (14.8)	25 (4.1)
Three	62 (2.8)	61 (3.8)	1 (0.2)
Four or more	17 (0.8)	17 (1.0)	0 (0.0)
Time to retest (days)			
Test 1 to 2	174 (98–274)	174 (98–274)	168 (90–284)
Test 2 to 3	71 (39–100)	71 (39–100)	53 (53–53)
Test 3 to 4	40 (39–71)	40 (39–71)	
First test result			
Low < (256)	1555 (69.5)	1555 (95.7)	0 (0.0)
High ≥ 256	682 (30.5)	70 (4.3)	612 (100.0)
Second test result			
Low < 1:256	288 (83.5)	288 (90.3)	0 (0.0)
High ≥ 1:256	57 (16.5)	31 (9.7)	26 (100.00)
Third test result			
Low < 1:256	72 (91.1)	72 (92.3)	0 (0.0)
High ≥ 1:256	7 (8.9)	6 (7.7)	1 (100.0)

* Data are reported as number (%), mean (±SD), or median (IQR).

Statistical analysis

Descriptive statistics are reported as mean ± standard deviation (SD), for continuous measures, or as frequency and percent for categorical measures. Changes in titers upon repeat testing were analyzed using generalized estimating equation (GEE) models for unbalanced repeated measures multivariable linear regression. GEE models account for the fact that the data for this study are not independent and that each individual can contribute a different number of observations to data. The interval values assigned to the

TABLE 2. Unadjusted contingency table for donor high-/low-titer status for each test*

Test number	Number	Low titer < 256	High titer ≥ 256	p value†
Anti-A				
1	2237	1638 (73.2)	516 (23.1)	<0.001
2	345	295 (85.5)	49 (14.2)	
3	79	74 (93.7)	5 (6.3)	
4 or more	17	17 (100.0)	0 (0.0)	
Anti-B				
1	2237	1979 (88.5)	208 (9.3)	0.01
2	345	328 (95.1)	17 (4.9)	
3	79	75 (94.9)	4 (5.1)	
4 or more	17	17 (100.0)	0 (0.0)	
Anti-A and -B				
1	2237	1555 (69.5)	682 (30.5)	<0.001
2	345	288 (83.5)	57 (16.5)	
3	79	72 (91.1)	7 (8.9)	
4 or more	17	17 (100.0)	0 (0.0)	

* Data are reported as number (%).
† Based on unadjusted, repeated-measures logistic regression analysis

titers met distributional assumptions for linear regression, and results are reported as coefficients with 95% confidence intervals (CIs) and p values. Analysis of the low-titer donor status was analyzed using GEE models for unbalanced repeated-measures multivariable logistic regression. Results of multivariable adjusted logistic regression analysis are reported as adjusted odds ratios (AORs), 95% CI, and p values. All analyses were performed using computer software (SAS, Version 9.4, SAS Institute).

RESULTS

Descriptive statistics are presented in (Table 1). During the study period 2237 blood donors had their anti-A and -B titers performed, of whom 2120 (94.8%) were group O. The mean age was 26.5 ± 6.5, and 2197 (98.2%) were male and 40 (1.8%) were female. The majority of donors were tested in Georgia (1283, 57.4%). A total of 1892 (84.5%) soldiers were tested only one time, while 266 (11.9%) had two tests, 62 (2.8%) had three tests, and 17 (0.8%) had four or more tests; only one person had more than four tests. The number of donors who were ever considered low titer (total low titers) during the study period was 1625 (72.6%), and of these low-titer donors, 1555 (95.7%) were qualified as such on their first test.

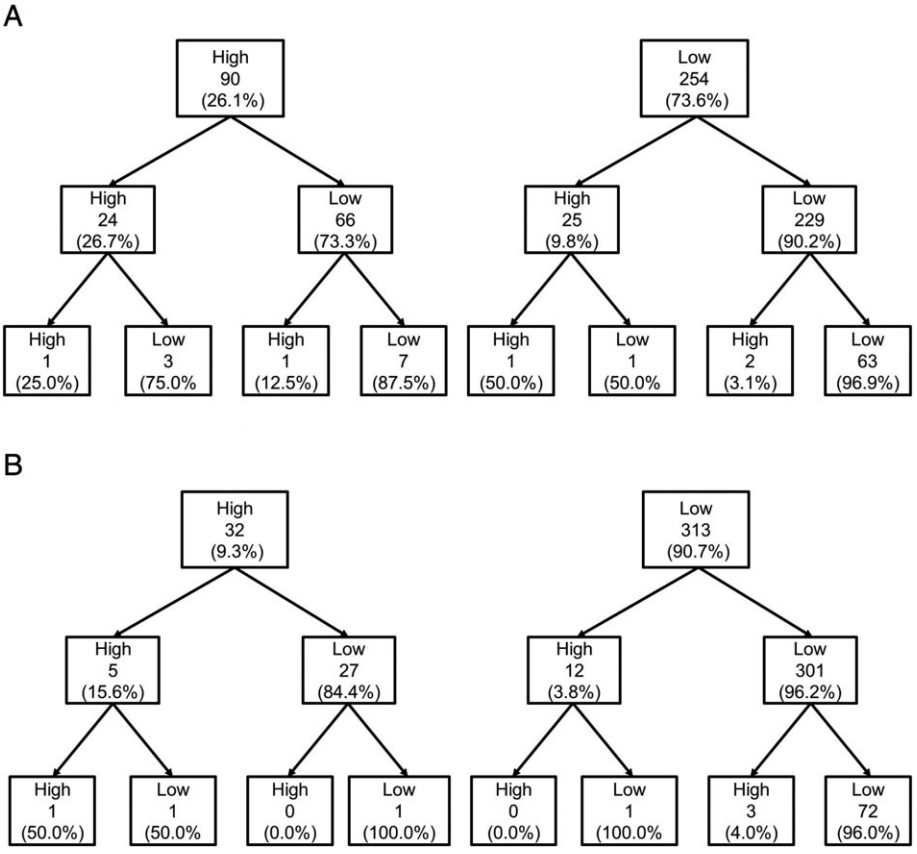

Fig. 1. Progression of anti-A and anti-B titers over time in group O donors. Each row represents the results of donors being serially tested. Not all donors underwent the final round of testing. (A) Titer A—threshold transitions (n = 344). One case had a noninterpretable result for first test. (B) Titer B—threshold transitions (n = 345).

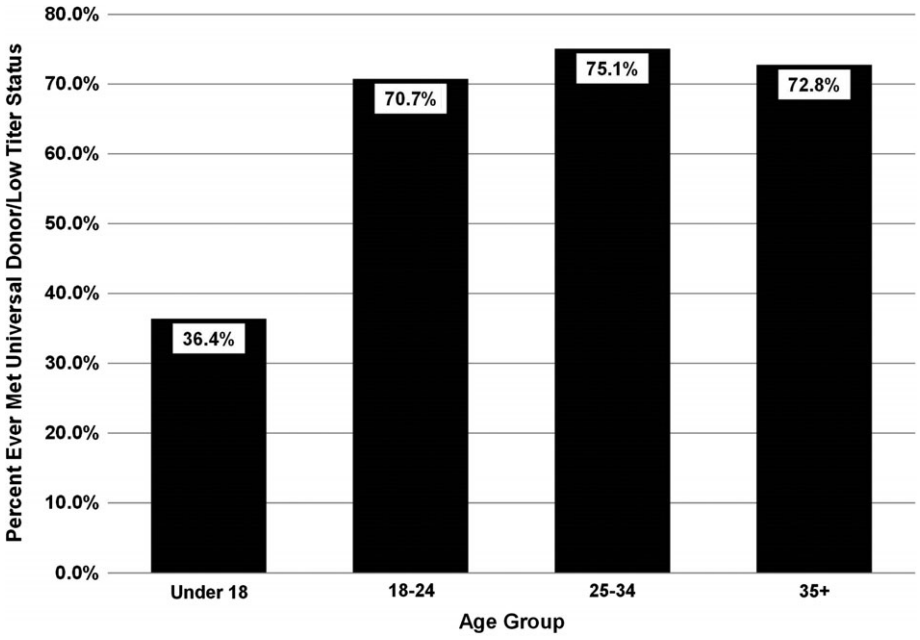

Fig. 2. Percentage of donors who ever met universal-donor/low-titer status by age.

The percentage of low-titer donors increased with the number of tests conducted (Table 2). A total of 69.5% of all donors screened met low-titer donor criteria on the first test. The percentage of donors meeting ROLO donor criteria increased to 83.5% on the second test, 91.1% on the third test, and 100% on the fourth and fifth tests. Donors tended to have higher test results with their anti-A titer, with 73.2% meeting low-titer criteria on the first test, compared with anti-B titer, in which 88.5% met low-titer criteria on the first test (Table 2, Fig. 1). Additionally, younger donors, particularly individuals under 18 years of age, were less likely to be low titer (36.4%), compared with individuals 18 to 24 years of age (70.7%), 25 to 34 years of age (75.1%), and 35 years and older (72.8%; Fig. 2).

Results of multivariable, GEE regression models of titer level changes over successive anti-A and anti-B titer tests are reported in Table 3 and Fig. 3. Titer levels tend to decrease with subsequent testing, even when controlling for age, sex, seasonality, and geographic location; however, the effect was stronger for anti-A titers (coefficient, −0.22; 95% CI, −0.30 to −0.14; $p < 0.001$) than anti-B titers (coefficient, −0.06; 95% CI, −0.14 to 0.01; $p = 0.09$). The titer of anti-A (coefficient, −0.04; 95% CI,

TABLE 3. Results of GEE repeated-measures multivariable regression analysis of A and B titer levels

	A Titer		B Titer	
Variables	Coefficient (95% CI)	p value	Coefficient (95% CI)	p value
Intercept	8.70 (8.34 to 9.05)	<0.001	7.34 (6.97 to 7.70)	<0.001
Number of tests performed	−0.22 (−0.30 to −0.14)	<0.001	−0.06 (−0.14 to 0.01)	0.09
Age	−0.04 (−0.06 to −0.03)	<0.001	−0.02 (−0.04 to −0.01)	<0.001
Sex				
Male (ref)				
Female	−0.04 (−0.56 to 0.47)	0.87	0.55 (−0.04 to 1.14)	0.07
Season				
Winter (ref)				
Spring	0.51 (0.23 to 0.78)	<0.001	−0.35 (−0.53 to −0.18)	<0.001
Summer	0.08 (−0.12 to 0.28)	0.45	0.35 (0.08 to 0.62)	0.01
Fall	−0.26 (−0.45 to −0.06)	0.01	−0.18 (−0.35 to 0.00)	0.05
State				
GA (ref)				
CA	0.46 (0.02 to 0.90)	0.04	−0.26 (−0.61 to 0.10)	0.15
CO	−0.15 (−0.47 to 0.16)	0.35	−0.67 (−0.99 to −0.34)	<0.001
KY	−0.02 (−0.35 to 0.31)	0.92	0.04 (−0.29 to 0.37)	0.80
TX	0.31 (−0.12 to 0.74)	0.15	0.00 (−0.38 to 0.38)	0.98
WA	−0.18 (−0.35 to −0.01)	0.04	−0.33 (−0.49 to −0.17)	<0.001
All other (FL, HI, IL, NC, OK, Guam, VA)	0.01 (−0.40 to 0.41)	0.97	0.33 (−0.02 to 0.67)	0.06

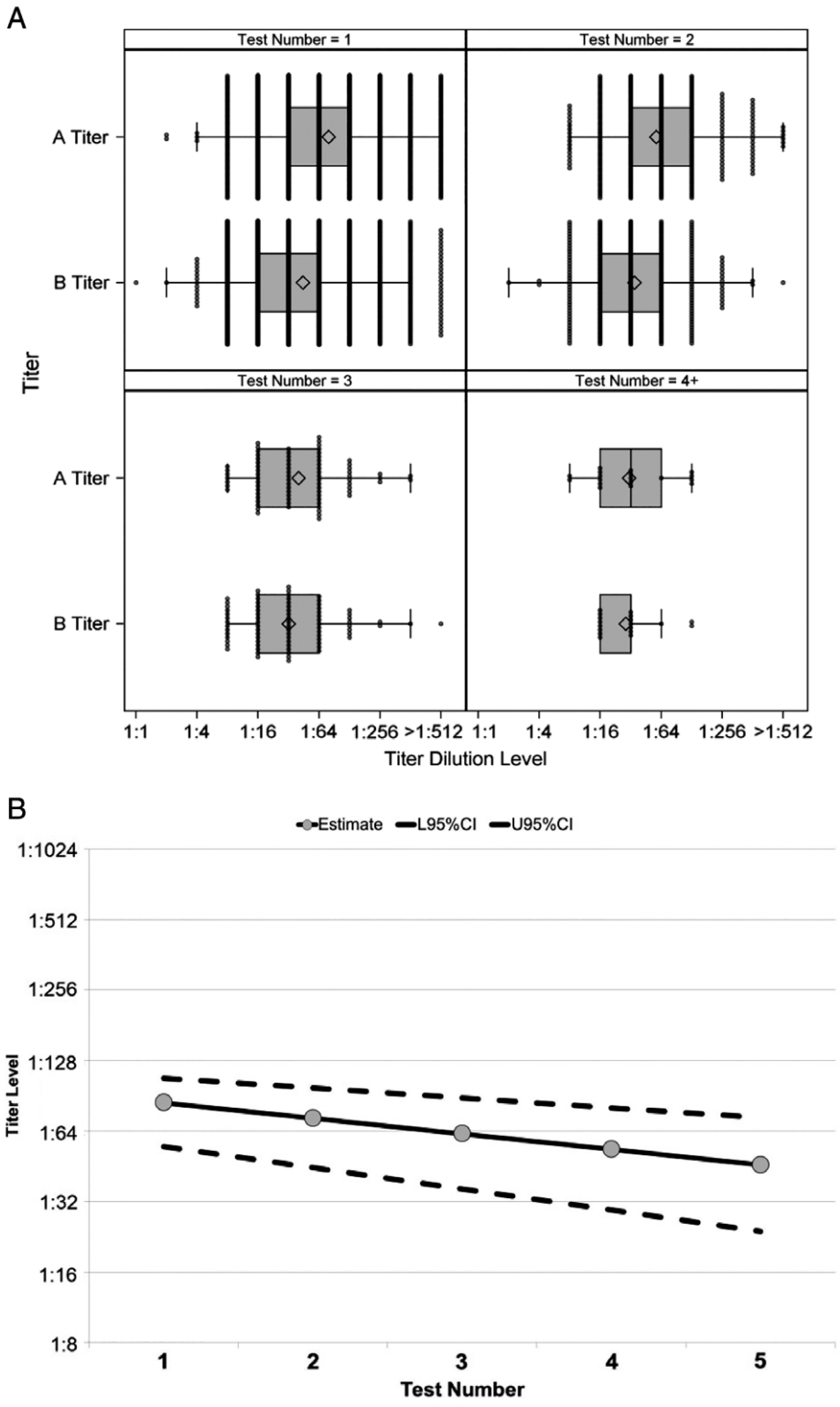

Fig. 3. Distribution of titer levels by number of tests. (A) Titer level boxplot distributions. (B) GEE estimates for titer A levels by number of tests.

–0.06 to –0.03; $p < 0.001$) and anti-B (coefficient, –0.02; 95% CI, –0.04 to –0.01; $p < 0.001$) levels tend to be lower with increased age. Anti-A titers levels did not differ significantly between males and females (coefficient, –0.04; 95% CI, –0.56 to 0.47; $p = 0.87$), but anti-B titers trended toward higher levels for females compared with males (coefficient, 0.55; 95% CI, –0.04

TABLE 4. Results of GEE repeated-measures multivariable logistic regression analysis of universal-donor/low-titer status

	Low-titer (<256) donor status	
Variables	AOR (95% CI)	p value
Number of tests	1.66 (1.30 to 2.11)	<0.001
Age	1.03 (1.02 to 1.05)	<0.001
Sex		
Male (ref)		
Female	0.43 (0.21 to 0.91)	0.03
Season		
Winter (ref)		
Spring	0.83 (0.57 to 1.23)	0.36
Summer	1.16 (0.90 to 1.51)	0.25
Fall	1.58 (1.22 to 2.04)	0.001
State		
GA (ref)		
CA	0.76 (0.44 to 1.31)	0.32
CO	1.66 (0.99 to 2.77)	0.05
KY	1.16 (0.70 to 1.90)	0.57
TX	0.82 (0.45 to 1.48)	0.51
WA	1.28 (1.01 to 1.62)	0.04
All other (FL, HI, IL, NC, OK, Guam, VA)	0.10 (0.06 to 0.17)	<0.001

to 1.14; p = 0.07). The anti-A titers were significantly higher in the spring months (coefficient, 0.51; 95% CI, 0.23 to 0.78; p < 0.001) and significantly lower in the fall months (coefficient, −0.26; 95% CI, –0.45 to –0.06; p = 0.01), compared with the winter months. In contrast, anti-B titers were significantly lower in the spring (coefficient, −0.35; 95% CI, –0.53 to –0.18; p < 0.001) and fall months (coefficient, −0.18; 95% CI, –0.35 to 0.00; p = 0.05) and significantly higher in the summer months (coefficient, 0.35; 95% CI, 0.08 to 0.62; p = 0.01), compared with the winter months. Similarly, geographic differences in titer levels were inconsistent between anti-A and -B titers. The anti-A titers were higher for individuals tested in California (coefficient, 0.46; 95% CI, 0.02 to 0.90; p = 0.04) and lower for individuals tested in Washington (coefficient, −0.18; 95% CI, –0.35 to –0.01; p = 0.04), compared with individuals tested in Georgia. Anti-A titers were not different for individuals tested in any of the other states. Anti-B titers were significantly lower for individuals tested in Colorado (coefficient, −0.67; 95% CI, –0.99 to –0.34; p < 0.001) and Washington (coefficient, −0.33; 95% CI, –0.49 to –0.17; p < 0.001), compared with individuals tested in Georgia.

Table 4 lists the results of multivariable logistic regression analysis of low-titer status. The odds of meeting low-titer criteria were increased by 66% with each successive test (AOR, 1.66; 95% CI, 1.30 to 2.11; p < 0.001). The estimated probability of meeting low-titer criteria increased significantly from the first test to the fifth test (Fig. 4). Older age was also associated with higher odds of meeting low-titer criteria (AOR, 1.03; 95% CI, 1.02 to 1.05; p < 0.001). Individuals tested in the fall months had 58% higher odds of meeting low-titer criteria (AOR, 1.58; 95% CI, 1.22 to 2.04; p = 0.001). Similarly, individuals tested in Colorado (AOR, 1.66; 95% CI, 0.99 to 2.77; p = 0.05) and Washington (AOR, 1.28; 95% CI, 1.01 to 1.62; p = 0.04) had 66 and 28% higher odds of meeting low-titer criteria, respectively, compared with individuals tested in Georgia.

DISCUSSION

The group O donors evaluated during the study period underwent titer testing as part of routine unit readiness procedures, after completion of training, or as preparation for deployment. During this period, personnel, including those who had previously been identified as high titer, were

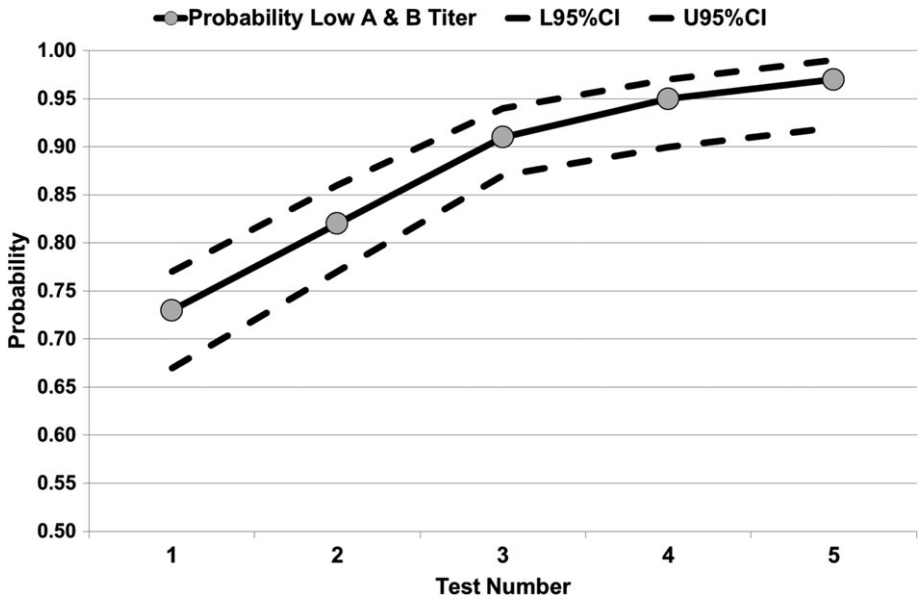

Fig. 4. Probability of low titer with repeat testing.

routinely rescreened and titered per unit protocol, since the degree of fluctuation of titers was unknown. This study has demonstrated that while titers can and do change over time, there is a trend toward decreasing titers over time and with increasing age. Furthermore, 90% of donors initially designated as low titer were found to have low titers on repeat testing and approximately three-quarters of donors with high titers on initial testing converted to low titer over time. These data are also consistent with a previous study that demonstrated the US military has between a 70% and 75% low-titer status.[3] It is known that anti-A and -B titers decrease over time, but it was an unexpected finding that this decrease could be observed over only an approximately 18-month period; a recent Danish study evaluated immunoglobulin (Ig)G and IgM titers of 56 non–group AB subjects over an approximately 1-year period.[4] In that study, each participant had their titer levels determined four times over a mean period of 43 weeks (range, 38–56 weeks) and it was found that the antibody titers over this period of time were generally stable with the majority of the repeat titers within one dilution of the initial measurement.[4] The Danish study had fewer subjects than in the current study but they included groups A and B individuals, which this study did not evaluate. These Danish investigators also evaluated the titers of anti-A and -B in people receiving regular hemodialysis and found that the antibody titers were likewise also stable over time in these patients.[7] Historically, titers have been noted to change over time with exposure to vaccines.[8] However, the study by Berseus[9] evaluating the titer changes within a population for WB donation demonstrated that titers are stable with newer vaccines, which was consistent with the findings of an American study that found no changes in HLA antibody titers or titers of anti-A and -B after receipt of the 2009 influenza vaccine.[10] Thus, it was unclear why a decline in antibody titer was detected among some initially high-titer donors; however, this finding supports performing repeat testing on high-titer donors in case they have converted to low titer and could therefore be utilized as WB donors.

A limitation of this study is its retrospective nature. Not all donors were retested for a third time, and so while trends in titer status over time are apparent, a more complete description of the natural history of titers was not possible with these data. In addition, this study was primarily based on young healthy males in the US military. Although the demographic composition of the US military is diverse, it would be beneficial to test the broader US civilian population for titer changes over time. Maintaining records of low-titer group O donors could be useful in disasters and other situations where emergency blood is needed. Finally, the possibility that the change in titers was caused by testing donors with different reagent RBCs cannot be excluded, although all testing was performed using in-date, FDA-approved reagent RBCs in laboratories where titer testing is routinely performed.

CONCLUSION

There is no current standard for frequency of titer testing when preparing for a low-titer group O walking blood bank. Current military unit practices typically rely on repeat titer testing before each deployment, which incurs significant costs. In addition, it is impractical for units to repeat titer testing in the deployed environment. This study suggests that up to 10% of donors classified as low titer on initial testing may be found to have higher titers on subsequent testing. However, repeat testing and increasing age are associated with increased likelihood of low-titer status. These results can inform the risk/benefit assessments of those planning walking blood banks.

CONFLICTS OF INTEREST

The authors have disclosed no conflicts of interest.

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