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Can the Interval Between Antibody Identifications be Increased for Alloimmunized Patients?

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Abstract

Background—New alloantibody formation is unpredictable in patients who have been previously alloimmunized. Pretransfusion testing is designed to detect these antibodies while antibody identification (ABI) techniques are designed to identify the specificity of the antibody. Pretransfusion testing intervals are prescribed by regulatory and accrediting agencies, intervals for ABI in alloimmunized patients are not. Our institution evaluated the safety of increasing the interval from every 72-hours to 14-days. The current 72-hour interval was chosen at our institution to align with AABB standard 5.14.3.2 which requires a pretransfusion specimen drawn within 3-days of the scheduled transfusion for potentially immunized patients.

Study Design and Methods—Over 2 years, all ABI entries in the laboratory information system were screened. All cases of alloimmunized patients with an additional antibody specificity that developed within 14-days of a previous ABI were reviewed and confirmed by four transfusion medicine physicians.

Results—Initially, 8948 entries were screened. Thirty patients were identified to have formed 33 newly identified clinically significant alloantibodies within 14-days. After further categorization, only 13 antibodies (0.15% of all ABI, 0.47% of alloimmunized patients examined) were deemed to be newly formed clinically significant antibodies that would have led to a change in transfusion practice.

Discussion—Retrospective analysis of ABI results over a 2-year period revealed that 0.47% of previously alloimmunized patients that have samples for pretransfusion testing develop a new

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clinically significant alloantibody in 14-days or less. While there would be significant resource advantages to increasing the duration between repeat ABI, it does not outweigh the risk of a potential hemolytic transfusion reaction.

Keywords

Immunohematology; Transfusion Practice; Antibody Identification Techniques

Introduction

In the United States, the overall prevalence of RBC alloimmunization is estimated to be approximately 1–3% in the general hospital population, 5% or more in multiply transfused patients and multiparous females, and can be greater than 20% in patients with transfusion dependent diseases, such as sickle cell anemia or thalassemia.^{1–7} The formation of a new red cell alloantibody is unpredictable in patients who have been previously transfused or pregnant and the alloimmunization rate has been reported to be between 1–10% and even higher (20%) in frequently transfused populations (Sickle Cell disease, Thalassemia).⁸ Pretransfusion testing, specifically the antibody screen, is designed to detect these antibodies, and antibody identification techniques are designed to identify the specific antibody. In an effort to provide compatible blood possible to all patients, blood banks perform a number of required pretransfusion assays. Pretransfusion compatibility-testing requirements are set forth by accreditation and regulatory organizations. This testing includes ABO grouping, RhD typing, and assays for the detection of unexpected antibodies. If an unexpected antibody is detected, additional testing both serological and molecular may be employed for specific alloantibody identification.

While there are published standards specifying the interval for pretransfusion testing (AABB Standard 5.14.3.2), there are no widely accepted time intervals for repeat antibody identification (ABI) testing in previously alloimmunized patients. The 29th edition of AABB Standard 5.14.3.3, states that “in patients with previously identified clinically significant antibodies, methods of testing shall be those that identify additional clinically significant antibodies.”⁹ This statement is not prescriptive as to how a blood bank is supposed to meet this standard. Published surveys exist that describe the different methods used to identify antibodies in alloimmunized patients, however, none report the frequency with which these serologic work-ups are performed at various laboratories or what prompts repeat work-ups.^{10,11}

While most pretransfusion assays can easily be performed using automated technology, antibody identifications often require manual processes that are labor intensive and time consuming. Faced with rising costs, increased workload, and a diminishing workforce, a solution is required to avoid repetitive, non-contributory antibody identification investigations for frequently transfused alloimmunized patients.

Transfusion and pregnancy history within the last 3 months is often not available and therefore all patients at our institution must have a sample drawn for pretransfusion testing within 72 hours of a scheduled transfusion to align with AABB standard 5.14.3.2.⁹ For patients with a positive antibody screen, ABI is performed every 72 hours as well. Our

laboratory decided to explore the possibility of increasing the interval between antibody identifications in previously alloimmunized patients from every 72 hours to 14 days. By decreasing the number of ABIs performed we hypothesized that not only would we reduce costs, but we could also increase productivity by allowing technologists to perform other tasks, decrease the turn-around time for issuing blood products in alloimmunized patients, and improve patient satisfaction. Fourteen days was chosen as a cut-off based on a survey¹² of common blood bank practices at multiple different United States academic medical centers.

To evaluate the risk of increasing the time between ABIs, we performed a retrospective review to determine the time interval between development of newly identified alloantibodies in previously immunized patients and the number of clinically significant alloantibodies that could potentially have been missed if the interval was extended to 14 days. In addition, we determined the reduction in ABIs that could be achieved with this change and performed an informal cost saving analysis.

Materials and Methods

All samples collected for ABO/Rh type and antibody detection testing, were collected in ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes and initial testing was performed using automated gel methodology (Ortho Provue System, Ortho-Clinical Diagnostics, Raritan, NJ). Samples with a positive antibody detection test were transferred to the antibody investigation area of the blood bank laboratory for further evaluation. The antibody investigations (ABI) were performed using either manual gel or tube methods or a combination of both. Abbreviated panel testing using selected cell “mini” panels was performed when possible. Depending on the complexity of the investigation, one or more of the following panel cells may have been used: Ortho-Clinical Diagnostics untreated and ficin treated panels, Panocell-20 panels (Immucor, Inc, Norcross, GA), or Biotestcell-I panels (Biotest Pharmaceuticals Corporation, Boca Raton, FL).

Using Crystal Reports (SAP America Inc, Newton Square, PA), a list of all antibody investigations performed over a two year period, September 2010–September 2012, was obtained from the laboratory information system and the data were analyzed in Microsoft Office Excel (Microsoft Corporation, Redmond WA).

Within Excel, ABIs were grouped according to patient name and medical record number. Within each patient, the ABIs were further grouped by date and time. The time delta between each ABI performed was calculated and any ABIs that were greater than 14 days from the previous investigation were excluded from further analysis. Any samples that had an additional result reported at a later date/time, for example samples that were sent to a reference laboratory, were merged if the antibody was present in the original sample despite the results being entered at a different time.

Next, each ABI was manually reviewed to assess the clinical significance of the results. Any ABI that only contained clinically insignificant antibodies that would not impact the blood component chosen for the patient or their transfusion management was also excluded from

further analysis. For the purpose of the study the following antibodies or patterns of reactivity were excluded: warm/cold autoantibodies, nonspecific reactivity, passively acquired anti-D, and alloantibodies against M (IgM), N (IgM), Le^a, Le^b, Lu^a, Lu^b, P(IgM), Bg, and Knops system.

The final data set, which contained ABIs with new clinically significant alloantibodies identified less than 14 days from the previous ABI, was submitted to three American Board of Pathology certified transfusion medicine physicians and one transfusion medicine fellow for independent review. During the review each antibody was classified according to the six following categories: 1) new clinically significant alloantibody 2) new low prevalence antibody; antigen not present on previous panels 3) new clinically significant alloantibody not fully ruled out on previous ABI, but special blood product requirements were in place to provide antigen negative products 4) new antibody that was missed on previous ABI 5) incorrect diagnosis of new alloantibody 6) antibody not fully ruled out on previous or current ABI; unable to definitively diagnose. After independent review, the reviewers met to discuss and resolve any conflicts. A consensus was obtained in all cases reviewed.

Finally, the antibody screen for all samples with a new alloantibody in the final data set were evaluated to determine if there was a change in the antibody screen results (increased or decreased strength of reactivity or change in screening cell reactivity pattern) from the previous sample.

Results

8948 antibody investigations, which corresponded to 2792 patients, were performed over a two year period in our institution. Six hundred-eighteen (618) patients were identified who had an ABI with a newly identified alloantibody within 1–14 days of a previous ABI (when that antibody had been ruled out). After all exclusion criteria were applied, 30 patients (with 33 antibodies) remained for evaluation. Table 1 lists the 33 new antibodies that were identified on the ABIs and their frequency. The 33 antibodies were further divided into the previously described categories (Table 2), 13 antibodies were determined to be newly formed clinically significant alloantibodies.

Of the 13 new clinically significant alloantibodies, 10 occurred within 7 days of a previous ABI which had ruled the antibody out and the remaining three occurred within 14 days (Table 3). Six of the 13 new antibodies were within the Kidd system (Jk(a) or Jk(b)). Five of the 13 patients had an unknown transfusion or pregnancy history. The remainder had a history of transfusion prior to the development of the new antibody (Table 3). In addition, 5 of the ABI with new antibodies showed no change in the antibody screen; and for one of the ABIs, the screen results actually became weaker (Table 3). One of the newly identified antibodies, anti-Jk(a), was identified on a ficin panel only.

Discussion

In previously alloimmunized patients who are pregnant or recently transfused, increasing the time between antibody identification investigations could potentially lead to a failure to detect the development of an additional, newly developed, clinically significant antibody and

result in an adverse patient event related to transfusion of red blood cells. However, it is impractical to perform an ABI daily on every alloimmunized patient. Therefore, a defined interval for repeat testing has to be established and due to a lack of regulatory guidance or literature establishing the safest interval, blood banks must set their own intervals.

The ability to safely increase the interval between ABIs could lead to significant cost savings. In an attempt to estimate the savings we might expect, we referred to the cost analysis and resource utilization HI-STAR study.¹³ The study divided serologic investigations at four major medical centers covering varied geographical regions within the United States into 19 categories based on diagnosis. The categories ranged from obstetrics (\$69), with low cost serologic work-ups to autoimmune hemolytic anemia (AIHA) with highest cost individual patient work-ups (\$1490). They reported a mean total cost of \$180 for 15 of the 19 diagnostic categories. However, they failed to mention the duration between repeat serologic work-ups at any of the institutions.

If our ABI interval increased from every 72 hours to every 14 days, we would reduce the number of ABIs performed annually by 250. We performed a cost-reduction analysis to determine the financial impact of the ABI reduction. Our institution, patient population, and diagnostic categories closely resemble those reported in the HI-STAR study, thus we used the results from this study to estimate our cost-savings. If we used a mean ABI cost of \$180/ABI we would expect to save our institution a minimum of \$45,000/year. This figure does not take into account the most costly work-ups on patients with AIHA or more than 5 previous transfusions.¹³ A formal cost analysis at our institution would most likely identify higher cost savings considering our large and complex oncology and thalassemia patient populations, both populations that frequently develop autoimmune hemolytic anemia.

Effects on patient safety must be evaluated prior to implementing changes based on projected cost savings. We investigated what the potential risk would be if we were to increase our ABIs to 14 days. Over a two year period, we performed over 8,000 ABI in our institution. During that period, 33 antibodies were initially identified as occurring within 14 days of a previous ABI (Table 2). However, upon further investigation approximately one third of the results (categories 2, 4, 5 and 6, Table 2) would not have changed transfusion practice in the corresponding patient. In another third (category 3, Table 2), a potentially clinically significant alloantibody could not be ruled out, but special requirements were in place to provide antigen negative units. Therefore, no patient harm would have occurred if the antibody had been definitively identified at a later date.

Our study indicates that increasing the ABI interval to 14 days could result in delayed identification of new alloantibody specificity in 13 patients. This represents 0.5% of all ABI performed or 0.47% of all alloimmunized patients that have a type and screen sent to our blood bank laboratory. Furthermore, 2 of the 13 antibodies developed within 72 hours of a previous specimen. While this data may suggest that decreasing the time interval to less than 72 hours between ABI may increase detection of new alloantibody specificities, the increased amount of testing becomes impractical. An alternative approach that could result in reduced frequency of ABI testing might be to perform an ABI only when there is a change in the antibody screen results for immunized patients as a number of institutions

have previously reported¹²; however, we found that only 8 of the 13 samples were associated with a change in the screen results. Therefore, 5 new antibodies could still have been missed during the antibody investigation process.

A full serologic crossmatch is considered the final step to identify incompatibilities between donor and patient, and must be performed with a current specimen (drawn within 72 hours for patients that have been transfused or pregnant within the prior 3 months) for every RBC unit transfused. Most institutions report the use of gel crossmatch or tube crossmatch with an enhancement agent.¹⁴ This theoretically allows a final opportunity to detect a new clinically significant alloantibody that might have been missed due to an absence of change in the antibody screen. In one retrospective study¹⁵, investigators reported the risk associated with using antibody screen plus IAT crossmatch for detection of additional clinically significant antibodies in previously alloimmunized patients without an ABI. They identified 19 new alloantibodies in 16 patients that were considered clinically significant. The authors reported that in 8 of the patients, the new antibodies were detectable by the antibody screen. In 7 of the patients, the new alloantibodies were detected by the IAT crossmatch, and in 1 patient, a new anti-S was masked in the crossmatch by a warm autoantibody. The authors concluded that new ABI should be performed when the possibility of a new antibody may be masked. A number of factors such as autoantibodies, multiple alloantibodies causing most donor units to be incompatible, or antibodies to high incidence antigens could lead to an expected incompatible IAT crossmatch and an inability to detect the presence of new reactivity. In order to reduce dependence on IAT crossmatch and complicated ABI procedures for patients with warm autoantibodies, our institutional practice is to obtain red cell antigen genotype testing and transfuse antigen negative blood. While this algorithm meets current accrediting agency requirements, the risk of failure to detect a clinically significant alloantibody remains and a potentially incompatible unit could be issued. One additional caveat about reliance on absence of antibody screen reactivity change is that there can be lot-to-lot variability in the antigen profile of the screening cells which could result in “false positive” antibody screen changes. Due to the retrospective nature of our study, we are unable to determine whether a similar algorithm would have identified incompatibility in the 5 of 13 patients for whom the antibody screen did not change. We performed an ABI for each patient and used antigen negative blood for the IAT crossmatch.

In addition to the limitations inherent in any retrospective review, our study has some additional limitations. First, because the data were collected retrospectively and because the current interval for ABI at our institution is every 72 hours, we could not determine how many patients developed new antibodies in less than 72 hours. Second, we are making the assumption that all 13 antibodies would lead to adverse events in the patient, possibly overestimating the risk associated with increasing the interval to 14 days, although six of the antibodies were in the Kidd system. Kidd antibodies are known for their rapid evanescence, robust anamnestic response after antigen re-exposure, and by their ability to cause intravascular hemolysis, and thus are known as one of the most clinically significant blood group systems. While our institution is a large academic medical center with an ethnically diverse patient population, a large oncology service, stem cell transplant program, and large Thalassemia service, the results of our study may not be applicable to all institutions.

Depending on the patient population and the laboratory methods employed (i.e. gel versus tube versus solid phase), the alloimmunization rates may differ between institutions.

In summary, despite the potential cost savings, our study found that there was a small but potentially significant risk of failing to detect the development of a new alloantibody in previously immunized patients if the ABI interval was increased to 14 days. After discussion between administrative staff and medical directors, it was decided that increasing the interval to 14 days could significantly compromise patient safety and we continue to perform ABI every three days when a new type and screen is submitted. However, we intend to revisit this question by using other methods such as the IAT crossmatch of candidate antigen negative RBC units prior to performing ABI to determine if this simple testing intervention can mitigate the risk of increasing the testing interval of ABI.

References

1. Walker RH, Lin DT, Hartrick MB. Alloimmunization following blood transfusion. *Arch Pathol Lab Med.* 1989; 113:254–261. [PubMed: 2493235]
2. Winters JL, et al. RBC alloantibody specificity and antigen potency in Olmsted County, Minnesota. *Transfusion.* 2001; 41:1413–1420. [PubMed: 11724988]
3. Hoeltge GA, Domen RE, Rybicki LA, Schaffer PA. Multiple red cell transfusions and alloimmunization. Experience with 6996 antibodies detected in a total of 159,262 patients from 1985 to 1993. *Arch Pathol Lab Med.* 1995; 119:42–45. [PubMed: 7802552]
4. Spielmann W, Seidl S. Prevalence of irregular red cell antibodies and their significance in blood transfusion and antenatal care. *Vox Sang.* 1974; 26:551–559. [PubMed: 4212267]
5. Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. *Transfusion.* 2002; 42:37–43. [PubMed: 11896310]
6. Singer ST, et al. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. *Blood.* 2000; 96:3369–3373. [PubMed: 11071629]
7. Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. *Transfusion.* 2012; 52:2220–2224. DOI: 10.1111/j.1537-2995.2012.03570.x [PubMed: 22339270]
8. Sood R, Makroo RN, Riana V, Rosamma NL. Detection of alloimmunization to ensure safer transfusion practice. *Asian J Transfus Sci.* 2013; 7:135–139. DOI: 10.4103/0973-6247.115577 [PubMed: 24014944]
9. Standards for Blood Banks and Transfusion Services. 28. AABB; 2012.
10. Shulman IA, Downes KA, Sazama K, Maffei LM. Pretransfusion compatibility testing for red blood cell administration. *Curr Opin Hematol.* 2001; 8:397–404. [PubMed: 11604582]
11. Maffei LM, Johnson ST, Shulman IA, Steiner EA. Survey on pretransfusion testing. *Transfusion.* 1998; 38:343–349. [PubMed: 9595016]
12. Famuyiwa L, Degtyaryova D, Crowley K, Cushing M. A Survey of Current Practice in Immunohematology. *Transfusion.* 2010; 50:163A.
13. Mazonson P, et al. The HI-STAR study: resource utilization and costs associated with serologic testing for antibody-positive patients at four United States medical centers. *Transfusion.* 2014; 54:271–277. DOI: 10.1111/trf.12176 [PubMed: 23550807]
14. Downes KA, Shulman IA. Pretransfusion testing practices in North America, 2005–2010: an analysis of the College Of American Pathologists Interlaboratory Comparison Program J-survey data, 2005–2010. *Arch Pathol Lab Med.* 2012; 136:294–300. DOI: 10.5858/2011-0127-CPR.1 [PubMed: 22372905]

15. Judd WJ, Butch SH. Repeat Antibody Identification Studies: How Much Is Enough? *Transfusion*. 2002; 42:20S.

Table 1

New alloantibodies identified within 14 days of a previous ABL.

Antibody Identification	Frequency
Anti-E	9
Anti-Jk(a)	5
Anti-C	5
Anti-K	3
Anti-Fy(a)	4
Anti-Jk(b)	2
Anti-Kp(a)	2
Anti-s	1
Anti-Fy(b)	1
Anti-Fy(3)	1
TOTAL	33

Table 2

Antibody diagnostic categories and frequencies.

	Category	Number of Antibodies
1	New clinically significant alloantibody	13
2	New antibody to low prevalence antigen; antigen not present on previous ABID	2
3	New clinically significant alloantibody not fully ruled out on previous ABID, but special requirements already in place to avoid antigen	11
4	New antibody missed on previous ABID	1
5	Incorrect diagnosis of new alloantibody	3
6	Antibody could not be ruled out on previous or new specimen; unable to definitively diagnosis	3
	Total	33

Table 3

Antibody characteristics. Antibody screen results are graded on a scale from 0 to 4+. SC I= screening cell I, SC II= screening cell II, Y=Yes, U=Unknown

Patient	New Antibody	Transfused or pregnant in last 3 months?	Previous Antibodies	Interval Between Panels (days)	Previous Antibody Screen Results			New Antibody Screen Results		
					SC I	SC II		SC I	SC II	
1	C	Y	Fy ^a , E	11	No Change					
2	E	U	JK ^a	13	No Change					
3	Fy ^a	U	C, E, K	4	+	++		++		++
4	Fy ^a	Y	E, JK ^b	6	No Change					
5	Fy ^a	Y	Non-specific	5	+	0		++		0
6	Fy ^b	U	Non-specific, K	13	+	0		++		++
7	JK ^a	Y	E	5	0	+		++		++
8	JK ^a	Y	E, I	3	0	++		0		+
9	JK ^a	U	c, E	5	0	++		+		++++
10	JK ^a	Y	Fy ^a , Non-specific	6	No Change					
11	JK ^a	Y	E, C ^w , Fy ^a , Cold	6	No Change					
12	JK ^b	Y	K, Fy ^a , C	3	++	0		++		+
13	K	U	E	6	0	+		+		0