Time interval between antibody investigations among patients who demonstrate serial red cell antibody formation

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BACKGROUND: Current national standards for pretransfusion testing do not address the frequency or optimal time interval to repeat antibody identification testing for patients in whom antibodies have been previously detected.

STUDY DESIGN AND METHODS: A retrospective review was performed of patients with existing red blood cell (RBC) antibodies who subsequently developed new antibody specificities. Data were drawn from a single institution where the antibody investigation was repeated if the screen suggested a new antibody or if 14 days had elapsed since the previous investigation. Clinically insignificant or drug-dependent antibodies were excluded. Among cases in which new antibodies were detected within 30 days of a previous sample that already demonstrated existing antibodies, the median and lower 95% confidence intervals for the number of days between the detection of the existing and new antibodies were determined.

RESULTS: Over a 9-year period, among 2114 patients with more than 1 antibody, 699 (33%) had serially detected antibodies from separate samples. Among 152 patients whose subsequent antibody was detected within 30 days of the existing antibodies, the median time interval to detection of the new antibody was 13 days. The lower 95% confidence interval was 1 day. By Day 3, 18% of the new antibodies had already appeared.

CONCLUSION: In patients who form multiple antibodies, the serial emergence of clinically significant antibodies is common. In some patients, detection of a new specificity occurs in a sample drawn shortly after the sample that demonstrated the first antibody. These results have implications for the frequency of pretransfusion testing.

Pretransfusion testing for the detection of red blood cell (RBC) antibodies is an essential component of providing safe transfusions to patients. The AABB Standards for pretransfusion testing state that for patients who have a history of RBC exposure through transfusion or pregnancy in the preceding 3 months, a sample must be collected and tested within 3 days of anticipated transfusion. Once an antibody is detected, however, the issue of when to reinvestigate for the possible formation of new additional antibodies is less clear. In a previously alloimmunized patient, a positive antibody screen on a new sample does not necessarily suggest a newly formed antibody. Suspicion for a new antibody depends on the antigen frequency of the original antibody, the number of cells on the screen, the antigen frequency of newly formed antibody, and crossmatch results. Although the 30th edition of the AABB Standards states that "in patients with previously identified clinically significant alloantibodies, methods of testing shall be those that identify additional clinically significant antibodies,"[1] no statement is made regarding when to repeat antibody identification panel testing. We could find no standard from any agency on the frequency of RBC antibody identification. As a result, local hospital policies vary regarding when to repeat an antibody identification panel.

Reported rates of RBC alloimmunization vary depending on the patient population. Alloimmunization rates range from 1% to 3% in the general population to 15% to 59% in

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patients with myelodysplastic syndromes.[2] Data are sparse, however, regarding the risk of formation of subsequent additional antibodies. Patients who have previously developed RBC alloantibodies appear to be at increased risk for developing additional antibodies, which may be related to genetics, repeated exposure from chronic transfusions, and/or a predisposition to new antibody formation in the inflammatory milieu of prior sensitization.[2–5] A retrospective analysis of over 1000 patients diagnosed with malignant myeloproliferative and lymphoproliferative diseases found that once a patient had formed an antibody, the probability of forming additional antibodies increased threefold.[3] In patients without hematologic-oncologic diseases, the risk of subsequent antibody formation has been reported to be more than 20 times greater than the risk of initial alloimmunization.[6]

Recently, Goss et al.[7] reported a retrospective analysis of patients with existing RBC antibodies in whom additional new antibodies were identified. In their program, repeat antibody identification was routinely performed every 3 days. Among 2792 patients with existing antibodies, prior to study exclusions, 618 (22%) were found to have a newly identified antibody within 14 days of the sample in which the preexisting antibodies were identified.[7,8] To provide additional information on the time to the appearance of additional RBC antibodies in patients with existing antibodies, we performed a retrospective single-center analysis of antibody identification.

STUDY DESIGN AND METHODS

Samples sent to Massachusetts General Hospital blood bank for type and screen are collected in ethylenediaminetetraacetic acid–anticoagulated tubes. Three-cell antibody screens are performed using a column agglutination (gel) technology (ID Microtyping System, Ortho Clinical Diagnostics). A first-time, positive antibody screen is reflexed to a panel test for antibody identification. Commercially available RBC panels (Panocell, Immucor; Resolve Panel, Ortho Clinical Diagnostics) were used for antibody identification. Antibody enhancement reagents (low-ionic-strength solution, polyethylene glycol, enzyme-treated cells), adsorption, and inhibition techniques were used to resolve difficult cases. Antiglobulin phase testing was done using an anti–immunoglobulin G reagent (Immucor). All antibody screening and identification methods were unchanged throughout the 9-year study period.

All blood bank samples were assigned a 3-day expiration time regardless of RBC exposure with the exception of patients from the preadmission testing clinic who had not been transfused or pregnant within the preceding 3 months. These policies were consistently applied throughout the 9-year period from which the data were generated.

The Massachusetts General Hospital policy for patients with previously identified antibodies is that subsequent type and screen samples sent within 14 days from the prior sample are not reinvestigated using an antibody identification panel unless the antibody screen changes to suggest an additional antibody or crossmatch results suggest an additional antibody, or if requested by the transfusion service physician. The time window of 14 days was chosen arbitrarily. The policy for antibody reinvestigation was developed prior to the study time frame and was stable throughout.

Crystal Reports was used to aggregate data regarding the patient medical record numbers, antibody identification, and dates of the specimen collection and entry into the blood bank management system (HCLL Blood Bank and Transfusion Software, Mediware) over a 9-year period (October 1, 2008, to September 29, 2017). A 9-year period was selected to generate approximately 1000 antibody identifications. For a given patient, unique antibody identifications are entered into the blood bank information system once at the time of initial detection. As such, for a patient with serial antibody identifications, the dates of entry were used to determine the time interval between the detection of an existing antibody and detection of the new antibodies. These time intervals were subsequently confirmed/reconciled with the associated specimen collection dates. Time intervals of "0 days," which represented the detection of multiple antibodies on the same sample, were excluded. To determine the median number of days (and lower confidence interval) between the identifications of the existing and new antibodies, we restricted our analysis to patients whose subsequent antibody was detected on a new sample within 30 days of the first antibody. Thirty days was chosen because our primary study question focused on how quickly second antibodies would be detected. Long intervals between sample collections would not accurately reflect the time interval between an initial antibody and the ability to detect a second antibody.

We excluded from analysis antibody reactivity due to daratumumab, passive anti-D due to Rh immunoglobulin injection, and cold-reactive autoantibodies (I, H, IH, "cold agglutinin"). Patients who were placed on phenotype matching protocols (patients with sickle cell disease or patients with strongly reactive autoantibodies) were few and were included. Among patients whose second antibody was identified within 30 days of the initial antibody, the RBC transfusion history, additional testing (direct antiglobulin test, eluate), and record of transfusion reaction evaluations were recorded.

For nonexcluded cases, we determined the lower 95% confidence interval for the median number of days between the initial and subsequent antibody identification among patients whose second antibody was identified within 30 days of the initial antibody on a new sample. The lower 95% confidence interval was used to estimate the time interval required to identify 95% of patients with detectable subsequent antibodies. Statistical analysis and graphic presentation

were performed using computer software (Excel, Microsoft; the Open-Epi software; and GraphPad Prism version 7.04, GraphPad Software).

RESULTS

Between October 1, 2008, and September 29, 2017, a total of 9665 antibodies were identified among 6196 patients (Fig. 1). Of these, 699 patients had 984 new antibodies identified 1 or more days after the demonstration of preexisting antibodies. Among these 699 patients, there were 192 in whom the new antibodies were detected within 30 days of a prior sample in which they were not detected. After exclusion of clinically insignificant new antibodies, there remained 152 patients with 167 clinically significant antibodies that were identified within 30 days of an earlier sample in which the antibodies were absent.

Clinical features of these 152 patients are shown in Table 1, and their transfusion history is categorized in Table 2. Not surprisingly, among the 152 patients, the largest percentage (36%) of patients with a new second antibody occurred among those with a history of remote RBC exposure who received a transfusion between the first and second antibody identification. However, as shown in Table 2, the transfusion history alone is not reliable to identify all those at risk for development of a second antibody within 30 days of the first antibody. As expected and as

TABLE 1. Demographics of 152 patients with a new antibody specificity identified within 30 days of an initial antibody identification

Median age in years (IQR)	68 (53.5–77)
Number of females (%)	86 (57)
Number of males (%)	65 (43)
RBC transfusion within 90 days of initial antibody (%)	63 (41)
Hematologic malignancy (%)	47 (31)
Sickle cell disease (%)	1 (<1)
Transfusion reaction investigations (%)	53 (35)

IQR = interquartile range.

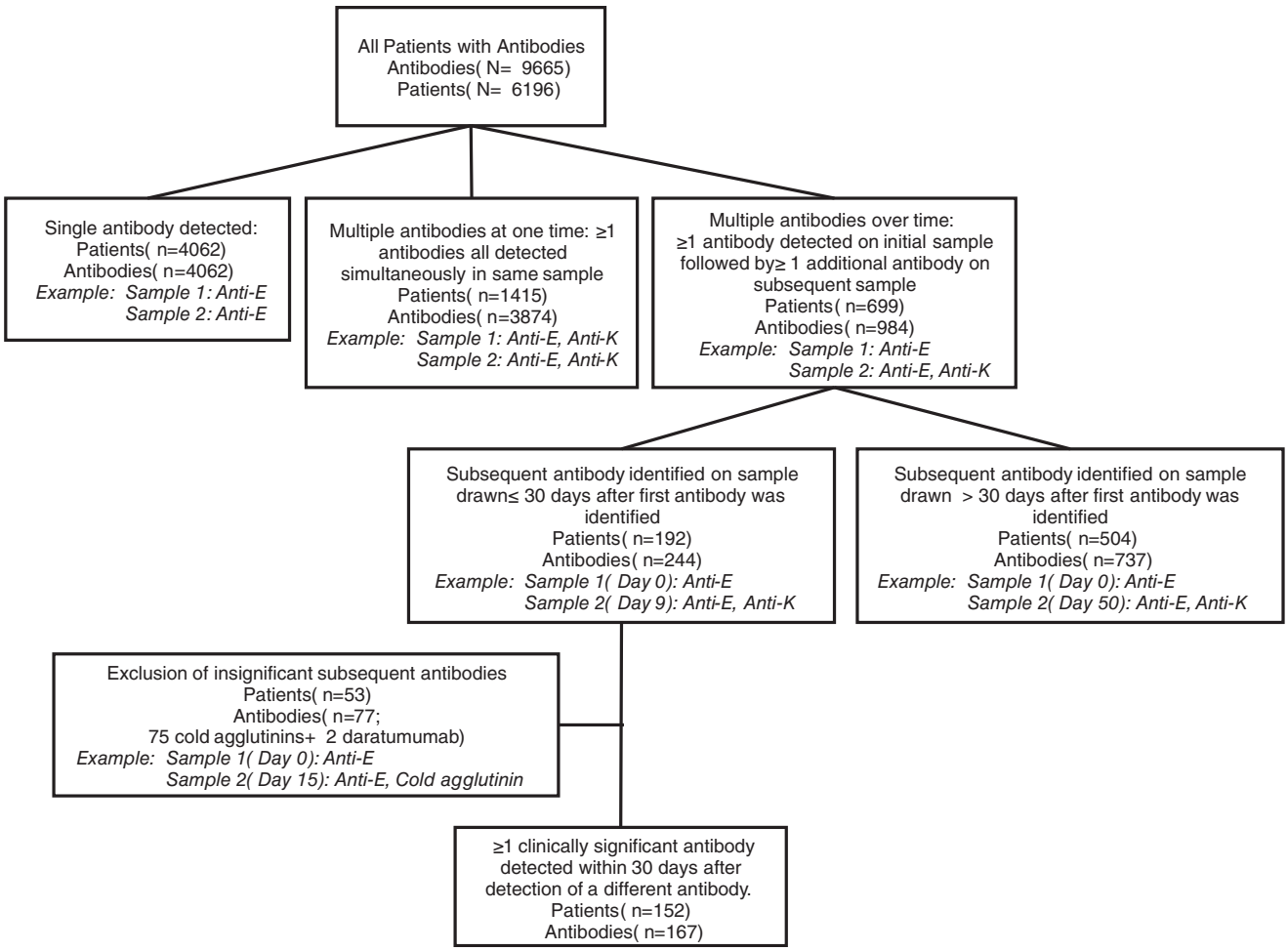

Fig. 1. Number of patients and antibodies detected between October 1, 2008, and September 29, 2017, and patients with new antibodies on subsequent samples, following restriction of time intervals to 30 or fewer days and application of exclusion criteria.

TABLE 2. RBC transfusion history of 152 patients who had at least one new antibody within 30 days of an earlier positive sample

Known history of RBC transfusion before detection of initial antibody	<90 days from last known RBC transfusion to detection of initial antibody	RBC transfusion between initial and subsequent antibody detection	Patients, n (%)
No	NA	No	20 (13)
		Yes	17 (11)
Yes	No	No	18 (12)
		Yes	34 (22)
	Yes	No	9 (6)
		Yes	54 (36)

NA = not applicable.

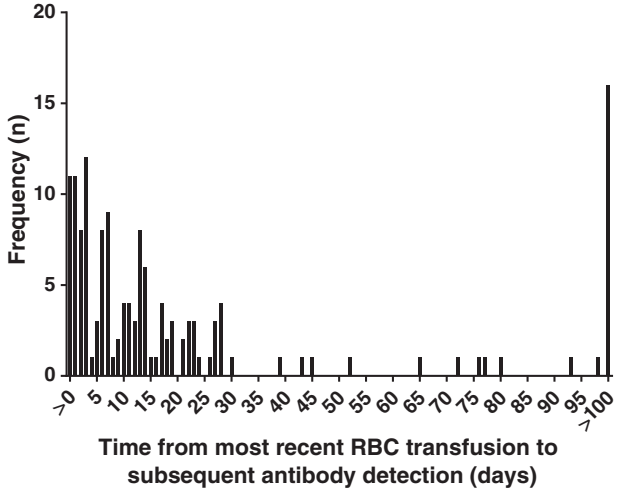

Fig. 2. Time (days) from most recent RBC transfusion to identification of new antibody among patients with a serial antibody formation. Of 147 new antibody identification episodes, 121 occurred within 30 days after a RBC transfusion.

shown in Fig. 2, most patients making a new second antibody were recently transfused.

Among the 152 patients suitable for analysis, the median time interval to new antibody identification was 13 days, the interquartile range was 6 to 19 days, and the range was 1 to 30 days (Fig. 3). The lower 95% confidence interval was 1 day (Fig. 4). A 3-day interval between samples represented the 18th percentile, suggesting that by waiting until Day 3 to repanel and reassess selection of antigen-negative units for transfusion, approximately 18% of cases would have already had a second antibody detectable. The specificities of the new antibodies are shown in Table 3. Additional immunohematologic testing was common among the 152 patients (167 antibody investigations) who demonstrated subsequent antibody identification within 30 days of the initial antibody workup. Results of additional testing are presented in Table S1 of the supporting information in the online version of this paper.

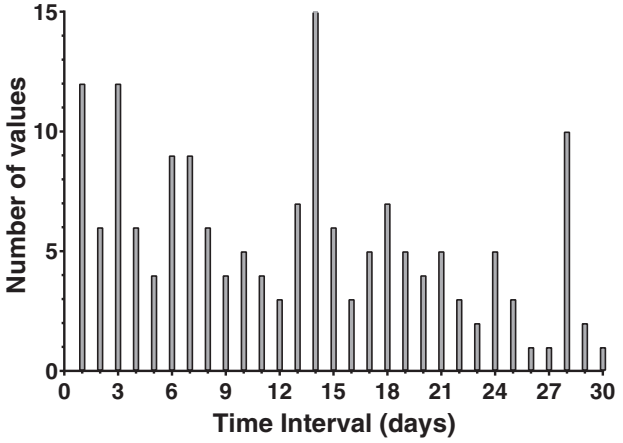

Fig. 3. Histogram of time interval (days) to subsequent antibody detection. N = 167. Median time interval of 13 days, interquartile range of 6 to 19 days, and range of 1 to 30 days.

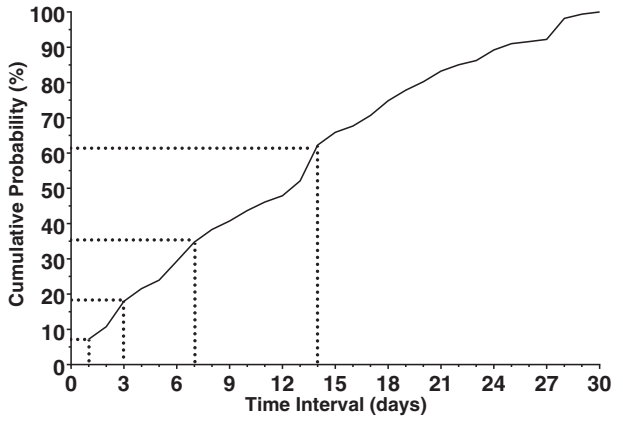

Fig. 4. Cumulative probability (%): time interval (days) to subsequent antibody detection. The lower 95% confidence interval is 1 day. The 18th, 35th, and 61st percentiles are 3 7, and 14 days, respectively. These data suggest that by waiting until Day 3, 7, or 14 to repanel, approximately 18%, 35%, and 61% of cases would have already had a second antibody detectable.

DISCUSSION

In this retrospective analysis of 6196 alloimmunized patients, 2114 (34%) made more than one antibody, a proportion that is similar to that of other reports.[9,10] Among patients in whom multiple antibodies were detected, one third (699 of 2114) developed these antibodies serially over time, confirming that serial antibody detection is quite common.[9,10] Among 152 patients in whom serial antibody detection occurred within 30 days, the median time interval to the appearance of the new antibody was approximately 13 days, with a lower 95% confidence interval of just 1 day.

Based on our analysis, at least 18% of new alloantibodies were detectable before Day 4. Using a standard

TABLE 3. New antibodies that were identified within 14 days of a prior antibody and their frequencies

Antibody identification	Frequency
Warm autoantibody	26
K	20
Jk(a)	19
E	14
C	13
Bg	12
Low frequency	10
c	10
S	9
Fy(a)	7
Wra	6
Jk(b)	6
Cw	5
Le(a)	3
D	3
V	2
Lua	2
e	2
Js(a)	2
G	2
Fy(b)	2
N	1
M	1
k (cellano)	1
Kp(a)	1
HTLA	1

HTLA = high titer, low affinity.

3-day outdate for samples, such early-appearing additional antibodies would go unrecognized during the initial 3 days after the first sample if no new sample were investigated. Waiting for 1 or 2 weeks until repeating the antibody investigation on a new sample increases the percentage of patients who have a second antibody that could have been detected earlier to approximately 35% and 61%, respectively.

The detection of these additional antibodies would influence selection of donor units for transfusion. Our data demonstrate that some alloimmunized patients are at high risk for early additional alloantibody formation and that accurate pretransfusion testing may require more frequent investigation in these patients compared with nonimmunized patients or remotely immunized patients. While the majority (121 of 168, 72%) of early second antibody identifications occurred within 30 days of a known RBC transfusion (Fig. 2), patients can receive transfusions at other facilities, and the transfusion history alone was not a completely reliable way to identify all individuals demonstrating serial antibody formation.

The true time interval between the ability to identify the first and second antibody is likely to be even shorter than our results indicate. We may have not identified a subsequent antibody in the shortest possible interval either because no sample was sent to the laboratory for testing or because our policy resulted in no antibody evaluation for 14 days if the antibody screen and crossmatch failed to suggest a new specificity. Without these restrictions, the time interval to the detection of a subsequent antibody specificity would likely have been shorter.

Our results are consistent with prior studies. A recent study of RBC alloimmunization rates and transfusion requirements in a large cohort of patients with myelodysplastic syndrome found that one-half of alloimmunized patients have multiple alloantibodies and that the number of units transfused in these patients was significantly higher than among nonalloimmunized patients.[11] Individual alloimmunized patients were also found to have increased numbers of RBC transfusions following alloimmunization. These findings, along with the short interval to subsequent antibody detection, highlight the importance of further studies to determine the optimal pretransfusion testing strategy for these higher-risk patients. Additionally, in the aforementioned study, a significantly higher proportion of alloimmunized patients developed autoantibodies compared with nonalloimmunized patients, most of which were detected within a short period of alloimmunization. We found the same association, observing that the most frequent subsequent antibody identified within 30 days was a warm autoantibody, suggesting that alloimmunization may provoke autoantibody formation.

It is not known what immunological drivers favor RBC sensitization. Identification of triggers for serial antibody formation would allow focus of increased pretransfusion testing on those patients most at risk for serial antibody formation. Additional data of the time interval between serial antibody identification could be used to make more informed recommendations for antibody investigation among at-risk patients. Studies such as these can provide a body of evidence for best recommendations for pretransfusion immunohematology evaluation of the sensitized transfusion recipient.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Additional testing (direct antiglobulin test and/or eluate) and transfusion reaction evaluations for patients with new antibodies detected within 30 days of a previously identified antibody. Of 47 eluates performed, 11 were positive for the new alloantibody, 10 were pan-reactive consistent with a new autoantibody, and 26 were negative for the new antibody identified in the plasma.