Patient Epidemiology Factors of Unidentified Positive Reactivity in Solid-Phase and Test Tube PEG Antibody Detection Methods

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Abstract

Background: Several publications have reported an increase in nonspecific reactions when automated technologies such as solid-phase are used for the detection of red blood cell antibodies. There is little known about the patient's epidemiology factors surrounding nonspecific reactions and the capability of these nonspecific reactions to transition into a detectable antibody.

Study Design and Methods: A retrospective review was performed in an academic medical center using the test tube (t-PEG) method before switching over to solid-phase for the detection and identification of antibodies. Patients with an initial negative antibody detection test with subsequent unidentified (UID) reactivity were analyzed.

Results: Our data shows a significant increase in UID reactions in solid-phase (0.17%) when compared to the t-PEG (0.04%) method for the detection of antibodies. We also observed significance in the gender distribution of positive UID reactions between test methods. Our data also shows significance between methods in the distribution of diagnoses such as chronic/autoimmune disease, cancer, OBGYN, surgery and trauma patients. 25% of patients had their UID reactivity disappear in which an auto or alloantibody (anti-E, -C, -K, -Jk^{a}, -D, -Le^{a}, -Ch and -McC^{a}) were identified on a subsequent detection test.

Conclusion: When solid-phase is used for antibody identification there is greater sensitivity towards UID reactivity when compared to the manual t-PEG method. Patient diagnoses and gender may explain the prevalence of UID reactivity with respect to the method used. Finally, UID reactivity should not be overlooked due to a small percentage of reactions transitioning into clinically significant antibodies.

Introduction

Pretransfusion testing is a critical component of transfusion medicine and is comprised of several tests including ABO group, Rh type, antibody detection, antibody identification and compatibility testing. These tests help identify clinically significant antibodies in the patient's plasma which could destroy incompatible donor red blood cells by intra or extravascular hemolysis. Traditionally, transfusion services conducted pretransfusion testing in test tubes with saline medium with several enhancement agents such as polyethylene glycol (PEG) and low ionic strength saline (LISS).[1] While each enhancement agent has its limitations, the goal is to increase the sensitivity of antibody detection and identification tests.[2]

Over the last 20 years, new instrumentation and methods have been developed to help improve sensitivity and reduce subjectivity when identifying clinically significant antibodies during pretransfusion testing. The solid-phase RBC adherence assay (SPRCA) and gel technology are two such methods developed to provide transfusion services with sensitive, reliable, and standardized methodology for the detection and identification of antibodies. Several studies have shown SPRCA and gel methods to be more sensitive than the test tube PEG (t-PEG) method; however, these same reports also claim an increase in the detection of nonspecific reactions.[3-5] The work of Liu and colleagues analyzed nonspecific antibody reactions in gel and observed a subset of nonspecific reactions transition over time to clinically significant antibodies.[6]

There is little published about what causes nonspecific reactions in solid-phase and even less about the patient's medical history surrounding the nonspecific reaction.[1-6] We hypothesize certain patient epidemiology factors may cause nonspecific reactions when solid-phase detection and identification methods are used and that a certain subset of nonspecific reactions will transition into a detectable antibody.

Materials and Methods

Study Design and Data Collection

This retrospective study examined records approved by the Internal Review Board at a large academic tertiary care facility between January 1, 2009 and December 31, 2012. During the study, two different pretransfusion testing methods were in use. The t-PEG method was used from January 1, 2009 until April 20, 2011 and on April 21, 2011 the SPRCA on Echo® (Immucor, Nocross, GA) was implemented. Electronic medical records were reviewed from patients who had at least two detection tests. The first detection test must have been negative and their next detection test must have been recorded as unidentified reactivity (UID). For each patient that met the above criteria the following variables were collected: date of negative and a positive screen recorded as an unidentified reaction (UID), gender, age, ethnicity, ABO group, Rh type, indirect and direct antiglobulin (IAT and DAT) results, hematocrit and hemoglobin values within 24 hours of a UID reaction, number of transfusions, type of transfusion products, date the UID reaction disappeared, and if an antibody was detected on a subsequent screen.

Patient epidemiology factors were recorded as the primary diagnosis surrounding the order for a type and detection test. Duplicate and missing medical records along with patients that did not have a record log were excluded from the analysis.

Pretransfusion Testing

For the t-PEG method, one drop of reagent RBCs, two drops of patient serum, and two drops of PEG reagent (Immucor, Norcross, GA) was added to a tube, mixed and incubated for fifteen minutes at 37˚C, washed and then two drops of monoclonal anti human globulin IgG (Immucor, Norcross, GA) were added. The sample was centrifuged and the RBC button was read for agglutination and scored according to standard protocols. The solid-phase method conducted on Echo® was preformed according to the manufacturer's directions. Briefly, the screen and antibody identification was performed automatically using the Capture-R ready screen (3). When either the t-PEG or Echo® detection test was positive an antibody identification panel was conducted and either an antibody or UID was recorded (Figure 1). When UID reactivity was recorded in solid-phase the samples were retested by the t-PEG method. DATs were performed with polyspecific IgG and C3 antihuman globulin (Immucor, Norcross, GA) using test tube methods and results were recorded.

Data Analysis

Data frequency was analyzed by a two tailed Fisher Exact test and considered significant if the p value is ≤ 0.05.

Results

Frequency of UID reactions detected during pretransfusion testing for t-PEG and SPRCA

During the study period 40,541 t-PEG and 50,288 solid-phase detection tests were conducted. We observed a significant increase in the frequency of UID reactions in solid-phase (0.17%) when conducted on the Echo® compared to the manual t-PEG (0.04%) method. Of the 101 UID reactions that our study identified, 83.2% of the UID reactions were observed when solid-phase was used as a method for detection while 16.8% UID reactivity was observed when the t-PEG method was used (Table 1). Of the 84 UID reactions that were detected with Echo®, only 3 UID reactions (3.5%) occurred in both solid-phase and the backup t-PEG method.

Patient epidemiology features of UID reactions in t-PEG and SPRCA

Our data shows significance in the distribution of diagnoses between those patients with a UID in t-PEG from those patients with a UID in solid-phase (Table 1). The majority of UID reactivity detected in t-PEG was from surgery and trauma patients (58.8%) (Table 1). The majority of UID reactivity detected in solid-phase was from cancer (solid and heme) (30.9%), chronic/autoimmune disease (21.5%) and OB patients (21.4%) (Table1). Within OB patients, 6 out of the total 19 patients (32%) were Rh negative and had the potential for a passive anti-D to

be detected during the routine pretransfusion screening at the time the UID was generated. 5 out of the 6 (83%) patients had Rh Immune globulin (RHIG) administrated about two months before UID was observed (data not shown). All 6 Rh negative patients UID reactions were detected by solid-phase and not by t-PEG. Of the 19 OBGYN patients only 1 patient had a UID at the time t-PEG was used for pretransfusion screening and was not a RhIG candidate. There were 3 patients with a HLA antibody detected during the time of the negative and UID detection tests, the UID reactivity was not observed by the backup t-PEG method.

Laboratory features of UID reactions in t-PEG and SPRCA

Our data revealed significance in association between the method used for pretransfusion testing and the gender distribution of positive UID reactions. We observed females at a 2.2:1 ratio when compared to males with the majority of the reactions occurring in solid-phase (Table 1). The mean age of the patients who produced UID reactions in t-PEG and solid-phase was 54 and 51 years, respectively. The ABO group, Rh type and ethnicity for all patients involved in the study have been recorded and no significance was observed (Table 1). Hemoglobin and hematocrits of 85 patients were recorded and the average hemoglobin of 9.9 g/dl and average HCT of 30.9%.

There were 10 patients out of the 101 UID reactions observed who had a positive direct antiglobulin test (DAT) at the time the UID was recorded. During the t-PEG method pretransfusion screening period a total of 2 cases were identified and 8 cases were identified during the solid-phase testing period. All 10 cases were positive with polyspecific reagent and the strength of the reactions range from weak to 1+. Of the 8 cases with a positive DAT at the time UID reactivity was detected by solid-phase, 3 cases had a negative eluate. Of these 3 cases, 1 patient was being treated with vancomycin and piperacillin, drugs known to elicit drug dependent antibodies.[7]

The potential of UID reactions to evolve into antibodies

We observed 25 patients (25%) out of the 101 in which the UID reactivity disappeared and either a warm autoantibody, cold antibody or an alloantibody was identified on a subsequent screen or identification panel (Table 2). There was no significant difference between patients who were or were not transfused during the interval of the UID reaction and an identifiable antibody.

For the t-PEG method there were 6 patients in which their UID reactivity disappeared and on a subsequent screen or several screens later an anti-Le^{a}, anti-E, anti-Jk^{a}, anti-D or warm autoantibody were identified. For the solid-phase method there were 19 patients in which their UID reactivity disappeared and on a subsequent or several screens later an anti-C, anti-E, anti-K, anti-McC^{a} and anti-Ch, passive anti-D, cold antibody or warm autoantibody were identified.

Of the remaining 76 patients with UID reactivity there were 36 with no follow up screens as of September 2014. 34 patients with a follow up detection test transitioned into a negative result. 5 patients had multiple UID reactions before disappearing and 1 patient had UID reactivity on every detection test after the initial UID.

Discussion

One major goal of transfusion services is to standardize testing to obtain reproducible results while decreasing subjectivity. According to the College of American Pathologists J-survey conducted from 2005-2010, the majority of North American laboratories are using automated technology for the identification of antibodies such as gel or SPCRA.[8] It has been noted that the tube-LISS method has decreased in use from 38.6% in 2005 to 21.1% in 2010 and that gel technology has increased from 43.7% in 2005 to 64.3% in 2010.[8] SPRCA recorded use was 2.3% in 2005 and increased to 3.4% in 2010.[8] While automated technology allows for standardization a major disadvantage of this technology that is reported is specificity.[3] It is still not clear if UID reactivity generated by solid-phase and other automatic methods should be accepted as a limitation of the method or if there is a specific cause.

We observed from our data UID reactivity happens in both t-PEG and solid-phase however our data shows a significant increase in the number of UID reactivity occurring in solid-phase when compared to t-PEG. This is similar to a report by Yamada et al. who also observed an increase in nonspecific reactivity when testing was conducted by SPRCA versus t-PEG.[3] We also found significance in association between t-PEG and solid-phase and the gender distribution of positive UID reactions. This agrees with Liu and colleagues who reported nonspecific reactivity during pretransfusion testing with gel at a ratio of 2:1 females vs. males.[6] Future studies should be conducted to comprehend why women would be more susceptible to UID reactivity in solid-phase when compared to the t-PEG method.

To date there has been little published on patient epidemiology factors and the correlation of UID reactivity. From our data we identified significance in the association of the antibody detection method and the distribution of patient diagnoses. When the manual t-PEG method was used for the detection and identification of antibodies, surgery/trauma patients had the majority of UID reactivity recorded. When solid-phase was used, the majority of UID reactivity was from cancer, chronic/autoimmune disease and OB patients. Garozzo et al. reported the incidence of positive samples by solid-phase was lower in surgery patients (1.9%) and higher in patients with blood disorders (13.1%), but did not conclude why.[13] These epidemiology factors surrounding UID reactivity point to patients who have an overactive or abnormal immune system which could be generating factors in their plasma that interact with the reagents in SPRCA more so than reagents used in t-PEG.[14] Of interest, several patients diagnosed with chronic/autoimmune disease had lupus which is characterized by antiphospholipid and antinuclear antibodies. In this situation it is plausible that the antihuman globulin reagent could bind to the F_c region of antiphospholipid antibodies bound to red blood cell membranes causing unidentified reactivity. In addition, studies which analyze medications and UID reactivity should be considered to see if there is a correlation.

We did observe a patient who was being treated with medications know to elicit a drug dependent antibody at the time the UID was observed.

We also believe that Rh negative female patients who are given RhIG may cause some UID reactions in solid-phase. We found that 83% of the Rh negative OBGYN patients were given RhIG within 2 months of their initial UID reaction. All cases with RhIG were detected in solid-phase and not by the t-PEG method. Mikesell et al. reported SPRCA assay on Echo® was more sensitive and able to detect RhIG 4-5 months after injection when compared to gel and test tube which was about 3-4 months.[12]

To date there are few publications on SPRCA that examine the possibility of nonspecific reactions being an early detection marker or the transition of alloantibodies or autoantibodies. With our data, 25% of UID reactions disappeared when tracked past the initial UID screen and a specific auto or alloantibody was identified. We believe the patient group which did not receive transfusions between the recorded UID and the detected antibody is the purest group to evaluate UID reactivity transitioning to a newly formed antibody. This group did not have any transfusions between the recorded UID reactivity and their next detection test. The antibodies in this group consisted of warm autoantibodies, a cold antibody and alloantibodies (2 anti-Le^a, 1 anti-C, 1 anti-Jk^a, 3 anti-E and passive anti-D). The majority of UID reactivity transitioned into either a warm autoantibody or anti-E. Liu and colleagues observed 31% of UID reactivity in gel transitioned to a detectable antibody in which anti-E was the most common.[6] Yamada et al. and Garozzo et al. both observed solid-phase to be more sensitive for the detection of antibodies in the Rh system. [3,13]

This study had several limitations and will need future studies to conclude any other possible trends or significant data surrounding patient epidemiology factors. The overall patient population analyzed was small with only 101 out of almost 100,000 detection tests. Our study criteria only looked at patients at one hospital with an initial negative detection test that also had a second screen recorded as UID. As such, this report did not capture all UID reactivity generated by solid-phase or t-PEG and may have revealed different epidemiology factors. We also cannot rule out the small chance that a patient was transfused at another hospital during the interval of the initial negative screen and the UID reactivity.

In conclusion, we observed a significant increase in UID reactivity after transitioning the antibody detection method from t-PEG to SPRCA conducted on the Echo®. We believe patient epidemiology factors such as gender, cancer, chronic/autoimmune disease, surgery and trauma play a role in generating unidentified reactivity. We also believe a small subset of UID reactivity is not actually nonspecific and an allo or autoantibody will be identified on future detection tests. If UID reactivity on the Echo® transitioned; warm autoantibodies, anti-E, anti-C, cold antibodies, anti-K and passive anti-D were observed. If there was a transition of UID reactivity in t-PEG; warm autoantibodies, anti-Le^a, anti-E, anti-Jk^a, and anti-D were observed. This study is the first to describe specific patient epidemiology factors surrounding UID reactivity in both t-PEG and solid-phase and more studies will need to be conducted to help elucidate why these patients may be more sensitive to solid-phase testing.

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Figure 1. Detection of UID Reactivity in Solid-Phase

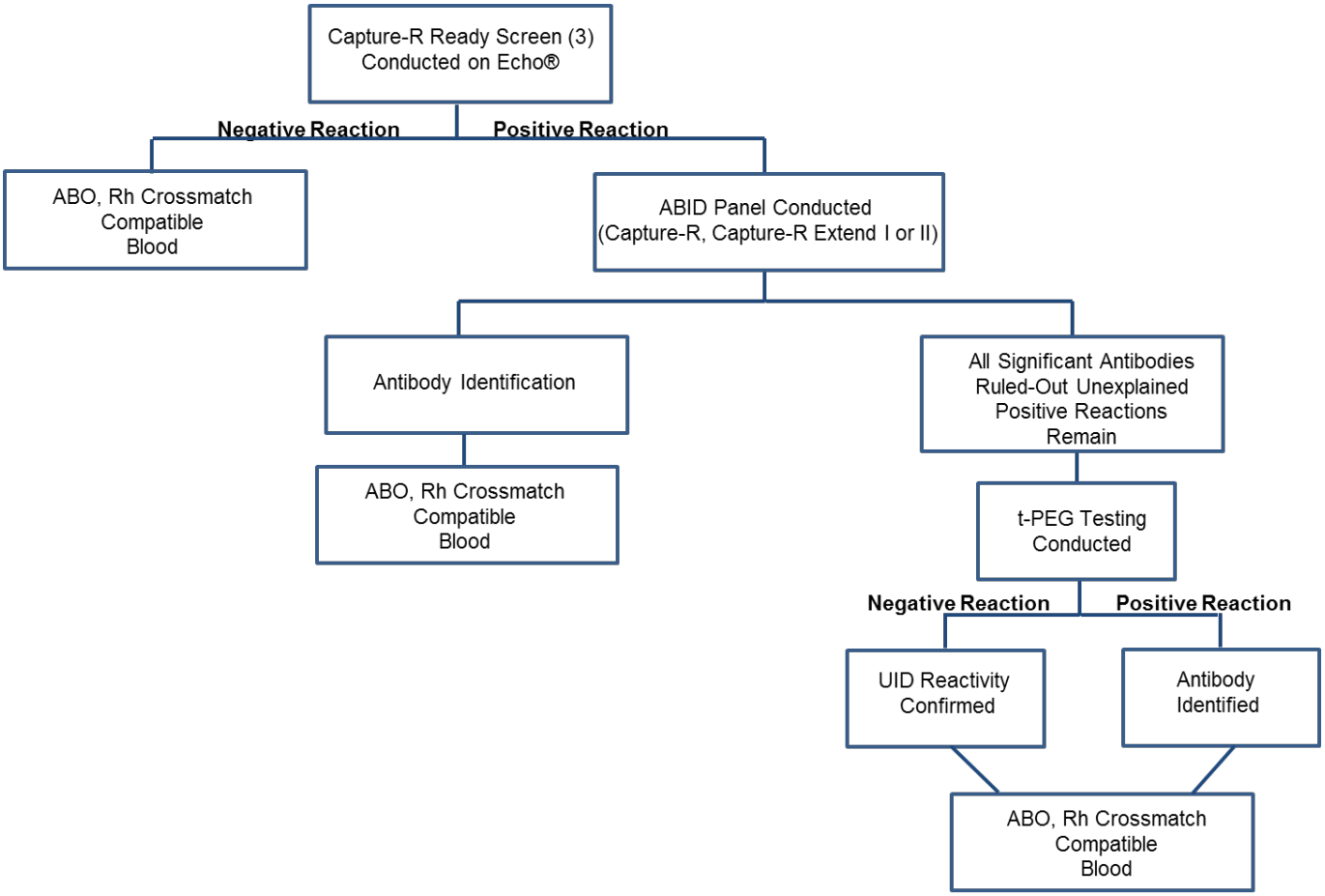

Table 1. Patient Epidemiology Factors Surrounding UID Reactivity in Solid-phase and t-PEG

	t-PEG		Echo	
Total number of Patients with UID (n = 101)	**n=**	**%**	**n=**	**%**
UID Reactivity Observed	17	16.8	84[#]	83.2
Gender				
Females	8	47.1	62*	73.8
Males	9	52.9	22	26.2
Age				
Average Age of Female	53±19	N/A	49±17	N/A
Average Age of Male	55±16	N/A	53±14	N/A
ABO Group				
O Positive	6	35.3	35	41.7
O Negative	1	5.9	10	11.9
A Positive	7	41.2	21	25.0
A Negative	1	5.9	3	3.6
B Positive	1	5.9	7	8.3
B Negative	0	0.0	1	1.2
AB Positive	1	5.9	7	8.3
AB Negative	0	0.0	0	0.0
Ethnicity				
Caucasian	14	82.4	57	67.9
African American	1	5.9	19	22.6
Asian/Hispanic/Alaskan/Native	1	5.9	6	7.1
Not Recorded	1	5.9	2	2.4
Patient Epidemiology Factors				
Chronic/Autoimmune Disease	1	5.9	18[#]	21.5
Cancer	3	17.6	26[#]	30.9
OB Patient	1	5.9	18[#]	21.4
Surgery/Trauma	10[#]	58.8	11	13.1
Genetic Disease	1	5.9	6	7.2
Other	1	5.9	5	5.9

Table 1. Overall epidemiology factors surrounding UID reactivity in solid-phase and t-PEG. We observed a statistical significance in UID reactivity in solid-phase when compared to the t-PEG method $^{\#}$ = $p \leq 0.0001$. We also observed significance in association between the method used and the gender distribution of positive UID reactions, * $p = 0.0427$. The Fisher's Exact test was used to calculate statistical differences.

Table 2. Antibody Specificity Detected after UID Reactivity

	No TxF between UID and Ab Detection		TxF between UID and Ab Detection	
Antibody Detected	**n**	**%**	**n**	**%**
Warm Autoantibody	3	25	3	23.2
Cold Autoantibody	0	0	1	7.6
Cold Antibody	2	16.7	0	0
Allo Antibody	7	58.3	9	69.2