ORIGINAL REPORT

Evaluation of solid-phase panreactivity with negative direct antiglobulin testing

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Solid-phase red cell adherence (SPRCA) is a sensitive platform for antibody detection, but nonspecific reactions may occur. One pattern of apparent nonspecific reactivity is a panagglutinin with a negative direct antiglobulin test (DAT). The purpose of this study was to define the clinical characteristics of patients with these nonspecific reactions and their associated serologic findings. Twenty patients with panreactive SPRCA testing results were identified between November 2022 and May 2023. In addition to panagglutinins, these patients had (1) a negative polyethylene glycol (PEG) antibody detection test, (2) a negative PEG autocontrol, and (3) a negative DAT. The strength of SPRCA panreactivity and the results of eluate testing (by tube and SPRCA) were studied. Clinical characteristics of patients included age, sex, and primary diagnosis. Each patient was also assessed for evidence of hemolysis. Fourteen female and six male patients were evaluated (average age 44 years). Primary diagnoses included pregnancy (n = 10), acute bleeding (n = 4), orthopedic (n = 3), and other (n = 3). There was no clinical or laboratory evidence of hemolysis. The predominant strength of SPRCA panreactivity was evenly distributed across reaction grades (1+ to 3+). Fifty-five percent of the eluates tested in PEG showed panreactivity, consistent with warm-reactive autoantibodies, while 85 percent of eluates tested by SPRCA were panreactive. Six discrepant cases, in which PEG eluate testing was negative and solid-phase eluate testing showed panreactivity, were associated with weak solid-phase plasma panreactivity (1+). In addition, the reactivity strengths of the eluates tested by SPRCA were invariably more strongly reactive than those eluates tested in PEG. Panagglutination is a distinct SPRCA-only plasma reactivity pattern. Despite a negative PEG tube and DAT, most panagglutinins are warm-reactive autoantibodies. Fortunately, these "interfering" panagglutinins do not appear to be clinically significant and are easily managed by an alternative testing method such as PEG. ***Immunohematology*** **2023;39:151–154. DOI: 10.2478/immunohematology-2023-022.**

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Blood group antibody detection testing is a critical component of pre-transfusion testing in that the accurate detection and identification of antibodies can reduce the risk of hemolytic transfusion reactions. A variety of antibody identification platforms have been introduced into routine immunohematology practice including tube-based methods, column agglutination (gel and glass beads), and solid-phase red cell adherence (SPRCA), including a method that uses protein A. Each technology has inherent benefits and drawbacks as well as relative sensitivities and specificities with respect to blood group antibody detection.[1]

The Providence Oregon Regional Laboratories (eight hospital labs and a centralized regional blood bank) routinely use SPRCA for antibody detection. When an antibody detection test is positive in one of the eight hospital-based laboratories, that sample is sent to the regional blood bank for further evaluation, including antibody identification. Through this approach, it became apparent that SPRCA testing was a sensitive method for antibody detection, but there appeared to be an unexpectedly high rate of apparent nonspecific reactions (i.e., positive antibody detection tests that did not result in identification of a defined blood group antibody specificity). This finding led to the routine practice of performing a concurrent antibody detection test by tube with polyethylene glycol (PEG) enhancement, whenever apparent nonspecific (NSP) SPRCA reactivity was identified.

We recently performed an assessment of our regional experience with SPRCA, paying particular attention to NSP reactivity.[2] In our assessment, we characterized the serologic features of patients with this reactivity, noting three distinct patterns on panel testing: (1) no reactivity (57.2% of cases), (2) panreactivity (26.0% of cases), and (3) sporadic reactivity (16.8% of cases). Because warm-reactive autoantibodies were the fourth most identified antibody type in the laboratory (NSP being the most common), a pilot investigation of panreactive NSP samples was undertaken to determine if the antibodies were, in fact, warm-reactive autoantibodies despite negative direct antiglobulin test (DAT) results. To this end, 10 random patient specimens with panreactive SPRCA testing, negative tube testing, and nonreactive DATs underwent an elution procedure. Of these, six of 10 specimens had panreactive eluates, while four of 10 were nonreactive, indicating that at least a portion of panreactive NSP antibodies represented warm-reactive autoantibodies below the limit of antibody detection by tube, both by the DAT and the indirect antiglobulin test (IAT). The current study was designed to further

explore panreactive NSP antibodies, characterizing their clinical and serologic features.

Materials and Methods

Indirect Antiglobulin Testing

Automated SPRCA assays were performed according to the manufacturer's instructions on either generation of the automated testing device (Galileo Echo or Echo Lumena; Immucor, Norcross, GA) using the SPRCA assay for antibody detection [Capture-R Ready-Screen (3); Immucor]. An automated, 14-cell antibody identification panel (Capture-R Ready-ID; Immucor) was performed on each specimen with a positive antibody detection test. A PEG-tube method antibody detection test (Gamma PeG; Immucor) was performed if no discernable antibody specificity could be identified or if all reactions were positive. If the PEG-tube antibody detection test was negative, the results were attributed to NSP reactivity. If positive, a PEG-tube antibody identification panel was performed.

The PEG-tube antibody detection test was performed by adding two drops of patient plasma, one drop of reagent red blood cells (RBCs) (Panoscreen I, II, and III; Immucor), and two drops of PEG (Gamma PeG; Immucor) to a tube, incubating for 10–15 minutes at 37°C, washing four times with phosphate-buffered saline, and adding two drops of monoclonal anti-IgG (Gamma-clone Anti-IgG; Immucor). The tubes were then centrifuged, and the RBC buttons were examined for agglutination. Of note, an autocontrol was performed with each PEG-tube antibody detection test.

Direct Antiglobulin Testing

Per standard practice, a DAT was performed on each specimen with an SPRCA-positive antibody detection test with polyspecific antihuman globulin (AHG) using a gel card [Anti-Human Globulin Anti-IgG,-C3d Polyspecific (Rabbit) MTS Anti-IgG,-C3d Card; Ortho Clinical Diagnostics, Raritan, NJ]. If positive, monospecific DATs were performed [Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG Card; Ortho Clinical Diagnostics and Anti-C3b,-C3d Gamma-clone; Immucor]. Eluates were prepared (Gamma ELU-KIT II; Immucor) according to the manufacturer's instructions. Eluates were assessed by both SPRCA and PEG-tube methods as described earlier. A three-cell product (Panoscreen I, II, and III; Immucor)) was used for this testing to preserve reagents and eluate.

Data Extraction

An electronic data repository was established to serologically characterize panreactive SPRCA agglutinins and correlate the reactivity with patients' clinical characteristics. Twenty consecutive samples were identified, all of which had negative DATs. Serologic characteristics included the following: (1) strength of SPRCA panreactivity, (2) results of tube testing with PEG enhancement, and (3) results of eluate testing (by tube and SPRCA). Clinical characteristics of the cohort of patients with NSP reactivity were obtained from the electronic medical record (EMR) (Epic; Verona, WI) and included each patient's age, gender, and primary diagnosis. In addition, each patient was assessed for evidence of hemolysis by interrogating laboratory values (hemoglobin [Hb]/hematocrit [Hct], reticulocyte count, lactate dehydrogenase [LDH], haptoglobin, and total bilirubin) and clinical notes. For this study, similar patients were grouped together into diagnostic categories.

Results

Twenty consecutive patients with panreactive NSP antibodies were identified between November 2022 and May 2023. In addition to their panagglutinins, these patients were chosen for study because they all had (1) a negative PEG-tube antibody detection test, (2) a negative PEG-tube autocontrol, and (3) a negative DAT. A summation of patient demographic data is found in Table 1. Half of the patients were pregnant

Table 1. Study patient demographic data

Demographic characteristic	Value	Percentage of total (*n*)
Gender, *n*		
Female	14	70
Male	6	30
Average age, years (range)		
Overall	44 (23–76)	100 (20)
Female patients	37 (23–62)	70 (14)
Female patients minus pregnant patients	51 (39–62)	20 (4)
Male patients	62 (45–76)	30 (6)
Primary diagnosis, *n*		
Pregnancy	10	50
Acute bleeding	4	20
Orthopedic	3	15
Other*	3	15

*Cardiogenic shock, prostate cancer, septic shock.

women, which resulted in a lower mean age of the female study patients. Although it appears that both women, in general, and pregnant women, in particular, are overrepresented in the study, these demographic features are representative of the total distribution of patient types evaluated by the laboratory (data not shown).

A summation of the serologic data evaluated in the study is found in Table 2. The overall, predominant strength of SPRCA panreactivity was evenly distributed across reaction grades (1+ to 3+). Eluate testing revealed that 55 percent of the eluates tested by PEG-tube were panreactive and consistent with warm-reactive autoantibodies. However, 85 percent of eluates tested in the SPRCA environment were panreactive and consistent with warm-reactive autoantibodies. Six discrepant cases were associated with relatively weak SPRCA plasma reactivity (1+). In these cases, PEG-tube eluate testing was negative and SPRCA eluate testing demonstrated a panagglutinin. In addition, the strength of reactivity of the eluates tested by SPRCA were invariably more strongly reactive (1+ to 2+ greater strength of reactivity) than eluates tested by PEG-tube (data not shown). Interestingly, there were three eluates that were nonreactive by both PEG-tube and SPRCA. All three had strongly reactive SPRCA plasma reactivity (3+).

Table 2. Study patient serologic data

Test name	Number	Percentage of total
SPRCA panel reactivity*		
1+	7	35
2+	6	30
3+	7	35
Eluate testing		
PEG	11 panagglutinins	55
SPRCA	17 panagglutinins	85

*The strength of reactivity represents the predominant overall strength of all red blood cells tested.

SPRCA = solid-phase red cell adherence; PEG = polyethylene glycol.

Finally, each study patient's electronic medical record was interrogated for evidence of hemolysis by reviewing narrative reports (e.g., consultation and progress notes) and laboratory results (Hb/Hct, reticulocyte count, bilirubin, haptoglobin, and LDH values). There was no clinical or laboratory evidence of hemolysis. Although there were gaps in the laboratory values obtained for each patient, 16 of 20 had normal or near-normal Hb and Hct values. The remaining four patients were anemic (Hct values in the 20s; normal range 36–46% for adult women and 41–53% for adult men), but each patient had evidence of active bleeding. Thus, although the vast majority of SPRCA panagglutinins represent warm-reactive autoantibodies, none were associated with overt hemolysis (i.e., warm autoimmune hemolytic anemia).

Discussion

SPRCA assays have a long history of use as a primary platform for blood group antibody detection testing because of their sensitivity and conduciveness to automated testing methods.[3] However, sensitive antibody identification must be balanced against the detection of clinically insignificant antibodies and nonspecific reactions. Several publications have acknowledged that, although SPRCA methods are quite sensitive, they are also prone to a higher rate of nonspecific reactions than other platforms.[4–6] One pattern of such reactivity is panagglutination, although the exact mechanism for this phenomenon remains unclear.[3,7,8] In addition, SPRCA assays appear to be more sensitive for the detection of warm-reactive autoantibodies than other primary antibody detection methods.[4,6] In a previous study of NSP antibodies, the authors identified an interesting pattern of reactivity: panreactive SPRCA plasma testing with a negative PEG-tube test and a negative DAT.[2] A preliminary study indicated that at least a portion of these antibodies actually represented warm-reactive autoantibodies.[2] In the current study, we extend those findings by studying a greater number of cases correlated with both patient clinical characteristics and more expansive serologic findings.

The most notable finding in our study is that even with a negative DAT, the vast majority of SPRCA panagglutinins are warm-reactive autoantibodies (based on a panreactive eluate). This finding is surprising, but perhaps should not be given the fact that (1) SPRCA testing is sensitive for warm-reactive autoantibodies and (2) relatively speaking, SPRCA plasma testing may be more sensitive than a standard gel- or tube-based DAT. Fortunately, these particular "SPRCA-only warm-reactive autoantibodies" do not appear to be associated with clinical hemolysis. This observation likely results from there being too little immunoglobulin bound to the patient's own RBCs (i.e., a negative DAT) to result in overt extravascular hemolysis. Finally, panagglutinating antibodies of this type are easily managed through use of an alternative antibody detection method. Specifically, these antibodies had completely negative antibody detection tests by PEG-tube, making the detection of underlying alloantibodies straightforward.

A final and interesting serologic finding identified in this study is seen in the three case subjects who had nonreactive eluate testing in SPRCA and PEG-tube testing environments. Two of these three patients were pregnant, and the third patient had active bleeding (hematuria). All, however, had 3+ plasma testing using SPRCA. We can only speculate as to what these panagglutinins represent, but the most likely possibilities include (1) a warm-reactive autoantibody despite negative eluate testing; (2) an antibody to a high-prevalence blood group antigen, including high-titer, low-avidity reactivity, that is only demonstrable in SPRCA; and (3) an antibody or other agglutinin that binds a component of the SPRCA testing matrix (e.g., a component of the solubilized internal RBC membrane).[9] Of these, the second and third possibilities seem most likely.

Like all studies, our study has strengths and weaknesses that must be acknowledged. Because our sample size is relatively small (n = 20), corroborative studies should be performed to verify our findings, but it is difficult to believe that additional samples would lead to radically different results, especially since the current results are consistent with results obtained in a pilot study.[2] Another potential weakness is that the patients selected for study, although consecutive in nature, represent a convenience sample of those case subjects who presented to the laboratory in the 7-month time frame of the study. It could be that other patients chosen at other times might have yielded different results. Another limitation to the study is the fact that DATs were performed using gel testing instead of tube methods. This difference in testing adds another variable when comparing PEG-tube antibody detection test results with SPRCA results. Some of these patients may have had a positive DAT using a tube method. Finally, it would have been interesting to follow the 10 pregnant study patients to determine how these panagglutinins affected their neonates, once delivered. It seems unlikely that neonates would be at increased risk for hemolysis or even a positive DAT, particularly because their mothers were unaffected, but this analysis was beyond the scope of the current study.

In conclusion, nonspecific serologic reactivity appears to be more common in SPRCA testing than other antibody detection platforms. This finding is likely due to the enhanced sensitivity of SPRCA for IgG antibodies. A distinct SPRCA-only plasma reactivity pattern is panagglutination. Despite negative PEG-tube testing and DAT results, the majority of these antibodies appear to be warm-reactive autoantibodies. Fortunately, these "interfering" panagglutinins do not appear to be clinically significant, with respect to hemolysis, and they are easily managed by turning to an alternative testing method such as PEG-tube testing. Further investigation is warranted to better understand the clinical and serologic features of nonspecific SPRCA antibodies, particularly antibodies that are ultimately determined to be warm-reactive autoantibodies.

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