The incidence of red cell alloantibodies underlying panreactive warm autoantibodies

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A recognized hazard of administering blood transfusions to patients with panreactive warm autoantibodies is that alloantibodies may be masked. Studies have shown the incidence of underlying alloantibodies to be 30 to 40 percent. Adsorption procedures can be used to remove autoantibodies and allow detection and identification of underlying alloantibodies. This study contains data from 126 patients referred to the Red Cell Immunohaematology laboratory at the National Blood Service, Newcastle upon Tyne, United Kingdom. These patients were from the northeast of England, a population for which data have not previously been reported. Samples identified as containing panreactive warm autoantibodies were subjected to adsorption procedures (95 by alloadsorption and 31 by autoadsorption). Absorbed sera were then tested to identify underlying alloantibodies. Of 126 samples, 39 (31%) contained a total of 61 RBC alloantibodies; 15 (12%) contained 2 or more antibody specificities; and 14 (11%) contained alloantibodies not found within the Rh or Kell blood group systems. Antibodies identified included the following specificities: E (19), D (9), c (7), C (6), S (5), Fy^a (3), Jk^a (2), Jk^b (2), K (2), Kp^a (2), Fy^b, C^w, N, and f (ce). This study reinforces the value of adsorption studies, whether using autologous or allogeneic RBCs, when panreactive warm autoantibodies are present. In addition, this study confirms that it is not appropriate in these cases simply to issue blood which is "least incompatible" or Rh phenotype- and K antigen-matched.
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One of the recognized hazards of administering blood transfusions to patients with panreactive warm autoantibodies is the failure to detect clinically significant alloantibodies that may be masked.[1,2] Traditionally, some blood banks have had policies of crossmatching and issuing blood that is "least incompatible" or "as compatible" as the patient's own RBCs, or they have issued blood that is Rh phenotype- and K antigen-matched.[3] Several methods may be used to remove free autoantibody from the patient's plasma to allow the detection and identification of any underlying alloantibodies.[4,5] Adsorption, whether using autologous or allogeneic enzyme-treated RBCs, is the method of choice within the National Blood Service (NBS) in the United Kingdom. British Committee for Standards in Haematology guidelines recommend that these techniques be used in such cases.[6] This study was performed over 18 months in the Red Cell Immunohaematology laboratory at the NBS in Newcastle. The region has 18 National Health Service (NHS) hospital blood banks, serving a population of 2.9 million.

Materials and Methods

Our alloadsorption method uses at least two sets of strongly papain-treated donor RBCs selected to have complementary expression of important RBC antigens. It is our usual practice to use Group O, rr, K– and Group O, R_1R_1, K– RBCs, one RBC sample being Jk(a–b+) and the other being Jk(a+b–). Reagent RBCs for adsorption were obtained from the UK NBS central reagents unit. Autoadsorption uses the patient's papain-treated RBCs.

Alloadsorption was performed on more cases as there was a limited supply of patients' RBCs to use to perform autoadsorption, due to severe anemia or low sample volumes. Serial adsorptions were performed by incubating 1 mL of the patient's plasma with 1 mL of the papain-treated packed RBCs at 37°C; the absorbed plasma was then retrieved by centrifugation. A maximum of 4 aliquots of allogeneic RBCs, or typically 2 aliquots of autologous RBCs, were used for this process. The number of autoadsorption procedures was dependent on the volume of RBCs available from the patient sample. Absorbed plasma was then investigated to detect and identify any underlying alloantibodies, using reagent RBCs suspended in low-ionic-strength red cell preservative solution (RCPS; Inverclyde Biologicals, UK) by tube IAT. Where possible, phenotype studies were used to confirm that the identified antibodies were alloantibodies.

Results

Samples from 130 individual patients were referred for investigation of panreactive autoantibodies by autoadsorption or alloadsorption procedures. Data from four samples in which we failed to successfully remove the autoantibody were omitted from this study. Alloadsorption procedures were performed on 95 samples and autoadsorption procedures on 31 samples. Thirty-nine of 126 samples (31%) contained a total of 61 RBC alloantibodies (Table 1); 87 samples (69%) contained no underlying alloantibodies. Fifteen samples (12%) contained two or more specificities (Table 2). Fourteen samples (11%) contained alloantibodies with specificities for antigens outside the Rh or Kell blood group systems.

Of the 61 alloantibodies identified, all but 4 (anti-Kp^a [2], anti-N [1], and anti-C^w [1]) would require the selection of antigen-negative RBCs for crossmatching in the UK.[6] Of 95 alloadsorbed samples, 33 (35%) contained alloantibodies, whereas 6 of 31 (19%) autoadsorbed samples contained alloantibodies.

All but six antibodies (anti-Fy^a [3], anti-Kp^a [2], and anti-Fy^b [1]) were confirmed as alloantibodies, using phenotyping studies. Phenotyping studies were not undertaken for these six patients as their RBC samples had strongly positive DATs. With only typing reagents requiring the use of AHG available and without a local procedure for removing autoantibody for typing, test results would be unreliable. These six antibodies were therefore assumed to be probable alloantibodies. Autoantibodies of these specificities are rarely reported.

Table 1. Underlying alloantibody specificities

Antibody specificity	Number identified
Anti-E	19
Anti-D	9
Anti-C	7
Anti-c	6
Anti-S	5
Anti-Fy^a	3
Anti-Jk^a	2
Anti-Jk^b	2
Anti-K	2
Anti-Kp^a	2
Anti-Fy^b	1
Anti-C^w	1
Anti-N	1
Anti-f (ce)	1
Total	61

Table 2. Multiple alloantibody specificities

Antibody specificity	Number of samples
Anti-D, -C	3
Anti-c, -E	3
Anti-E, -Kp^a	1
Anti-E, -Fy^a, -Jk^a	1
Anti-S, -N	1
Anti-Fy^b, -f(ce)	1
Anti-c, -E, -K, -Fy^a	1
Anti-C, -Fy^a, -Jk^b	1
Anti-D, -C, -E, -S	1
Anti-E, -K, -Jk^a	1
Anti-C, -Jk^b	1

Discussion

Limitations and advantages of the adsorption procedures

1. Alloadsorption

Alloadsorption procedures were preferentially performed in the cases described, but a number of factors must be borne in mind when interpreting results. Alloadsorption procedures require a minimum of two individual RBC samples with a complementary antigenic profile at key antigens, in our study, Rh, K, Jk^a, and Jk^b antigens, and at least 2 mL of available patient plasma or serum. Papain treatment of the adsorption RBCs removes enzyme-labile antigens, effectively rendering the RBCs negative for such antigens. By this procedure we ensured that underlying alloantibodies such as anti-S, -s, -Fy^a, -Fy^b, -M, and -N remained behind after adsorption. We acknowledge that many workers have found that the s antigen is not readily destroyed.[7] Although not every batch of reagent RBCs is specifically tested for antigen destruction, the process was thoroughly validated when introduced by the UK NBS reagents unit. Although no patients in this study were found to have underlying anti-s at the time of collating data, the patient referred to in Table 2 with anti-C, -Fy^a, and -Jk^b did subsequently produce an underlying anti-s. The enzyme treatment usually increases the uptake and removal of autoantibody, although there were some exceptions to this in our study (four patients) when autoantibody removal was less effective.

Our standard protocol does not include the use of e– adsorption RBCs. In our experience the benefits of including RBCs of the R_2R_2 phenotype are outweighed by the practical difficulties of obtaining suitable RBCs

and the increased volume of plasma required. However, RBCs of the R_2R_2 phenotype are used when the initial investigation suggests they may be useful. The policy of matching the Rh phenotype of the donor units to patients with autoantibodies decreases the risk of an underlying alloanti-e causing a transfusion reaction.

Recently transfused patients may give misleading or inconclusive Rh phenotyping results. In such cases it is important to establish the transfusion history and make an individual assessment of the most appropriate Rh phenotype to select. Where possible it is good practice to prospectively establish the extended phenotype of patients likely to be multiply transfused.

Alloadsorption procedures carry the risk that an alloantibody to a high-prevalence blood group antigen can be adsorbed by the procedure and not detected when screening or matching blood. We believe our findings show that it is better to carry out the adsorption procedures, being aware of this risk, than not to adsorb at all. We have experience of one case (not included in this study) where an underlying anti-Vel was not detected following alloadsorption (data not published).

2. Autoadsorption

Autoadsorption uses the patient's own RBCs to remove autoantibody so that underlying alloantibodies can be tested for. The advantages of autoadsorption are that it does not remove any alloantibodies and it requires adsorption of only a single aliquot of patient's plasma, considerably reducing the volume required. The number of tests performed is also reduced since the tests need not be carried out in duplicate, or triplicate as in the case of alloadsorptions.

Autoadsorption is not without its problems, however. The patient's RBCs often are heavily coated with antibody, with reduced capacity for further antibody uptake. Patients may also be anemic, sometimes very anemic, with few RBCs available for autoadsorption. In our experience, anything less than two equal-volume (RBCs:plasma) adsorptions does not remove sufficient autoantibody to be of investigative value. The relatively large amount of plasma available from anemic patients lends itself more to alloadsorption.

Autoadsorption is inappropriate for the recently transfused patient. If the procedure is inadvertently applied to samples from such patients there is a risk that alloantibodies may be adsorbed in vitro onto transfused RBCs. Our protocol for patients transfused within the last 3 months is to perform alloadsorption and to prepare an eluate to detect alloantibodies bound in vivo.[8]

Transfusion practices

Transfusion in the presence of panreactive warm autoantibodies can be a complicated and dangerous proposition.[9] In our study, adsorption techniques excluded the presence of alloantibodies in 69 percent of our patients. In the patients found to have alloantibodies, adsorption studies allowed the selection of appropriate RBCs for transfusion. In this group, Rh and Kell phenotype matching alone would have exposed 14 patients (11%) to the risk of a significant transfusion reaction.

We believe this study reinforces the value of adsorption studies when panreactive warm autoantibodies are present and concurs with the findings of similar studies. Our findings also show that it is not appropriate in these cases simply to issue RBCs that are "least incompatible" or Rh phenotype- and K antigen-matched.

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