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First Indian study to establish safety of immediate-spin crossmatch for red blood cell transfusion in antibody screen-negative recipients

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Abstract:

BACKGROUND AND OBJECTIVES: The US Food and Drug Administration and American Association of Blood Banks approved the type and screen approach in 1980s, long after antibody screen (AS) was introduced in 1950s. The present study omits conventional anti-human globulin (AHG) crossmatch and replaces it with immediate-spin (IS) crossmatch as part of pretransfusion testing in AS-negative patients to study the safety and effectiveness of IS crossmatch in recipients.

MATERIALS AND METHODS: This prospective longitudinal study was conducted on over 5000 red cell units transfused to AS-negative patients admitted to the hospital. Pretransfusion testing comprised blood grouping and AS followed by IS crossmatch, at the time of issue of red cell unit. The patients were transfused IS compatible red cell units. AHG crossmatch was performed posttransfusion for all red cell units. Any incompatible AHG crossmatch was followed up as suspected transfusion reaction.

RESULTS: A total of 5023 red cell units were transfused to 2402 patients with negative AS. 99.7% IS compatible red cell units were also compatible on posttransfusion AHG crossmatch. Anti-P1 alloantibody was identified in one patient who was transfused two IS crossmatch compatible units but later both units were incompatible on AHG crossmatch. There was no clinical or serological sign of hemolysis in the patient.

CONCLUSION: In AS-negative patients, IS crossmatch is as safe as conventional AHG crossmatch and can, therefore, replace conventional AHG crossmatch protocol.

Key words:

Anti-human globulin, immediate-spin crossmatch, pretransfusion testing, red blood cell transfusion, type and screen

Antibody screen (AS) was established as a part of pretransfusion testing in early 1960s.[1] Several workers, then questioned the importance of performing the anti-human globulin (AHG) phase of crossmatch. This resulted in introduction of type and screen (TS) approach, wherein any patient with AS negative on AHG phase could be transfused red cell unit after compatible immediate-spin (IS; aka abbreviated/room temperature) crosssmatch.[2] The US Food and Drug Administration and AABB approved the TS approach in 1982.[2] One of the first studies conducted by Oberman *et al*.[3] demonstrated the safety of TS approach in patients requiring massive transfusions. Other studies followed and Pinkerton *et al*.[4] demonstrated the absence of delayed hemolytic transfusion reactions in patients receiving blood transfusion using TS approach. Heddle *et al*.[5] conducted the first prospective study by omitting the conventional AHG crossmatch in AS-negative patients. Although the authors found 27 transfused red cell units as incompatible by the subsequent AHG crossmatch, none of these patients had clinical or serological evidence of hemolysis. Authors, therefore, concluded that the AHG crossmatch could be omitted from pretransfusion testing without putting patients at risk. A recently conducted study by Lee *et al*.[6] demonstrated the safety of TS approach in patients with autoantibodies and anti-human leukocyte antigen antibodies, where the presence of alloantibody was ruled out.

Indian researchers including Pathak *et al*.[7] and Agrawal[8] also conducted studies comparing conventional AHG crossmatch and AS in parallel and did not report any discrepancy between

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them. Both concluded that TS method of compatibility could be safe, cost-effective, and beneficial to transfusion services in India.

On the basis of AABB standards allowing AS negative and IS crossmatch approach,[2] paper of Heddle *et al*.[5] and two published reports from India corroborating safety of AS[7,8] prompted us to omit AHG crossmatch in AS-negative patients in our institutional pretransfusion testing protocol. This was duly approved by the Independent Ethics Committee before the change in protocol was ushered in. AS-negative patients were issued red cell units after compatible IS crossmatch. With an aim to study the safety and effectiveness of IS crossmatch in comparison to conventional AHG crossmatch in AS-negative patients, AHG crossmatch was also performed on the subsequent day and compared with IS crossmatch in a consecutive cohort of 2396 patients.

Materials and Methods

Study setting

This was a prospective, longitudinal study conducted in the Department of Transfusion Medicine of a large tertiary care hospital in North India over a period of 3 months from January 2014 to March 2014. The study population comprised all patients admitted to the hospital and requiring red blood cell (RBC) transfusion.

Patient selection

The patients who had blood group (ABO and Rh) and AS completed and had negative AS were included in the study. Following patients were excluded:

- Any patient with positive AS
- Any patient who had incompatible IS crossmatch after negative AS.

"Red cell" transfusion

For the study, red cells were made from 450-ml whole blood collected from the healthy blood donors, in triple bags with 63-ml citrate phosphate dextrose anticoagulant. The red cells were prepared by centrifugation of whole blood, removal of plasma, and resuspension of the red blood corpuscles in 100 mL of an additive solution containing sodium chloride, adenine, glucose, and mannitol solution. The final component had a hematocrit of 55–65% (0.55–0.65) and a total volume of around 300 mL. Direct antiglobulin test (DAT) is done on all donor samples and red cell units with positive DAT are discarded as an institutional practice.

Immunohematology techniques (antibody screen, immediate-spin, and anti-human globulin)

Column agglutination technology (CAT) was used to perform patient and donor blood grouping, patient AS and identification (AI), donor DAT, IS and AHG crossmatch. Forward ABO/Rh grouping was done on ABD cards (Ortho Clinical Diagnostics, Johnson and Johnson, USA). Reverse grouping was done using in-house pooled A-cell, B-cell, and O-cell on reverse diluent cards (Ortho Clinical Diagnostics, Johnson and Johnson, USA). AS was performed using low-ionic strength solution-based CAT, the patient's plasma was screened for irregular antibodies using commercially available three-cell reagent panel (R1wR1, R2R2, and rr phenotype; 0.8% Surgiscreen, Ortho Clinical Diagnostics, Johnson and Johnson, USA). Positive AS test was followed by antibody identification using eleven-cell identification panel resolve Panel A and B (Ortho Clinical Diagnostics, Johnson and Johnson, USA). The donor DAT was performed on AHG cards. IS and AHG crossmatch for compatibility testing was carried out on reverse diluent cards and AHG cards, respectively. The antigen-antibody reaction on the cards was interpreted according to the manufacturer's instructions. Reaction strength of zero was considered negative or compatible.

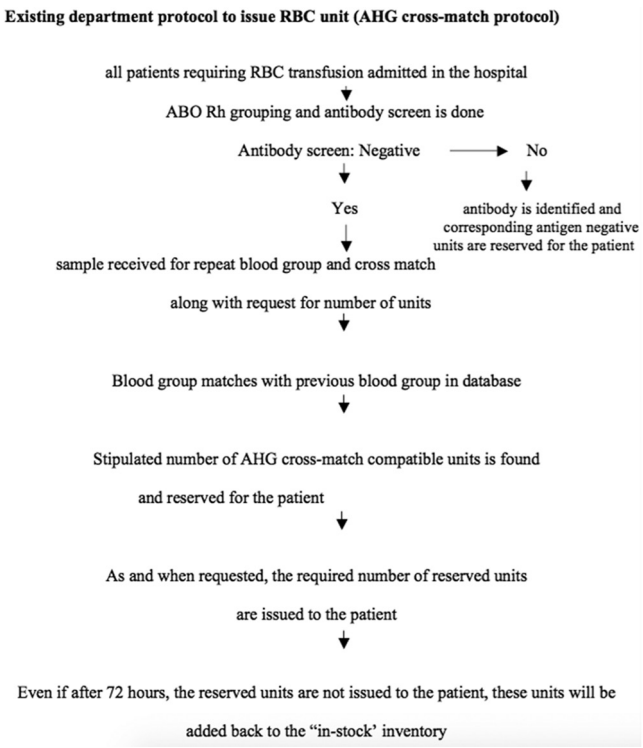

Figure 1: *Existing conventional protocol*

Existing conventional protocol [type and screen and anti-human globulin crossmatch]

All patients admitted to the hospital underwent "type and screen" which comprised blood grouping and irregular AS with the three-cell panel. There was a conventional protocol [Figure 1] of performing AHG crossmatch at the time of receiving blood component request; the red cell units were reserved and kept in a separate refrigerator called "cross-matched" units. The units were, then, physically issued against an "issue-slip" sent from the clinical areas. The units that were reserved but not physically issued to the patients; 72 h from the time of receiving the "request," were returned to the main inventory.

Study protocol [type and screen and immediate-spin crossmatch]

With this new study protocol [Figure 2], blood unit was issued to patients with negative AS and after compatible IS crossmatch. The red cell unit(s) was issued as and when required against an "issue-slip" sent from clinical areas. Unlike the conventional protocol, the stipulated numbers of red cell units were not AHG cross-matched and reserved in

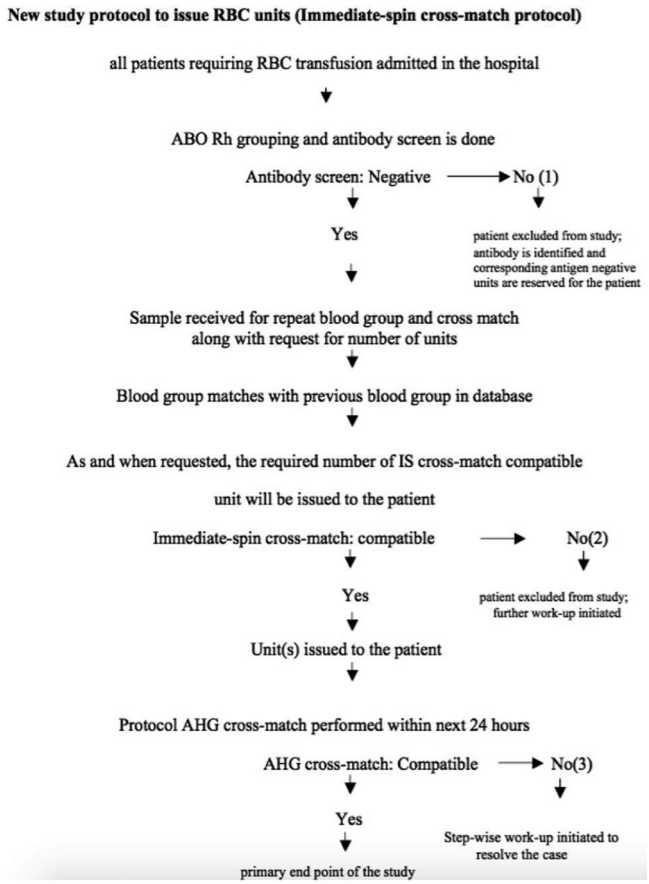

Figure 2: *Study protocol*

the patient's name at the time of receiving initial requisition form. If there was no history of transfusion, AS performed within the last 3 months was valid. However, if the history of transfusion was present, AS was repeated within 3 days of transfusion. The patients with positive AS were excluded from the study. Alloantibody was identified and corresponding antigen-negative AHG crossmatch compatible units were transfused to patients. Furthermore, the patients who had incompatible IS crossmatch after negative AS were also excluded from the study. Immunohematological work-up was performed to identify the reason of incompatibility. The patient was transfused appropriate AHG compatible blood unit.

Discordant patient results: Workup of suspected transfusion reaction

Red cell units that were compatible on both IS crossmatch and posttransfusion AHG crossmatch were called concordant units. Any red cell unit with compatible IS crossmatch and incompatible AHG crossmatch was termed as a discordant unit. Incompatible posttransfusion AHG crossmatch warranted immediate clinical and serological follow-up of the patient. Immunohematological work-up was performed to identify the reason of incompatibility. Any eventful transfusion was followed as a suspected transfusion reaction. A transfusion reaction work-up consisted of rechecking the patient blood group, AS, and DAT with pre- and post-transfusion samples. The donor blood group was also rechecked with blood bag segment kept at the time of issue of the unit. The major crossmatch was performed with both pre- and post-transfusion samples. The patient's first urine sample, postsuspected transfusion reaction, was also sent for routine testing, microscopy, and hemoglobinuria.

Sample size calculation

Using confidence level = 99%, precision (d) = ±1%, the formula for calculating sample size:

$$n = \frac{(Z_\alpha^2 PQ)}{d^2}$$

where $Z\alpha$ = value of standard normal variate corresponding to α level of significance, P = likely value of parameter, $Q = 1 - P$, and d = margin of errors which is a measure of precision.

The sample size (red cell units for transfusion) for the study was calculated as 4884.

Statistical analysis

The analysis included profiling of patients on different demographic and laboratory parameters, etc. Quantitative data will be presented in terms of means and standard deviation. Categorical data will be presented in terms of absolute number and percentages. Sensitivity and specificity will be used for diagnostic test evaluation. SPSS software (Version 24.0; IBM, Bengaluru, Karnataka, India) will be used for analysis.

Ethical clearance

The Independent Ethics Committee approved the study with changes in pretransfusion testing protocol.

Results

During the study period, 2413 patients required red cell transfusion and were included in the study. Eleven patients were excluded from the study on the account of positive AS. A total of 2402 patients with negative AS were included in the study and formed the study cohort. These patients were transfused a total of 5023 red cell units. Each red cell unit transfused to the patient was considered as a separate "episode" of transfusion. A majority of patients included in the study were admitted under surgical specialties (55.5%); followed by medical specialties (40.3%) and critical care (4.2%). Demographic and exclusion details are described in Table 1.

Safety of type and screen and immediate-spin crossmatch

A total of 5012 RBC units were issued to 2396 patients after compatible IS crossmatch and initial negative AS. When posttransfusion protocol AHG crossmatch was performed, 13 units were found to be incompatible. On further work-up, 11 units were found to be false positive on repeat testing (2 replicates). The two units that were compatible on IS crossmatch and later, incompatible on posttransfusion AHG crossmatch, were issued and transfused to a single patient [Table 2]. The patient was a 35-year-old female suffering from neuromyelitis optica. No acute transfusion reaction was reported at that time. Fresh immunohematological work-up of the patient revealed anti-P1 alloantibody. The IgG titer (using the conventional tube technique) was 2 with pretransfusion sample and fresh posttransfusion sample. Clinical and laboratory follow-up of the patient on posttransfusion day 1 and day 7 did not show any sign of hemolysis [Table 3]. Sensitivity

Table 1: Patient demography and exclusion details

Parameter	Value
Total number of patients included in the study	2413
Male (%)	1623 (67.2)
Female (%)	790 (32.8)
Total number of red cell units transfused	5023
Indication for transfusion (%)	
Anemia	58
Surgery	35.3
Blood loss	8.8
Percentage of patients with history of transfusion	8.4
Percentage of females with history of pregnancy	68.9 (*n*=543)
Number of patients excluded on the basis of positive antibody screen	11
Number of patients excluded on the basis of incompatible IS crossmatch	6
Anti-M	2
Anti-Lewis (a)	1
Anti-Lewis (b)	1
Cold autoantibody	2

IS= Immediate-spin

Table 2: Discordant patient results

Parameter	Value
Total number of red cell units incompatible on posttransfusion AHG crossmatch	13
False positive (incompatible on posttransfusion AHG crossmatch due to technical error) (%)	11 (84.6)
True positive (incompatible on repeat posttransfusion AHG crossmatch also) (%)	2 (15.4)
Number of patients followed on the basis of incompatible posttransfusion AHG crossmatch (true positive)	1 (anti-P1 alloantibody identified)

AHG= Anti-human globulin

Table 3: Posttransfusion work-up of patient who was transfused discordant red cell units

Parameter	Day 1	Day 7	Reference values
Urine R and M for RBC	Negative	Negative	0-2 per high power field
Activated partial thromboplastin time (s)	22.7	23.1	26.3-39.4
Prothrombin time (s)	9.5	8.9	12.7-15.4
International normalized ratio	1	0.90	0.8-1.2
Hemoglobin	11	11.1	12-15.8 (mg/dl)
Lactate dehydrogenase	330	323	115-221 (U/l)
Unconjugated bilirubin	0.4	0.1	0.2-0.9 (mg/dl)
Conjugated bilirubin	0.1	0.2	0.1-0.4 (mg/dl)
Total bilirubin	0.7	0.6	0.3-1.3 (mg/dl)
Urine hemoglobin	Negative	Negative	Negative
Plasma hemoglobin	Negative	Negative	0.6-5.0 (mg/dl)

RBC= Red blood cell, R and M= Routine and microscopy

and specificity of IS crossmatch were 99.9% (99.70–99.99%; 95% confidence interval [CI]) and 80% (28.36–99.49%; 95% CI), respectively. Positive predictive value was 99.96% (99.77–100%; 95% CI) and negative predictive value was 66.67% (22.8–95.6%; 95% CI).

Discussion

In the present study, authors have demonstrated the safety of IS crossmatch in comparison to conventional AHG crossmatch in AS-negative patients. Authors prospectively omitted the AHG crossmatch from the routine pretransfusion testing in AS-negative patients, to demonstrate that IS crossmatch can replace AHG crossmatch without any adverse outcome in the patients.

Over a period of 3 months, 5012 IS crossmatch compatible red cell units were transfused to 2396 AS patients. None of the patients had any form of transfusion reaction. Posttransfusion AHG crossmatch was concordant with IS crossmatch in 99.7% samples that correlate with 98.4% concordance demonstrated by Heddle *et al*.[5] However, a lower rate of false-positivity in the present study (0.17%) as compared to Heddle *et al*. (1.2%) is probably due to the improved specificity of AHG reagent and CAT used in this study.

Although a single patient was transfused both the AHG incompatible red cell units, the patient showed no clinical signs of acute hemolytic transfusion reaction or demonstrable serological signs of hemolysis. Fresh posttransfusion immunohematological work-up revealed anti-P1 alloantibody. Anti-P1 is known to be responsible for incompatible AHG crossmatch after being missed in AS, and this result is consistent with the previous studies.[3,5,9] This result can be explained by the varied expression of P1-antigen on reagent red cells and deterioration during storage along with weak anti-P1 that may be responsible for negative AS and compatible IS crossmatch.[10] Anti-P1 is typically a weak, cold-reactive IgM antibody optimally reacting at 4°C and is usually not seen in routine testing and. therefore, considered clinically insignificant. Rare cases of Anti-P1, reactive at 37°C, have been reported to cause *in vivo* destruction of red cells. Both immediate and delayed hemolysis have been reported.[11,12] It is an acceptable approach to transfuse units that are crossmatch compatible at the antiglobulin phase, without typing for P1.[10]

Most Indian blood centers issue blood units on the basis of AHG crossmatch. Quite a few have also begun TS in addition to AHG crossmatch. In fact, this conventional AHG crossmatch protocol was there at authors' institute before this study was planned and executed. Indian workers, Pathak *et al*.[7] and Agrawal[8] have reported 100% concordance between AS and AHG crossmatch in 354 patients and 45373 patients, respectively. Although Chaudhary *et al*.[13] did report one case (one in 12 cases) where conventional AHG crossmatch was incompatible while AS was negative. However, the study could not establish the specificity of the alloantibody. Further, the authors only speculated a possible transfusion reaction in the absence of any actual transfusion.

Since no hemolytic transfusion reaction was reported during the entire duration of the study and 99.7% red cell units transfused was concordant, the authors continued with the study protocol for all the patients with AS negative admitted to the hospital. Till date, more than 35,000 red cell units have been transfused following the TS protocol and no hemolytic transfusion reaction has been reported.

Conclusion

In AS-negative patients, IS crossmatch is as safe as conventional AHG crossmatch and can, therefore, replace conventional AHG crossmatch protocol.

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Conflicts of interest
There are no conflicts of interest.

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