Conference Report

An immunohematological ‘Wet’ workshop

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Abstract:
A practical workshop on ‘Immunohematology’ was conducted in conjunction with the Indian Society of Blood Transfusion and Immunohaematology annual scientific program. The participants, from many parts of India, were able to obtain valuable practice in key areas of blood group serology and by the end of the workshop were able to carry out ‘tube’ techniques for antibody detection and identification. Column agglutination methods were also demonstrated. A preliminary questionnaire was completed by participants. Results showed a wide variety in types of pretransfusion (serologic) testing being performed. Less than half of the participants had encountered hemolytic transfusion reactions. The program was rated as excellent by most participants in response to a postworkshop evaluation questionnaire, with requests for longer and more frequent workshops. Safety of blood for transfusion depends on maintenance of high standards of both microbiological and immunohematological performance by the blood bank staff.

Key words:
Blood group serology, education, immunohematology

Ensuring the safety of all blood collected for transfusion has been a priority issue in many countries. Over recent years, the advance of numerous infectious diseases has necessitated considerable expenditure to introduce sophisticated screening techniques on blood donations. There has been so much focus on this work, driven in large part by public concern, that it is often overlooked that the safety of blood also depends upon its serological testing. Accurate blood grouping, testing for blood group antibodies, careful cross-matching and the use of appropriate controls, as well as great care, in carrying out immunohematological procedures are all necessary if safe blood is to be available for patients.

In India, there is great variation in the standard of procedures carried out in immunohematology laboratories, which are commonly associated with blood banks. Blood grouping techniques vary greatly and are often carried out without appropriate controls. Blood matching procedures also are not standardized throughout the country despite the availability of a well-prepared manual. Only a minority of laboratories carry out routine screening for unexpected antibodies in donors or patients, and even fewer can accurately identify blood group antibodies or carry out phenotyping of red cells. Reasons for such a situation are complex but are related to education in the specialty, lack of resources and an administration system that has not always appreciated the importance of immunohematology in protecting patients from the risks of transfusion of blood. As increased numbers of Indians have access to increasingly sophisticated medical care, the number of patients with previous transfusions will rise; therefore, the need for accurate serologic testing can be expected to increase, as well as an effort to improve knowledge and practical abilities of medical and laboratory staff in blood group serology; and at the behest of the Indian Society of Blood Transfusion and Immunohaematology (ISBTI), a 3-day ‘wet’ workshop was held at Ahmedabad in November 2006.

Procedures

The workshop was prepared and organized by three overseas consultants in conjunction with staff of the Prathama Blood Centre in Ahmedabad. Most of the equipment and serological reagents were sourced from overseas, largely donated by commercial firms. The ISBTI called for nominations for the workshop from active blood bank workers, and eventually 15 doctors and 9 laboratory scientists were admitted to the course. Thirteen centers from various areas of India were represented, with some institutions sending multiple staff members. Before the course commenced, all participants were sent a CD containing recorded lectures, case studies and procedures on blood group serology. At the workshop, all were supplied with manuals of techniques, a book of blood typing problems and other printed information.

Laboratory space was provided at the Prathama Blood Centre to accommodate 12 workstations – each with their own Serofuge™, a high-speed,
bench-top centrifuge. Gloves, plastic pipettes and all other disposables were supplied as required, and attendees were expected to work through a series of exercises, starting from basic techniques and building up to red cell antibody identification.

1. ‘Tube’ testing for the detection of red cell antibodies was the main technique taught. This is recognized as being the ‘gold’ standard of immunohematology methods but does require a fair measure of skill, attention to detail and accurate documentation, as well as the availability of a high-speed calibrated serofuge.

2. In addition, there was opportunity to assess a column agglutination technique (CAT) as one bench was set up to demonstrate Diamed gel methods.

Participants were all able to carry out laboratory tests using reagents prepared in advance. Practical sessions were supplemented by lectures, informal talks and individual tutoring where required.

As a preliminary, a questionnaire was circulated among participants to provide a baseline on the immunohematological procedures being carried out in the institutions represented. After the seminar, an evaluation questionnaire was completed by most participants.

**Results**

**Preliminary questionnaire**

Previous immunohematological experience of the participants varied greatly, with nine having less than 2 years’ experience in blood banking. On the other hand, six had experience extending 10-20 years. The size of the institutions represented varied, with five collecting between 20,000 and 40,000 blood units/year; and the remainder, below 20,000 units/year. Sixty-four percent of respondents performed routine blood grouping, this including both doctors and scientists.

An analysis of the routine methods of blood grouping and cross-matching utilized revealed 4 using slide testing; 11, tube methods; 3 had moved to CAT methods; with 1 laboratory using automated equipment. Some laboratories used several methods. For cross-matching of blood, approximately equal numbers used the tube technique and CAT method, with some laboratories using both procedures depending on circumstances. For resolution of cross-matching problems, 18 respondents performed additional cross matches until compatible blood was found. Six attempted to identify the antibody and 3 referred the problem to another laboratory. There was some overlap of replies to this question, with different replies from the same center, indicating that standard procedures varied.

Red cell antibody detection (screening) tests were performed in about half the laboratories. These tests were done either by tube or CAT, about equally. Twenty participants reported standard operating procedures as being available in their laboratory. Most centers appeared to have access to the Indian Technical Manual, as well as a variety of other textbooks. Questions were asked regarding clinical problems encountered. The results may partly represent the length of experience of participants.

Thirty-seven percent of centers identified hemolytic transfusion reactions but the remainder did not. Only 12 of 22 attendees reported having seen a case of autoimmune hemolytic anemia, and only 11 of 21 recognized a case of hemolytic disease of the newborn.

**Program**

The workshop commenced with a short lecture on immunohematology test methods, followed by a bench exercise in the preparation of cell suspensions and grading of agglutination reactions. This was followed by an exercise using different antibody detection methods on the ‘same antibody’-containing serum, including the low ionic strength saline (LISS) method. At the same time, demonstrations of the column gel technique were provided. Participants proceeded to identify the specificity of the ‘same antibody’-containing serum and by the end of the day had understood the procedures and analysis required.

On the next day, a lecture was given on important aspects of quality control, as well as information on the rationale for the type-and-screen methods of compatibility testing. At the bench, participants then carried out several type-and-screen procedures followed by abbreviated cross-matches (no indirect antiglobulin test). They were also required to identify the cause of any incompatibilities, including a mistyped unit of blood and identification of a cold-reactive antibody. There were also discussions on the problems of ABO discrepancies and how to resolve these.

For the third day, there was further work on antibody identification, particularly relating to situations where multiple antibodies and autoantibodies were present. In particular, an elution technique was done on a patient with a positive direct antiglobulin test. A number of prepared clinical problems were discussed and this proved popular. Some limited red cell phenotyping was performed. Each day was very busy and the work extended into the evenings. There was time for all individuals to carry out the specific procedures, and the availability of a good range of test reagents was a great asset to the workshop.

**Evaluation Questionnaires**

These were distributed quite late in the workshop, resulting in only 13 replies being received. The majority graded the workshop as ‘excellent’ and the other two as ‘good.’ Most participants found that all the exercises taught were useful, with some remarking that it was the first time that they had received such coordinated teaching on antibody detection. The case report studies were appreciated and the method of personal teaching was valuable. No attendee thought that there were parts of the workshop that were not useful.

There were numerous subjects that attendees thought could have been also covered. These included antisera preparation, cryopreservation and thawing of red cells, plasma fractionation, quality control and calibration of equipment, preparation of red cell antibody identification panels, autoantibody workups and use of various types of antihuman globulin reagents.

A question was asked as to what procedures individual attendees would be able to put into place in their own laboratory subsequent
to the workshop. Most felt they would be able to set up antibody screening and identification, although some felt they would first need additional training. Several mentioned that the problem of poor availability of red cell screening and identification panels would need to be solved before antibody screening could be introduced routinely. It is anticipated that additional training and work to identify Indian donors for use as antibody screening cells and identification panels will have to be performed.

Other comments included the need for more such workshops, and it was suggested that they should be held six-monthly in various parts of India. It was proposed that workshops directed towards beginners in serology be combined with advanced courses for others. Also suggested were different workshops for technical and medical staff but with discussions between the two different groups. Quite a number of the participants suggested that the time allocated for the workshop was too short, with one attendee proposing that 15 days would be better! Others considered that ABO typing and for the workshop was too short, with one attendee proposing that 15 days would be better! Others considered that ABO typing and quality control deserved additional time allocation.

There were a number of suggestions as to improvements in the space available and the availability of disposable items. Overall the comments made were very favorable, with many stating how much they enjoyed the workshop.

**Discussion**

Immunohematology workshops tend to be a series of lectures rather than having a practical component. This workshop was the opposite, requiring each participant to carry out all the technical procedures under supervision with a minimum of lectures. The availability of a preliminary CD containing basic serological information, as well as other printed referral material, meant that participants could come well prepared for the technical exercises.

It was apparent that relatively few centers in India are performing red cell antibody detection tests and identification procedures confirming to acceptable international standards. As this is a basic requirement for the availability of 'safe blood,' the workshop emphasis was timely. To avoid many transfusion reactions and complications, the need for routine antibody detection is paramount. It was also clear that the standards and quality control procedures used in many blood banks may need improvement; and considerable re-training of staff is needed.

There are various methods of performing blood group serology, with 'tube' techniques being generally accepted as the 'gold standard.' The technique, to be reliable, requires considerable attention to detail but when performed carefully is very sensitive in the detection of blood group antibodies. Few centers in India are able to perform the technique well, and there is a need for the development of numerous reference centers throughout the country to enable serological problems to be resolved. The attendees at this course may well provide an important staff resource to initiate a higher standard of blood grouping serology.

There are problems in instituting widespread antibody detection in India. Most of the equipment and reagents, with the exception of CAT materials, were not easily available from Indian sources and were specially imported for the workshop. Although some of these items were left in India, the need for new equipment that facilitates reliable techniques to be introduced is evident. The poor availability of relatively inexpensive red cell screening and identification panels is also a major problem. The best solution for this is to produce Indian-sourced reagents which are more appropriate for serology in India than foreign-sourced reagents, particularly for antibody detection. Another problem is the paucity of reliable blood group data from India as this is essential to understand the distribution of blood group antigens and antibodies in the various areas of the country.

It was perplexing to find that such a low number of participants had seen the clinical complications of red cell antibody presence that include hemolytic transfusion reactions, hemolytic disease of the newborn and immune hemolytic anemias. It is likely that some of these clinical conditions are under-diagnosed, partly because many Indian laboratories are not able to perform the required special types of laboratory investigations. Attention to this is required as it is unlikely that these clinical conditions occur so infrequently in India.

Quality assurance has been a growth area in immunohematology in the past decade. Participants in this workshop were keen to upgrade this area of work and some were surprised to see how little quality assurance was performed, even by quite large blood banks. Workshops on this subject would be useful, with an emphasis on such matters as reliable ABO and Rh typing, independent quality control surveys and reagent preparation and control.

One of the pleasures of this workshop was to see the evident enthusiasm of many of the participants. They were eager to learn, were appreciative of the teachers and were willing to concentrate for quite a many days on the work in hand. It is hoped that they will receive the required administrative support when they return to their laboratories, as they could make a considerable difference to the safety margins for transfused blood in their centers.

During the workshop, the CAT gel method was demonstrated and many of the tests were performed in parallel with tube tests. There was a good concordance of results. These types of procedures are now being widely used in many countries and, although imported and relatively expensive, they are more easily quality controlled and are gaining popularity. Although many centers regard them as too expensive for routine use, this may not be a contraindication for their use as other expenses in the laboratory may show a considerable decrease. Staff numbers can be often reduced, quality control is easier, safety margins are improved, staff training needs are diminished, documentation is easier and administrative costs can be lessened. The results of these tests are now very good; and for many laboratories, their use, particularly for cross-matching, provides a fair measure of safety.

Workshops of this kind are very demanding on the teachers, and much preparation is required if they are to be successful. It is clear there is a need for similar workshops in other parts of India, and the ISBTI is to be congratulated on facilitating this innovative program. It may result in a safer blood supply for the country, along with directing attention to the many other aspects of the blood supply system that also need significant improvement, particularly on the voluntary donor side.
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Reference

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