NOTE DE L’ÉDITEUR
FRENCH EDITOR’S NOTE

SÉCURITÉ TRANSFUSIONNELLE EN AFRIQUE :
tellement de données à trouver, à éditer et à exploiter

BLOOD SAFETY IN AFRICA: so much data to find, to publish and to use

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Actuellement, un premier groupe de données scientifiques sont connues et se précisent progressivement dans un grand nombre de pays. Elles révèlent entre autres un approvisionnement insuffisant en produits sanguins et médicaments dérivés du sang, une forte prévalence des infections transmissibles par la Transfusion (ITT), une prédilection des donneurs familiaux, l’insuffisance d’efficacité des techniques utilisées pour le dépistage des ITT, un risque résiduel encore fortement élevé et un usage clinique inapproprié du sang. D’autre part, elles rapportent les premières caractéristiques sociologiques et hématologiques générales du donneur de sang africain notamment sur les motivations du don, les phénotypes érythrocytaires, le taux d’hémoglobine, les souches virales. Enfin, elles rapportent les coûts relativement élevés de la mise en œuvre des programmes d’approvisionnement auprès des donneurs volontaires non rémunérés.

TROUVER PLUS DE DONNÉES

Durant ces deux dernières décades, un deuxième groupe de données ont été rapportés de façon parcellaire, insuffisamment pour mener des actions de grande ampleur. Ce sont par exemple des travaux sur l’identification des facteurs de risque chez le

In the field of blood transfusion, the first frequently reported research activities are certainly those of the British scientist William Harvey in his book *Exercitatio Anatomica de Motu Cordis and Sanguinis in Animalibus* and of the Frenchman Jean Baptiste Denis in his book *Lettres*. In 1616, the first described completely and in detail the systemic circulation and properties of blood; in 1667, the second transfused about twelve ounces of sheep’s blood into a 15-year old boy. In Africa, it was about 70 years ago that the first work was published on the characteristics of blood transfusion and the blood donor. Indeed, from the beginning of the 1940s, some journals published data on anemia and on red blood cell phenotypes of African blood donors. Other older information may exist but remains unfortunately not easily accessible for most of us, and is thus not useful. However, in spite of the tumult of civil wars, of colonization and decolonization, in spite of precarious conditions related to poverty, many research activities were carried out by our predecessors and contributed to enrich our knowledge on blood safety in Africa.

Over the years the volume of knowledge related to blood transfusion and transfusion safety challenges in African countries has accumulated. Studies reveal an insufficient supply of blood and blood products, a high prevalence of transfusion transmissible infections (TTI), a large proportion of family replacement donors, a lack of effectiveness of techniques to detect TTI in blood donations, a persisting residual risk of transmission and the inappropriate clinical use of blood. In addition, this first group of data revealed the first sociological and hematologic characteristics of African blood donors, and a relatively high cost of blood supply systems based on voluntary non-remunerated blood donation.

FINDING MORE DATA

During these two last decades, a second group of data was partially reported but seems not enough to initiate actions of great impact. For instance: risk factors of TTI notably related to tropical parasitic diseases or to local habits (traditional scarification, polygamy...). Other data are currently discussed and not yet concluded such as infectious risk related to family replacement donation. Similarly, data on patient blood management, haemovigilance and indications for the use of various blood products in Africa are still partially known. Other capital data are almost unknown, notably data concerning the use of physico-chemical techniques for the reduction of pathogens, or concerning bone marrow grafts in Africa.
SPREADING SCIENTIFIC OBSERVATIONS

What is the essential common point between these groups of data? They do not serve blood transfusion in Africa if they are neither spread nor used. The verbal spread of information (teaching, scientific discussions) and the written word (publication) are essential to share scientific observations. The aim of our journal, Africa Sanguine, is initially to contribute to the education of specialists and students by publication of reviews and technical reports. It is also to publish the results of original studies in all the fields of transfusion medicine, in order to contribute to the better understanding of African specificities. Through communication and publications in several languages, the journal intends to improve good blood transfusion practices for the benefit of patients who need transfusions.

USING PUBLISHED DATA

It is well-known: to know and to be aware is good but to act in consequence is better. Non-exploited data, still many, await a greater will of stakeholders. Thanks to the data already available, many programmes supported by governments and international organizations were implemented. They were also used for the evaluation of the impact of actions undertaken. Amongst activities related to research, to the spreading of information and/or to the good use of data, we choose our contribution to the improvement of blood safety in Africa. Without moderation!

REFERENCES


donneur de sang africain : les risques transfusionnels spécifiques liés aux parasitoses tropicales ou aux coutumes locales (scarification, polygamie, saignées traditionnelles...) ont été peu décrites. D’autres données sont controversées comme par exemple le risque infectieux lié au don familial qui fait actuellement l’objet de discussion [6]. Il en est de même pour les données sur la gestion du receveur de produit sanguin notamment l’hémovigilance et les indications des produits sanguins stables et labiles. D’autres données capitales sont presque inexistantes : par exemple, on ne dispose presque pas d’expérience et de données sur l’usage des techniques de réduction physicochimique des pathogènes, ou sur l’usage des médicaments dérivés du sang et leurs conséquences chez le receveur, ou encore sur les greffes de moelle osseuse en Afrique.

DIFFUSER LES DONNÉES TROUVÉES

Quel est le point commun essentiel entre ces trois groupes de données ? Elles ne servent absolument pas la transfusion sanguine en Afrique si elles ne sont ni diffusées ni exploitées. Les diffusions orale (enseignement, discussions scientifiques) et écrite (publications) sont essentielles à cet objectif. Le rôle de notre journal, Africa Sanguine, est d’abord de contribuer à l’éducation des praticiens et des étudiants par la publication des revues et articles de synthèse portant sur les données actuelles en particulier, celles spécifiques à la transfusion sanguine en Afrique. Son rôle est aussi de publier les résultats de travaux originaux, dans divers domaines, susceptibles d’apporter une contribution notable à la compréhension les spécificités de la transfusion en Afrique en vue de leur amélioration. Par la communication et le partage des informations scientifiques, le journal contribue donc en plusieurs langues à l’amélioration de la pratique transfusionnelle pour le bien du receveur de produits sanguins.

EXPLOITER LES DONNÉES DIFFUSÉES

C’est bien connu : savoir et prendre conscience c’est bien, mais agir en conséquence c’est mieux. Les données non exploitées, encore nombreuses, attendent une plus grande volonté de acteurs du domaine. Grâce aux données déjà disponibles, de vastes programmes supportés par les gouvernements et les organisations partenaires ont vu le jour. Elles ont également servies à l’évaluation de l’impact de cette action.

Trouver, diffuser ou/et exploiter, nous choisissons notre contribution à l’amélioration de la transfusion sanguine sur notre continent. Sans modération.

RÉFÉRENCES

MEETING STABILITY AND CONTINUITY OF THE BLOOD SUPPLY OVER THE YEAR: A unique approach combining the liaison role of school teachers with the establishment of community resource persons - could the Uganda experience serve as an example for other African countries?

STABILITE ET CONTINUITE DE L’APPROVISIONNEMENT EN SANG AU COURS DE L’ANNEE: Une approche unique combinant le rôle de liaison des enseignants d’école avec l’établissement des personnes ressources de la communauté - l’expérience de l’oUganda peut-elle servir d’exemple pour les autres pays Africains?

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ABBREVIATIONS
AfSBT: Africa Society for Blood Transfusion;
UBTS: Uganda Blood Transfusion Service;
URCS: Uganda Red Cross Society;
BDR: Uganda Blood Donor Recruiters;
CRP: Community Resource Person;
IEC: Information, Education, Communication;
BD: Blood Donation;
M&E: Monitoring & Evaluation;
SCS: Sanquin Consulting Services, the Netherlands;
MoU: Memorandum of Understanding;
PEPFAR: President’s Emergency Plan for AIDS Relief. 5 year project funding from the USA government to selected African countries in the period 2005-2010

BACKGROUND
At present secondary schools and their students are an important source of voluntary blood donors in a substantial number of African and other countries, for reasons of safety and accessibility1,2. In a recent publication3 in a previous issue of Africa Sanguine, official journal of the AfSBT, the authors described a nationwide assessment among school teachers in Uganda assessing teachers’ knowledge, attitudes, motivation on BD issues, organisation of BD sessions, and the performance practice of mobile collection teams visiting schools (M&E).

The results of the assessment were evaluated in a workshop for BDR, and the staff of UBTS and URCS. This was then followed by a further evaluation in four Ugandan regions. BDR, the regional staff of UBTS and URCS, together with invited teachers from regional schools that had participated in the initial assessment were participants in these workshops.

The results of the assessment and evaluation workshops showed great commitment and motivation of teachers towards the practice of BD by secondary school students. In general there is a very positive attitude towards BD. The teachers were eager to receive correct information about BD practises and showed their will to participate in workshops organised by UBTS and URCS to become more educated about BD.

A number of practical steps were taken to sustain the interest and commitment of teachers involved. One of the conclusions derived from the assessment and evaluation workshops was that in order to be able to improve the organisation of this communication process between the stakeholders it is necessary to build a strong partnership between teachers and UBTS and URCS. This should be done by organising a ‘Teachers Liaison’ and related Task Force3.
CONTEXT

Les établissements scolaires secondaires actuels et leurs étudiants sont une source importante des donneurs volontaires de sang dans un nombre substantiel de pays africains et autres, pour des raisons de sécurité et d’accessibilité. Dans une publication récente, le Journal officiel de la Société Africaine de Transfusion Sanguine, les auteurs ont décrit une évaluation nationale faite parmi des enseignants d’établissements scolaires en Ouganda, analysant leurs connaissances, attitudes, et motivation sur des questions de don de sang (DS), l’organisation des sessions de don de sang, et la pratique des équipes de collecte mobile visitant les écoles.

Les résultats de cette évaluation ont été analysés dans un atelier pour les recruteurs de donneurs de sang (RDS), du personnel de l’UBTS et de l’URCS. Ceci a été suivi d’une autre évaluation dans quatre régions ougandaises. Les RDS, le personnel régional de l’UBTS et de l’URCS, ainsi que les enseignants invités des écoles régionales qui avaient participés à l’évaluation initiale étaient des participants à ces ateliers.

NEW APPROACH AND CHALLENGES

In the past, UBTS and URCS tended to concentrate their efforts on schools as the strongest blood donor source. This resulted in a problem for continuity in meeting hospital blood demands for transfusion in Uganda, due to the fall in blood collections during school holidays. As a consequence a joint concept ‘Creating Community Awareness through Community Resource Persons - CRP’ was developed by the URCS / UBTS with PEPFAR funding and technical support from SCS. The idea is to build on the results of the school teacher assessment (survey) and the already realised Teachers Liaison and related Task Force.

The initial setup of this project was that for its realisation it should divided in three phases.

FIRST PHASE

The first phase was a pilot project. In this pilot the goal was to increase the number of blood collections. This was supported by the development of a MoU. According to this MoU the School Teachers act as UBTS/URCS Community Resource Persons (CRP) in their own Community.

- Pilot Area
  
  Hoima sub region was selected based on existing good relationships and structures already built by the BDRC with the school teachers in that region. At that time our desire to implement CRP in due time and preferably country wide, strongly influenced the decision to pilot in the three districts of Hoima collection centres of Kibale, Masindi and Hoima.

- Mission Dates
  
  The CRP awareness project was initiated in three planned teachers association Task Force meetings organised in 2010 in Kibale (14-17 Feb), Hoima (18-21 Feb), and Masindi (22-25 Feb). The meetings involved 20 participants each.

- Objectives of the Meeting
  
  This was a plenary meeting for schools teachers, where UBTS and URCS informed all stakeholders about the CRP awareness approach in order to form a strong working group from among the stakeholders.

Specific objectives included:

a) To define to the participants the MoU between UBTS and URCS and use it as a stepping stone for the MoU with teachers.

b) To share findings of the URCS/UBTS assessment from 2006 with the teachers and the key stakeholder of the CRP awareness project.

c) To share the URCS/UBTS implementation plans with the stakeholders of the CRP awareness project.

d) To gain support and commitment of the CRP/leaders on the importance of donating blood.

e) To form small working groups to draft the MoU between URCS/UBTS and the CRP (Teachers) that will be further discussed in the 2nd phase after participants had agreed with continuation of the CRP awareness project.

MEETING DISCUSSION AND AGREEMENT

- The MOU between UBTS and URCS was discussed with participants. The participants were asked to use the good working arrangement between the UBTS and URCS (MoU) to forge a third line of partnership with the UBTS/URCS.

- URCS/UBTS teachers’ assessment findings: These URCS/UBTS - SCS assessment findings from the teachers were discussed with the key stakeholders who were invited to the CRP awareness meeting. The attendees agreed that the findings were true and representative of what they themselves thought about blood donation in the different districts. These aspects were published in the recent article. The participants henceforth agreed and pledged their support for the programme and agreed that they would be available for this proposed project.

- URCS/UBTS implementation plans: The participants were told that the meeting was a plenary meeting of schools teachers; UBTS and URCS arranged to inform the teachers present about the CRP awareness project being proposed to be implemented in partnership with all of them. Background information as provided, was to ensure that a sustained partnership was formed and formalised in a way that would help to mobilise blood donors and ensure availability of blood around the country. A strong working group with a high level of commitment was to be formed by the end of the meeting to discuss four major areas that would be further discussed in the second phase plenary meeting proposed later in 2010.
SECOND PHASE

- The second phase was implemented and coincided with the Club 25 implementation launched in September 2010.
- Feedback given to SCS, which has been supporting the project, involved the need for speedy response and action.

PILOT AREA

Once again, the pilot area was in Hoima Sub region where all parties work in synergy i.e. teachers, local leaders, health workers and elders in the region. The region consists of the three districts of Hoima collection centres of Kibale, and Masindi.

MISSION DATES

The CRP project awareness second stage was implemented from 30 August to 1 September 2010 and participants were members that came from the three districts named.

OBJECTIVES OF THE MEETING

This was a meeting for the foundation for implementation of the school teachers’ liaison; local leaders, health workers, UBTS and URCS synergy to develop a framework and draft the MOUs for discussion and adoption by all stakeholders. This formed the basis for strengthening and formalising the working approaches purposefully to achieve the requirements of the CRP.

SECOND PHASE ACHIEVEMENTS

a) A drafted MOU between UBTS / URCS and CRP was duly adopted for implementation
b) All assessment findings of 2006 with teachers and key people involved in the CRP awareness project were shared and discussed.
c) Implementation plans were shared with stakeholders of the CRP awareness project and roles/activities defined
d) The CRP/leaders’ full support and involvement in promoting the importance and role of donating blood was achieved by the adoption of the MoU.
e) Sensitization and mobilization of community members has improved in the region as a result of the CRP efforts taking place.
f) The CRP has already registered a remarkable improvement in collection of safe blood within the region from the previous target of 760 units per month to over 1000 safe units of blood a month.

SPECIFIC AND AGREED STAKEHOLDER ROLES/ACTIVITIES

a) Formation of blood donor clubs within the schools and communities
b) Training of student leaders, youth leaders and community on matters of UBTS and URCS
c) Monitoring and evaluation of CRP activities
d) Locate suitable venues for blood donation
e) Organizing tailored and appealing blood donation programmes such as plays, drama, blood donation songs
f) Engage various forms of communication i.e. TV/Radio talk shows, debates, drama, newspaper advertisements.
KEY EMERGING ISSUES TO CRP IMPLEMENTATION

CRP members appealed for operational funds to cater for transport and meals while in the field.

a) Members requested that there should be provision of appropriate transport i.e. bicycles and or motorcycles if possible to move with ease within the communities and further requested for clear identification as CRP officials within their respective communities

b) CRP members also requested a clear link between UBTS/URCS through provision of telephones to aid mobilisation and giving prompt feedback

c) CRPs requested UBTS/URCS management to formally link project members and government for recognition during implementation of the project objectives; this preferably by provision of official introductory letters.

d) Members requested provision of IEC materials, mega Phones, blood donor dissemination charts and facilitation whenever CRP members and community meetings are to be held

CHALLENGES OF THE CRP IMPLEMENTATION

a) The CRP project was initially financed by the UBTS technical assistance provider SCS (PEPFAR I funded the no-cost extension ended September 2010). This presented a big challenge to the CRP project since not all stages could be completed by members of the CRP who were learning to perform their roles in line with the MOU.

b) Publicity and advocacy to champion the objectives of the CRP is still very low and needs resources.

c) The CRP promoter/champion SCS support services came to an end.

d) There is need to translate the present IEC materials into all the local languages to gain full support of community members while educating the public on the importance of donating blood to save the many lives of those in need.

e) The project lacks transport for the core initiators UBTS/URCS. Transport would help officials to monitor all the activities of CRP in the pilot area and when it comes to implement the approach in other regions.

WAY FORWARD

a) There is a need to secure financial support for CRP in order to guarantee continuity

b) There is a need for continuation of technical support from SCS, which initiated and assisted this project in Uganda from the start to the second phase of implementation.

c) In the near future there is a possible need to share and learn good practices of the pilot CRP blood donor mobilisation approach in Uganda with other countries that may gain interest in this new development.

d) There is need for UBTS/URCS to suggest a comprehensive fundable proposal preferably with technical support from SCS, to be made available to donors for funding to help achieve the long term sustainable CRP project objectives.

CONCLUSION

This project has already generated great enthusiasm amongst the community members and all stakeholders to the CRP. So far the implementation to Phase II has been successful in this pilot project. The authors are pleased to mention that all stakeholders are very committed to this CRP project and in Hoima pilot area collections from voluntary donors have more than tripled since the first meeting.

For sustainability and evaluation of previous findings and experience, a next phase (III) of the pilot needs to be implemented, that subsequently can be followed by the roll-out of the CRP approach in other regions and collection centres in Uganda. The authors expect that successful implementation and embedding will take a long time to transform blood donor recruitment practices in the whole of Uganda.

The technical assistance of SCS for the project is very much appreciated, and the authors hope that they will be able to continue their involvement in the project.

The MoU is available for interested readers on request. At a later date the authors will publish the trend of blood collections to assess whether or not they can justify the role of CRP.

ACKNOWLEDGEMENT

The authors thank the staff and co-workers of the UBTS and URCS for their efforts to make the assessment and related workshops a success.

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REFERENCES


POTASSIUM LOAD OF STORED BLOOD for intra-operative transfusion at the Lagos University Teaching Hospital, Nigeria

ABSTRACT

BACKGROUND

Storage of blood depletes adenine triphosphate (ATP) synthesis resulting in decreased potassium pump activity and leakage of intracellular potassium into plasma. Hyperkalaemia can occur resulting in cardiac arrhythmias. We determined the potassium content of stored blood for intra-operative transfusion and the effect if any on patients' serum potassium levels.

METHODS

All blood for intra-operative transfusion was prospectively studied and the age of the blood documented. The potassium content of the blood was estimated by flame photometry. The patient's potassium level was estimated pre-operatively and one hour after the completion of the blood transfusion.

RESULTS

Ninety-eight units of CPD-A blood were studied. The mean age was 9.47 ± 7.7 days (range 1-33) with 58.2% aged <1 week, 21.4% aged 1-2 weeks, 14.3% aged 2-3 weeks, 3.1% aged 3-4 weeks and 4-5 weeks respectively. The mean potassium content was 13.15 ± 8.16 mmol/L. This increased with each week of storage from 9.84 ± 4.05 mmol/L in the 1st week to 14.77 ± 6.85 mmol/L in the 2nd week, 15.28 ± 6.72 mmol/L in the 3rd week, 41.77 ± 9.33 mmol/L in the 4th week and 26.16 ± 8.66 mmol/L in the 5th week of storage.

There were non-significant changes in patient potassium levels after the blood transfusion (pre-operative 3.94 ± 0.51, post-transfusion 4.23 ± 0.68, p=0.09).

CONCLUSION

There was substantial potassium load in stored blood transfused during surgery which increased with age of storage. This may be of consequence in critically ill patients or when massive blood transfusion is given.

KEYWORDS:

Intra-operative, blood transfusion, potassium level

MOTS-CLÉS

Intra-opératoire, transfusion sanguine, taux de potassium

CHARGE POTASSIQUE DANS LE SANG CONSERVE pour la transfusion intra-opératoire au Centre Hospitalier Universitaire de Lagos, Nigeria

RÉSUMÉ

CONTEXTE

La conservation du sang réduit la synthèse de l’adénine triphosphate (ATP), ce qui résulte à la diminution de l’activité de la pompe potassique et la fuite du potassium intracellulaire vers le plasma. L’hyperkaliémie peut survenir et résulter à des arythmies cardiaques. Nous avons déterminé la quantité de potassium dans le sang conservé et destiné à la transfusion intra-opératoire et les effets, s’il y en a, sur le taux sérique de potassium chez les patients.

MÉTHODES

Tous les produits sanguins destinés à la transfusion intra-opératoire ont été étudiés et leurs âges documentés. La quantité de potassium dans le sang a été estimée par la photométrie de flamme. Le taux de potassium des patients a été estimé en pré-opératoire et une heure après la fin de la transfusion sanguine.

RÉSULTATS

Quatre vingt dix-huit unités de sang prélevées sur CPD-A ont été étudiées. L’âge moyen a été de 9,47 ± 7,7 jours (variant de 1 à 33 jours) avec 58,2% âgés < 1 semaine, 21,4% âgés de 1 à 2 semaines, 14,3% âgés de 2 à 3 semaines, 3,1% âgés de 3 à 4 semaines et de 4 à 5 semaines respectivement. Le taux moyen de potassium était de 13,15 ± 8,16 mmol/L. Ce taux a augmenté au cours de chaque semaine de conservation, de 9,84 ± 4,05 mmol/L dans la 1ère semaine à 14,77 ± 6,85 mmol/L dans la 2ème semaine, 15,28 ± 6,72 mmol/L dans la 3ème semaine, 41,77 ± 9,33 mmol/L dans la 4ème semaine et 26,16 ± 8,66 mmol/L dans la 5ème semaine de conservation. Il n’y avait pas de changement significatif du taux de potassium du patient après la transfusion sanguine (pré-opératoire : 3,94 ± 0,51, post-transfusionnel : 4,23 ± 0,68, p=0,09).

CONCLUSION

Il y avait une charge potassique substantielle dans le sang conservé et transfusé au cours de la chirurgie, laquelle charge augmentait avec l’âge du sang conservé. Ceci peut avoir une conséquence chez des patients en phase critique ou lorsque une transfusion sanguine massive est effectuée.
INTRODUCTION

Moderate to major surgery are usually attended with significant blood loss. Blood transfusions are often required to maintain blood volume and preserve oxygen carrying capacity. Stored whole blood has numerous complications one of which is hyperkalaemia. As storage progresses there is a fall in ATP synthesis leading to decrease energy-dependant potassium pump and leakage of intracellular potassium into plasma. A considerable concentration gradient exists between intra-cellular potassium (100-150 mmol/L) and plasma potassium (3.5-5.5 mmol/L) which encourages diffusion of potassium out of the red blood cells. The amount of potassium in banked blood is directly proportional to its duration of storage with levels in excess of 30 mmol/L documented in blood more than two weeks old. Ordinarily this potassium load should be re-distributed into the intra-cellular space within 30 minutes with minimal effect on the recipient. When this physiological response is overwhelmed, the resulting hyperkalaemia leads to increased resting membrane potential and cardiac arrest.

The majority of cases of hyperkalaemia have been documented after massive blood transfusion of stored blood or in neonates and children. Rapid blood transfusion of a moderate volume of blood at a rate in excess of 0.4 ml/kg/min and the use of a pressure infuser device have also been reported to result in significant hyperkalaemia and cardiac arrest. The contribution of blood filters and blood warmers have not been consistent.

Our hypothesis is that the potassium level would increase as time of storage of blood increased.

This study therefore set out to determine the magnitude of increase in potassium with increasing storage of blood provided for intra-operative blood transfusion at the Lagos University Teaching Hospital and to investigate whether this significantly affected the patients’ immediate post-transfusion potassium level.

PATIENTS AND METHODS

The study was conducted at Lagos University Teaching Hospital, Lagos, Nigeria between March and October 2010 after Institutional Health Research and Ethics Committee approval and patient informed consent. Our hospital is a 760-bed tertiary hospital with eight operating theatres suites providing services for all major surgical specialties including neurosurgery, maxillo-facial and plastic and reconstructive surgery. The hospital blood bank has a capacity for storage of 750 units of blood. An estimated 10 000 units of blood are collected and screened annually. Blood is issued by the blood bank as whole blood but recently facilities have been made available for blood component separation into packed red cells, fresh frozen plasma and platelet concentrate.

All patients for elective surgery had pre-operative plasma potassium estimated 24 hours before surgery as part of the routine pre-operative investigations. During the intra-operative period, the patients were then recruited into the study after the decision to transfuse had been made by the attending anaesthetist and demographic data was obtained. All patients on medications known to alter potassium were excluded.

The blood for transfusion was run through a blood giving set (Dana blood transfusion set, Shanghai Kindly Enterprise Development Group Ltd, Shanghai, China) and 5 ml blood run into a plain (no anticoagulant) sample bottle for potassium estimation from the distal end of the blood transfusion line before going through any warming or pressure devices. A patient sample obtained using the standard method of venepuncture from a forearm/ hand vein one hour after completion of transfusion was put in a lithium heparin bottle. All blood samples were immediately transported to the laboratory taking care to avoid unnecessary agitation. Upon receipt, the sample was immediately centrifuged to prevent lysis of the red cells. Potassium estimation was determined within one hour of receipt of the sample using the flame photometry method (SEAC Semi-automated flame photometer FP 20, Biotecnica, Italy) which involves the principle of nebulisation of a test solution into a flame which vaporizes and excites the atoms causing the release of photons at wavelengths measured by the photodetector.

The number of units of blood transfused, rate of transfusion, age and storage medium of the blood and blood potassium level were documented. The ECG was monitored continuously to identify any ECG changes during transfusion.

STATISTICAL METHODS

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 15.0. Numerical data was expressed as mean ±SD and categorical data as frequencies. Students’ t-test was used to compare potassium content at different ages of storage while comparison of pre and post-transfusion potassium levels was achieved using the paired t-test. A p-value <0.05 was taken as statistically significant.

RESULTS

Fifty patients were studied with a mean age of 34.35 ± 22.66 years (range of 0.25 to 75 years). Fifty-six percent of the patients were females. Table 1 indicates the various specialties for which surgery was performed.

Ninety-eight units of CPD-A whole blood were transfused with a median of 2 units. Twelve paediatric patients (24%) had less than one unit of blood transfused while one patient (2%) received ten units of blood. The mean duration of transfusion was 49.92 ± 19.59 minutes (range of 15 to 90 minutes).

The mean pre-operative potassium level of all patients was 3.94 ± 0.51 mmol/L (range 3.2 to 5.10). After transfusion, the mean post-operative potassium level increased to 4.23 ± 0.68 mmol/L (range 2.90 - 5.10). This difference was not statistically significant (p=0.09). Four patients recorded a fall in potassium after transfusion.

The mean age of all the blood transfused was 9.47 ± 7.7 days (range 1-33) with 57 units (58.2%) aged one week, 21 (21.4%) aged between one to two weeks, 14 (14.3%) aged between two to three weeks, three (3.1%) aged between three to four weeks and three (3.1%) aged between four to five weeks. (Figure 1)
The mean potassium content of all the blood studied was 13.15 ± 8.16 mmol/L (range of 4.8-48). For each week of storage, the mean potassium content was 9.84 ± 4.05 mmol/L in the first week (range 4.8-23.4), 14.77 ± 6.85 mmol/L in the second week (range 4.8-30.4), 15.28 ± 6.72 mmol/L in the third week (range 7.4-25.4), 41.77 ± 9.33 mmol/L in the fourth week (range 31-48) and 26.16 ± 8.66 mmol/L in the fifth week (range 18.39-35.4). The mean potassium level was significantly higher with each week of storage compared to the level obtained on blood in the first week of storage. (Figure 2). Statistical significance however was not determined with blood aged 4 and 5 weeks due to small sample size.

![Figure 2: Potassium content of transfused blood](image)

At second and third weeks of storage, mean potassium differed significantly compared to first week of storage.

**DISCUSSION**

Our study has shown that the potassium content of stored blood at the Lagos University Teaching Hospital increases with age of storage and that significant increases occur after the first week of storage. Mean values of potassium obtained as storage progressed is in agreement with the wide range reported in the literature. Though Carvalho and Smith reported much higher values with SAG-M stored blood and packed red blood cells (RBC) and Robinson did not demonstrate any significant relationship between duration of storage and potassium levels, most authors agree that the potassium values have a wide variability during storage. This makes comparison between studies difficult.

We observed a non-significant rise in serum potassium to a mean of 4.23 ± 0.68 mmol/L one hour after completion of the blood transfusion. Other workers have also reported non-significant changes in patient potassium level using a blood gas analyser. This may be because the potassium load is normally re-distributed into the intra-cellular space within 30 minutes with minimal effect on the recipient unless low-cardiac output states occur. Carvalho reported intra-operative potassium values of 9.5 mmol/L which decreased to 6 mmol/L one hour post-transfusion and 5.1 mmol/L after three hours. We were unable to determine intra-operative potassium levels in our study due to the non-availability of a blood gas analyser. The risk of development of hyperkalaemia during blood transfusion depends on the potassium concentration of the transfused blood and the volume and rate of transfusion; being greater with massive blood transfusion. Other researchers have quoted a rate of blood transfusion more than 0.4 ml/kg/min or greater than 120 ml/min1 to be associated with the development of hyperkalaemia and the possibility of a cardiac arrest. We had a mean transfusion duration of 49.92 minutes which equates to 10 ml/min in our patients. This relatively slow rate of transfusion is likely to be responsible for the lack of hyperkalaemia seen in our patients. The extent of tissue trauma during surgery has also been implicated. Other influencing factors include circulating blood volume, cardiac output and acid-base status as well as the site of administration of blood with administration through the internal jugular vein more likely to result in cardiac complications.

In our institution, blood for transfusion is most often whole blood compared to packed RBC as documented in literature. Michael et al observed that stored whole blood (SWB) and stored packed cells (SPC) had similar rate of increase in potassium levels though SWB had an overall greater level than SPC. They documented that packed RBC prepared from stored whole blood had the least concentration of potassium and recommended that removal of supernatant plasma from SWB just before transfusion, may help lower potassium administered. We had a mean potassium concentration of 9.47 mmol/L with a range of 4.8-23.4 mmol/L at one week of storage compared with mean values of 20.5 mmol/L and 19 mmol/L obtained from packed RBC at similar storage time. Brown suggested that potassium diffusion out of cells plateaus at 20 days of storage implying that there is a limit to the value that potassium may rise to during storage. We also demonstrated a steady increase in potassium levels up to three weeks of storage. With only a few units of blood aged four and five weeks amongst our sample population, probably due to dispensing policies of our blood bank, statistical comparison at these ages of storage is therefore unreliable.

No ECG changes of hyperkalaemia were observed amongst our patients. As potassium levels increase, the most common initial observation in the ECG is the appearance of peaked T waves. This can be erroneously counted as QRS complexes by the ECG resulting in an ECG rate double that of the pulse rate. This emphasises the importance of standard intra-operative monitoring throughout any anaesthetic and especially if blood is being transfused. Other ECG changes that may occur include widened QRS complex, prolonged PR interval, loss of P wave, loss of R wave amplitude, ST depression progressing to ventricular fibrillation or asystole.

Known methods of reducing potassium load during transfusion include pre-operative washing of the red cells, intra-operative washing of red cells using cell salvage equipment and the use of in-line potassium filters.

One limitation of this study was that the age of the blood was grouped into weeks as opposed to days and it could be that some of the blood units were nearer one end of the time frame than others. This may result in some units having greater potassium content which would affect the mean of that group. Also it would have been ideal to insert an arterial cannula and perform potassium estimation by blood gas analyser. This would have enabled us to more frequent potassium estimations as well as estimate the influence if any of acid-base disturbances on the post-transfusion levels. Unfortunately, this technology is not available in our hospital. Though all our surgeries performed were either intermediate or major surgery, this study did not investigate the influence of the invasiveness of surgery and its attendant tissue trauma on the patient's post-transfusion potassium levels. Notwithstanding, the mean post-transfusion potassium level did not differ significantly from the pre-operative values.
CONCLUSION

From our study we can conclude that whole blood supplied by our blood bank for intra-operative transfusion contain a significant amount of potassium above the physiological range and that this increases with the duration of storage. This however did not translate into significant potassium changes amongst our elective surgical patients. It is however recommended that when massive blood transfusion is anticipated, effort should be made to obtain fresh blood in order to minimise the risks of hyperkalaemia. All blood transfusions should also be performed under standard intra-operative monitoring.

Conflict of Interest Statement
I declare that there is no financial or personal relationship(s) which may have inappropriately influenced the writing of this paper.

Table 1: Surgical specialties of patients who had blood transfusion

<table>
<thead>
<tr>
<th>Surgical Specialty</th>
<th>No. of patients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Surgery</td>
<td>12</td>
<td>(24%)</td>
</tr>
<tr>
<td>Maxilla-facial</td>
<td>5</td>
<td>(10%)</td>
</tr>
<tr>
<td>Orthopaedics/ Trauma</td>
<td>6</td>
<td>(12%)</td>
</tr>
<tr>
<td>Plastics&amp; Reconstructive</td>
<td>8</td>
<td>(16%)</td>
</tr>
<tr>
<td>Gynaecology</td>
<td>6</td>
<td>(12%)</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>3</td>
<td>(6%)</td>
</tr>
<tr>
<td>Urology</td>
<td>2</td>
<td>(4%)</td>
</tr>
<tr>
<td>Paediatric Surgery</td>
<td>5</td>
<td>(10%)</td>
</tr>
<tr>
<td>Obstetrics</td>
<td>3</td>
<td>(6%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>50</strong></td>
<td><strong>(100%)</strong></td>
</tr>
</tbody>
</table>

REFERENCES

MCS+ Apheresis System
Platelet collection
Red Cell collection
Plasma collection

QWALYS® 3
High performance automated blood grouping system
FREELYS® Mini lab
Compact blood grouping workstation

Cell Saver 5+
Autologous Blood Recovery System

Tubing Sealers
Sterile Connection Devices
Tubing Strippers

Blood Collection Bags
Leukodepletion filters
Blood processing equipment
Pathogen Inactivation Systems

Blood Collection and Processing
Electronic Plasma Expressors
Blood Collection Mixers

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BLOOD SAFETY AND ADEQUACY IN MAURITIUS: Knowledge, Attitude, and Practice (KAP) Survey in a small island nation

SECURITE TRANSFUSIONNELLE ET ADEQUATION DU SANG A L’ILE MAURICE : Enquete CAP (Connaissance, Attitude et Pratique) dans une petite nation insulaire

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KEYWORDS:
Mauritius, KAP, Africa, blood safety, blood donation, small island nation

MOTS-CLÉS
Ile Maurice, CAP, Afrique, Sécurité transfusionnelle, don de sang, petite nation insulaire

ABSTRACT

BACKGROUND AND OBJECTIVES
Reorientation of Mauritian economic activities into medical tourism has caused a drastic increase in the demand for safe blood and blood products11. A widespread Knowledge, Attitude, and Practice (KAP) sample survey provides the clues on how to expand, and retain the pool of regular, voluntary blood donors9. The main focus was to increase safety and adequacy of blood, maximize efficiency of donor recruitment programmes13 and provide empirical demographic data about Mauritian donors and non-donors.

MATERIALS AND METHODS
A randomized, cross-sectional study was carried out on a population of 200 blood donors and 200 non-donors, who were approached in several regions of the island. A KAP questionnaire, adapted to the Mauritian practices, was designed, pre-tested, and used to harvest information from the population.

RESULTS
It has been found that males represent 82% of Mauritian blood donors while non-donors are predominantly young female adults with disproportionate fear of the phlebotomy process. Socio-demographic profile, ethnicity, socio-economic profile, awareness, and knowledge are the 5 principal factors determining willingness to donate blood. Altruism was lacking in the population sample.

CONCLUSION
Ageing of blood donors, in tandem with misconceptions from these subjects represent a direct threat to blood safety. Targeting young adults, an untapped pool of blood donors, may be the answer to improved safety, and long-term sustainability of a safe blood supply in Mauritius.

RÉSUMÉ

CONTEXTE ET OBJECTIFS
La réorientation des activités économiques mauriciennes vers le tourisme médical a causé une augmentation drastique de la demande de sang et de produits sanguins sûrs. Une enquête CAP (Connaissance, Attitude et Pratique) élargie fournit des indices sur la manière d’augmenter et de retenir un pool de donneurs de sang volontaires et réguliers. Le principal objectif de l’enquête a été d’améliorer la sécurité et l’adéquation du sang, maximiser les programmes de recrutement des donneurs et disposer des données démographiques empiriques sur les donneurs et non-donneurs mauriciens.

MATÉRIELS ET MÉTHODES
Une étude transversale, randomisée a été réalisée sur une population de 200 donneurs de sang et 200 non -donneurs approchés dans plusieurs régions de l’île. Un questionnaire CAP, adapté aux pratiques des mauriciens, a été élaboré, pré-testé et utilisé pour récolter des informations auprès de la population.

RÉSULTATS
Les résultats révèlent que les personnes de sexe masculin représentent 82% des donneurs de sang mauricien alors que les non-donneurs sont majoritairement les jeunes femmes adultes ayant une peur disproportionnée du processus de phlébotomie. Le profil démographique, l’appartenance ethnique, le profil socio-économique, la prise de conscience, et la connaissance sont les 5 principaux facteurs qui influencent la volonté de donner du sang. L’altruisme manquait dans cet échantillon de la population.

CONCLUSION
Le vieillissement des donneurs de sang couplé à la méconnaissance du don de sang par ces sujets représentent une menace directe à la sécurité transfusionnelle. Cibler les jeunes adultes, un pool inexploité de donneurs de sang, pourrait être la solution pour l’amélioration de la sécurité et la pérennisation sur le long terme d’un approvisionnement en sang sûr dans l’île Maurice.
INTRODUCTION

Blood transfusion, or the transfusion of its component parts, is a well-established and important medical intervention. Irrespective of the standard of the health care system, therapeutic transfusion of blood finds its use where compensatory blood infusion is vital to sustain life. The success of blood transfusion therapy has fuelled an incessant drive in demand for blood and blood products. Ageing population, increasing complexity of novel surgical procedures, as well as increase in disease burden all augment the need for blood.

Meeting the need for blood has become increasingly challenging due to the risk of contamination by transfusion transmissible Infections (TTIs). Safe blood and blood products must reach the patient timeously. This requires the input of significant resources and logistics. Maintaining an equitable and sufficient supply of safe blood is therefore an ongoing challenge.

Blood safety and adequacy is a growing cause for concern for Small Island Developing States (SIDS). Mauritius, being a geographically remote island off the eastern coast of Madagascar, is challenged to be both self-sufficient in providing an adequate supply of safe blood to its 1.2 million inhabitants and to cater for the local emerging medical tourism industry. Indeed, in 2008, an economical and medical reorientation was announced in the national budget speech. Medical tourism was already a priority for Mauritian government, and represented an investment of more than MUR 2 billion in a sophisticated private medical facility. A target influx of 1 million international tourists was also set for the 2009-2010 period.

The Mauritian population has diverse ethnic origins and, by extension, diverse ethnic-specific illnesses. Thus, Mauritius has a major disease burden with regards to Non-Communicable Diseases (NCDs), namely Type 2 Diabetes, Hypertension, and the associated target organ pathologies. Global estimates for prevalence of diabetes for 2010 have ranked Mauritius in the top 5 countries, with a projected progression to the third place in 2030. NCDs represent a considerable economic burden for Mauritius. Due to scant natural resources, the Mauritian economy relies heavily on the local workforce, and NCDs, through absenteeism and loss of productivity, only hamper economic development. NCDs also drain considerable financial resources from the national budget since the local health care system is free and accessible to residents.

Over the years, Mauritius has witnessed a growing demand for blood and blood products linked to NCD complications, practice of novel medical interventions, and natural demographical ageing and expansion. The Mauritian National Blood Transfusion Service (NBTS) has been able to meet that demand. Recently, the NBTS has expressed its concern over blood safety as a result of the budget-related medical and economic changes. Increased prevalence of blood-borne diseases, increased intravenous drug use, changing norms and practices leading to normalization of high-risk behaviours e.g. unprotected sex, have all increased the threat to blood safety. With the setting up of a local medical tourism hub, Mauritius saw its number of acute hospital beds increase. This generated greater demands for blood and blood products while raising expectations in terms of the quality of reliable blood products. This caused the NBTS to function under considerable strain as it was compelled to supply a greater volume of blood whilst maintaining the integrity of its quality control chain.

Due to the vulnerability of Mauritius, the island has the compounded responsibility to equip its health care system with the tools to respond to periods of acute needs. Mauritius is not insulated from an eventual force majeure, since risks of tsunamis secondary to earthquakes in the South-East Asian region are real. It is understood that meeting demands for blood products is central to sustain such a disaster recovery scheme.

Projections for 2012 estimate a blood deficit of 33% using the number of acute beds as a parameter. However, more active private and public renal dialysis procedures, haematology and oncology departments, compounded by an increase in disease burden, higher risks of epidemics related to travel and tourism, and the rise of the middle class to access high-end private medical care shall all favour more blood demand. Thus, the projected deficit of 33% is thought to be an underestimate.

It became evident that the NBTS had to increase both the adequacy and safety of its blood supply in both the short and long term due to added responsibilities. The Mauritian NBTS has always relied on the goodwill, and voluntarism, within the local population to meet the demand for blood. However, the need for a shift from passive recruitment to a more proactive stance is more acutely needed to remedy the predicted blood deficit. Profiling of the blood donors and non-donor, and a proper knowledge of the positive and negative motivational factors is important in increasing recruitment and retention of blood donors. Understanding these dynamics not only empowers the NBTS with more material to orient its focus, but also improves understanding of the financial impact of these influences on the production of a safe unit of blood. Hence a Knowledge, Attitude and Practice (KAP) study within the local context is relevant.

MATERIALS AND METHODS

Research Method

A randomized, cross-sectional sample survey study design was used to evaluate differences between blood donors and non-donors. The study was from May 2008 to February 2009, with data collection lasting over a 2-month period from December 2008 to January 2009.

Ethical Clearance

The study was awarded ethical clearance without amendment by the Ethical Committee of the Ministry of Health and Quality of Life (MoHQL) of the Government of Mauritius. The MoHQL Ethical Committee uses the Declaration of Helsinki (1964) as a blueprint during their exercise. The study received the full support of the Mauritian NBTS in collaboration with the Department of Medicine from the University of Mauritius.

Research population

Participants in the study were residents of Mauritius with Mauritian nationality. Subjects had to meet the donor-eligible range of 18 to 65 years. Younger blood donors, who need authorisation from their guardians, were excluded from the study. Since the study had a descriptive basis, a sample of 200 blood donors and 200 non-donors was found to be adequate. Blood donors represented the NBTS database of 35,000 blood donors (end of 2008). Non-donors fulfilled similar nationality and age criteria for comparative reasons.
Survey Instrument
Due to the multi-ethnic and multi-cultural background of the Mauritian population, a questionnaire relating to the diversity of local beliefs and practices had to be designed. This was done only after different opinions were sought on the topic of blood donation and blood safety. This process was carried over a month and involved individuals from diverse background and fields of expertise. Following this consultative process, a draft survey instrument was prepared with technical support from the NBTS. This questionnaire was pre-tested using a sample of 10 blood donors and 10 non-donors from various age groups. Their feedback on the data collection process as well as their thoughts on the questionnaire was noted and the questionnaire modified after discussions. The equivalent translation of each question was standardized in Mauritian Creole - the local dialect - so as to improve understanding for individuals with little or no education with the help of trained research assistants.

The final survey instrument was a 3-page questionnaire with standardised checkbox style questions which required approximately 20 minutes for completion. The questionnaire was anonymous, hence, no subject identifiers was sought. It consisted of six sections:
1. Information clause and consent
2. Personal details
3. Knowledge
4. Blood safety
5. Attitudes and practices
6. Willingness to give blood

Personal information included demographical data like gender, ethnicity, and education. Knowledge and blood safety assessed the knowledge of the individual on aspects of blood donation. Responses to these questions were transformed so that correct answers were awarded a higher score. Attitudes and practices aimed at diagnosing misconceptions around the blood donation practice and determining the reasons which influence individuals to give blood or not. The last section had the primary purpose of determining motivational factors which might contribute towards increasing the pool of blood donors.

SURVEY

Quality control
Consultation with individuals from various backgrounds helped in minimizing the risk of experimental error and this ensured that the questionnaire targeted subjects from different ethnic background as well as levels of understanding. Pre-testing of the survey instrument in a small population and the feedback ensured that the flow of questions was appropriate. While we believed that the questionnaire was self-explicit, concerns over the misinterpretations and the length of the survey instrument was raised. Hence, it was decided that the help of trained research assistants was compulsory. Since data entry was done manually, proof-reading of the questionnaires by a second individual helped minimize typographical mistakes.

Homogeneity
People of different regions of the island, ethnicity, social status and other demographic variables converge to Port-Louis, the capital, for work. This confluence of population was tapped for the purpose of this study. Blood donors and non-donors were approached at a “Mega Blood Donation” event organized by the MoHQL in collaboration with the Blood Donor’s Association which groups people of diverse backgrounds. More participants from all the 9 districts were reached though mobile blood donation caravans.

Statistical analysis
Statistical Package for Social Sciences v.17.24 was used to perform basic descriptive analysis as well as inferential analysis. In the latter, the sample was tested for adequacy, normality, validity, and reliability. Further analysis yielded 5 main components influencing blood donor status. These 5 components were graded according to their level of importance.

RESULTS

Demographic characteristics
Table 1 provides details of the demographic characteristics of the population, including profile, gender, age, marital status, ethnic group, education, income and type of residence.

Profiling
The typical Mauritian blood donor (Table 1) is a 38-year-old male of Indian origin with average to high education and income while the typical non-donor is a 29-year-old single female of equal education and income. Blood donors scored better marks when tested about knowledge on blood donation than their non-donor counterparts. 74.5% of blood donors also obtained grade ‘B’ and above for knowledge on blood safety as compared to 67.4% of non-donors. When using ethnicity and residence as parameters, results were similar for both groups.

Positive motivational factors
Knowledge on blood donation and blood safety are clearly the first positive motivational factors noted among blood donors. 80.5% of blood donors are motivated by the will of “helping others”. 7% gave blood because of peer pressure, and 4.5% did so in order to test for their HIV status. 54.4% of regular blood donors admitted that they did so because of the “personal satisfaction” and “sense of responsibility” that they obtained post-donation. Also, 34.4% of regular blood donors listed “pride” while 31.2% listed “feel sorry for the sick” as their motivational factors. 44.4% of regular blood donors gave blood in order “to monitor my health”.

Altruism and incentives for blood donation
Altruism defined by Fernandez-Montoya (1997)8, as being “behaviour that promotes the well-being of others without consciously taking account of self interest”, is lacking among the pool of blood donors, regular or not. The scarcity of altruistic practice7 was further consolidated when the participants were queried about possible incentives for giving blood in the future3. While 55.5% of subjects would have liked to meet the recipients of their blood, up to 50.0% of them affirmed that issuing of certificates of donation would encourage them to give blood and 11.5% would want financial remuneration for blood donation5,6. However, both blood donors (73.5%) and non-donors (65.5%) listed “more information regarding blood donation” as being possible incentives for future donations. The conflicting nature of attitudes towards altruism was noted since the totality of the blood donors motivated by the will of “helping others” also wanted a token so as to further encourage them to give blood in the future. This initial diagnosis of lack of altruism needs to be tested using alternative methods since our questions were not screened for possible bias20.

It is worth noting that previous sensitisation to blood donation, e.g. a relative needing blood transfusion, did not seem to have an effect on blood donors giving blood more regularly. This refuted a hypothesis on whether regular blood donors are motivated by personal witness of the need for blood.
### Table 1: Characteristics of donors and non-donors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Blood donor</th>
<th>Non-donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage registered over total population (1.2m)</td>
<td>2.92 (35,000 registered blood donors)</td>
<td>97.08</td>
</tr>
<tr>
<td>Sample</td>
<td>200 (of which 80% regular blood donor)</td>
<td>200</td>
</tr>
<tr>
<td>Profile</td>
<td>38 years old married males of Indian origin with average to advanced education and average to high income.</td>
<td>29 years old single females of Indian origin with average to advanced education and average to high income.</td>
</tr>
<tr>
<td>Gender</td>
<td>$\sigma = 82%$ (164) $\varphi = 18%$ (36)</td>
<td>$\sigma = 35%$ (70) $\varphi = 65%$ (130)</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>18-30 = 32.5%</td>
<td>18-30 = 64.5%</td>
</tr>
<tr>
<td></td>
<td>31-45 = 40.0%</td>
<td>31-45 = 22.0%</td>
</tr>
<tr>
<td></td>
<td>46-60 = 27.5%</td>
<td>46-60 = 13.5%</td>
</tr>
<tr>
<td>Marital status “married”</td>
<td>74.5%</td>
<td>41.0%</td>
</tr>
<tr>
<td>Ethnic group*</td>
<td>Indian origin: 81.5% (163)</td>
<td>Indian origin: 78.0% (156)</td>
</tr>
<tr>
<td></td>
<td>African origin: 11.0% (22)</td>
<td>African origin: 9.5% (19)</td>
</tr>
<tr>
<td></td>
<td>European origin: 3.0% (6)</td>
<td>European origin: 2.5% (5)</td>
</tr>
<tr>
<td></td>
<td>Chinese origin: 1.0% (2)</td>
<td>Chinese origin: 0.5% (1)</td>
</tr>
<tr>
<td></td>
<td>Mixed origins: 3.5% (7)</td>
<td>Mixed origins: 9.5% (19)</td>
</tr>
<tr>
<td>Level of education</td>
<td>No academic education: 2.5% (5)</td>
<td>No academic education: 1.0% (2)</td>
</tr>
<tr>
<td></td>
<td>Primary education: 12.0% (24)</td>
<td>Primary education: 13.5% (27)</td>
</tr>
<tr>
<td></td>
<td>School Certificate: 31.5% (63)</td>
<td>School Certificate: 24.0% (48)</td>
</tr>
<tr>
<td></td>
<td>Higher School Cert: 23.0% (46)</td>
<td>Higher School Cert: 37.5% (75)</td>
</tr>
<tr>
<td></td>
<td>Certificate/Diploma: 9.5% (19)</td>
<td>Certificate/Diploma: 12.0% (24)</td>
</tr>
<tr>
<td></td>
<td>Degree: 21.5% (43)</td>
<td>Degree: 12.0% (24)</td>
</tr>
<tr>
<td>Net monthly income</td>
<td>Low (&lt;$300) 25.0% (50)</td>
<td>Low ($300) 34.0% (68)</td>
</tr>
<tr>
<td></td>
<td>Medium ($300-760) 51.5% (103)</td>
<td>Medium ($300-760) 46.0% (92)</td>
</tr>
<tr>
<td></td>
<td>High (&gt;760) 23.5% (47)</td>
<td>High (&gt;760) 20.0% (40)</td>
</tr>
<tr>
<td>Residence</td>
<td>Rural: 55.0% (110)</td>
<td>Rural: 55.5% (111)</td>
</tr>
<tr>
<td></td>
<td>Urban: 45.0% (90)</td>
<td>Urban: 44.5% (89)</td>
</tr>
</tbody>
</table>

(a) Ethnic distribution closely reflects the general population demographics of the Island of Mauritius.

### Negative motivational factors

Occasional and one-time blood donors listed “not having time” (37.5%), “not motivated anymore” (32.5%) and “no one asked me again” (25.0%) as the reasons for not giving blood regularly. No participant checked “Do not believe in saving lives” as a response.

46.5 % of non-blood donors believe that their health does not permit them to give blood while 42.5% and 24.5% reported being scared of the needle and blood respectively. 31.0% of non-donors reported that they were “never asked” to give blood. An average of 16.0% of non-donors also affirmed that they do not like the environment of the blood donation centre as well as not having enough free time from work as possible reasons for not giving blood.

### Misconceptions

A minority of participants believed in facts about blood donation that were not accurate (e.g. blood donation cleanses the mind). This had 2 consequences:

1. Positive misconceptions - promoted blood donation
2. Negative misconceptions - discouraged individuals from giving blood

### Inferential Analysis

The sample parameters passed statistical tests for adequacy, normality, validity and reliability. Rotated component matrix showed the following factors according to their decreasing loadings (degree of influence) - the grouped variables were named as shown in Table 2.

### Table 2: Factors affecting blood donation (in order of priority)

<table>
<thead>
<tr>
<th>Priority</th>
<th>Factor</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Socio-demographic profile</td>
<td>Age group, Marital status, Gender</td>
</tr>
<tr>
<td>2</td>
<td>Ethnicity</td>
<td>Ethnic belonging, Residential area</td>
</tr>
<tr>
<td>3</td>
<td>Socio-economic profile</td>
<td>Income, Level of education</td>
</tr>
<tr>
<td>4</td>
<td>Unawareness</td>
<td>Misconception among participants, Sensitization to blood donation</td>
</tr>
<tr>
<td>5</td>
<td>Knowledge</td>
<td>Knowledge on blood donation, Blood safety</td>
</tr>
</tbody>
</table>

The factors in Table 2 were found to influence blood donor status of the participants, and, in theory, could potentially be used to predict the tendency of a certain population to give blood if the dependent parameters are available.
DISCUSSION

Mauritian blood donors are predominantly married, responsible, and proud males in the late thirties, with an average schooling, and sound knowledge regarding blood donation. On the other hand, non-donors are single, equally educated, female young adults, who are scared of the needle, with an average knowledge on blood donation and who believe that they are not fit for blood donation. Ethnic belonging as well as income levels 4,5,6 do not influence blood donor status as individual, independent factors. 2.92% of blood donors for a small island with ambitions for medical tourism is low as compared to 4-6% in the US10. Female involvement in blood donation is disturbingly low. 80% of voluntary, non-remunerated blood donors form the bulk of the participants. Mauritian blood donors are benevolent rather than altruistic supporting arguments from Ferguson et al (2008)7.

Inferential analysis showed the following:

1. Socio-demographic profile
A rough marker of common behaviour for specific age groups, gender, and marital status was of importance in determining blood donor status. This suggests that multi-level approaches have to be used to tap into the potential blood donors for maximum effect.

2. Ethnicity
It is found that there is a strong influence of group behaviour in the Mauritian. This may be explained partly by the willingness to form part of the larger majority of people at a specific place and time or it could be due to involvement of socio-cultural organizations and religious groups during blood donation campaigns. This strongly correlates with the predominance of individuals of Indian descent forming the majority of the blood donor pool as well as the demographic majority.

3. Socio-economic profile
Improved literacy is synonymous to greater receptivity to sensitization campaigns. Also this might be a marker for social status, so that higher status would implicitly induce a greater debt and responsibility towards society.

4. Unawareness
The influence of inaccurate knowledge about blood donation (e.g. blood donation cleanses the mind) still prevails and are both a threat to blood donor status, and blood safety1.

5. Knowledge
More accurate knowledge on blood donation reinforces decision-making capacities. This not only promotes the sense of responsibility and loyalty, but also pushes the individual to overcome negative motivational factors like fear.

CONCLUSION

The existing Mauritian blood donor population (2.92% of general population) cannot fuel the demand for blood products of the existing health care system, cater for the increase in disease burden, support the ambition of an emerging medical tourism sector, and cushion the shock in the case of a natural calamity. Profiling the Mauritian population with respect to blood donation and blood safety empowers the NBTS21 with fresh data so as to allow for expansion of the blood donor pool. The focus of this process should be to tap into the segment of female young-adults whilst adopting the approach of group behaviour. This would not only increase the blood donor pool, but effectively combat the image that females are least likely to give blood because of “unfavourable health conditions”. Modern tools for disseminating information should be used to educate, convince, and retain blood donors. This is crucial for sustainability of the modern health care practice in Mauritius.

Acknowledgements
The authors acknowledge Mr Bhoooshansingh Gunesh, Mr Ramprakash Beeharly, Mr S Ramsamy, Mr Jeanoody, Mrs. B. Armstrong, and all individuals who contributed to this project.

REFERENCES


Additional reading


ISOLATED IgM ANTI-HBc ANTIBODY AND CURRENT ESTIMATES OF SOME INFECTIOUS DISEASE MARKERS among blood donors in the semi-arid region of Nigeria

ANTICORPS ISOLE IgM ANTI-HBc ET ESTIMATION ACTUELLE DE CERTAINS MARQUEURS DE MALADIES INFECTIEUSES PARMI les donneurs de sang de la région semi-ariade du Nigeria

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KEYWORDS
isolated anti-HBc-IgM, infectious diseases, hepatitis B virus, blood donors, Maiduguri

MOTS-CLÉS
Anticorps isolé anti-HBc-IgM, maladies infectieuses, virus de l’hépatite virale B, donneurs de sang, Maiduguri

ABSTRACT

BACKGROUND AND AIMS
Laboratory screening for the diagnosis of hepatitis B virus (HBV) infection in blood donors currently consists of testing for hepatitis B surface antigen (HBsAg) alone. The prevalence of isolated IgM anti-HBc is not yet known in the semi-arid region of Nigeria.

OBJECTIVES
To determine the seroprevalence of IgM antibody to hepatitis B core antigen (IgM anti-HBc) and other infectious agent markers like HBsAg, anti-HCV, anti-HIV-1/2 and syphilis tests among Maiduguri blood donors.

MATERIALS AND METHODS
In a cross sectional study from October 2010 to January 2011, 266 blood donors were tested for these infectious disease markers using standard enzyme linked immunosorbent assays (ELISA) procedures for IgM anti-HBc and rapid sandwich ELISA tests for HCV, HIV-1/2, HBsAg and syphilis as contained in the manufacturer’s standard operating procedures.

RÉSUMÉ

CONTEXTE ET BUTS
Le dépistage au laboratoire pour le diagnostic de l’infection par le virus de l’hépatite B (VHB) chez les donneurs de sang consiste couramment au seul test de recherche de l’antigène de surface du virus (AgHBs). La prévalence de l’anticorps isolé anti-HBc IgM n’est pas encore connue dans la région semi-ariade du Nigeria.

OBJECTIFS
Déterminer la séroprévalence de l’anticorps IgM dirigé contre l’antigène du core du virus de l’hépatite B (anticorps anti-HBc IgM) et celles des marqueurs d’autres agents infectieux comme l’AgHBs, l’anti-VHC, l’anti-VIH-1/2 et de la syphilis parmi les donneurs de sang de Maiduguri

MATÉRIELLS ET MÉTHODES
Dans une étude transversale entre Octobre 2010 et Janvier 2011, 266 donneurs de sang ont été testés pour ces marqueurs de maladies infectieuses en utilisant les procédures standards des tests ELISA (enzyme linked immunosorbent assay) pour l’anti-HBc IgM et les tests ELISA sandwich rapides pour le VHC, le VIH-1/2, l’AgHBs et la syphilis selon les procédures standards opératoires du fabricant.
RESULTS
The prevalence of various infectious markers obtained were as follows: HBsAg (8.6%); anti-HCV (1.5%); anti-HIV-1 (2.6%) and IgM anti-HBc (18.4%). There was a zero percent prevalence of Syphilis in this donor population. The proportion of isolated IgM anti-HBc was 18.1%. Performance indices for HBsAg were as follows: sensitivity (10.2%), specificity (91.7%), positive predictive value (PPV) (21.7%), negative predictive value (81.9%), and efficiency (76.7%). The prevalence of IgM anti-HBc was higher among first time blood donors (21.4%), and in some ethnic groups.

CONCLUSION
There is a high prevalence of isolated IgM anti-HBc antibody among blood donors in Maiduguri. The sensitivity of HBsAg was found to be very low and as such many recent HBV infections may be missed during pre-transfusion screening. We recommend that IgM anti-HBc screening be included as a mandatory pre-transfusion screening test in University of Maiduguri Teaching Hospital.

INTRODUCTION
Laboratory screening for the diagnosis of hepatitis B virus (HBV) infection in asymptomatic individuals such as blood donors generally consist of testing for HBsAg, hepatitis B surface antibody (anti-HBs) and hepatitis B core antibody (anti-HBc). Those with a positive HBsAg are usually requested to follow-up laboratory testing to define the stage of the hepatitis B infection. The follow-up tests include testing for the presence of IgM antibodies to hepatitis B core antigen (IgM anti-HBc), Hepatitis B envelope antigen (HBeAg), Hepatitis B envelope antibody (anti-HBeAg) and hepatitis B DNA. In a setting where HBV infection is acute, HBsAg typically becomes detectable 4 to 8 weeks after infection. Shortly thereafter, IgM anti-HBc appears in the blood. Thus the diagnosis of acute hepatitis B is generally made by the simultaneous detection of HBsAg and IgM anti-HBc. Rarely, acute hepatitis B may be diagnosed during the period when HBsAg titre has declined below detectable levels and anti-HBs has not yet appeared. In this window period, the diagnosis of acute HBV infection is based on the presence of IgM anti-HBc. These patients will thus have isolated anti-HBc as the only marker of acute HBV infection is not known. This study was therefore aimed at screening the donor population in the North-East region of Nigeria for the presence of IgM anti-HBc and also to provide an update on current estimates of the prevalence of some transfusion transmissible infection markers.

RÉSULTATS
Les prévalences obtenues pour les divers marqueurs infectieux étaient les suivantes : AgHBs (8,6%); anti-VHC (1,5%); anti-VIH-1 (2,6%) et anti-HBc IgM (18,4%). La prévalence de la syphilis a été de zéro dans cette population de donneurs. La proportion d’anticorps isolé anti Hbc IgM était de 18,1%. Les indices de performances pour l’AgHBs étaient les suivantes : sensibilité (10,2%), spécificité (91,7%), valeur prédictive positive (VPP) (21,7%), valeur prédictive négative (81,9%), et efficacité (76,7%). La prévalence de l’anti-HBc IgM a été plus élevée chez les nouveaux donneurs (21,4%), et dans certains groupes ethniques.

CONCLUSIONS
La prévalence de l’anti-HBc IgM est élevée parmi les donneurs de sang à Maiduguri. La sensibilité de l’AgHBs a été trouvée très basse et ainsi, plusieurs infections à VHB pourraient être non détectées lors du dépistage pré-transfusionnel. Nous recommandons que le dépistage de l’anti HBc IgM soit inclus comme un test pré-transfusionnel obligatoire au Centre Hospitalier et Universitaire de Maiduguri.

SUBJECTS AND METHODS
Study Area
This study was conducted in the Blood bank in collaboration with immunology department of University of Maiduguri Teaching hospital (UMTH). UMTH is the largest referral hospital in the north eastern region of Nigeria. The patients who attend this hospital come from diverse ethnic groups in Nigeria, neighbouring countries such as Cameroon, Chad, and Niger republic. Maiduguri is the capital of Borno state; it lies at latitude 11°5’N and longitude 13°5’E. Borno state has an area of 65,436 km² and is the largest state of the federation in terms of land mass and is the only state in Nigeria that shares international borders with three countries, namely, Republics of Niger to the North, Chad to the north-east and Cameroon to the East. Within the country its neighbours are Adamawa to the south, Yobe to the west and Bauchi to the south-west. Therefore, data from this hospital can be representative of subjects of various socio-economic groups. Subjects recruited into this study were voluntary donors whose blood samples were collected in blood bank unit of the Haematology laboratory. The information about subjects such as names, gender, age, frequency of donation, and information about their ethnic group were obtained directly from the donors during sample collection.

Subjects and Sample Size
A total of 266 allogeneic voluntary blood donors’ samples as well as their demographical data were collected and processed with the consent of the subjects after obtaining ethical approval from the ethics committee of the hospital management. Any disorder(s) that might affect either the donor or the recipients were used as a criterion for temporary deferment or complete exclusion from the study.

Materials and Methods
The haematological investigations were done according to standard manual haematological procedures while HBsAg, anti-HCV, anti-HIV-1/2, anti-syphilis and IgM anti-HBc were determined using commercially available kits. IgM anti-HBc was determined using solid phase ELISA kits (96 microwells for each kit) bought directly from Clinotech, Canada. These ELISA tests were done in duplicate and the average OD taken before a sample was reported reactive or non-reactive. HBsAg, antibodies to Treponema pallidum and anti-
HCV were detected using Clinotech strips (Clinotech Diagnostics, Canada). Samples were screened for anti-HIV 1 and 2 using Determine HIV 1/2 test kits (Abbott, Japan Co. Ltd, Germany) and Immunocomb (Organics, Israel). All reactive samples were confirmed using Clinotech diagnostic ELISA kits. Antibodies to Treponema pallidum were confirmed with Treponema pallidum haemagglutination test (TPHA, Lorne Laboratories Ltd, UK). A result was considered positive if both the first and second tests were reactive. The study was a cross sectional survey, so follow up samples were not obtained from reactive donors for retesting. The manufacturers' standard operating procedures were strictly followed for the performance of all tests.

Specimen collection
A 5 ml sample of venous blood was collected from each subject, 2 ml of this being dispensed into an ethylene diamine tetracetic acid (EDTA) test tube for haemoglobin estimation and the remainder being allowed to clot and retract in a plain (dry) tube. The samples were labelled with subject name, age, gender and the laboratory number. The plain specimen tube content was spun at 1200 g for 10 minutes. The serum was transferred into a labelled cryovial and stored frozen at -70 °C prior to testing.

Table 1: Demographic characteristics of the blood donors

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number (n = 266)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (Years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>7</td>
<td>2.6</td>
</tr>
<tr>
<td>20 - 24</td>
<td>63</td>
<td>23.7</td>
</tr>
<tr>
<td>25 - 29</td>
<td>70</td>
<td>26.3</td>
</tr>
<tr>
<td>30 - 34</td>
<td>48</td>
<td>18.0</td>
</tr>
<tr>
<td>35 - 39</td>
<td>29</td>
<td>10.9</td>
</tr>
<tr>
<td>40 and above</td>
<td>49</td>
<td>18.4</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>244</td>
<td>91.7</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>Category of donors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First time</td>
<td>187</td>
<td>70.3</td>
</tr>
<tr>
<td>Repeat</td>
<td>79</td>
<td>29.7</td>
</tr>
<tr>
<td><strong>Transfusion history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously transfused</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Never been transfused</td>
<td>262</td>
<td>98.5</td>
</tr>
<tr>
<td><strong>Major tribes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babur</td>
<td>20</td>
<td>7.5</td>
</tr>
<tr>
<td>Fulani</td>
<td>24</td>
<td>9.0</td>
</tr>
<tr>
<td>Hausa</td>
<td>43</td>
<td>16.2</td>
</tr>
<tr>
<td>Ibra</td>
<td>6</td>
<td>2.3</td>
</tr>
<tr>
<td>Kanuri</td>
<td>80</td>
<td>30.1</td>
</tr>
<tr>
<td>Marghi</td>
<td>14</td>
<td>5.3</td>
</tr>
<tr>
<td>Michika</td>
<td>7</td>
<td>2.6</td>
</tr>
<tr>
<td>Shuwa</td>
<td>28</td>
<td>10.5</td>
</tr>
<tr>
<td>Yoruba</td>
<td>6</td>
<td>2.3</td>
</tr>
<tr>
<td>Others</td>
<td>38</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Table 2: Frequencies of some transmissible infectious diseases markers of the blood donors

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. Reactive (n = 266)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HIV-1</td>
<td>7</td>
<td>2.6</td>
</tr>
<tr>
<td>HBsAg</td>
<td>23</td>
<td>8.6</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Anti-Syphilis</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>49</td>
<td>18.4</td>
</tr>
</tbody>
</table>

HIV = Human Immunodeficiency Virus
HBsAg = Hepatitis B Surface Antigen
HCV = Hepatitis C virus
IgM-HBc = Hepatitis B Core antibody

Table 3: Influence of age on the prevalence of the infectious disease markers

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>HIV</th>
<th>HBsAg</th>
<th>HCV</th>
<th>Syphilis</th>
<th>IgM anti-HBc</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20 (n = 7)</td>
<td>0 (0.0)</td>
<td>2 (28.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>20-24 (n = 63)</td>
<td>2 (3.2)</td>
<td>1 (1.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>12 (19.1)</td>
</tr>
<tr>
<td>25-29 (n = 70)</td>
<td>0 (0.0)</td>
<td>9 (12.9)</td>
<td>1 (1.4)</td>
<td>0 (0.0)</td>
<td>11 (15.7)</td>
</tr>
<tr>
<td>30-34 (n = 48)</td>
<td>1 (2.1)</td>
<td>4 (8.3)</td>
<td>1 (2.1)</td>
<td>0 (0.0)</td>
<td>10 (20.8)</td>
</tr>
<tr>
<td>35-39 (n = 29)</td>
<td>1 (3.4)</td>
<td>5 (17.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>≥40 (n = 49)</td>
<td>3 (6.1)</td>
<td>2 (4.1)</td>
<td>2 (4.1)</td>
<td>0 (0.0)</td>
<td>12 (24.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7 (2.6)</td>
<td>23 (8.6)</td>
<td>4 (1.5)</td>
<td>0 (0.0)</td>
<td>49 (18.4)</td>
</tr>
</tbody>
</table>

Pearson Chi-Square (X²) = 4.616**

Prevalence was calculated within age group
ns = not significant (P>0.05)
* = significant at P < 0.02

Table 4: Influence of frequency of donation on the prevalence of infectious disease markers

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Infectious disease markers: % reactive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>HBsAg</td>
</tr>
<tr>
<td>First time (n = 187)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Repeat (n = 79)</td>
<td>5 (6.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>7 (2.6)</td>
</tr>
</tbody>
</table>

Pearson Chi-Square (X²) = 5.996**

Prevalence was calculated within age group
ns = not significant

** = Significant at P < 0.05
ns = Not significant
Table 3 shows the influence of age on the prevalence of infectious disease markers. Age was shown to exert significant influence on HBsAg (\(2 = 4.616, P < 0.02\)). In Table 4, frequency of donation was found to exert an influence on the prevalence of HIV (\(2 = 3.992, P < 0.05\)) and IgM anti-HBc (\(2 = 3.694, P < 0.05\)). High prevalence rates in HIV and HCV were found among repeated blood donors (6.3% and 3.8% respectively) while the prevalence of IgM anti-HBc was higher among first time blood donors (21.4%). Ethnic origin was found to exert significant influence only in the prevalence of IgM anti-HBe with 5 out of 7 Michika donors (71.4%) being affected. The results are shown in Tables 4 and 5: frequency of donation and tribe respectively.

The performance indices of HBsAg as a screening test for hepatitis B infection were evaluated using IgM anti-HBc as the gold standard. The following results were obtained as shown in Table 6: sensitivity (10.2%), specificity (91.7%), positive predictive value (PPV) (21.7%), negative predictive value (NPV) (81.9%) and efficiency (76.7%). The proportion of isolated IgM anti-HBc was 18.1%. Table 7 shows the prevalence of anaemia (at a cut off HB of <10g/dl) in the donor population; 3.4% had values below the cut off. The anaemia was found to be both age and sex dependent (\(2 = 13.549, P = 0.01\) for age and \(2 = 7.713, P = 0.005\) for sex).

Table 5: Effect of tribal origin on the prevalence of infectious disease markers

<table>
<thead>
<tr>
<th>Major tribes</th>
<th>Infectious disease markers: % reactive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV</td>
</tr>
<tr>
<td>Babur (n = 20)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Fulani (n = 24)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Hausa (n = 43)</td>
<td>2 (4.7)</td>
</tr>
<tr>
<td>Ibra (n = 6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Kanuri (n = 80)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Marghi (n = 14)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Michika (n = 7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Shuwa (n = 28)</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>Yoruba (n = 6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Others (n = 38)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td>Total</td>
<td>7 (2.6)</td>
</tr>
</tbody>
</table>

(\(\chi^2\) value) = 3.895*<br>ns = not significant<br>zero (0) = not detected

Table 6: Performance indices of HBsAg as screening test using IgM anti-HBc as gold standard

<table>
<thead>
<tr>
<th>Data</th>
<th>IgM anti-HBc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
</tr>
<tr>
<td>HBsAg Positive</td>
<td>5</td>
</tr>
<tr>
<td>HBsAg Negative</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
</tr>
</tbody>
</table>

Sensitivity of HBsAg = 5/49 x 100 = 10.2%<br>Specificity of HBsAg = 199/217 x 100 = 91.7%<br>Positive predictive value (PPV) = 5/23 x 100 = 21.7%<br>Negative predictive value (NPV) = 199/243 x 100 = 81.9%<br>Efficiency = 5 + 199/266 x 100 = 76.7%<br>Isolated IgM anti-HBc = 44/243 x 100 = 18.1%<br>

**DISCUSSION**

This research was mainly aimed at determining the prevalence of isolated antibody to hepatitis B core antigen (IgM anti-HBc) in blood donors and to update information on the prevalence of other transfusion transmissible infections in the semi arid region of Nigeria. The prevalence of isolated IgM anti-HBc was 18.1%. The finding of isolated anti-HBc may be as a result of many factors notably among them being:

- Presence of IgM anti-HBc during the “window period” following acute HBV infection
- Infection with HBV without persistent viraemia
- Remote infection with persistent “occult” infection

Anti-HBc is the earliest antibody to develop in response to acute HBV infection, appearing in IgM form as early as 12 weeks after infection. IgM anti-HBc may be the only marker present during the “window period,” when antigenemia with HBsAg has resolved and anti-HBs has not yet developed. Such patients often have other laboratory evidence of acute hepatitis B infection, including increased hepatic aminotransferase levels or hyperbilirubinemia. The most common scenario for detecting isolated anti-HBc is probably that of resolved hepatitis B infection with high titers of anti-HBs, particularly in populations at high risk for HBV infection. This conclusion is largely derived from the observation that many individuals with isolated anti-HBc do not develop evidence of new hepatitis B infection despite repeated exposures. Because most such patients will also have negative HBV DNA test, it is difficult to firmly establish this diagnosis of prior infection. In this scenario, detecting anti-HBe would support the diagnosis of prior infection.

Sometimes, patients with hepatitis B infection has actively replicating HBV (at low-levels), but without the production of detectable HBsAg. This is an unusual clinical situation, and the biological basis for occult HBV infection remains poorly understood. This situation occurs with or without detectable anti-HBs and anti-HBc; tests for HBeAg and anti-HBe are typically non-reactive. Many patients with occult HBV will have anti-HBc as the only serologic marker to suggest HBV infection. The diagnosis requires measurement of detectable HBV DNA and the infection is considered chronic, since HBV is
actively produced and detectable in serum. The major limitation of this work was the lack of facilities for HBV DNA testing to confirm the presence of occult HBV infection. Since there are no comparable reports in this region on the prevalence of IgM anti-HBc, it is difficult to corroborate the finding of a high prevalence of IgM anti-HBc as shown in this study.

Previous studies in Nigeria have shown that hepatitis B virus infection occurs at different proportions among blood donors and general population. In this study, HBsAg prevalence rate of 8.6% was obtained. This is quite high when compared with 1.1% by Ejele et al in the Niger Uetia area14 and 1.2% by Kagu et al in the North Eastern Nigeria15. In Pakistan, 2.2% was reported by Bhatti et al20 while in some African countries like Kenya and Tanzania, 4.0% and 8.8% were reported by Abdalla et al21 and Matee et al22 respectively.

Contrastingly, a higher prevalence rate than that obtained in this study has been reported in some parts of Nigeria as follows: 10.6% in South-South region of Nigeria by Esumeh et al, and 13.2% by Basola et al23 in Ibadan, South-West region of Nigeria. The HCV prevalence of 1.5% obtained in this study is close to earlier reports by Abdalla et al21, Ejele et al, Jeremiah et al23 and exactly the same with Matee et al22 in Tanzania. These figures suggest that hepatitis B and C virus infections are endemic in Nigeria and in sub-Saharan Africa and could be a contributory factor to the high prevalence of isolated IgM anti-HBc. It is possible that many of the blood donors who tested negative for HBsAg may actually be in their serological window period and thus constitute a high risk for the transmission of hepatitis to recipients. The wide differences in the HBV and HCV infection rate among blood donors in the different regions within Nigeria, and outside Nigeria may be due to differences in geographical locations, age range of donors sample sizes, the period of time the studies were carried out, and the different socio-cultural practices such as sexual behaviour, marriage practices, circumcision, scarification and tattooing which take place in these regions. Access to healthcare, immunization practices, and laboratory test reagents used may also be contributory factors. The incorporation of assays for other serological markers of HBV infection, such as IgM anti-HBc, an indicator of early exposure to HBV infection, may help to reduce the risk of post transfusion hepatitis.

In this study, the prevalence rate of HIV obtained was 2.6%, which is lower when compared with the 4.0% rate found in previous work of Ajayi et al, conducted on blood donors in Maiduguri, Nigeria. This may suggest a declining rate of HIV prevalence in the region. The previous work by Ajayi et al was a ten year’s experience of the prevalence of HIV infection among blood donors in Maiduguri and after three years this study has recorded 2.6% prevalence which is a reduction a little above half of the previous value of 4.0%. The implication is that though there is a reduction, HIV infection is still endemic in our locality and this could be a contributory factor for a high prevalence of isolated IgM anti-HBc in blood donors. Even though the prevalence of isolated IgM anti-HBc and some infectious agents were seen to be selectively high among certain tribes, the small sample size of those ethnic groups may have contributed to this high prevalence. Another study with large sample sizes in these affected tribes will be necessary before any meaningful conclusion can be drawn.

CONCLUSION

There is high prevalence of HBV and isolated IgM anti-HBc in Maiduguri blood donor population. This study has also shown that screening of blood samples using HBsAg as the only test is not sufficient in protecting blood recipients from HBV infection. Thus, the inclusion of IgM anti-HBc as an additional test is hereby advocated.

REFERENCES

7. Douglas DD, Talseth HF, Rakele J, Rabe D. Absence of hepatitis B virus DNA detected by polymerase chain reaction in blood donors who are hepatitis B surface antigen negative and antibody to hepatitis B core antigen positive from a United States population with a low prevalence of hepatitis B serological markers. Transfusion 1993; 33: 212-6
SEVERE VASOVAGAL REACTION NECESSITATING INTUBATION OF A BLOOD DONOR: case report and literature review

REACTION VASOVAGAL SEVERE NECESSITANT UNE INTUBATION DU DONNEUR DE SANG: rapport sur un cas et revue de la littérature

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INTRODUCTION

Blood donation is usually well tolerated, but occasionally adverse reactions may occur during donation, immediately afterwards or some time later. Blood donation reactions, which are usually of variable severity, can be divided into local and systemic reactions. In localized reactions, complications directly relate to the insertion of the needle for venous access and some of these are characterized by extravasations from the vein such as haematoma while others are characterized by pain arising from nerve or tendon injury.

Systemic blood donation reactions occur almost always due to a vasovagal reaction (VVR). A VVR refers to the presence of any of the following symptoms and signs during or shortly after a blood donation: dizziness, weakness, diaphoresis, apprehension, pallor, hypotension, and bradycardia which may progress to loss of consciousness (a faint). Progress to syncope and tonic clonic seizures are rare. The reaction is generated by the autonomic nervous system and further stimulated by psychological factors and the volume of blood removed relative to the donor’s total blood volume.

Several studies have reinforced the fact that blood donation is a very safe procedure though a few have reported that adverse events and complaints during or after donation may be more common than previously thought.

INTRODUCTION

Le don de sang est habituellement bien toléré, mais occasionnellement, des réactions adverses peuvent survenir au cours du don, immédiatement après ou un peu plus tard. Les réactions au don de sang, qui sont habituellement de sévérité variable, peuvent être divisées en réactions locales et réactions systémiques. Dans les réactions locales, les complications sont directement liées à l’introduction de l’aiguille dans la veine et certaines d’entre elles sont caractérisées par la douleur suite à la lésion d’un nerf ou d’un tendon. Les réactions systémiques au don de sang surviennent presque toujours à la suite d’une réaction vaso-vagale (RVV). La RVV correspond à la présence de n’importe quel signe et symptôme pendant ou immédiatement après un don de sang : vertiges, fatigue, diaphorèse, inquiétude, pâleur, hypotension, et bradycardie qui peuvent évoluer vers une perte de conscience (évanouissement). L’évolution vers la syncope et les convulsions tonico-cloniques sont rares. La réaction est générée par le système nerveux autonome et ensuite stimulée par les facteurs psychologiques et par le volume de sang prélevé relativement au volume de sang total du donneur.

Plusieurs études ont renforcé le fait que le don de sang est une procédure très sûre, malgré que quelques unes ont rapporté que les effets adverses et les plaintes au cours et après le don de sang peuvent être plus fréquents que les estimations précédentes.
VVRs are the most common adverse events related to blood donation that negatively impact on blood donor return rate. A VVR can be triggered by pain, sight of blood and anxiety. Most suffer only minor symptoms, but a few have a more severe course with symptoms like loss of consciousness, convulsions or incontinence. We report a severe VVR in a young first-time male donor that progressed to convulsive syncope with laryngospasm that necessitated endotracheal intubation.

CASE REPORT

A 19 year old well-built male donor, weighing 75 kg visited a blood donor centre for voluntary blood donation on a warm afternoon after a very active day. He had had lunch approximately 4 hours before the donation and was a first-time donor. He had no symptoms suggestive of cardiovascular disease or any other medical history of note. He was used to doing heavy exercises and had been very active throughout the day as a volunteer student bus driver in the organization of the blood donation drive. Pre donation questionnaire and physical examination certified him fit for blood donation. Phlebotomy was done and blood was collected into a 450 ml blood bag. With a good flow, 450 ml was collected within 10 minutes. Immediately after completion of blood collection, he started complaining of uneasiness; he became restless and started hyperventilating with muscular spasm in some parts of the body. His pulse and blood pressure were normal at 98 bpm and 130/70 respectively. Random blood sugar was also normal at 112 mg%. He was made to re-breathe his expired air with the aid of a paper bag but muscular spasm became generalized and he became increasingly agitated. An immediate biochemical test revealed hypocalaemia of 1.31 mmol/L, that was corrected by administration of 10% calcium gluconate. Other biochemical parameters were within reference range, but spasm continued. He was admitted to the intensive care unit (ICU) but generalized spasm continued which had to be aborted with midazolam repeatedly. His O₂ saturation was 99% at room air, and EEG, ECG and brain computer tomography showed no abnormality. Subsequent serial examination of electrolytes including magnesium and ionized calcium were normal.

He was kept sedated but spasms continued, were intermittent, non-stereotypical and affecting the whole body. There was no tonic component during episode of spasm and no loss of consciousness. There was also an increase in ventilation but vital signs remained stable during episodes of generalized spasm.

On the second day of admission to ICU, the spasms became more frequent and persistent with associated laryngospasm on an occasion which necessitated endotracheal intubation. He also had a short lapse into unconsciousness during this episode. He was however extubated after a few hours when he regained consciousness and was intolerant of the endotracheal tube. Serial electrolytes remained essentially normal. By the third day, frequency of spasm decreased remarkably and he was eventually discharged after 7 days in ICU.

DISCUSSION / LITERATURE REVIEW

Blood donors are the mainstay of any transfusion service. Regular flow of donors is essential and any untoward reaction could frighten away first-time donors and endanger donor return. Donor reactions have a negative impact on the blood donor return rates. Therefore interventions to prevent and to ameliorate these reactions have great potential to improve return rates.

Based on available reports, 11% to 21% of the blood donor population may have a reaction or injury from their whole blood donation. This includes 9% to 16% who develop a bruise or haematoma, 2% to 5% who have VVRs and less than 0.5% who develop other injuries or reactions including antecubital nerve injuries (irritations), and arterial punctures 15-18. Although some studies have documented the prevalence of VVRs to be between 2% and 5%, Rohra et al documented a higher rate.

VVRs, the most common systemic donor reaction are graded severe or non-severe based on the requirement for treatment and on outcome. It may also be immediate or delayed with or without injury based on the reaction occurring before or after the donor has left the donation site. Some of the most severe complications of VVR seen in relation to blood donation are accidents in donors who lose consciousness after leaving the donation site. Approximately 5% of VVRs are syncopal and progress to loss of consciousness in about 0.08-0.34% of donors. Common symptoms and signs of a VVR are pallor, light-headedness, anxiety, diaphoresis (perspiration), hyperventilation, irregular breathing, weakness, nausea, vomiting, hypotension, and bradycardia. A few have a more severe course with symptoms like loss of consciousness and seizure activity (tetany or tonic clonic convulsive movement with loss of bladder or bowel control). The symptoms of a VVR usually develop suddenly and generally tend to occur at the end of the phlebotomy or shortly after its completion but in about 3% to 15% of VVRs, the reaction occurs after the donor has left the donation site.

Pathophysiology

VVR is thought to occur in a biphasic mode. In the prodrome, the donor’s heart rate is increased and blood pressure is slightly to moderately elevated which are normal responses to stress and blood volume reduction. However, in a VVR, this response is followed by vascular vasodilatation and bradycardia. The vascular vasodilatation causes hypotension and the onset of symptoms. Whatever triggers the VVR affects the hypothalamic portion of the brain through two different neural pathways that then causes the symptoms through the autonomic nervous system. In the first and central pathway, emotions, pain, or stress directly affect the brain, which then stimulates the hypothalamus. This pathway explains why a donor can faint when just thinking about the donation. The second and peripheral pathway refers to the effects of peripheral
pressure receptors, known as baroreceptors, on the hypothalamus. Baroreceptors are found in the aorta, carotid arteries, and the left ventricle of the heart. The ventricular baroreceptors when stimulated by forceful heart contractions or heart distension initiate what is known as the Bezold–Jarisch reflex. This refers to increased parasympathetic vasovagal output to the heart and decreased sympathetic output to the vasculature in the muscles and viscera. The net result is bradycardia and peripheral pooling of blood which causes hypotension and the onset of symptoms. If the hypotension decreases the brain’s blood supply, the donor becomes dizzy, weak, and anxious and when the systolic blood pressure falls below 70 mm to 80 mm Hg, the donor faints. The more severe or longer the hypotension, the more likely the development of either tetany or convulsions. Both result from cerebral hypoxia. Incontinence can also occur but is less common. What ends a VVR is increased blood supply to the brain. This results when the donor falls to the ground or the collection staff place the donor in a flat or legs-up Trendelenburg position. Eventually the muscular tone in the donor’s vasculature returns to normal, and the donor recovers.

Severe VVR and other severe outcomes of blood donation

All these are rare and are defined as reactions requiring hospitalization. Most studies on whole blood donation have focused on acute reactions particularly the vasomotor related responses and have concluded that most reactions are mild and of vasovagal origin. Though rare, more severe VVR resulting in tetany, convulsion, incontinence, and other severe complications like arterial puncture, myocardial infarction, a variety of serious local injuries that include phlebitis, compartment syndrome, arteriovenous fistula and pseudoaneurysm have also been reported. One author described hospitalizations from 4.1 million whole blood donations. The incidence was approximately 1 in 200 000 for allogeneic whole blood donation and 1 in 17 000 for autologous whole blood donation. The most common adverse events causing the hospitalization were VVR (73%), angina (15%), and a variety of arm injuries (15%).

Predictive factors for VVR

The likelihood of a blood donor sustaining a VVR is unknown. One cannot predict with certainty which donors will or will not have a VVR. However, there are psychological, physical, demographic, and environmental factors that place a donor at a high risk for a reaction. These factors have a low predictive value, so even when present, the donor is still unlikely to have a reaction. A frequent donor may on occasion have a reaction after many asymptomatic donations. Several studies have evaluated these factors affecting donor reaction rates. Many of these studies were done during or shortly after World War II.

Psychological factors

VVR can be triggered by pain, fear, or any strong emotion. There is a strong psychogenic component associated with blood donation related VVR. For instance, some blood donors faint even before blood collection begins. This is an obvious psychological reaction based on a phobic fear of needles, pain, or the sight of blood. Epidemic fainting, when donors faint in response to seeing other donors faint have also been reported.

Anxiety, hyperventilation and first-time donors

Hyperventilation is more common in anxious first-time donors, and when present, it can cause or potentiate a donor reaction. Hyperventilation can be recognized as faster breathing, deeper breaths, or an irregular breathing pattern. As a result of hyperventilation, excessive carbon dioxide is exhaled, which causes a decrease in the body’s carbon dioxide level and an increase in pH. Both decreased carbon dioxide levels and elevated pH can cause cerebrovascular constriction which decreases the blood supply to the brain. Over breathing can also cause the calcium levels to drop which may result in numbness and tingling usually in both arms or around the mouth, spasms or cramps of the hands and feet and muscle twitching. It takes only 1 or 2 minutes of hyperventilation to cause a 40% decrease in the brain’s blood supply which will in turn potentiate dizziness or fainting.

Young age

Young blood donors, especially those under the age of 20, are much more likely to faint than donors over the age of 30. In a recent multicentre study, severe donor reaction rate was 10 to 14 fold higher in donors under 20 years. Tomasulo et al. evaluated first-time donors and showed clearly that age itself was a key factor in donor reactions. His study showed that first-time male donors under the age of 50 had a reaction rate of 1.1%, whereas first-time male donors under the age of 30 had a reaction rate of 5.3%. This represents a fivefold increase in the donor reaction rate in younger donors. Neurologic studies have shown that elderly subjects have fewer VVRs when subjected to a postural tilt test that is designed to induce VVRs in susceptible individuals compared with young subjects.

Low donor weight

Low donor weight is another factor associated with increased donor reaction rates. Donors who weigh close to 50 kg, the minimum acceptable weight, have a higher probability of a reaction when 450 ml of blood is collected. Similarly, low body weight is largely responsible for reaction rate differences between males and females.

Volume of blood collected

Collection of a large volume of blood also influences donor reaction rates. In a study, when the volume collected was changed from 440-540 ml, the reaction rate increased from 3.8% to 8.5%. In an earlier study, changing the volume collected from 250-500 ml in donors who weighed less than 50 kg (150 lb) was largely responsible for a doubling of the reaction rate, from 2.3% to 5.7%.

Individual susceptibility

A history of a past donor reaction places the donor at higher risk for another reaction. A study found that donors who fainted were much more likely to have had a history of fainting during a blood donation than controls; 30-43% versus 1-5%. Nonetheless, having had one reaction does not mean the donor will have a second reaction, and donors with a history of a reaction are generally permitted to donate. In contrast, a frequent donor may on occasion have a reaction after many asymptomatic donations. Some authors have concluded that susceptibility to VVR may be associated with decreased baroreceptor sensitivity and several studies have shown that baroreceptor sensitivity is decreased in healthy young subjects when such individuals are physically or psychologically stressed.
Attitude of collection staff
Some studies have also shown that a very important factor influencing donor reaction rate is the attitude and actions of the collection staff. These studies have shown that spending more time with donors decreases the rate of VVR. Maloney in 1946 noted lower reaction rates when more attention was given to donors and when rest times after the phlebotomy were longer. Oga et al. noted that talkative, personable nurses had a lower donor reaction rate than non-communicative nurses; 0.8% versus 1.3%.

Other factors
In addition to the above, studies outside of the blood donation setting have noted that exhaustive exercise and certain medications or drugs such as nitrates, furosemide, and alcohol can increase the rate of VVRs.

Management of VVRs
Management of VVR varies depending on whether it is mild or severe. For mild reactions, it is important to observe the early symptoms because it is possible to reverse the reaction. The collection staff’s first and second actions in a mild reaction is to place the donor in a Trendelenburg position and to try to change the donor’s breathing pattern by having the donor cough, hold his breath for 5 seconds, or breathe into a paper bag. The last method is preferred because it increases the donor’s carbon dioxide level.

A third action is to physically stimulate the donor with a cold towel around the neck and/or use ammonia salts. Nausea can be treated with cola soft drink syrup, which inhibits vomiting. Talking to the donor and diverting the donor’s attention can also alleviate or reverse the reaction.

Severe reactions with syncope, hypotension, and thready pulse can be frightening. When tetany, convulsions, or uncontrolled movements occur, the needle should be removed immediately to prevent harm to the donor. One should also keep the donor from falling off the bed and make sure the airway is intact. After an intact airway is assured, it is important to get the donor into a Trendelenburg position. Cardiopulmonary resuscitation is rarely if ever needed. Administration of intravenous fluids can also ameliorate the reaction. Blood pressure, pulse, skin colour, and mental status should be monitored closely until the donor is fully recovered.

After the reaction, the donor’s status should be assessed. Donors who have convulsions may hurt themselves; tongue, lip, and needle injuries are possible but rare. Any serious injury such as a fracture or laceration should be referred for immediate medical attention. Head injuries should be referred to an emergency centre if there is suspicion of a skull fracture or internal injury or if the donor’s mental status has changed. If donors with head trauma are sent home, they should be watched carefully by family members for 24 hours for any neurologic change, which should precipitate an immediate visit to the emergency room. Donors should be well-orientated before being sent home.

Complications of severe VVR
Serious and significant complications of severe VVR include fractures and/or lacerations which may occur when a donor faints and falls, hitting a hard or sharp surface during the fall. One author reported scalp and head lacerations, chipped teeth, nose fractures, skull fractures, mandible fractures, finger fractures, fracture of the malar bone, and fracture of the humerus bone in a cohort of donors with severe VVR.

The authors have not seen any reported blood donation-related VVR causing donor death.

Prevention
Some donors are at higher risk for a VVR; they include young donors, first-time donors, donors who weigh less than 50 kg, and donors with estimated blood volume less than 3 500 ml. Although at higher risk, such donors may be accepted for blood donation because of the lack of serious sequelae from a donor reaction, the fact that most donors in these risk groups will do fine, and because of the chronic need for blood. However, to reduce the likelihood of VVR and blood volume depletion, less volume of blood should be collected from donors weighing less than 50 kg or with an estimated blood volume of less than 3 500 ml.

Donor reactions can be prevented by careful and attentive care of the donor from the time the process begins and until the donor departs from the blood donor clinic. Talking to the donor, displaying empathy, and being very observant while taking care of physical needs inspires confidence and keeps the donor from thinking too much about the donation. The donor’s desire to speedily complete the donation process must be balanced with sufficient post donation rest to minimize reactions. It is critical that phlebotomists be aware of each donor’s mental and physical status so reactions can be quickly recognized and reversed.

SUMMARY
In this case report of a severe VVR in a first-time young male donor necessitating endotracheal intubation and review of predictive factors of VVRs related to blood donation, VVRs are noted to be the most common systemic reaction that will be observed daily in a busy blood collection centre. Factors definitely associated with increased VVR rates include young age, history of previous reaction, anxious and hyperventilating first-time donor, low weight and inattentive or non-communicative phlebotomist.

VVR can progress to the severe form with loss of consciousness, development of abnormal movement, tetany, tonic clonic convulsive movement and loss of bladder and or bowel control. Severe VVRs are rare but incidences can cause panic among the personnel in blood centre and produce a negative effect on the donor population especially the first-time donors and blood donor return rate. This case highlights the need for blood bank staff to be aware of possibilities of severe reaction in donors requiring hospitalization. Through good management, a blood donor organization can minimize the incidence of syncope by making the donation experience as pleasant as possible, identifying those at risk and taking necessary precautions to reduce its occurrence.
REFERENCES


REVIEW OF STRATEGIES FOR SCREENING BLOOD DONORS FOR HEPATITIS B VIRUS MARKERS: need for increasing sensitivity of detecting early infection in Nigeria

REVUE DES STRATEGIES DE DEPISTAGE DES DONNEURS DE SANG POUR LES MARQUEURS DU VIRUS DE L'HEPATITE VIRALE B: necessite d'augmenter la sensibilite pour la detection precoce de l'infection

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MOTS-CLÉS
Virus de l’hépatite viral B (VHB), infections transmissibles par la transfusion (ITT), Dépistage Génomique Viral (DGV), Nigéria.

ABSTRACT
Hepatitis B virus (HBV) is a Hepadnaviridae that has affected about 2 billion people worldwide with over 350 million people as chronic carriers of the virus. It is about the most common infection transmitted by blood transfusion especially in developing countries where less sensitive modalities for screening are often used. A number of tests are available for screening blood donors for HBV but none of these gives a zero risk. However some have higher sensitivity and specificity especially when sensitive markers of HBV infection are combined for testing in conjunction with good donor selection criteria, aiming at reducing the risk to near zero. In Nigeria where the infection is hyperendemic, there is still laxity in trying to curtail this risk and some centres are not able to screen. For those that do screen, many still use the latex agglutination while a few use ELISA based methods. The new NN BTS is still struggling to meet the blood demand using voluntary non-renumerated donors and there is difficulty in donor selection mainly because of lack of confidential environment during campaigns for such voluntary blood donations.

In the absence of expensive tests such as Nucleic acid test (NAT) which is the most sensitive assay method for determining the level of HBV DNA, there is the need to include tests that will increase the sensitivity of viral detection in Nigeria. ORTHO Hbc ELISA for HBV anti-core antibody has high sensitivity and specificity. Additionally, it is simple, ELISA based and is therefore recommended as an additional test to the HBsAg test in blood donor testing in Nigeria.

RÉSUMÉ
Le virus de l’hépatite B (VHB) est un Hepadnaviridae qui affecte plus de 2 milliards de personnes avec 350 millions de personnes porteuses chroniques du virus. C’est l’infection la plus transmise par la transfusion en particulier dans les pays en développement où les moyens de dépistage les moins sensibles sont habituellement utilisées. Nombre de tests sont disponibles pour le dépistage du VHB chez les donneurs de sang mais aucun ne donnent un risque zéro. Cependant, certains ont une plus grande sensibilité et spécificité, en particulier lorsque les marqueurs de l’infection à VHB sont combinés dans le même test, en association avec de bons critères de sélection du donneur, ceci visant à réduire le risque jusqu’à presque zéro. Au Nigéria, où l’infection est hyper endémique, il existe toujours un laxisme dans la tentative de réduire le risque et certains centres ne sont pas à mesure de faire le dépistage. Pour ceux qui font le dépistage, plusieurs d’entre eux continuent à utiliser l’agglutination sur latex pendant que quelques uns utilisent des méthodes basées sur le principe ELISA. Le nouveau CNTS continue à se battre pour satisfaire la demande en sang en utilisant des donneurs volontaires et non rémunérés, et il ya des difficultés dans la sélection des donneurs, principalement à cause du manque d’un environnement confidentiel au cours des campagnes destinées à de tels donneurs de sang volontaires.

En l’absence de test couteux comme le Dépistage Génomique Viral (DGV) qui est la méthode de dépistage la plus sensible pour déterminer le taux d’ADN du VHB, il est nécessaire d’introduire des tests qui augmenteront la sensibilité de la détection virale au Nigéria. ORTHO Hbc ELISA pour l’anticorps anti-core du VHB a une sensibilité et une spécificité élevées. De plus, ce test est simplement basé sur la méthode ELISA et est par conséquent recommandé comme un test additionnel au test de l’AgHBs au cours du dépistage du donneur de sang au Nigéria.
INTRODUCTION

Hepatitis B virus (Australia antigen) was discovered by Blumberg et al. in 1965, and a few years later, Dane visualized the hepatitis B virus (HBV) virion and called it the Dane particle. It is a partially double-stranded circular DNA consisting of a core capsid which contains viral DNA surrounded by an envelope containing surface antigen. It belongs to the Hepadnaviridae family of viruses. Figure 1 shows the structure of Hepatitis B virus.

Figure 1: Hepatitis B Virus

Over 350 million people are chronically infected with this virus worldwide and it is one of the most important viruses transmitted by blood transfusion affecting about 1.205,000 donations in the United Kingdom. Other modes of transmission of the virus include sexual contact, close interpersonal contact through blood and body secretions, mother to baby (perinatal), IV drug abuse and unsafe traditional practices including tattoos and ear piercing.

The use of Hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) screening tests has been the basis of HBV screening in many countries and this has significantly reduced but not eliminated transfusion transmitted HBV infection.

Nigeria, one of the most populous nations in Africa is considered hyperendemic for HBV infection with prevalence of HBsAg in the adult population including replacement blood donors ranging between 5 and 25%. By age 40, a large number of the population is expected to have been in contact with the virus. The carrier rate is 10.4% in black Africans and 15% in young volunteer blood donors. Donors usually present amidst family members to donate for a relative. Screening questions for possible risks associated with infection transmission such as sexual contact, homosexuality and the like are difficult to ask or be answered truthfully. This therefore leaves the burden on good laboratory screening tests to detect infected blood. These tests are currently not at the optimal level because most of those used in the developed nations where the prevalence is less than 1% of the population are too expensive and therefore unaffordable for hyperendemic regions with poor resources. Current testing strategies for Hepatitis in Nigeria are by hepatitis B surface antigen (HBsAg) screening using either Latex method or Enzyme Immunosorbent Assay (ELISA). Other tests on blood donors employed in the country include screening for human immunodeficiency virus type 1 and 2 (HIV 1 & 2) and hepatitis C virus (HCV).

MARKERS OF HEPATITIS B VIRUS (HBV) INFECTION IN BLOOD DONORS

HBsAg and anti-HBs

The diagnosis of hepatitis B infection is made primarily by detecting HBsAg which appears within four weeks of exposure and indicates active viral infection. Recovery results in elimination of HBsAg and appearance of antibodies to HBsAg (anti-HBs) in about four months. Chronic infection is defined by persisting HBsAg for more than six months. The presence of HBsAg mutants with altered expression of HBsAg may cause false negative results with some test kits. Successful vaccination against hepatitis B virus also results in measurable anti-HBs in the blood.

Anti-HBc

Hepatitis B core antigen is found in the liver and cannot be detected in the blood. Its presence in large amounts indicates an ongoing and active replication of the virus. Antibody to hepatitis B core antigen, (anti-HBc), is detectable in the blood either as IgM anti-HBc in acute infection and lasts for up to six months after the onset of symptoms, or as IgG anti-HBc which develops during the course of the acute hepatitis B viral infection and persists for life, and is a marker of past or current viral replication. Therefore, IgM anti-HBc can be specifically used to diagnose an acute hepatitis B viral infection and the use of total anti-HBc as a diagnostic test may not be helpful (Hollinger, 2008). Anti-HBc usually persists after the disappearance of HBsAg and before the appearance of detectable anti-HBs. In the absence of information about any other hepatitis B virus (HBV) marker, it must be considered that the infection may have resolved, leaving the person immune. Anti-HBc may be the only serologic marker of hepatitis B viral infection and potentially infectious at this stage. This is therefore an important marker especially in endemic populations.

It is also important to mention the window period as it relates to acute HBV infection. This is a situation that occurs during acute disease when the surface antigen has disappeared and IgM anti-HBc is yet to be produced. During this period, the only way one can make definitive diagnosis is by the use of HBV DNA using PCR which is expensive for routine use in blood donor screening.

Hepatitis B e Antigen (HBeAg)

HBeAg signifies active viral replication and infectivity whereas anti-HBe indicates an inactive state of the virus and less likelihood of transmission. HBV DNA may undergo a particular structural change, called a pre-core mutation resulting in its inability to produce HBeAg, even though the virus is actively replicating. Individuals with this mutation are HBeAg negative but infectious.

Hepatitis B virus DNA

High levels of HBV DNA indicate ongoing viral replication and activity while low or undetectable levels of HBV DNA are associated with the inactive phase of hepatitis B viral infection. Several different laboratory tests are available to measure HBV DNA. NAT using polymerase chain reaction (PCR) is the most sensitive assay method for determining the level of HBV DNA. It detects minute amounts of HBV as it amplifies the material that is being measured up to a billion times. It can measure as few as 50 to 100 copies (particles) of hepatitis B in blood following amplification. Patients with dormant disease can thus test positive. Active disease shows several billion copies/ml of blood. This test is therefore too sensitive for practical diagnostic use.
PCR hybridization assay is less sensitive and measures the viral material without amplification and can detect hepatitis B virus DNA only in active infection. Figure 2 shows the typical serological response of HBV infection by Hollinger while table 1 interprets the results of tests used in detection of HBV.

**Figure 2: The serologic/ immunologic response to HBV**

### ANTI-HBc SCREENING TESTS FOR BLOOD DONORS

**ORTHO HBc ELISA Test System**

This is an indirect enzyme immunoassay for the detection of total antibodies to Hepatitis B core in human serum or plasma. The test involves addition of sample or control to their allocated wells. The sample is allowed to react with rHBcAg during incubation and then washed to remove unbound material, a conjugate is added and also allowed to bind to antibody present and after washing, the reaction is stopped by addition of sulphuric acid and read at 490 nm with 630 nm reference filter within 60 minutes. Specificity is 99.7% with 97.8% sensitivity. This test is simple, semi-automated and relatively cheap. It does not require a large or dedicated area and may be done using ELISA machines.

**ABBOTT PRISM HB core assay**

This is an in vitro chemiluminescent immunoassay (ChLIA) for the qualitative detection of total antibody to hepatitis B core antigen (anti-HBc) in human serum and plasma in blood donors. The test is dependent on the use of solid phase microparticles coated with recombinant HBc antigen. Serum or plasma samples (a calibrator and control) and cystein solution are incubated. Anti-HBc present in the sample binds to the rHBcAg on the microparticles. This is the first step in the two-step competitive/ ChLIA assay. The reaction mixture is transferred to the glass fibre matrix of the reaction tray using the Transfer Wash. In the second stage, acridium (labelled anti-HBc conjugate) is added and incubated. This binds to rHBcAg that has not been blocked by human anti-HBc in the sample. The unbound conjugate is washed and chemiluminescence is emitted when alkaline hydrogen peroxidase solution is added. The amount of light detected is inversely proportional to the amount of anti-HBc in the sample. The test is automated and expensive, requires specialised training and maintenance. Reagents may not be readily available in this setting and a dedicated building is required to accommodate the large Abbot Prism. Specificity for this test in low risk volunteer whole blood donor population is 99.88%, with 99.49% sensitivity.

**AxSYM CORE**

This is a microparticle Enzyme Immunoassay (MEIA) which uses coated recombinant HBcAg (rHBcAg) particles for the detection of anti-HBc in human plasma. Sample is incubated with rHBcAg to form an antigen-antibody immune complex. A conjugate is then added which binds rHBcAg coated microparticles not bound to anti-HBc in the sample. Following addition of a substrate, the resulting fluorescence is measured by MEIA. The amount of fluorescence is determined by comparison to a cut off rate for anti-HBc provided. This test is automated and requires expensive machines to measure the fluorescence. Sensitivity - can detect less than 1 PEI unit/ml against a reference standard. Specificity 99.9%.

**ARCHITECT-Chemiluminescent microparticle Immunoassay (CMIA)**

This uses chemiluminescence for qualitative detection of total anti-HBc. Human serum or plasma is added to microparticles coated with rHBcAg. Presence of anti-HBc results in chemiluminescence following addition of a conjugate. The amount of light emitted is directly related to the amount of anti-HBc present in the sample. This assay is quick and automated but may give false positive reactions with a sensitivity of 98% and specificity of 99%.

### Table 1: Interpretation of a typical infection with Hepatitis B virus

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>IgM anti-HBc</th>
<th>IgG anti-HBc</th>
<th>HBeAg</th>
<th>Anti-HBe</th>
<th>HBV DNA/ (viral load)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+ (low)</td>
<td>Early phase of acute infection/Incubation</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+ (high)</td>
<td>Later phase of acute infection/acute hepatitis</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+ (high)</td>
<td>Later phase of acute infection/ HBsAg -ve</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+/+</td>
<td>+</td>
<td>-</td>
<td>+ (low)</td>
<td>Recovery/ Inactive HBsAg carrier</td>
</tr>
<tr>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Successful vaccination</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>+ (high)</td>
<td>Chronic infection/ pre-core mutant</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+/+</td>
<td>-</td>
<td>+/-</td>
<td>High/low</td>
<td>Chronic infection/ inactive phase</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>High/low</td>
<td>Occult infection</td>
</tr>
<tr>
<td>+</td>
<td>++</td>
<td>+/-</td>
<td>+/+</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>Recovery, false positive</td>
</tr>
</tbody>
</table>

(+ = positive; - = negative)
Serologic testing for HBsAg and anti-HBc have been used as the foundation of blood screening in many countries, while HBV NAT was recently developed to detect HBsAg- and anti-HBc-negative blood units donated during early acute infection. However, screening of blood donors for anti-HBc has been shown to be the only indicator of healthy but infectious carriers, in whom anti-HBs, anti-HB and even HBV-DNA may be negative, likely due to an infected donor with low viraemia forming immune complexes with HBV and anti-HBs, that is only detected by anti-HBc testing. Indeed, even single-sample HBV NAT may not substitute for anti-HBc screening, as some donors have isolated-anti-HBc with extremely low DNA levels undetectable by standard single-sample NAT, and who have been associated with transfusion-transmitted HBV.

The test that will best suit our purpose is the ORTHO HBc ELISA Test System that measures IgM anti-HBc in serum or plasma. This test is chosen mainly because it is semi-automated, sensitive, specific and does not require “robotics” or dedicated areas. The test is relatively cheap and there are already existing facilities for ELISA tests and thus there is no extra cost of buying a new machine. However, most of the studies done for estimation of anti-HBc among blood donors, have used kits for total anti-HBc (both IgG and IgM). IgG anti-HBc may be positive in an affected individual who has had past infection with HBV, even in the presence of protective levels of anti-HBs antibodies, and therefore may not be infective. IgM anti-HBc is a marker of recent infection and is therefore considered to be a more specific marker for HBV infection during the window period. Studies have shown that all blood units positive for anti-HBc may not be infectious, especially if the donor sera have an adequate titre of anti-HBs. 35 All anti-HBc positive donors should thus be tested for anti-HBs, and those with high levels considered as immune to avoid unnecessary donor deferral and/or anxiety.

The possibility of chronic ill health resulting from infection with HBV puts the blood transfusion service to at increasing search for tests that would completely eliminate the risks involved in transfusion of infected blood to recipients especially those that are immunocompromised. It is therefore important to be part of eliminating or decreasing the prevalence rate of hepatitis and other transfusion transmitted diseases by doing all that is possible to achieve this. Routine IgM anti-HBc screening of blood donors could prevent some transfusion-transmitted HBV infections. An active campaign for HBV vaccination should be incorporated into the campaign for blood donation. It is hoped that the current childhood immunization plan will see future generations free of HBV infection and thus less of a challenge to the transfusion service. This opens a window of research that will establish the number of blood donors, qualified by the current HBV screening protocol, but positive for HBV DNA (when facilities allow). It is also important to establish infectivity of the transfusion of blood containing BV DNA. This information will be helpful in determining whether new tests, such as NAT, are required in the future. Before then, it calls for judicious use of blood and blood products if only to prevent the current, emerging and re-emerging infections.

**REFERENCES**


BIORISK MANAGEMENT:
A relatively new concept for Blood Services

David Chama
Contributor and member of the AfSBT
Blood Transfusion Service, Zambia

INTRODUCTION

Biorisk management is relatively new to blood transfusion establishments in Africa. Traditional emphasis on staff safety has been limited to the provision and use of laboratory coats and examination gloves. Biorisk management has a much broader scope. The World Health Organization (WHO) and other organizations such as the United States Centers for Disease Control and Prevention (CDC) are placing emphasis on broadening the scope of safety and security in the workplace. We know that work-related infections have occurred as a result of exposure in the laboratory. In some instances, it may be that workers are expected to manage laboratory-generated waste without appropriate containment barriers. Biorisk management should therefore be promoted and WHO and CDC have recommended the implementation of its concepts as a hallmark of quality accreditation in laboratories.

Biorisk management extends beyond laboratory safety. This article describes a stepwise process related to daily practices and suggests alignment of these to suit biorisk management concepts. In following issues of Africa Sanguine, a practical approach will be described, with details on various concepts under biorisk management. Commonly used terminologies follow:

Biorisk Management
• This is a compound word that encompasses both biosafety and biosecurity concepts
• The AMP model is a systematic way of implementing the concept. The acronym relates to Assessment, Mitigation and Performance
• Biosafety refers to prevention of employee exposure to occupationally acquired infections which may occur due to accidental contact. Biosafety also includes prevention of release of organisms into the environment using appropriate safety measures
• Achievement of safety is made possible through a combination of appropriate mitigation measures or controls: Elimination or substitution Engineering controls Processes and procedures Use of personal protective equipment

Biosecurity
This refers to the protection of biological agents from loss, theft or misuse. Under a biosecurity programme, emphasis is placed on access control to areas where micro-organisms are stored, so that inventory, data security and transportation are all administered correctly.

Waste management
This programme emphasizes the need for knowledge on the nature of waste and how to handle different types of waste. Waste segregation is described, and the management of different types of spillages which may occur in the laboratory, such as chemicals, or blood spills.

Transportation of infectious substances
This refers to categorizing, packaging, labelling and transportation of infectious substances in accordance with United Nations regulations. Transportation of substances may be by road, air or sea. Organizations such as International Air Travel Association (IATA) or Universal Parcel Union (UPU) or International Civil Aviation Organization (ICAO) are familiar with the process of transportation of infectious substances. They provide shipping instructions and courses on how this is done safely and securely.

CONCLUSION

Importance of biosafety and biosecurity programme
• In order to achieve biosafety and biosecurity, collaborative efforts are encouraged with agencies within and outside the country.
• Staff safety should take priority in the laboratory. Security of pathogen containing material is equally important.
• As part of the preparation for accreditation, a laboratory needs to address all aspects of biorisk management.
Planning for our congress next year in Mauritius is going ahead and by the time this newsletter reaches you, we will have the website up and running, providing information on registration, submission of abstracts and an outline of the scientific programme and social activities.

This will be the 6th international meeting of the AfSBT – the first was in Durban, South Africa in 1999, Tunis, Tunisia in 2002, Lagos, Nigeria in 2004, Cape Town, South Africa in 2006 and Nairobi Kenya in 2009. Those of you who attended our 5th meeting in Nairobi will agree that it was a wonderful success, enjoyed by all, and our Mauritian congress should prove just as exciting. We hope that as you read this, you are making plans to attend!

This congress is going to be unique; oral presentations will be delivered in either French or English, with two sets of slides – one in French and the other in English. After the presentation, questions/discussion will be handled via the moderator, who will be bilingual. In this way simultaneous translation will be avoided. For poster presentations, the author will give the overview in either French or English and the moderator will lead the discussion in both languages, depending on the interest and needs of the delegates attending.

Information at time of going to print:

Theme of the congress:
Training and Quality Lead to Safe and Sustainable Blood Services

Chairs, Local Organizing Committee:
Dr Janaki Sonoo, Head, National BTS

Chairs, Scientific Committees:
Local: Dr Mohammad Iqbal Issack;
International: Beryl Armstrong

Congress Organizer:

Congress website address:
www.afsbt-mauritius.org

Congress Venue:
Hotel Le Meridien http://www.lemeridien-mauritius.com/

Contact email addresses:
janaki.sonoo@gmail.com; beryl.armstrong@nbisa.org.za

PROGRAMME OVERVIEW
- ISBT Academy Education Day
- Step-Wise Accreditation Forum
- AfSBT General Assembly
- Scientific programme:
  - Plenary sessions
  - ... presentations in French and English
  - Poster presentations
  - ... discussions in French and English
  - AfSBT Committee meetings
  - Short symposia / breakfast sessions
  - Trade exhibition
  - Social programme
  - Pre and post congress tours
  - Research - special session
  - PEPFAR - special session

Scientific Programme offers vein-to-vein presentation subjects:
- Blood donor recruitment, selection and screening
- Donations for blood group and TTIs
- Immunohaematology
- Blood component preparation and rational use
- Quality and blood safety from donor to patient
- Computer traceability
- Haemovigilance
- Education and training in transfusion medicine
- Organization and management
- Sustainability of services
- Challenges for island services

AFSBT MEMBERSHIP COMMITTEE ESTABLISHED
It was recently agreed by the AfSBT Board that a Membership Committee should be constituted and managed regionally, with each of the 4 AfSBT regions being involved in the retention and expansion of membership within their Region. The Vice Presidents (VPs) of the Regions have been appointed the Chairs, and the Management Office of the Society is their advisory, guidance and administrative support. After consulting the VPs and our membership database (because committee members must be paid up individual members of the AfSBT), we extended invitations to selected members. All accepted enthusiastically and now constitute the committee membership as listed below – they will extend their recruitment retention and expansion efforts, via their VPs, throughout their Region.
In the meantime the management office is preparing a promotional brochure, which will have been distributed by the time of publication of this issue of the journal. It is hoped that committee members find it beneficial in helping with membership drives.

**Administrative Chair:**
David Chama

**Administrative support:**
Beryl Armstrong, Leesha Raman, Conilynn Venter

**Region: RAFTS**
Chair: Séidou Konate (Côte d’Ivoire): plasmaci@yahoo.fr
- Kamel Boukef (Tunisia)
- Kouao Diane (Côte d’Ivoire)

**Region: Ecowas**
Chair: Banji Adewuyi (Nigeria): jowuy@yahoo.co.uk
- Sulaimon Akanmu (Nigeria)
- Justina Kordai Ansah (Ghana)

**Region: EAC**
Chair: Jamilla Rajab (Kenya): naimjamilla@yahoo.com
- Joseph Wang’endo (Kenya)
- Edith Mbabazi (Uganda)
- Senet Ibrahim (Eritrea)

**Region: SADC**
Chair: Gabriel Muyinda (Zambia): gabriel.muyinda@znbts.gov.zm
- Ravi Reddy (South Africa)
- Lucy Marowa (Zimbabwe)

Email contact addresses have been added to the list above, for your information and use, should you wish to contact your Regional VP in connection with membership matters.

The Management Office continues to be responsible for the maintenance of the membership database, issuing of membership cards and arranging for member’s only website access. The email address, for membership record queries and problems with internet access to the members’ only section, is beryl.armstrong@nbisa.org.za.

The banking account of the AfSBT remains, as before, in South Africa.

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The step-wise accreditation project continues to enjoy general financial support from the National Bioproducts Institute of South Africa. Specific support was provided by ICCBBA; for assessor fees, documentation and training, and travel costs pertaining to the first test site.

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Anglophone SADC countries were approached and invited to be considered by the AfSBT, as a pilot site for testing the programme. The countries included in this advice were those who – to the best of our knowledge, do not have any sort of accreditation or certification already. The pilot site will provide us with the opportunity to fine-tune the programme and improve it before considering a second pilot site and then a general roll-out.

The countries selected as the first and second pilot sites are Namibia and Malawi. At the time of going to print, it is planned to assess Namibia in April 2012 and Malawi in July/August 2012.

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Beryl Armstrong

**French Editor:**
Claude Tayou Tagny

**Committee:**
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**Administrative support:**
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E D U C A T I O N

This issue of Africa Sanguine continues the printing of a section from Introduction to Blood Transfusion Technology by B Armstrong et al. Hard copies of the publication Introduction to Blood Transfusion Technology may be requested by emailing the Editor at Beryl.Armstrong@nbisa.org.za.

H O W  C A N  Y O U  G E T  I N V O L V E D ?

We are providing you with an opportunity to have your questions, problem areas, queries or comments answered by the authors of this book. We will publish correspondence and replies in the journal. You are encouraged to participate and make use of this opportunity to share your ideas, thoughts or views.

Submit correspondence to the Editor: Beryl.Armstrong@nbisa.org.za or leesha@klprojects.co.za

E x t r a c t  f r o m  t h e  P u b l i c a t i o n :


Authors: B. Armstrong, J. Hardwick, L. Raman, E. Smart, R. Wilkinson
ISBT Science Series Volume 3 Issue 2 (June 2008)
ISSN 1751-2816 (Print) / ISSN 1751-2824 (Online)
Website address: http://www3.interscience.wiley.com/journal/120090113/issue


Elizabeth Smart, Beryl Armstrong

Acknowledgement of this extracted section is given to the following:

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- International Society for Blood Transfusion
- Blackwell Publishing

B L O O D  G R O U P  S Y S T E M S

Elizabeth Smart, Beryl Armstrong

I N T R O D U C T I O N

This section will cover the major blood group systems, some of the other blood group systems and will also include information on HLA and notes on platelet antigens. Although some references will be made to the molecular structures, the detailed molecular structures and recent advances in DNA technology are not within the scope of this publication.

L E A R N I N G  O B J E C T I V E S

By the end of the section, the student should be able to describe the following in simple terms:

- Blood group terminology
- Functions of blood groups
- ABO and H blood group systems
  - ABO grouping
  - Inheritance of ABO blood groups
  - ABO blood group frequencies
  - Production of ABO antigens
  - H-deficient phenotypes and Bombay O phenotype
- Subgroups within the ABO system
- ABO system antibodies
- Clinical significance of ABO system
  - Clinical significance in transfusion
  - Clinical significance in haemolytic disease of the fetus and newborn
- Lectins (plant agglutinins)
- ABH secretion
- Unique features of ABO system
- Rh blood group system
Rh genetics and inheritance
- Molecular studies
- Terminology
- Frequencies
Rh typing
Rh antigens
Clinical significance of Rh system
- Clinical significance in transfusion
- Clinical significance in haemolytic disease of the fetus and newborn
Unique features of Rh system
Other major blood group systems (MNS, P, Kell, Duffy, Kidd, Lewis, Lutheran):
- Date of discovery
- Well known antigens
- Antigen frequencies
- Antibody characteristics
- Significance in transfusion
- Significance in haemolytic disease of the fetus and newborn
- Features and practical application
MNS
p
Kell
Duffy
Kidd
Lewis
Lutheran
I blood group system
Additional blood group systems/collections/antibodies reacting with high and low frequency antigens
Polyagglutination
Human leucocyte antigen system
- Disease association
- Transplantation
- Transfusion
- Pregnancy
- Parentage Testing
Human platelet antigen system
Fetomaternal alloimmune thrombocytopenia

BLOOD GROUP TERMINOLOGY
Currently 29 different blood group systems are known of which nine are considered to be the major blood group systems.

In addition there are various blood group antigens which have been allocated to collections, low incidence antigens (700 Series) or high incidence antigens (900 Series) according to the International Society of Blood Transfusion (ISBT) Committee on Terminology for the Red Cell Surface Antigens, which is the committee responsible for the terminology and allocation of antigens to the appropriate system.

There are strict criteria for:
- The allocation of blood group antigens to a new blood group system (the antigen must be shown to be an inherited character defined by a human alloantibody, the gene encoding it must have been identified and sequenced, and the chromosomal location must be known).
- The allocation of a new specificity to an existing system.
- The establishment of a collection (requires two or more antigens that are related serologically, biochemically or genetically, but do not fulfil the requirements for a blood group system).
- Inclusion into the 700 series (incidence of less than 1% of the population and distinct from other systems and collections).
- Inclusion into the 900 series (incidence of >90% in most populations tested and distinct from other high frequency antigens).

Various terminologies have been used to describe the different blood group systems and their antigens and respective antibodies ever since the ABO blood group system was first discovered in 1900. In 1980 an ISBT committee was tasked to devise a genetically based numerical terminology for red cell antigens. This is an ongoing process and new information regarding the antigens and apparent new antigens are reviewed by the committee on a regular basis.

The numerical terminology was primarily designed to facilitate computer input. The alternative/popular terminologies are commonly used, both in everyday communication, in the laboratories and in publications.

Note: The term group or type can be used interchangeably when discussing blood grouping or typing. Further notes on Rh terminology will be found in the Rh section.

The number of antigens per blood group system, collection, and series varies tremendously from 1 in the P system to more than 30 in the Rh and MNS systems.

Table 6.1 shows the ‘major’ blood group systems. Note that H antigen is in a separate system, H Blood Group System 018, and is not part of the ABO system.

Table 6.2 gives information on the blood group systems other than the nine major systems.

Table 6.1: ‘Major’ blood group systems (9 of 29)

<table>
<thead>
<tr>
<th>ISBT No</th>
<th>Blood group system name</th>
<th>Major antigens</th>
<th>Chromosome location no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>ABO</td>
<td>A, B, A, B, A_</td>
<td>9</td>
</tr>
<tr>
<td>002</td>
<td>MNS</td>
<td>M, N, S, s, U</td>
<td>4</td>
</tr>
<tr>
<td>003</td>
<td>P</td>
<td>P1</td>
<td>22</td>
</tr>
<tr>
<td>004</td>
<td>Rh</td>
<td>D, C, E, c, e</td>
<td>1</td>
</tr>
<tr>
<td>005</td>
<td>Lutheran</td>
<td>Lu*, Lu^</td>
<td>19</td>
</tr>
<tr>
<td>006</td>
<td>Kell</td>
<td>K, k, Kp^, Kp, Js, Js^</td>
<td>7</td>
</tr>
<tr>
<td>007</td>
<td>Lewis</td>
<td>Le*, Le^</td>
<td>19</td>
</tr>
<tr>
<td>008</td>
<td>Duffy</td>
<td>Fy*, Fy_b, Fy3</td>
<td>1</td>
</tr>
<tr>
<td>009</td>
<td>Kidd</td>
<td>Jk*, Jk^, Jk3</td>
<td>18</td>
</tr>
</tbody>
</table>
FUNCTIONS OF BLOOD GROUPS

The structures of the different blood group systems and their antigens have been studied extensively, and a wealth of information is available particularly since the development of molecular genetic techniques. Less is known about the actual function of the blood groups. The red cell is a complex structure and the red cell membrane contains many surface proteins that are anchored to the membrane or cross the lipid bilayer one or more times. Many of the proteins on the surface of the red cells are polymorphic and carry the different blood groups. The functions of some of the red cell membrane proteins have been identified, and other functions have been deduced from the structures of the protein. Studies on the null phenotypes which occur in most blood group systems have contributed to the information. The ABO, H, I, P and blood groups are carbohydrate structures on the red cell membrane glycolipids and glycoproteins and less is known about their function. Table 6.3 provides a list of the functions of the blood groups.

<table>
<thead>
<tr>
<th>ISBT No</th>
<th>Blood group system name</th>
<th>Major antigens</th>
<th>Chromosome location no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>010</td>
<td>Diego</td>
<td>D^a, D^b, Wr^a, Wr^b</td>
<td>17</td>
</tr>
<tr>
<td>011</td>
<td>Yt</td>
<td>Yt^a, Yt^b</td>
<td>7</td>
</tr>
<tr>
<td>012</td>
<td>Xg</td>
<td>Xg^a</td>
<td>X</td>
</tr>
<tr>
<td>013</td>
<td>Sciana</td>
<td>Sc1, Sc2</td>
<td>1</td>
</tr>
<tr>
<td>014</td>
<td>Dombrock</td>
<td>Do^a, Do^b, Gy^a, Hy, Jo^a</td>
<td>12</td>
</tr>
<tr>
<td>015</td>
<td>Colton</td>
<td>Co^a, Co^b, Co3</td>
<td>7</td>
</tr>
<tr>
<td>016</td>
<td>Landsteiner-Wiener</td>
<td>LW</td>
<td>19</td>
</tr>
<tr>
<td>017</td>
<td>Chido/Rodgers</td>
<td>CH/RG</td>
<td>6</td>
</tr>
<tr>
<td>018</td>
<td>H</td>
<td>H</td>
<td>19</td>
</tr>
<tr>
<td>019</td>
<td>Kx</td>
<td>Kx</td>
<td>X</td>
</tr>
<tr>
<td>020</td>
<td>Gerbich</td>
<td>Ge2, Ge3, Ge4</td>
<td>2</td>
</tr>
<tr>
<td>021</td>
<td>Cromer</td>
<td>Cr</td>
<td>1</td>
</tr>
<tr>
<td>022</td>
<td>Knops</td>
<td>Kn^a, Kn^b</td>
<td>1</td>
</tr>
<tr>
<td>023</td>
<td>Indian</td>
<td>In^a, In^b</td>
<td>11</td>
</tr>
<tr>
<td>024</td>
<td>Ok</td>
<td>Ok^a</td>
<td>19</td>
</tr>
<tr>
<td>025</td>
<td>Ralph</td>
<td>MER2</td>
<td>11</td>
</tr>
<tr>
<td>026</td>
<td>John Milton Hagan</td>
<td>JMH</td>
<td>15</td>
</tr>
<tr>
<td>027</td>
<td>J</td>
<td>J</td>
<td>6</td>
</tr>
<tr>
<td>028</td>
<td>Globoside</td>
<td>P</td>
<td>3</td>
</tr>
<tr>
<td>029</td>
<td>Gill</td>
<td>GIL</td>
<td>9</td>
</tr>
</tbody>
</table>

The format for describing the blood group systems in this publication is as follows:

Antigen frequencies are given as approximate percentages, simply to make them easier to remember. This will sometimes result in the total being slightly more or less than 100%. The figures for black people apply to published data, or to surveys done in southern Africa.

- When an antibody is stated to cause haemolytic disease of the fetus and newborn (HDFN) and when it has an optimum reaction temperature of +37°C using the IAT technique, it is presumed to be a type IgG antibody.
- When an antibody is described as a saline agglutinin and when it reacts best at cold temperatures, it is presumed to be a type IgM antibody.

The two most clinically significant blood group systems are the ABO and the Rh blood group systems.

**ABO AND H BLOOD GROUP SYSTEMS**

Although the ABO and H are two different blood group systems genetically they will be described together as they are closely related, both at the biochemical and phenotype level.

The ABO system is the most important blood group system in transfusion therapy and was the first blood group system to be discovered. This great contribution to medicine was made by Landsteiner in 1900 when he observed that ‘the serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals’. The following year, in 1901, Landsteiner was able to recognize two antigens on the red cells, by separating and mixing the cells and sera of several individuals. He called the antigens A and B. Those individuals with the A antigen on their red cells were called group A; those with the B antigen, group B. Many individuals lack the A and the B antigens and these individuals were termed group C which was later termed group O. The least common group, called AB, was found by several of Landsteiner’s students in 1902. Group AB individuals have both A and B antigens on their red cells. Landsteiner found that the serum of an individual always contained antibodies to the antigen, which was not present on that individual’s red cells. Thus group A individuals will have anti-B antibodies in their serum and group B individuals will have anti-A antibodies in their serum. These facts became known as Landsteiner’s Rule which states:

‘(In the ABO system) the antibody to the antigen lacking on the red cells is always present in the serum or plasma.’

The regular presence of anti-A and/or anti-B antibodies means that it is critical for patient safety and good transfusion practice that ABO groups are performed, recorded and interpreted correctly. ABO incompatibilities are responsible for the majority of serious and/or fatal transfusion reactions and are usually caused by technical, clerical or administrative errors.

**ABO GROUPING**

The ABO system is unique in that whenever the A or B antigens are not present on the red cells, the corresponding antibody is present in the serum/plasma. Anti-A and anti-B alloagglutinins are therefore often referred to as being ‘naturally occurring’.

The two most clinically significant blood group systems are the ABO and the Rh blood group systems.

**FUNCTIONS OF BLOOD GROUPS**

The structures of the different blood group systems and their antigens have been studied extensively, and a wealth of information is available particularly since the development of molecular genetic techniques. Less is known about the actual function of the blood groups. The red cell is a complex structure and the red cell membrane contains many surface proteins that are anchored to the membrane or cross the lipid bilayer one or more times. Many of the proteins on the surface of the red cells are polymorphic and carry the different blood groups. The functions of some of the red cell membrane proteins have been identified, and other functions have been deduced from the structures of the protein. Studies on the null phenotypes which occur in most blood group systems have contributed to the information. The ABO, H, I, P blood groups are carbohydrate structures on the red cell membrane glycolipids and glycoproteins and less is known about their function. Table 6.3 provides a list of the functions of the blood groups.
**ABO grouping can therefore be performed by:**

- Typing the red cells for the presence or absence of the A and/or B antigens. This is known as forward grouping.
- Testing the serum/plasma for the presence or absence of anti-A and/or anti-B antibodies. This is known as reverse grouping.
- The forward and reverse grouping results should correlate; refer to Landsteiner’s rule.

The general population can then be divided into four ABO groups as shown in Table 6.4, based on the forward and reverse grouping.

<table>
<thead>
<tr>
<th>ABO Group</th>
<th>Antigens on red cells</th>
<th>Antibodies in serum/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>O</td>
<td>None</td>
<td>Anti-A,B</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>None</td>
</tr>
</tbody>
</table>

It should be noted that the anti-A,B produced by a group O individual is different from anti-A+B, which is a mixture of anti-A from one source and anti-B from another source. Anti-A,B detected in group O individuals is an antibody that will react with group A and group B cells. The monoclonal anti-A,B reagents available commercially will detect the weak A group A\textsubscript{x}.

**Inheritance of the ABO blood groups**

The ABO genes are located on chromosome number 9. The inheritance in the ABO system is controlled by means of various alleles A\textsuperscript{1} and A\textsuperscript{2}, B and O and a series of rare alleles A\textsuperscript{3}, A\textsuperscript{4} and A\textsuperscript{n} (etc). The O allele (which does not produce an antigenic product) is recessive to the A and B alleles, which are co-dominant. The ABO phenotype is shown by ABO grouping laboratory tests on a blood specimen but this is not necessarily the genotype of the individual. For example, blood of phenotype A\textsuperscript{1} can represent one of several possible genotypes: A\textsuperscript{1}A\textsuperscript{1}, A\textsuperscript{1}A\textsuperscript{2}, A\textsuperscript{2}A\textsuperscript{1}, A\textsuperscript{2}A\textsuperscript{2}, A\textsuperscript{1}A\textsuperscript{n}, (etc) or A\textsuperscript{n}O.

Although each individual has two ABO genes, serologic tests do not reveal the O allele in the A and B phenotypes, nor can an allele producing a weak form of A be recognized if an allele higher in the scale of A antigen production is simultaneously present. The genotype can however be determined by DNA analysis of the gene or may be determined by family studies. Table 6.5 shows ABO blood group/phenotype with possible genotypes (simplified) including some of the rare alleles.

<table>
<thead>
<tr>
<th>Blood group/phenotype</th>
<th>Possible genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A\textsubscript{1}</td>
<td>A\textsuperscript{1}A\textsuperscript{1}, A\textsuperscript{1}A\textsuperscript{2}, A\textsuperscript{1}A\textsuperscript{3}, A\textsuperscript{1}A\textsuperscript{n}, A\textsuperscript{1}O</td>
</tr>
<tr>
<td>A\textsubscript{2}</td>
<td>A\textsuperscript{2}A\textsuperscript{1}, A\textsuperscript{2}A\textsuperscript{2}, A\textsuperscript{2}A\textsuperscript{3}, A\textsuperscript{2}A\textsuperscript{n}, A\textsuperscript{2}O</td>
</tr>
<tr>
<td>B</td>
<td>BB, BO</td>
</tr>
<tr>
<td>AB</td>
<td>A\textsuperscript{1}B, A\textsuperscript{2}B, A\textsuperscript{1}B, A\textsuperscript{2}B</td>
</tr>
<tr>
<td>O</td>
<td>OO</td>
</tr>
</tbody>
</table>

**ABO BLOOD GROUP FREQUENCIES**

Many populations have been studied world-wide and it has been shown that the frequency of the ABO blood group genes varies between different populations. Note the variation shown in Table 6.6 as an example of ABO blood group distribution.

<table>
<thead>
<tr>
<th>Group</th>
<th>White</th>
<th>Black</th>
<th>Indian</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>59</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>19</td>
<td>32</td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**Production of ABO antigens**

The ABO red cell antigens expressed on the red cells are dependent on the presence of both the H gene, and the A,B and O genes. The loci for the H and ABO genes are not linked although they are related and they are therefore two separate blood group systems. The H, A and B genes do not code directly for red cell antigens, but for enzymes known as transferases. The H-transferase adds the sugar L-fucose to a precursor substrate which is a carbohydrate chain already present on the red cell membrane. Once this has been performed, the A- and B-transferases can act. The A-transferase adds another sugar called N-acetyl-D-galactosamine, which results in the expression of A antigen on the red cells. Similarly, the B-transferase adds the sugar D-galactose and the cells then also express B antigen. These red cells type as group AB.

Group A antigen is expressed when H- and A-transferases are the two enzymes present; group B antigen is expressed when the H- and B-transferases are the enzymes present, and in group O only H-transferase is present. Figure 6.1 shows a simplified diagram to indicate the structural differences in the molecules that result in ABO antigen expression.

**Figure 6.1:** Simplified diagram to indicate structural differences in ABH antigen composition
The expression of A, B or AB antigens results in a relative masking of the H antigen. Thus A1, B or A1B cells express only small quantities of H.

The A’ allele is less effective than the A’ allele in masking the H determinant, and A1 cells therefore have considerably more H antigen and less A antigen than do A2 cells. The O allele in double dose leads to the expression of H specificity alone, resulting in group O individuals having abundant H antigen.

The amount of H antigen that is present in red cells of different groups, from left or right in decreasing order, is as follows: most H antigen: O → Weak A → A2 → A1B → B → A1 → A1B → least H antigen.

The A, B and H antigens are detectable long before birth. The ABH antigen strength usually peaks at about 2 and 4 years of age and then remains relatively constant in most individuals. It may not be possible to distinguish between group A1 and A2 groups at birth as the antigens may not yet be fully expressed.

H-deficient phenotypes

Although the ABO and H are two different blood group systems genetically (H Blood Group System: Number 018), they are closely related at the biochemical and phenotype level. The H-deficient phenotypes are very rare and include a total deficiency in H antigen (Bombay or O phenotype) or a partial deficiency (Parabombay).

Bombay O phenotype

The Bombay or O phenotype, in which the cells lack the H antigen, arises when the individual has not inherited the very common gene H. As there is no H gene present, the H-transferase enzyme is absent. The precursor substance remains unchanged and no molecules of L-fucose are present on the precursor substrate in the red cell membrane. The individual may have inherited A and/or B genes, which code normally for the appropriate transferases. However, without the single terminal carbohydrate (sugar) L-fucose at the end of the substrate protein, these transferases are non-reactive. The Bombay O phenotype therefore results when the individual has inherited a double dose of a rare recessive allele, known as h. The gene h does not code for H-transferase. Individuals who have inherited HH or Hh genes produce normal amounts of H-transferase.

Bombay O individuals are extremely rare. Those who were originally shown to carry the trait were Indians whose ancestors originated in Bombay, hence the name Bombay O. Their cells are not agglutinated by anti-A, -B, -A,B or -H. Bombay O individuals have powerful anti-A, -A and -B antibodies in their serum. They should therefore be transfused only with type O blood. Table 6.7 shows the difference between blood group O and blood group O.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>Anti-H</th>
<th>A cells</th>
<th>B cells</th>
<th>O cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>O_h</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 6.7: Differences in blood groups O and O

SUBGROUPS WITHIN THE ABO SYSTEM

Subgroups of A

About 10 years after the discovery of the ABO groups, the subgroups of A were described. It was observed that not all group A bloods tested gave similar results with anti-A. Those unusual bloods which gave weaker reactions became known as ‘weak A’. Furthermore, it was realized that the common A antigen occurred in two forms: A1 and A2. Later studies on transferase enzymes of group A1 and group A2 individuals showed that less antigenic sites are produced in group A1 individuals as the enzyme is less effective in converting the precursor H substance into A antigen. However, with the use of monoclonal anti-A blood grouping reagents, little if any difference between the reactions of group A1 and group A2 cells can be detected in the laboratory. About 99.9% of all group A bloods from white people and about 96% of group A bloods from black people, in one survey, were either group A1 or A2, with group A2 being more frequent than group A1 in both populations. A higher incidence of ‘weak’ A was detected in the black people.

The anti-A found in the serum/plasma of group B individuals consists of two separate antibody specificities, anti-A and anti-A1, the latter being specific for the A1 type. Group A or AB individuals who lack the A1 component may form an irregular, cold reacting anti-A1 antibody in their serum. The lectin Dolichos biflorus or monoclonal anti-A1 reagents, are usually used to type red cells for the A1 antigen.

Further subgroups of group A and subgroups of group B

A number of further subgroups of group A and subgroups of group B also occur. The subgroups are caused by genetic variations that result in a variety of weakened expressions of the antigens. The subgroups cannot be detected when inherited with a normal A or B gene. The subgroups may be detected in the laboratory when weak or unexpected negative results are obtained with the forward grouping and/or anomalous results with the reverse grouping.

Weak A

The term weak A covers a large range of reactivity, some bloods giving clear (although weak) results and other bloods giving such weak reactions that detection may prove difficult. The weak A types include A1, A2, A1*, A2*, A1w, A2w and A1w*. Weak A type A1 gives a characteristic mixed field agglutination pattern when tested against polyclonal anti-A and anti-A,B antiserum. However, strong agglutination is observed when using most monoclonal blood grouping reagents. Table 6.8 compares reactions between group A and subtypes. Anti-A1 may or may not be produced, although it is often produced by group A1 individuals. Note that type A1 shows mixed field agglutination with anti-A and anti-A,B and that type A1 reacts macroscopically with some polyclonal anti-A,B.
**Table 6.8: Comparison of reactions: group A and subtypes**

<table>
<thead>
<tr>
<th>Red cell phenotype</th>
<th>Monoclonal reagents</th>
<th>Lectin Anti- A</th>
<th>Reaction of serum/plasma with reagent red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-A,B</td>
</tr>
<tr>
<td>A₁</td>
<td>4</td>
<td>U</td>
<td>4</td>
</tr>
<tr>
<td>A₂</td>
<td>4</td>
<td>U</td>
<td>4</td>
</tr>
<tr>
<td>A₃</td>
<td>1 mf</td>
<td>0</td>
<td>1 mf</td>
</tr>
<tr>
<td>A₄</td>
<td>Micro +/-1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Weak AB**
In weak AB types, the B antigen may occur with any of the weak A subtypes.

**Weak**
Subgroups of group B are suspected when the expression of the B antigen is weak or cannot be easily detected. Subgroups of B are very rare and are found mainly in populations where the frequency of group B is high as in Far Eastern populations. The subgroup cannot be detected if inherited with a normal B allele. The weak B subgroup may be inherited with an A allele giving rise to a normal A weak B phenotype, ABweak.

**Acquired B**
This is caused by the action of enzymes which break down the group A antigen N-acetyl-D-galactosamine to galactosamine which is similar to the structure of the group B antigen immunodominant sugar (D-galactose). Some anti-B reagents react with this acquired group B antigen and a group A individual could be incorrectly grouped as group AB. It is important to select anti-B grouping reagents carefully to ensure that they do not react with acquired B cells. The condition is rare but may be associated with gastrointestinal bacterial disease or due to bacterial contamination of a blood sample. The individual’s red cells often become polyagglutinable.

**ABO system antibodies**
Healthy adults who lack a particular ABO group antigen on their red cells usually have the corresponding antibody in their serum due to stimulation from the environment, such as exposure to certain bacteria, or food which may contain A-, B-, or H-like substances. Additional exposure to the antigen can result in more potent antibody formation.

*This immune response may be prompted by:*
- Presence of ABO incompatible fetal red cells in the maternal circulation during pregnancy and at delivery.
- Injection of A or B substances which may be found in vaccines, either in the culture medium or in the micro-organisms themselves.
- The accidental transfusion or injection of ABO incompatible red cells.

Alloagglutinins that are weak or missing in adults may occur in weak subgroups of A or B, agammaglobulinaemia (patients with low levels of serum globulins), twin chimerism, old age or treatment with immunosuppressive drugs.

**Alloagglutinins in infants**
Alloagglutinins are not normally detected in newborn infants and develop after 3-6 months of life due to exposure to A- and B-like substances/antigens in the environment. If ABO antibodies are detected in neonatal blood samples, they are usually agglutinating IgG antibodies of maternal origin. Table 6.9 shows the grouping results of a group B newborn and an infant of 6 months of age.

**Table 6.9: Newborn and infant ABO grouping results**

<table>
<thead>
<tr>
<th>Age of infant</th>
<th>Newborn</th>
<th>Infant of 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Forward grouping</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-B</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Anti-A,B</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Reverse grouping</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A cells</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>B cells</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O cells</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Interpretation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>B</td>
<td>B</td>
</tr>
</tbody>
</table>

**Anti-A₁**
Individuals of phenotypes A₁, A,B and weaker subgroups of A may have anti-A₁ in their serum/plasma. This antibody will react with group A₁ cells. Anti-A₁ is usually a cold reacting antibody, which is usually not of clinical significance. As it seldom reacts above +25°C, it is unlikely to cause transfusion reactions or HDFN. It may however mask a clinically significant antibody.

Anti-A₁ occurs naturally in the serum/plasma of about 2% of A₁ individuals and 26% of A,B individuals. The antibody occurs more frequently as the strength of the A antigen decreases, therefore weak A (or weak AB) individuals are more likely to have anti-A₁ in their serum/plasma than A₁ (or A,B) individuals.

**Anti-H (other than Bombay anti-H, -A₁, -B)**
As individuals of group A₁, A,B and B have very little H antigen on their red cells, they sometimes develop anti-H in their serum/plasma. This antibody can be recognized by its strong reaction with group O red cells, a weaker reaction with group A₁ cells and usually a failure to react with group A₁ or group B red cells. Anti-H of this nature, which is formed by individuals who are not H-deficient, is usually benign and occurs naturally in the serum/plasma of some group A₁ and A₁,B individuals.
Group O serum

Group O serum is not a simple mixture of anti-A and anti-B. It cannot be separated by selective adsorption using either group A or group B cells and is a cross-reacting antibody generally known as anti-A,B. Various theories have been suggested to explain this cross-reactivity (including Wiener’s C theory) and it appears that the anti-A,B produced by group O individuals detects a structure common to both A and B antigens.

CLINICAL SIGNIFICANCE OF THE ABO SYSTEM

Clinical significance in transfusion

Of all the blood group systems, the ABO is the most important in transfusion because the alloagglutinins are normally present in the absence of the corresponding antigen. Strong reactions take place when incompatible bloods are mixed with each other, not only in vitro but also in vivo. Even an initial transfusion of group A blood into a group B patient may be disastrous, because the naturally occurring anti-A in the blood of the group B patient would react promptly with the incoming group A cells, causing agglutination and haemolysis of the donor cells and a likely haemolytic transfusion reaction.

‘Universal blood donor’

Individuals of blood group O are termed universal blood donors, as their blood can usually be safely infused into recipients of other ABO groups (heterologous group transfusion) because:

They do not have A or B antigens on their red cells to react with antibodies within the circulation of the recipient.

Their naturally occurring anti-A,B antibodies are not harmful to the red cells of the recipient if whole blood is transfused, providing the alloagglutinins are ‘low titre’ (lack ABO haemolysins).

However, blood from ‘high titre’ group O donors, which contains immune anti-A and/or -B, may only be transfused into group O recipients (homologous group transfusion). This is because these ‘dangerous’ universal donors have potent alloagglutinins with haemolyzing characteristics, which may cause severe haemolytic reactions when infused into recipients with A, B or AB antigens on their red cells. The risk of transfusing harmful anti-A and anti-B in group O whole blood can be reduced by the transfusion of group O red cell concentrates.

In practice, however, it is better to transfuse a patient with blood of the same ABO group (ABO identical) and to conserve stocks of group O blood for group O patients and for emergency use.

‘Universal recipient’

Broadly speaking, group A,B individuals are universal recipients because:

They have both A and B antigens on their red cells.

• They usually lack ABO antibodies in their serum.

• Therefore, irrespective of the ABO blood group antigens on the donor cells, there are no antibodies in the group AB recipient to react with them.

Likewise, provided that the alloagglutinins in the plasma of the donor are low titre, they will not be harmful to the A and B antigens on the red cells of the AB recipient.

Clinical significance in haemolytic disease of the fetus and newborn

Some individuals produce potent, high titre anti-A and/or anti-B, consisting of a mixture of IgM and IgG antibodies, with haemolysing characteristics in the presence of complement. This immune anti-A and/or -B in pregnant women can cause ABO HDFN with varying degrees of severity, although the fetus is rarely affected in utero. ABO HDFN typically develops within a few days of birth.

See Section 7: Haemolytic Diseases for more information.

LECTINS (PLANT AGGLUTININS)

Certain plant extracts (usually seeds) agglutinate human and animal red cells. Two names have been suggested for these plant agglutinins: phytagglutinins and lectins, the latter for those which show red cell specificity. Note that these substances are not antibodies. Some lectins are described below:

Lectin anti-A

The most useful anti-A is found in Dolichos biflorus: the extract strongly agglutinates A, and A, B cells; it reacts less strongly with A, B cells and very weakly with A, B cells. The extract can therefore be standardized by dilution as a specific anti-A, reagent.

Lectin anti-H

Lectin anti-H can be extracted from the seeds of Ulex europaeus or the common European gorse. Ulex is invaluable for the classification of group O secretor/non-secretor saliva (or for group O secretor status).

ABH SECRETION

In addition to being present on the red cells, A, B and H antigens are present on most other body cells as glycolipids. Blood group substances of the same ABO group as the red cells may also be found in the serum/plasma and are readily detectable in the saliva and other body fluids of most individuals.

The secretor status is controlled by the Se and se genes at the secretor locus. Se is the dominant gene and is responsible for the secretion of H. Approximately 75% of the general population secrete ABH substances (in the form of water soluble antigens) in abundance in all their body fluids. The ABO group of a secretor may be determined by testing the saliva to determine the presence or absence of A, B and H substance. The remaining 25% of the population are termed non-secretors. Table 6.10 shows the soluble antigens secreted according to ABO group.

Table 6.10: soluble ABH antigens according to ABO group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Soluble antigens present</th>
</tr>
</thead>
<tbody>
<tr>
<td>A secretor</td>
<td>A and H</td>
</tr>
<tr>
<td>B secretor</td>
<td>B and H</td>
</tr>
<tr>
<td>O secretor</td>
<td>Abundant H</td>
</tr>
<tr>
<td>AB secretor</td>
<td>A, B and a little H</td>
</tr>
<tr>
<td>Non-secretor</td>
<td>Not readily detectable</td>
</tr>
</tbody>
</table>
UNIQUE FEATURES OF THE ABO SYSTEM

The critical unique feature of the ABO blood group system is that unlike other blood group systems the anti-A and/or anti-B alloagglutinins are invariably present in the serum/plasma of every healthy adult when the corresponding antigen is absent.

As the ABO antigens are widely distributed throughout the body, the ABO group must be considered in organ transplant. Some organs e.g. kidney, must be ABO compatible. In bone marrow transplant, ABO incompatibility is acceptable due to lack of expression of ABO on stem cells, but precautions need to be taken such as removal of the unwanted donor red cells or plasma.

Note: Anomalous red cell typing may be seen post transplantation.

Practical application
The cornerstone of safe blood transfusion practice is to transfuse safe blood of the compatible ABO group. It is critical that the ABO group on all samples, whether from a patient or a donor, is correct, as ABO group mistyping can have fatal consequences.

Rh BLOOD GROUP SYSTEM

The discovery of the Rh systems by Landsteiner and Wiener in 1940, together with the work of Levine and Stetson in 1939, heralded the greatest discovery in the blood grouping field since Landsteiner described the ABO system in 1900.

In 1939 Levine and Stetson described how the mother of a stillborn fetus suffered a severe haemolytic reaction when transfused with her husband’s blood. The mother, who obviously lacked some ‘new’ antigen, must have become immunized by her fetus that possessed this antigen, having inherited it from the father. When the ABO compatible husband’s blood was transfused, the maternal antibody reacted with this same antigen on his red cells.

In 1940 Landsteiner and Wiener, having immunized rabbits with the blood of a rhesus monkey (Macaca mulatta), discovered that the resulting antibodies agglutinated not only the monkey red cells but also the red cells of about 85% of white people.

Later work, however, showed that the red cell antigens detected by the human-derived antibody and the animal antibody were not identical and belonged to two different blood group systems. The blood group system detected by the human-derived antibodies is now known as Rh (not Rhesus) and the antigen is called D. The antigen originally described by Landsteiner and Wiener is LW in the LW blood group system. The two systems are serologically, biochemically and genetically different from one another. The locus for the Rh genes is on chromosome 1 and is linked to the gene for elliptocytosis. The locus for LW is on chromosome 19.

It was soon realized that the Rh antibodies produced in humans were not as simple as they had first appeared, and that many sera contained antibodies to more than one specificity. Many related antigens were found by workers in England and in the United States of America. This led to the discovery of the five major Rh antigens, D, C, E, c, and e. The Rh blood group system has now been shown to be one of the most complex multi-allelic blood group systems with more than 50 antigenic specificities having been described.

Rh GENETICS AND INHERITANCE

Most individuals are either D+ or D- and the expression or absence of the D antigen on the red cells results from the presence or absence of an RHD gene. A D+ individual may inherit two RHD genes, one from each parent (homozygous) or one RHD gene from either parent (hemizygous). The two pairs of antithetical antigens C and c, and E and e are controlled by the various RHCE genes. The RHD and RHCE alleles are inherited as a gene complex or haplotype.

Two different theories were initially proposed for the genetics and inheritance of the Rh blood group system but these have been disproved by molecular genetic studies.

Fisher Race theory (UK – theory of 3 pairs of linked genes)
In 1943 the statistician Fisher, studying the results of Race and co-workers in England, noticed that some reactions were antithetical (opposite), and he therefore supposed that there were three sets of alleles involved: C and c, D and d, E and e. Fisher assumed that the three genes, if separable, must be very closely linked, for no crossing-over had been observed.

The CDE nomenclature was devised and although it did not accommodate subsequent complexities in the Rh system it was easy to use.

Although this theory suggests that antibodies to all the antigens described are able to be stimulated in individuals lacking the corresponding antigen, no anti-D has ever been found.

Wiener theory (USA – theory of multiple allelicomorphic genes)
This is a theory of multiple allelic genes occurring at a single chromosomal locus (rather than at three closely linked loci). One gene complex is inherited from the mother and one from the father. It was thought that each gene complex produced an agglutinogen which had several serologic specificities (blood factors or antigens). One agglutinogen could react with various antibodies because it had as part of its structure more than one antigen. The Rh-Hr nomenclature was developed to describe the gene complexes, agglutinogens and antigens.

Molecular studies

Following studies on the Rh blood group system at the DNA level, it has been shown that there are two Rh genes, RHD and RHCE at the Rh locus. The RHD gene produces the D antigen. At the RHCE gene locus, depending on the allele present, one of four alternative antigenic combinations are produced, namely ce, Ce, ce or Ce. D+ individuals inherit two Rh genes: RHD coupled with one of the alleles of RHCE from each parent. In most D- individuals, RHD is deleted, and individuals possess the RHCE gene only. As a result, most D- individuals lack the total RhD protein on their red cell membrane but this does not appear to have an adverse effect on the cell function. Note that many apparent D- individuals of African origin have a D antigen, which is not expressed due to the presence of the RHD pseudogene or inactive gene.
The RHD and RHCE locus genes produce separate proteins but are located in the red cell membrane next to each other, forming a complex of antigens. As the two gene loci are in such close proximity, many of the usual Rh variants are the result of various genetic occurrences within the two gene loci, such as unequal crossing over, or mutations. Both the RhD protein and the RhCcEe proteins comprise 400 amino acids each. Whereas D- individuals generally lack the entire RhD protein, the difference between ce and Ce proteins are differences in four amino acids, and ce and C proteins differ by one amino acid.

The presence or absence of the RHD gene, together with one of the four possible alleles of the RHCE gene, results in eight possible gene combinations or complexes. The Rh genotype is therefore a combination of any of the eight possible haplotypes. One Rh gene complex or haplotype is inherited from each parent.

**Table 6.11**

<table>
<thead>
<tr>
<th>Rh genes present</th>
<th>Gene complex/haplotype</th>
<th>Shorthand nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD gene</td>
<td>RHCE gene</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Ce</td>
<td>Dce</td>
</tr>
<tr>
<td>D</td>
<td>cE</td>
<td>DcE</td>
</tr>
<tr>
<td>U</td>
<td>ce</td>
<td>Uce</td>
</tr>
<tr>
<td>U</td>
<td>ce</td>
<td>Uce</td>
</tr>
<tr>
<td>d</td>
<td>le</td>
<td>dle</td>
</tr>
<tr>
<td>d</td>
<td>cb</td>
<td>dcb</td>
</tr>
<tr>
<td>d</td>
<td>ce</td>
<td>dce</td>
</tr>
<tr>
<td>d</td>
<td>lb</td>
<td>dlb</td>
</tr>
</tbody>
</table>

**Numerical terminology**

In 1962 Rosenfield introduced a new terminology for the Rh system based on a numerical system. Each antigen was numbered, as was the antibody detecting it. For example, the D antigen is Rh:1 and anti-D is anti-Rh1. Blood lacking the D antigen is noted as Rh:-1 (minus one). This numerical terminology lends itself to computerization. It is now the basis for the ISBT terminology for all the blood groups.

**Terminology hints**

The Rh terminology can be very confusing and there are several different ways of documenting Rh. Below are several points which may be of assistance when using the shorthand notation:

- Whenever D is present use the letter R together with the appropriate number or symbol (see points 2, 3 and 4 below).
- The C antigen is associated with either < or > (prime). If C occurs with D use . When D is absent use *(e.g. Dce = R<sub>1</sub> and Dce = r<sub>1</sub>).*
- The E antigen is associated with either ' or » (double prime). If E occurs with D use _. When D is absent use # (e.g. DcE = R<sub>2</sub> and DcE = r<sub>2</sub>).)
- When C and E are absent, but D is present the notation R<sub>1</sub> is used.
- The phenotypes dCe and DCE are both very rare, so it seems logical to use y and z to describe them (r<sup>y</sup> and r<sup>z</sup>).
- Genotypes are written in italics, and subscripts become superscripts (e.g. R<sub>1</sub> becomes R<sup>1</sup>).

**Frequencies**

The frequency of the eight possible Rh gene haplotypes varies between different populations. For example, the gene combination R<sup>y</sup> is seen more frequently in black people, particularly in sub-Saharan Africa, than in white people, whereas the haplotype r<sup>y</sup> is more frequent in white people than in black people. Table 6.12 gives the frequencies of some of the Rh genotypes in a UK population.

**Table 6.12**: Frequencies of some Rh genotypes in a UK population

<table>
<thead>
<tr>
<th>Rh genotype</th>
<th>Percentage frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sup&gt;y&lt;/sup&gt;R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>18</td>
</tr>
<tr>
<td>R&lt;sup&gt;y&lt;/sup&gt;R&lt;sup&gt;r&lt;/sup&gt;</td>
<td>13</td>
</tr>
<tr>
<td>R&lt;sup&gt;y&lt;/sup&gt;R&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>R&lt;sup&gt;r&lt;/sup&gt;R&lt;sup&gt;r&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>R&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>r&lt;sup&gt;y&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
<td>r&lt;sup&gt;r&lt;/sup&gt;</td>
<td>0.4</td>
</tr>
<tr>
<td>r&lt;sup&gt;r&lt;/sup&gt;</td>
<td>0.8</td>
</tr>
<tr>
<td>r&lt;sup&gt;y&lt;/sup&gt;r&lt;sup&gt;y&lt;/sup&gt;</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>r&lt;sup&gt;y&lt;/sup&gt;r&lt;sup&gt;r&lt;/sup&gt;</td>
<td>Very rare</td>
</tr>
<tr>
<td>r&lt;sup&gt;r&lt;/sup&gt;r&lt;sup&gt;y&lt;/sup&gt;</td>
<td>Very rare</td>
</tr>
</tbody>
</table>
Rh TYPING

D typing

The D antigen is the most clinically significant blood group antigen in the Rh system. Individuals are divided into D+ or D- based on D typing results which detect the presence or absence of the D antigen. The frequency of the D antigen varies in different populations, e.g., in a white population 85% are D+ and 15% D- but in the Far East it is extremely rare to type D-. Ideally a patient or donor sample should be tested with two different anti-D reagents and if the test results concur then the sample can be designated D+ or D-. As a result of the introduction of potent monoclonal anti-D typing reagents as routine blood grouping reagents, many red cells previously typed as weak D are now typed D+ and the typical former weak D types cannot be differentiated from D+ in routine tests.

Donor red cells should be typed for weak D either using a sensitive technique such as the indirect antiglobulin test (IAT) or by using the appropriate monoclonal blood grouping reagents, which are known to detect weak D.

Patients should be tested with the appropriate anti-D reagents. It is not necessary to detect the very weak D variants because if patients are typed D-, they should receive D- blood (see Rh antigens later in this section).

Terminology notes

It should be noted that there are various methods to describe the results of D typing and the Rh type, as indicated in the list below:

Result: D+ and D-

Description: D+ and D-
RhD-positive and RhD-negative
RhD positive and RhD negative
Rh-positive and Rh-negative
Rh positive and Rh negative
D-positive and D-negative

In this publication the terminology D+ and D- is used together with Rh-positive and Rh-negative where appropriate. The term Rh-positive indicates that the test result is D+. The term Rh-negative indicates that the test result is D-.

Rh phenotyping

The Rh phenotype can be determined by typing the red cells with specific reagents; anti-D, anti-C, anti-E, anti-c and anti-e in the laboratory. Positive and negative test results using these reagents denote the presence or absence of the Rh antigens and this is known as the Rh phenotype.

Table 6.13 shows the variation in percentage frequency of the various Rh phenotypes in one study of southern African populations. The symbol d is used to denote the absence of the D antigen.

It is not possible to determine the genotype of an individual from the red cell phenotype result but the most probable genotype can be deduced using the haplotype frequency data. The phenotypes shown in Table 6.13 do not reflect one specific genotype. For example, cells which are phenotypically R_{r}D may be genotypically R^{D}R^{d} or R^{d}R^{d}.

However, as the genotype incidence varies between different populations and ethnic groups, the ‘probable genotype’ result should be treated with reservation. It is an advantage to know the ethnicity of the individual being typed so that the appropriate frequencies can be used. The phenotype cannot determine if the red cell sample is homozygous or heterozygous for the RhD gene as there is no d gene product. Accurate genotype determination can only be established by the use of molecular genetic techniques or by informative family studies.

Table 6.14 shows an example of an Rh phenotype and the possible genotype options, using figures derived from UK statistics for a white population. The probable genotype can then be determined.

It should also be noted that the presence of the very rare variant genes such as --- (Rh_{null}), Uc- and Uc-- result in all or some of the Rh antigens missing from the red cells and this will affect the possible genotype calculations.

Rh ANTIGENS

The Rh antigens are coded for by the RH and RHCE genes, each of which produces a separate protein which is inserted into the red cell membrane. The RhD protein crosses the red cell membrane 12 times, giving rise to six extracellular domains. Despite many studies,
the exact function of the Rh proteins within the red cell membrane is unknown, but their structure suggests a transmembrane transporter function. The functions of the RhD and RhCcEe proteins appear similar. In cases of the very rare type Rhnull, the absence of the Rh proteins has shown that the red cells are abnormal morphologically and individuals often suffer from some degree of haemolytic anaemia as the red cells are abnormal in shape.

D antigen

The vast majority of all populations are D+. The D antigen is extremely immunogenic and is likely to stimulate antibody production in D- individuals exposed to the D antigen. It is the most clinically significant of the Rh antigens and plays a significant role in HDFN (refer to Section 7: Haemolytic diseases).

Weak D (previously termed D0)

Weak D describes a weaker form of D+, where fewer D antigen sites are present on the red cell as compared with a normal D+. Studies have shown that D+ red cells of the R/ phenotype have about 10,000 antigen sites per cell, while R/Rp phenotypes have about 30,000 sites. Weak D cells have far less than this number, although the number is variable. The weak D characteristic is usually the result of the inheritance of a genetic variation.

The term D0 for weak D is now obsolete. It was used to describe those forms of the D antigen that reacted weakly in laboratory tests when tested with different polyclonal anti-D reagents. With the use of monoclonal anti-D reagents many weak D types now type as D+ and cannot be distinguished from normal strength D+ in a routine laboratory.

The identification of a weak D type will depend on the anti-D reagents selected for use and the technique used. If two potent, type IgM agglutinating monoclonal anti-D reagents are used routinely for patient testing, most weak D samples will type D+. Only the weakest form of weak D will be identified as weak or may be typed as D- and such patients will receive D- blood. The weak D type should, however, be detected in donor samples by either proceeding to the second (IAT) phase of a monoclonal blend reagent or by using agglutinating monoclonal anti-D reagents specifically selected for the ability to detect weak D.

Partial D

In 1953 there was a report of a D+ individual who had anti-D in the serum/plasma. Since then, many examples of D+ with anti-D have been reported, although overall it is a rare occurrence.

The term partial D is used to describe the phenotype of those rare individuals, whose red cells lack one or more of the D epitopes. The D antigen is considered to be a mosaic of epitopes. If some D epitopes are missing, then the individual can make an antibody specific for the missing epitope/s if they are exposed to normal D+ cells. The anti-D produced in this way reacts with all normal D+ cells, which have all the epitopes, but fails to react with their own cells and cells of the same or similar partial D types.

In their studies Wiener and Unger used the Rh0, Rh4, RhC and RhE classification to describe the Rh mosaic antigen, but this terminology is now obsolete.

Tippett and Sanger used the Category Classification to describe partial D. Their original classification, using human sera, divided partial D bloods into six categories: I, II, III, IV, V and VI, with category VI containing fewer D epitopes than the others. The categories were shown to be inherited. The original framework of this study has allowed for the addition of further partial D types complexities, as more information became available. The use of epitope-specific monoclonal anti-D has enabled the partial D to be categorized further into categories II, IIIa, IIIb, IVa, IVb, Va, VI, VII and many others. Panels of monoclonal antibodies are now available to classify partial D types. The molecular structures of the many partial D types have been extensively studied and it has been shown that various RHD and RHD-CE-D, and RHCE-D-CE hybrid genes give rise to different partial D types.

Clinical significance of weak D and partial D

Weak D individuals do not usually produce anti-D although isolated cases have been reported whereas partial D type individuals may develop clinically significant anti-D.

Patients:
- Weak D patients very rarely form anti-D, and it is not considered necessary for weak D mothers who carry D+ infants to be given anti-D immunoglobulin.
- As routine anti-D typing reagents do not differentiate most partial D type bloods from normal D+ bloods, these rare individuals may be stimulated to produce anti-D if transfused with D+ blood. The anti-D can cause severe HDFN.
- A partial D mother will not be identified as a candidate for anti-D immunoglobulin. Should she deliver an infant with normal D and have a transplacental haemorrhage, she may be stimulated to develop anti-D to the missing epitope(s). Future pregnancies may be complicated by HDFN.

Donors:
- Blood donors should be tested for weak D, and weak D blood should be transfused only into D+ recipients.
- Most partial D blood donors will be typed as D+.

Trans effect of C

A weak D phenotype can occur as a result of the trans effect of C. If the haplotype encoding the D antigen is in trans (on the opposite chromosome) with a haplotype encoding C but not D (e.g. dCe), the expression of the D antigen may be weak. Family studies have shown that when the haplotype encoding the weak D is not in trans with C, then the D antigen is expressed normally. For example, Dce/dCe will type as weak D, while Dce/dce will type as normal D.

Cc and Ee antigens

The Cc and Ee antigens are less immunogenic than the D antigen and may demonstrate dosage effect depending on the reagent used. A number of variants, particularly variants of the c antigen, have been described such as the rare hrc and hrC, found mainly in black people.

C* antigen and anti-C*

Although the C* antigen was originally thought to be an allele of L; it has been shown to be an allele of the high frequency Rh antigen MAR. C*+ cells are usually C+. Anti-C* is not necessarily produced in response to a known red cell stimulus and may occur in combination with other antibodies to low frequency antigens.
G antigen and anti-G
Red cells which are C+ or D+ are generally G+, although very rare exceptions have been reported. The Rhc and RhD proteins share an amino acid sequence that is recognized by anti-G. Many anti-C+D sera may therefore contain anti-G. Anti-G will appear to be Anti-C+D in routine laboratory tests but anti-G can be separated from anti-D and anti-C by adsorption and elution studies, or identified by the use of rare cells.

Other Rh antigens
The Rh blood group system is a very complex system, and 49 different antigens, including the ones discussed previously, are recognized by the ISBT Committee on Terminology for Red Cell Surface Antigens.

CLINICAL SIGNIFICANCE OF Rh SYSTEM

Rh antibodies are nearly always immune type IgG antibodies that have been stimulated by exposure to foreign red blood cells either through pregnancy or transfusion.

Clinical significance in transfusion
The antibodies in the Rh blood group system can cause severe transfusion reactions and are second only to the ABO system in this regard. The transfusion of D+ blood into D- patients should be avoided, as the D antigen is highly immunogenic and can stimulate antibody production in the recipient. Group O (low titre) is considered to be the universal donor. The universal donor should also be D- if the blood is to be transfused into D- patients. However, in order to conserve supplies of D- blood, one may consider transfusing crossmatched D+ blood into male D- recipients in cases of severe blood shortage. The reasoning is that should anti-D be produced, it will not be able to cause HDFN.

Good laboratory practices should ensure that both patient and donor are correctly Rh typed and that incompatible transfusions resulting from the presence of Rh antibodies are avoided.

There is a risk that patients who lack the C, E, c or e antigens will be exposed to these antigens during transfusion. This occasionally leads to the production of the corresponding antibodies, but these antigens are far less immunogenic than the D antigen. For example, a recipient who has the Rh phenotype Dce/Dce may receive blood from a donor who is Dce/Dce. In this example, it is possible that the recipient may be stimulated to produce of anti-c and/or anti-E antibodies. Further transfusions of blood positive for c and/or E antigens may cause a haemolytic reaction if the antibody is not detected during crossmatching.

In the case of multi-transfused patients or patients on long term transfusion therapy, Rh phenotyped, matched blood should be provided i.e. the units selected should be matched to the patients’ Rh phenotype. The blood should also be matched for the K antigen in the Kell blood group system, which is strongly immunogenic. This will prevent antibodies being formed to these antigens which could complicate future transfusions or pregnancies.

When Rh antibodies are present, antigen negative crossmatch compatible blood should be transfused.

Clinical significance in haemolytic disease of the fetus and newborn
Rh antibodies, particularly anti-D and anti-c are capable of causing HDFN. (See Section 7: Haemolytic diseases for details). Anti-D is the most common cause of severe HDFN and may be combined with other Rh antibodies e.g. anti-D + anti-C.

Although ‘enzyme only’ reacting Rh antibodies may be detected (they often have anti-E specificity), the Rh antibodies that cause HDFN are type IgG and are best detected using iAT.

A fetomaternal haemorrhage (FMH), either during pregnancy or during delivery, acts in exactly the same way as a transfusion, although the volume of cells is much smaller. The mother may be exposed to antigens she lacks, but which the fetus has inherited from its father. The best example of this is the D- mother who has a FMH of D+ blood and develops anti-D, which may cause HDFN in subsequent pregnancies if the fetus is D+.

HDFN usually increases in severity with each succeeding D+ pregnancy. The anti-D antibodies increase in titre and avidity with every additional immunization of fetal cells, so that eventually they may be of sufficient potency to cause intrauterine death of the fetus. Before the advent of prophylaxis (preventative treatment) with Rh immunoglobulin, anti-D was responsible for about 90% of severe haemolytic disease cases.

Other Rh antibodies, particularly anti-c, may cause severe HDFN. For example, it is possible for an Rh-positive (Dce/Dce) mother who has been stimulated to produce anti-c, to give birth to an Rh-positive (Dce/dce) infant suffering from HDFN caused by anti-c.

UNIQUE FEATURES OF Rh SYSTEM

- The Rh blood group system is an extremely complex blood group system.
- Many variant antigens have been described.
- The antibodies are generally type IgG and are clinically significant.
- Anti-D is the most common cause of severe HDFN.

Practical application
1. It is essential that the laboratory system enables both patients and donors to be correctly Rh typed using the appropriate reagents.
2. Adequate antibody screening tests must be available to detect the clinically significant antibodies; in patients requiring transfusions, pregnant women and donor plasma.
3. Anti-D immunoglobulin should be given to all D- women who have not developed anti-D, antenatally and/or postnatally, the regime followed depending on the guidelines for the particular service/country.
4. If D+ blood was given to a D- patient with child bearing potential, whether in emergency or in error, the administration of anti-D immunoglobulin to the patient should be considered, the size of the dose depending on the volume of blood transfused.
OTHER MAJOR BLOOD GROUP SYSTEMS

Twenty nine different blood group systems have been recognized, including the two most important systems just described. In addition to the red cell antigens described in the ABO and Rh blood group systems, there are many more that can be detected on human red cells by specific antibodies and these are allocated to blood group systems, collections or series.

The succeeding pages will describe the other major blood group systems: MNS, P, Kell, Duffy, Kidd, Lewis, and Lutheran. Other systems of practical importance in the laboratory such as I will be described thereafter with some comments on the other systems/collections.

MNS BLOOD GROUP SYSTEM (discovered in 1927)

Well-known antigens: M, N, S, s, U
Number of antigens: 46 (2006).

Table 6.15 provides the MNS blood group frequencies excluding U.

Table 6.15: MNS blood group frequencies in percentage: excluding U which is a high frequency antigen

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+N-</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>M+N+</td>
<td>50</td>
<td>44</td>
</tr>
<tr>
<td>M-N+</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>S+s-</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>S+s+</td>
<td>44</td>
<td>28</td>
</tr>
<tr>
<td>S-s+</td>
<td>45</td>
<td>69</td>
</tr>
</tbody>
</table>

Although the MN types and Ss types are shown separately in the table, the two sets of alleles form a single blood group system. The MN antigens are situated on glycophorin A (GPA) and the Ss antigens on glycophorin B (GPB). There are two amino acid differences between the M and N on glycophorin A and a single amino acid difference between S and s on glycophorin B. Amino acid substitutions and hybrid GPA/GPB glycophorins result in many of the large number of antigens identified within this system e.g. M\(\text{a}\), Miltenberger, St\(\text{a}\), Dantu and others. The very rare type M\(\text{b}\) is the result of the absence of both red cell GPA and GPB.

The S and s antigens are associated with the high frequency antigen, U. The rare phenotype S-s-U occurs in black populations and a rare variant form of U gives rise to an S-s-U+ phenotype.

Antibody characteristics

1. Enzyme treatment of red cells denatures M, N and S antigens but not s and U, resulting in anti-M, anti-N and anti-S not usually being demonstrable by enzyme techniques.
2. Some examples of anti-M and anti-S are enhanced by lowering the pH of the serum.
3. M and N antibodies are usually cold agglutinins reacting by saline agglutination techniques. The antibodies seldom react above +20°C.
4. Occasionally, anti-M and anti-N react by IA/I.
5. Although anti-S may be naturally occurring, it is usually a complement-binding type IgG antibody. Anti-S is often produced together with antibodies to low frequency antigens or ‘private’ antigens.
6. Anti-s seldom occurs and may be demonstrable at temperatures below +20°C or by IAT.
7. Anti-U antibodies are usually demonstrable by IA/I, but some may react by enzyme methods.
8. Monoclonal anti-M, anti-N and anti-S reagents are available for red cell typing and a lectin anti-N (Vicia graminea or Vicia unijuga) is available for N typing.

Clinical significance

Transfusion

- Anti-M and anti-N are generally considered not to be of clinical significance and have rarely been the cause of transfusion reactions. IA/I crossmatch compatible blood is usually given.
- Anti-S, -s and -U are considered clinically significant and may cause moderate to severe transfusion reactions. Crossmatch compatible and antigen negative blood should be selected for transfusion.

Haemolytic disease of the fetus and newborn

- Anti-M and anti-N have occasionally caused mild HDFN but are not considered to be of obstetric significance.
- Anti-S, anti-s and anti-U may cause moderate to severe, even fatal HDFN.

Features

- The M and N antigens are within the glycophorin molecules of the red cell membrane.
- Anti-M and anti-N antibodies are not usually detected in tests using enzymes such as papain or bromelin.

Practical application

1. Anti-M and N are not considered to be clinically significant when they react at low temperatures. Examples that react at +37°C should be considered to be of possible clinical significance.
2. Anti-S and anti-s can cause severe transfusion reactions and HDFN.
3. U is a high frequency antigen and if a patient with anti-U requires blood it will be extremely difficult to find random U- donors. A rare donor registry needs to be consulted.

P BLOOD GROUP SYSTEM (discovered in 1927)

Well-known antigen: P
Number of antigens: 1 (2006)

P\(_{1}\) is the blood group antigen in the P system (ISBT 003) and the terminology P\(_{1}\) indicates the lack of P\(_{1}\) antigen. The associated antigens, P, P\(_{0}\) and LKE, are not included in the P blood group system but are mentioned here for convenience. The P and LKE antigens are controlled by genes at a different locus to that for P\(_{1}\) antigen. The P\(_{1}\) antigen has been allocated to the Globoside Blood Group System (028). P\(_{1}\) remains in Collection 209, symbol GLOB, until more information is available to allocate it to a specific system. LKE is also classified in Collection 209, GLOB.

The frequency of the P\(_{1}\) antigen varies in different populations as shown in Table 6.16.

Table 6.16: Frequency of P\(_{1}\) antigen in percentage

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(<em>{1}) (or P(</em>{2}))</td>
<td>79</td>
<td>94</td>
</tr>
<tr>
<td>P(<em>{1})- (or P(</em>{2}))</td>
<td>21</td>
<td>6</td>
</tr>
</tbody>
</table>
Antibody characteristics

1. Anti-P₁ is usually a type IgM antibody. It is commonly encountered as a cold agglutinin but occasionally reacts at +37°C. However, some rare examples may bind complement and react by IAT.
2. Anti-P₂ that binds complement and reacts by IAT may cause transfusion reactions.
3. Other antibodies that are not actually part of the P blood group system are noted here:
   (i) Anti-PP₁P* (previously called anti-Tj*) is a rare, potent antibody found in the very rare type p or minus/minus individuals. (P is the only antigen in the Globoside system ISBT 028). The antibody reacts at all temperatures by all methods and is frequently present as a haemolysin. It causes transfusion reactions, and is a potential cause of recurrent abortions. It rarely causes HDFN.
   (ii) Anti-P₁ is found in the serum of all P⁺ individuals and will haemolysed P₁ and P₂ cells in the presence of complement. Anti-P₁ is also found in cases of paroxysmal cold haemoglobinuria (PCH). PCH is a haemolytic disease which occurs mainly in children following a viral infection. The sera from such patients give a positive Donath-Landsteiner test.

Clinical significance

Transfusion
Anti-P₁ is not generally considered to be clinically significant even when reactive at +37°C and it is not usually necessary to select antigen negative blood. The blood crossmatched is considered compatible if negative by IAT.

Haemolytic disease of the fetus and newborn
Anti-P₁ has not been reported to cause HDFN.

Features

1. Anti-P₁ is usually not of clinical significance.
2. P₁ antigen is weakly expressed at birth.
3. P₁ substance can be found in various flatworms, and hydatid (tape worm) cysts in sheep livers. P₁ substance from avian sources, e.g. pigeon egg white can be used in inhibition tests.
4. The frequency and avidity of anti-P₁ is increased in P₁- individuals suffering from helminth infestations (parasitic worm e.g. hookworm).

Practical application

1. The P₁ antigen varies considerably in antigenic strength and known strong P₁+ cells should be used for antibody detection.
2. Anti-P and anti-PP₁Pk are antibodies to high frequency antigens and it is extremely difficult to find compatible blood for patients requiring blood transfusions.

KELL BLOOD GROUP SYSTEM (discovered in 1946)

Well-known antigens: K and k, Kp₁ and Kp₂, Js¹ and Js²

The Kell blood group system is extremely complex. Twenty four Kell system alleles have been identified at the KEL locus on chromosome 7. The Kell system is also associated with the Kx and the Gerbich blood group systems, which adds to its complexity.

The most commonly encountered antigens in the laboratory are the K and k antigens. After the ABO and Rh antigens, the K antigen is the most immunogenic.

Table 6.17 shows the Kell blood group frequencies.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺K⁻</td>
<td>0.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>K⁺K⁺</td>
<td>8.8</td>
<td>3.5</td>
</tr>
<tr>
<td>K⁻K⁺</td>
<td>91.0</td>
<td>96.5</td>
</tr>
</tbody>
</table>

Many different Kell and para-Kell antigens are produced and the frequency of the antigens, other than the high and low frequency antigens, varies greatly between populations. The well-known high frequency antigens are K, Kp₁ and Js².

The para-Kell antigens are very high frequency antigens that are absent from Ko cells, but have not been shown to be controlled by genes at the KEL locus, although they are located on the Kell glycoprotein. Depending on the specificity, the antibodies react with the corresponding para-Kell antigens, but not with K⁺ cells.

The K₁ type is the rare null type in the Kell system. Ko red cells lack all the Kell and para-Kell antigens. K₁ patients who have become immunized frequently produce anti-Ku, an antibody which reacts with all cells except K₁ cells. This makes the provision of compatible blood for such patients extremely difficult.

The chemical, dithiothreitol (DTT), destroys all the Kell and para-Kell antigens, so the detection of Kell antibodies is not possible if the following are used in laboratory tests:
- DTT to determine whether IgM or IgG antibodies are present in a sample.
- A ZZAP solution which contains DTT and an enzyme such as papain or bromelin (as used for auto adsorptions). See Glossary for information on ZZAP.

Red cells of the very rare McLeod phenotype show weakened expression of the Kell high frequency antigens, together with the para-Kell antigens and the K antigen if present. The degree of depression of the different antigens varies.

Depressed Kell system antigens have also been observed in individuals of the rare Ge⁻:2,-3 phenotype in the Gerbich system.

Antibody characteristics

1. Anti-K and anti-k are usually type IgG antibodies, reacting optimally by IAT and some examples react by enzyme techniques.
2. Some examples of anti-K will react at temperatures below +37°C.
3. Although anti-K is usually produced in response to stimulus by transfusion or pregnancy, ‘naturally occurring’ cases have been reported and are possibly due to bacterial infections such as *Escherichia coli*. This type of anti-K is usually transient.
4. Some examples of anti-K react more weakly in tests using LISS or polybrene.
5. If a patient develops anti-K it will be difficult to provide compatible blood due to the low frequency of k- blood. However, anti-k is a relatively rare antibody as few individuals are k-.

Clinical significance

Transfusion

Anti-K and anti-k are clinically significant and can cause severe haemolytic transfusion reactions and delayed transfusion reactions.

Haemolytic disease of the fetus and newborn

Anti-K antibodies differ from the other blood group system antibodies that cause HDFN as the antibodies appear to destroy the precursor red cells, causing severe anaemia, and often death of the fetus. High bilirubin levels are not a characteristic as the precursor cells are destroyed. Amniocentesis therefore does not give an indication of the severity of the disease.

Practical application

K- or k- (i.e. antigen negative) blood and crossmatch compatible blood should be provided to patients with anti-K or anti-k antibodies respectively. In K- patients requiring long term transfusion therapy, units matched for K as well as the various Rh antigens should be provided.

Kp and Kp

The Kp and Kp antigens result from the presence of two allelic co-dominant genes in the Kell blood group system. The Kp antigen is a low incidence antigen occurring in about less than 2% of individuals. The Kp antigen is a high incidence antigen occurring in more than 98% of individuals. The antibodies are IgG, reacting optimally by IAT and are clinically significant both in transfusion and HDFN. The provision of Kp(b-) blood to patients who have become immunized is difficult as most donors type Kp(b+). Antigen negative and IAT crossmatch compatible blood should be transfused when the intended recipient has the corresponding antibodies.

Js and Js

The Js antigen is found mainly in individuals of African origin. The incidence is approximately 20% in black people and less than 0.1% in white people. The Js antigen is a high incidence antigen found in more than 98% of individuals. The antibodies are type IgG reacting best by antiglobulin techniques. Anti-Js is seldom seen. All examples of anti-Js have been detected in black people. Both antibodies have caused HDFN and transfusion reactions. Antigen negative and IAT crossmatch compatible blood should be transfused. If the rare type Js(a-b-) blood is required it may be obtained through a rare donor registry.

General comment

The antibodies to the Kell system are usually of clinical significance and may cause severe or delayed transfusion reactions. The respective antigen negative and IAT crossmatch compatible blood should be transfused. If the antibody is to a high frequency antigen in the Kell blood group system it may be necessary to obtain the antigen negative blood from a rare donor registry.

### Duffy Blood Group System (Discovered in 1950)

**Well-known antigens:** Fy, Fy

**Number of antigens:** 6 (2006).

A pair of co-dominant allelic genes located on chromosome 1 at the Duffy blood group locus is responsible for the production of the \( Fy^a \) and \( Fy^b \) antigens.

The frequency of the Duffy blood group system antigens varies greatly between different populations. The gene \( Fy \), which is a silent recessive gene, is very rare in white populations but occurs frequently in black African populations, particularly in malaria areas. A homozygous expression of the \( Fy \) gene results in the \( Fy(a-b-) \) phenotype and it has been shown that these red cells are resistant to invasion by the malaria parasites *Plasmodium vivax* and *Plasmodium knowlesi*. It is therefore an advantage for individuals living in malaria areas to lack the Duffy red cell antigens. Table 6.18 shows the Duffy blood group frequencies.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Fy(a+b-) )</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>( Fy(a+b+) )</td>
<td>47</td>
<td>1</td>
</tr>
<tr>
<td>( Fy(a-b+) )</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>( Fy(a-b-) )</td>
<td>0</td>
<td>68</td>
</tr>
</tbody>
</table>

The \( Fy^a \) and \( Fy^b \) antigens are destroyed by the action of proteolytic enzymes. The antigens also progressively deteriorate within a short period of time when the cell suspensions are prepared in some saline or LISS solutions. The antigens are, however, well preserved in anticoagulant solutions and preservation solutions for red cells.

The Duffy protein spans the red cell membrane nine times and is 338 amino acids in length. The difference between \( Fy^a \) and \( Fy^b \) is due to one amino acid substitution within the protein.

**Antibody characteristics**

1. Anti-\( Fy^a \) and anti-\( Fy^b \) are type IgG and can be stimulated by transfusion and pregnancy.
2. Anti-\( Fy^a \) and anti-\( Fy^b \) antibodies react by antiglobulin technique and are not detected by enzyme techniques.
3. Anti-\( Fy \) is often detected together with other red cell antibodies, particularly Rh antibodies.
4. Anti-\( Fy \) antibodies are not frequently encountered.

**Clinical significance**

The Duffy system antibodies are capable of causing severe transfusion reactions and HDFN. Antigen negative and IAT crossmatch compatible blood should be transfused to patients with antibodies.
KIDD BLOOD GROUP SYSTEM (discovered in 1951)

Well-known antigens: Jk⁺, Jk⁻
Number of antigens: 3 (2006).

A pair of co-dominant allelic genes located on chromosome 18 at the Kidd blood group locus is responsible for the production of the Jk⁺ and Jk⁻ antigens.

The frequency of the Kidd blood group system antigens vary between different populations.

The Jk⁺ antigen occurs more frequently in black populations.

The rare Jk(a-b-) or Jk:-3 phenotype (the null type in the Kidd blood group system), has been reported in some Polynesian, South American and South African Indian populations. The urea lysis test is a useful test for screening for the rare Jk:-3 phenotype as Jk:-3 cells, unlike Jk(a+) and/or Jk(b+) cells, are resistant to lysis by 2M urea.

Table 6.19 shows the Kidd blood group frequencies.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jk(a+b-)</td>
<td>26</td>
<td>51</td>
<td>23</td>
</tr>
<tr>
<td>Jk(a+b+)</td>
<td>50</td>
<td>40</td>
<td>27</td>
</tr>
<tr>
<td>Jk(a-b+)</td>
<td>23</td>
<td>8</td>
<td>49</td>
</tr>
<tr>
<td>Jk(a-b-)/Jk:-3</td>
<td>Very rare</td>
<td>Very rare</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Antibody characteristics

1. Anti-Jk⁺ and anti-Jk⁻ are type IgG antibodies that activate complement and are stimulated by transfusion or pregnancy.
2. The antibodies react best by antiglobulin technique, although some weak examples react by enzyme techniques. Some examples may be complement dependent and require the presence of complement before they can be detected.
3. They are relatively rare antibodies and are most often present in serum containing other blood group antibodies.
4. The avidity and the titre of the antibodies often diminish rapidly and the antibodies may not be demonstrable in subsequent samples from the patient.
5. Anti-Jk3 can be a potent type IgG antibody detected by IAT.

Clinical significance

Transfusion

1. Both anti-Jk⁺ and anti-Jk⁻ (including weak examples) have caused severe haemolytic transfusion reactions and delayed transfusion reactions. Antigen negative and IAT crossmatch compatible blood should be transfused to patients with antibodies.
2. If further transfusions are required at a later date the antibodies may be difficult to detect at the time of the crossmatch, or may no longer be demonstrable. Antigen negative blood should be transfused to prevent a delayed transfusion reaction. Good records of patients with clinically significant antibodies should be kept so that the appropriate blood can be crossmatched.
3. Anti-Jk3 should be considered to be clinically significant. Compatible rare type Jk:-3 blood may need to be obtained from a rare donor registry.

Haemolytic disease of the fetus and newborn

The antibodies rarely cause HDFN.

LEWIS BLOOD GROUP SYSTEM (discovered in 1946)

Well-known antigens: Le⁺, Le⁻
Number of antigens: 6 (2006).

Leus antigens are not actually red cell antigens but are adsorbed onto the red cells from the plasma. The presence or absence of the Lea and Leb antigens is determined by genes at three different loci:

- H (or FUT1) gene: responsible for production of H substance (the precursor substance for the A and B antigens).
- Se (or FUT2) or secretor gene: enables the A, B and H antigens to be secreted.
- Le (or FUT3) gene: the symbol le is used to show the absence of the Le gene.

When the Le gene is inherited, the enzyme produced reacts with one of two possible H substrates. The preferred substrate is secreted H substance. This is produced when both H and Se genes are present. The secreted H substance is then converted into the Le⁺ antigen. If the secreted H substance is not present as a result of the inheritance of the sese genes (or because of the inheritance of the very rare hh or Bombay O₂ gene), the enzyme product of the Le gene acts on the precursor H substance instead, giving rise to the Le⁻ antigen.

If no Le gene is present, neither the Le⁺ or Le⁻ antigens are produced, resulting in the Le(a-b-) phenotype.

Table 6.20 provides a summary of the Lewis blood groups, together with their interactions with the ABH and secretor genes.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genes present</th>
<th>Comment (ABH)</th>
<th>Comment (Lewis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(a+b-)</td>
<td>Le sese</td>
<td>ABH non-secretor</td>
<td>Le gene product acts on H precursor substance</td>
</tr>
<tr>
<td>Le(a+b+)</td>
<td>Le Se</td>
<td>ABH secretor</td>
<td>Le gene product acts on secreted H substance</td>
</tr>
<tr>
<td>Le(a-b-)</td>
<td>le le Se</td>
<td>ABH secretor</td>
<td>No Lewis genes are present</td>
</tr>
<tr>
<td>Le(a-b-)</td>
<td>le le sese</td>
<td>ABH non-secretor</td>
<td></td>
</tr>
</tbody>
</table>

The Le⁺ and Le⁻ antigens are adsorbed onto the red cells from the plasma, but the antigenic strength is variable and may change e.g. pregnant women often type Le(a-b-). Cord cells type Le(a-b-) as the red cell Lewis antigens only develop during the first 12 to 15 months after birth.

The Le(a-b-) phenotype occurs more frequently in black people than in white people, as shown in table 6.21.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(a+b-)</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Le(a+b+)</td>
<td>72</td>
<td>55</td>
</tr>
<tr>
<td>Le(a-b-)</td>
<td>6</td>
<td>22</td>
</tr>
</tbody>
</table>
Antibody characteristics

1. Anti-Le\(^a\) and anti-Le\(^b\) are often type IgM naturally occurring antibodies. They are usually developed by type Le(a-b-) individuals.
2. The antibodies generally react best at temperatures below +37°C and react by saline and enzyme techniques. Some examples of anti-Le\(^a\) and anti-Le\(^b\) are type IgG and react by IAT.
3. Strong reacting type IgG antibodies may cause in vitro haemolysis in the presence of complement.
4. Some anti-Le\(^a\) antibodies react better with group O or group A\(_2\) Le(b+) cells than with group A\(_1\) or group B Le(b+) cells. These are termed anti-Le\(^a\) as they react with cells with the most H antigen. Anti-Le\(^b\) does not show a variation in reaction based on the ABO group of the Le(b+) cells being tested.

Clinical significance

Transfusion

1. The majority of Lewis antibodies do not cause transfusion reactions.
2. Generally patients with anti-Le\(^a\) and anti-Le\(^b\) which react at +37°C can be transfused with crossmatch compatible blood. There are, however, some rare examples of Lewis antibodies, which react strongly at +37°C by IAT and activate complement. These antibodies should be considered to be of potential clinical significance.

Haemolytic disease of the fetus and newborn

Lewis antibodies do not cause HDFN as fetal and newborn red cells lack Le\(^a\) and Le\(^b\) antigens.

Features

The Lewis antigens are adsorbed onto the red cells from the plasma.

Practical application

1. Most examples of Lewis antibodies are not of clinical importance but there are rare exceptions that are clinically significant.
2. It is not usually necessary to provide antigen negative blood for transfusion.
3. Le(a-b-) screening cells are useful when screening the sera of pregnant women for clinically significant antibodies, as Lewis antibodies (which are not of obstetric significance) will then not be detected.

LUTHERAN BLOOD GROUP SYSTEM

(discovered in 1945)

Well-known antigens: Lu\(^a\), Lu\(^b\)
Number of antigens: 19 (2006).

In addition to the two major antigens, Lu\(^a\) (low frequency) and Lu\(^b\) (high frequency), there are three further pairs of allelic co-dominant genes which control the Lu6 and Lu9 antigens, Lu8 and Lu14 antigens, and the Au\(^a\) and Au\(^b\) antigens. Ten other high frequency antigens are part of the system. No Lutheran antigens are demonstrable by routine laboratory testing on samples of the rare Lu(a-b-) type or null type.

The Lutheran locus is part of a linkage group situated on Chromosome 19, which includes the Secretor, Lewis, H and LW loci.

Table 6.22 shows the Lutheran blood group frequencies.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>White</th>
<th>Black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu(a+b-)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Lu(a+b+)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Lu(a-b+)</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Lu(a-b-)</td>
<td>Very rare</td>
<td>Very rare</td>
</tr>
</tbody>
</table>

The expression of Lutheran antigens is variable, and dosage effects may be seen.

Antibody characteristics

1. The antibodies may be stimulated by pregnancy or transfusion.
2. Anti-Lu\(^a\) is usually a type IgM antibody reacting by saline techniques. Reactions with anti-Lu\(^a\) often show a typical 'mixed field' result.
3. Anti-Lu\(^b\) is usually a type IgG antibody reacting best by IAT.
4. Anti-Lu\(^a\) is seldom seen and rarely causes a problem in the crossmatching laboratory as compatible blood can be easily found.

Clinical significance

Transfusion

1. Both antibodies have been reported to cause mild or delayed transfusion reactions.
2. IAT crossmatch compatible blood should be transfused.
3. The provision of Lu(b-) blood may be difficult but the antibody is seldom seen.

Haemolytic disease of the fetus and newborn

Lutheran antibodies have not been reported to cause severe HDFN as the antigens are only weakly expressed on cord cells.

I BLOOD GROUP SYSTEM (discovered in 1956)

Antigen: I
Number of antigens: 1

Although the I blood group system is not one of the major blood group systems it is included in this section as it is of practical importance. The I antigen is found on all normal adult red cells. The expression of I antigen varies with age and with disease, and the degree of expression varies considerably between individuals.

All cord cells type I\(^-\), i\(^+\) as the I antigen is not developed at birth. Cord cells may then be used as a source of I\(^-\) cells for antibody identification tests. During the first 2 years of life, the I antigen develops and replaces the i antigen, possibly due to the action of a transferase enzyme using the I antigen as a substrate.

Antibody characteristics

1. Anti-I antibodies are usually type IgM antibodies which occur frequently as cold reacting autoantibodies or cold agglutinins. They are seldom seen as an alloantibody.
2. However, potent auto-anti-I cold reacting antibodies can cause cold autoimmune haemolytic anaemia.
3. The antibodies may be detected at +37°C by enzyme or IAT methods but they are not normally clinically significant.
4. Anti-i is occasionally found in patients recovering from diseases such as infectious mononucleosis (glandular fever).
5. Anti-i may be associated with anti-H, forming antibodies with anti-HI specificity.
Clinical significance

Transfusion
1. Although most anti-I antibodies are not clinically significant, some examples of auto-anti-I with a wide thermal range can be seen in cold haemagglutinin disease. Should these patients require blood transfusions, they may be given the least incompatible blood, warmed in a validated blood warmer before infusion.
2. The patient’s samples may be difficult to type if the patient’s cells are autoagglutinated. The red cells may need to be washed with warm saline before testing.
3. Crossmatching must be very carefully performed to ensure that the auto-anti-I is not masking clinically significant antibodies.

Haemolytic disease of the fetus and newborn
I system antibodies have not been implicated in HDFN.

ADDITIONAL BLOOD GROUP SYSTEMS/COLLECTIONS/ANTIBODIES REACTING WITH HIGH AND LOW FREQUENCY ANTIGENS

The blood group systems discussed so far in this section are either of clinical or practical importance. However, patients may produce antibodies to many other blood group antigens. As the frequency of antigens may vary between population groups it may be informative to know the ethnicity of the patient particularly when trying to find compatible blood for a patient with antibodies to an apparent high frequency antigen or antigens. Such samples are usually referred to a reference laboratory.

Antibodies to high frequency antigens are usually detected when the serum/plasma reacts with all samples tested but the autoantibody control is negative. This indicates the presence of an alloantibody, not an autoantibody. Further tests are required to identify whether the antibodies are directed against a high frequency antigen or whether they are a mixture of antibodies of different specificities.

Antibodies to high frequency antigens which do not form part of a blood group system include anti-Vel, anti-Lan, and anti-Sd

Antibodies to the high frequency antigen, Kn, may be referred to as high titre low avidity (HTLA) antibodies. Chido/Rodgers antibodies to the high frequency antigens Chido/Rodgers react with the complement C4 protein which is found on red cells. The identification of these antibodies may be time consuming and not all are clinically significant.

The antibodies may be directed against the high frequency antigens mentioned previously in the various blood group systems. Many of these antibodies are clinically significant and can cause severe transfusion reactions and HDFN. It may be extremely difficult to find compatible, antigen negative blood and donations may have to be obtained from a local, national or international rare donor registry/frozen storage facility. Family members who would be suitable donors may also be tested to see if they lack the particular high frequency antigen, as siblings are more likely to be compatible than donors in the random population.

If the patient has an antibody to a low frequency antigen, the provision of compatible blood should not be a problem. Antibodies to low frequency antigens may be found by chance when crossmatching random units of blood or may be detected when using a commercial panel which includes a panel cell sample positive for a low frequency antigen.

Table 6.23 lists some of the rare phenotypes associated with different ethnic groups and the increased likelihood of patients producing antibodies of the corresponding specificity. Note that this should be used as a guideline only as there are always exceptions.

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Blood group system</th>
<th>Increased likelihood of rare type</th>
<th>Antibody specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>Rh</td>
<td>hr&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Anti-hr&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hr&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Anti-hr&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MNS</td>
<td>U</td>
<td>Anti-U</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J&lt;sub&gt;s(a+b-)&lt;/sub&gt;</td>
<td>Anti-J&lt;sub&gt;s&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Indian</td>
<td>H</td>
<td>Bombay O&lt;sub&gt;s&lt;/sub&gt;</td>
<td>Anti-H, -A, -B</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>In(a+b-)</td>
<td>Anti-In&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidd</td>
<td>J&lt;sub&gt;k(a-b)&lt;/sub&gt;</td>
<td>Anti-Jk&lt;sub&gt;k&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>Kell</td>
<td>K&lt;sup&gt;k&lt;/sup&gt;</td>
<td>Anti-k</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kp(a+b-)</td>
<td>Anti-Kp&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Vel</td>
<td>Vel-</td>
<td>Anti-Vel</td>
</tr>
<tr>
<td></td>
<td>Yta</td>
<td>Yt(a-b+)</td>
<td>Anti-Yt&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

POLYAGGLUTINATION

This is defined as the agglutination of red cells by serum/plasma from most normal adult humans, irrespective of the ABO groups involved.

Polyagglutination may be inherited or acquired. Acquired polyagglutination can be caused by the action of microbial enzymes on the red cells, as a result of infections such as septicaemia, and in bowel, gastric and respiratory infections among others. This action can result in the cleavage of sialic acid from glycoproteins and glycolipids, thus exposing the crypt antigens on the red cells that are not normally able to be detected. This is known as T-activation. T-activated cells are agglutinated by most normal sera as most sera contain IgM antibodies to these crypt antigens. T-activation can be readily detected using the lectin Arachis hypogaea or ‘peanut anti-T’. Acquired polyagglutination is usually transient, unlike inherited polyagglutination.

An example of inherited polyagglutination is the agglutination of red cells of the individuals of the rare Sd<sup>a+++</sup> or Cad type. The Sd<sup>a</sup> antigen occurs frequently and the strength of the antigen varies greatly with the strongest expression being seen in the rare Cad phenotype red cells.
HUMAN LEUCOCYTE ANTIGEN SYSTEM

The human leucocyte antigen (HLA) system, also known as the Major Histocompatibility Complex (MHC) is an extremely complex, polymorphic system.

The HLA antigens are produced as a result of genes located on the short arm of autosome number 6. The genes are located at what is known as the MHC locus. The MHC locus also contains the genes for some cytokines and various complement components. Gene products are found on the surface membranes of all nucleated cells, including solid organs, lymphocytes, granulocytes, monocytes and also on platelets although they are not nucleated. HLA antigens are not generally detected on red cells (as mature red cells are not nucleated), and red cells are not suitable for HLA typing. However, some HLA haemagglutinins, also known as Bg antibodies, may detect certain HLA antigens when expressed on the red cells.

The HLA antigens are the products of the HLA-A, -B, -C, -DR, -DQ, and DP genes. The original work on HLA was performed by serological techniques but now HLA loci genes are detected using DNA assays.

The HLA-A, -B, -C antigens are termed the class I antigens and the HLA-DR, -DQ and DP antigens, class II antigens, based on their biochemical structure.

Some of the HLA antigens share the same epitope and give rise to the many subtypes or splits. As many different terminologies were in use, the World Health Organization (WHO) Terminology Committee was established in 1967 to review and standardize the nomenclature.

As the HLA antigens are found on white blood cells and platelets, and also on tissue cells, this complicates the successful grafting of transplanted organs.

Disease association

The HLA-A and HLA-B (class I) antigens are expressed on all nucleated cells in the peripheral blood. The HLA-DR and HLA-DQ (class II antigens) are not expressed on granulocytes, unstimulated T-lymphocytes or on platelets. However, both play a major immunological role in the defence against disease.

The association between certain HLA phenotypes and the increased occurrence of a specific disease is statistically significant e.g. HLA-B27 and ankylosing spondylitis, and there are many other examples.

Transplantation

When foreign tissue is transplanted into an individual, the HLA antibodies formed as a result of the transplantation may lead to graft rejection in the case of a solid organ transplant, or graft versus host disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the rejection in the case of a solid organ transplant, or graft versus host disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant. It has been shown that HLA matching between the donor and recipient lessens the disease in the case of a bone marrow transplant.

Solid organ transplant

The patients are HLA typed and the results entered in a national registry. ABO matching is critical due to the presence of ABO antigens on body tissue. (It is not necessary for bone marrow transplantation as ABO groups are not expressed on stem cells) The HLA types of the patients are matched against the HLA type of each donor that becomes available. A pre-transplant microlymphocytotoxic crossmatch can be performed to see if the patient has pre-existing antibodies to the donor organ which would preclude transplantation.

Allogeneic bone marrow transplants

Potential donors are HLA typed in advance, and their results recorded on a donor panel. The donor panel can then be searched to find the best HLA match for a potential recipient. Although there may be many thousands of donors listed on the donor panel it is extremely difficult to find a matched donor.

Transfusion

If a patient is transfused with unfiltered blood products containing white cells and/or platelets the patient may become immunized to foreign HLA antigens. If the patient is transfused subsequently he/she may suffer febrile reactions. For this reason leucocyte-poor products should be transfused to patients on long term transfusion therapy. In some cases it may be necessary to transfuse immunized patients with HLA-matched blood products. The introduction of leucodepletion as a routine procedure in many services has reduced the exposure to HLA antigens.

Pregnancy

HLA antibodies are more frequently seen in women following pregnancy. White cell antibodies in multiparous blood donors have been responsible for transfusion related acute lung injury (TRALI) in transfusion recipients. See Section 14: Benefits and risks of transfusion for more details on TRALI.

Parentage Testing

The HLA system is a highly polymorphic and well-defined system. HLA typing is a useful tool in the range of laboratory tests used in parentage testing. As the frequency of the HLA genes varies greatly between different populations, the ethnicity of the individuals concerned must be considered when interpreting results.

HUMAN PLATELET ANTIGEN SYSTEMS

The molecular basis for the human platelet antigen (HPA) systems has been determined and a total of 24 platelet-specific alloantigens have been defined serologically. The systems are very polymorphic and the frequency of the genes varies in different populations according to the ethnic backgrounds. Exposure to foreign platelet antigens following pregnancy, transfusion, or organ transplant can result in alloimmunization. This can be responsible for organ transplant rejection or fetomaternal alloimmune thrombocytopenia (FMAIT).

The platelet antigens are located on the platelet membrane. Serological tests were originally used to study platelet antigens but it was extremely difficult to obtain specific antisera for the tests and many antisera also contained HLA antibodies. With the development of molecular testing, it is possible to determine the HPA genotype and many studies have now been performed.
The HPA-1a and HPA-5b are considered to be the most immunogenic platelet alloantigens in white people and most cases of FMAIT are due to anti-HPA-1a.

In cases where patients develop platelet refractoriness during transfusion therapy, it may be necessary to provide both HLA and HPA matched blood products.

**FETOMATERNAL ALLOIMMUNE THROMBOCYTOPAENIA**

1. The commonest cause of severe thrombocytopenia in the fetus and newborn is FMAIT.
2. Although FMAIT has been regarded as the platelet equivalent of HDFN, it occurs frequently during first pregnancies. In FMAIT, the antigen that stimulates the maternal antibody response is found on fetal platelets where the fetus has inherited an HPA type from the father, lacking in the mother.
3. The most common antigens to stimulate the production of platelet antibodies are HPA-1a and HPA-5b which are the most immunogenic platelet antigens. FMAIT can also be caused by low frequency HPA antigens.
4. Paternally inherited platelet antigens, lacking in the mother, may stimulate the production of IgG maternal antibodies as a result of FMH. These IgG antibodies are able to cross the placenta and sensitize fetal platelets, causing thrombocytopenia.
5. The diagnosis is usually made after birth when the neonate shows symptoms of thrombocytopenia, and platelet specific antibodies are detected in the maternal serum.

6. Because of severe thrombocytopenia, fetal/neonatal intracranial haemorrhage may occur.
7. The antenatal administration of intravenous immunoglobulin (a non-invasive procedure) to the mother may lead to an increase in circulating platelets in the fetus, reducing the likelihood of fetal intracranial haemorrhage. The reason for this is perhaps the modulation of maternal antibody production that may be induced by the administration of immunoglobulin.
8. Although the introduction of antenatal screening has been considered, there is generally a lack of optimal antenatal treatment. The safety of fetal blood sampling and platelet transfusion is also a concern. Most laboratories are unable to predict even severe cases of FMAIT.
9. As in the case of HDFN, premature induction of labour may also be recommended. It appears that caesarean section may be preferred to natural birth, in order to avoid cranial trauma at the time of delivery.
10. The practical problem is that most cases are diagnosed postnatally. Time is important as the condition should be treated promptly, preferably with HPA compatible platelets although random donor platelets, or (rarely) washed and irradiated maternal platelets, may be used. Most transfusion services do not have HPA matched platelets available as this would require the use of a panel of HPA typed donors available on call.

**SUMMARY OF SECTION: BLOOD GROUP SYSTEMS**

Table 6.24 summarizes the characteristics of the blood group systems and their corresponding antibodies. Haemolytic transfusion reaction has been abbreviated as HTR.

<table>
<thead>
<tr>
<th>System</th>
<th>Antibody</th>
<th>Predominant type</th>
<th>Clinical significance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>Anti-A</td>
<td>IgM/IgG</td>
<td>HTR and HDN</td>
<td>Can cause fatal TR and severe HDFN Antibodies may be haemolytic</td>
</tr>
<tr>
<td></td>
<td>Anti-B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-A,B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-A₁</td>
<td>Mainly IgM</td>
<td>Rare</td>
<td>Found in A₁ individuals</td>
</tr>
<tr>
<td></td>
<td>Anti-H</td>
<td>Mainly IgM</td>
<td>Low risk</td>
<td>Weakest reactions with group A₁ and B cells</td>
</tr>
<tr>
<td></td>
<td>Anti-H₁,B₁</td>
<td>IgM/IgG</td>
<td>HTR and HDN</td>
<td>Bombay O₁ type</td>
</tr>
<tr>
<td>H</td>
<td>Anti-D</td>
<td>Mainly IgG</td>
<td>HTR and HDN</td>
<td>Main cause of HDFN</td>
</tr>
<tr>
<td></td>
<td>Anti-C</td>
<td></td>
<td></td>
<td>Often occurs with anti-D</td>
</tr>
<tr>
<td></td>
<td>Anti-E</td>
<td>IgM/IgG</td>
<td>HTR and HDN</td>
<td>May be naturally occurring and react with enzymes only</td>
</tr>
<tr>
<td></td>
<td>Anti-c</td>
<td>IgM</td>
<td>HTR and HDN</td>
<td>Severe HDFN May be produced with anti-E</td>
</tr>
<tr>
<td></td>
<td>Anti-e</td>
<td></td>
<td></td>
<td>Not often seen. Variants may appear to be anti-e in an e+ individual</td>
</tr>
<tr>
<td>Rh</td>
<td>Anti-M</td>
<td></td>
<td>Rare TR</td>
<td>Antigen denatured by enzyme treatment</td>
</tr>
<tr>
<td></td>
<td>Anti-N</td>
<td></td>
<td>Low risk</td>
<td>May show dosage</td>
</tr>
<tr>
<td>MNS</td>
<td>Anti-S</td>
<td>HTR and HDFN rare</td>
<td>Antigen denatured by enzyme treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-s</td>
<td>HTR and HDN</td>
<td>Rare antibody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-U</td>
<td>HTR and HDFN</td>
<td>Antibody to high frequency antigen Compatible blood from rare donor registry</td>
<td></td>
</tr>
<tr>
<td>P₁ antigen</td>
<td>Anti-P₁</td>
<td>IgM</td>
<td>Low risk</td>
<td>Common cold agglutinin produced by P₁- individuals. P₁ substance used in inhibition tests</td>
</tr>
</tbody>
</table>
HTR and HDFN rare Antibody reactions and clinical significance variable

Antibody to high frequency antigen Compatible blood from rare donor registry

Antigen denatured by enzyme treatment

Antigen adsorbed from plasma onto red cells

Antigen denatured by enzyme treatment Rare antibody

Antigen loss in pregnancy

Produced by Le(a-b-) individuals

Antibody to high frequency antigen

Antigen denatured by enzyme treatment

May require complement for IAT detection

Soluble I antigen is secreted in human milk

HTR and HDFN

Very rare antibody

Antibodies formed as a result of transplantation may lead to delayed TR, and HDFN

Comments

HTR

HLA typing is a useful tool in the range of laboratory tests used in the diagnosis of alloimmunization. When foreign tissue is transplanted into an individual, the HLA antibodies formed as a result of transplantation may lead to graft rejection in the case of a solid organ transplant, or graft versus host disease in the case of a bone marrow transplant. The HLA antigens are the products of the HLA-A, -B, -C, -DR, -DQ, and -DP genes. As the HLA antigens are found on white blood cells and platelets, and also on tissue cells, this complicates the successful grafting of transplanted organs. It has been shown that the association between certain HLA phenotypes and the increased occurrence of a specific disease is statistically significant e.g. HLA-B27 and ankylosing spondylitis. When foreign tissue is transplanted into an individual, the HLA antibodies formed as a result of transplantation may lead to graft rejection in the case of a solid organ transplant, or graft versus host disease in the case of a bone marrow transplant. As the HLA system is extremely polymorphic it is difficult to find a match for transplantation. For solid organ transplants the HLA types of recipients are entered onto local registers to enable the patient to be matched against a donor as available. For allogeneic bone marrow transplants volunteer donors are HLA typed and their results entered on a national register. They are then matched against potential recipients but it is extremely difficult to find a match. The introduction of leucodepletion as a routine procedure in many services has reduced the patient’s exposure to HLA antigens. HLA antibodies are more frequently seen in women following pregnancy. White cell antibodies in multiparous blood donors have been responsible for TRALI in transfusion recipients. HLA typing is a useful tool in the range of laboratory tests used in the diagnosis of alloimmunization. When foreign tissue is transplanted into an individual, the HLA antibodies formed as a result of transplantation may lead to delayed TR, and HDFN. The HLA system is extremely polymorphic it is difficult to find a match for transplantation. For solid organ transplants the HLA types of recipients are entered onto local registers to enable the patient to be matched against a donor as available. For allogeneic bone marrow transplants volunteer donors are HLA typed and their results entered on a national register. They are then matched against potential recipients but it is extremely difficult to find a match. The introduction of leucodepletion as a routine procedure in many services has reduced the patient’s exposure to HLA antigens.
ADDITIONAL LEARNING ACTIVITIES

1. It is suggested that students use a medical dictionary and/or the Internet to clarify the meaning of words and phrases and to add to the information provided in this section. A list of key words that may be useful in this regard is provided below. It is also suggested that the student compare the published data on the Rh phenotypes and genotypes and note the variation in frequencies amongst the different populations and ethnic groups.

- ISBT Committee for Red Cell Surface Antigens
- Rh inheritance
- Rh immunoglobulin
- HDFN
- Rare donor registry
- HLA typing
- Transplantation
- Class I and class II antigens
- Graft rejection
- HPA typing
- Polymorphic

2. Use knowledge of the different blood group systems to design a set of screening cells for red cell antibody detection in the crossmatch laboratory, using donations from the blood transfusion service. Create a mini panel as for the panel cells decode using the selected screening cells. Which combinations of red cell phenotypes are easily obtainable? Which red cell phenotypes would be difficult to obtain and what are the reasons for this? What alternatives could be used?

3. If a strong IAT reacting antibody is detected in the crossmatch laboratory what facilities are available within the laboratory to identify the antibodies? How should one proceed if the antibody reacts strongly with all the reagent panel cells tested? How would knowing the patient’s ethnicity and/or history assist in the identification of the antibodies and finding compatible blood? Does the service/organization have access to a rare donor registry? If so, what rare blood types are available? If rare type blood is not available discuss other options to be considered.

JMS Blood Banking Bags and Equipment of the Highest Quality!

JMS started manufacturing blood bags in 1969. They have developed a long and laudable reputation for reliability, superior product quality and assurance. SSEM Mthembu Medical distributes the quality range of JMS blood banking bags and equipment, including the newly launched state-of-the-art touch screen HemoPress Automated Blood Separator, with an option for barcoding all operational stages of the production cycle.

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- Platelet Incubators & Agitators
- Hemosealer
- Hemoscale
- Comfort-Line Donation Chair

SSEM Mthembu Medical (Pty) Ltd. is the sole distributor of all JMS blood banking products in Sub-Saharan Africa.

For information or assistance contact Carol Collett on carolc@ssemmthembu.co.za or +27 72 807 2166.
The AfSBT Education committee was founded in the first quarter of 2011. The committee members were selected to provide a diverse set of skills and represent various sectors in the blood transfusion field.

The members are: Dr Claude Tayou Tagny (Cameroon), Mr David Ndakala (DRC), Prof André Loua (Guinee), Dr Mounirou Baby (Mali), Prof Kamel Boukef (Tunisia), Mr Jaonomaridimby Andrianavalona (Madagascar), Mr Francois Xavier Rwandamuriye (Rwanda), Dr Honorine Dahourou (Burkina Faso), Mr Idris Salii (Nigeria), Dr Justina Ansah (Ghana), Miss Mary Karithi, Mr Patrick Kisabei and Mr Gordon Kamande Wanyoike (Kenya), Dr Isaac Kajja (Uganda), Ms Senet Ibrahim (Eritrea), Ms Maleqhoa Nyopa (Lesotho), Mr Thom Mtung (Malawi), Mr Rob Wilkinson (Namibia), Dr Joseph Mulenga (Zambia), Mr Emmanuel Masvikeni (Zimbabwe), Mr Ivan Horner and Mrs Michele Breuninger (South Africa). The committee has been further strengthened by the recent addition of Claudine Hossenlopp and Arnaldo Santos, who have so willingly translated all the committee correspondence into French and Portuguese respectively.

The committee’s first task was to distribute the donated AABB Technical Manuals in a fair manner to countries and regions where they will be best used. A total of 35 motivational responses were received from 27 African countries for the 200 AABB Manuals that the AABB, ICCBBA and National Bioproducts Institute of South Africa collaboratively donated to Africa.

During the months August, September and October, the manuals made their way by airfreight or post to blood services, haematology departments and laboratories that completed a needs questionnaire. The recipients of these gifts have committed themselves to providing feedback over the following 18 months as to whether the AABB Manuals have improved knowledge and skills in the region. Table 1 shows the number of AABB Manuals distributed to various countries.

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namibia</td>
<td>4</td>
</tr>
<tr>
<td>Malawi</td>
<td>9</td>
</tr>
<tr>
<td>Rwanda</td>
<td>11</td>
</tr>
<tr>
<td>Mauritanie</td>
<td>3</td>
</tr>
<tr>
<td>Cameroon</td>
<td>7</td>
</tr>
<tr>
<td>Republic of Guinée</td>
<td>5</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>5</td>
</tr>
<tr>
<td>Lesotho</td>
<td>4</td>
</tr>
<tr>
<td>Guinea-Bissau</td>
<td>5</td>
</tr>
<tr>
<td>Tunisia</td>
<td>8</td>
</tr>
<tr>
<td>Nigeria</td>
<td>23</td>
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<tr>
<td>Ethiopia</td>
<td>5</td>
</tr>
<tr>
<td>Eritrea</td>
<td>4</td>
</tr>
<tr>
<td>Comores</td>
<td>3</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>5</td>
</tr>
<tr>
<td>The Gambia</td>
<td>4</td>
</tr>
<tr>
<td>Seychelles</td>
<td>4</td>
</tr>
<tr>
<td>Ghana</td>
<td>12</td>
</tr>
<tr>
<td>Algeria</td>
<td>5</td>
</tr>
<tr>
<td>Kenya</td>
<td>7</td>
</tr>
<tr>
<td>Tanzania</td>
<td>7</td>
</tr>
<tr>
<td>South Africa</td>
<td>3</td>
</tr>
<tr>
<td>RDC Congo</td>
<td>10</td>
</tr>
<tr>
<td>Toga</td>
<td>5</td>
</tr>
<tr>
<td>Zambia</td>
<td>9</td>
</tr>
</tbody>
</table>

With the help of the Education committee the manuals have been distributed to large countries such as Nigeria and even to islands off Africa such as Seychelles. Members of the committee have gone to great lengths to ensure that staff in their countries benefit from this generous gift. This exercise has been a good starting point for the committee which I believe will go from strength to strength as we head towards 2012 and the additional tasks that lie ahead before the AfSBT Congress in Mauritius.
## NEW MEMBERS joining the AfSBT

<table>
<thead>
<tr>
<th>Country</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zimbabwe</strong></td>
<td>Anastaasia Lubimbi</td>
</tr>
<tr>
<td></td>
<td>Sibonile Magwali</td>
</tr>
<tr>
<td></td>
<td>Doreen Kanoganga</td>
</tr>
<tr>
<td></td>
<td>Morleen Mutamba</td>
</tr>
<tr>
<td></td>
<td>Andrew Makunura</td>
</tr>
<tr>
<td></td>
<td>Solomon Dambire</td>
</tr>
<tr>
<td></td>
<td>Collins Timire</td>
</tr>
<tr>
<td><strong>Nigeria</strong></td>
<td>Haruna M Muktar</td>
</tr>
<tr>
<td></td>
<td>Waliu A Braimo</td>
</tr>
<tr>
<td></td>
<td>Patience Osinubi</td>
</tr>
<tr>
<td></td>
<td>Aishatu Suleiman</td>
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<tr>
<td></td>
<td>Ahmadu B Aliyu</td>
</tr>
<tr>
<td></td>
<td>Abdulaziz Hassan</td>
</tr>
<tr>
<td></td>
<td>Obadiyah D Damulak</td>
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<tr>
<td></td>
<td>Oyinwola Oni</td>
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<tr>
<td></td>
<td>Theresa Otu</td>
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## CORPORATE MEMBERS OF THE AfSBT

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ACKNOWLEDGEMENTS

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AfSBT also acknowledges translations from English into French, of abstract / background information as required for this issue, by Dr Claude Tayou Tagny (Cameroon).

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<th>Renewal ☐</th>
<th>Update of details ☐ (no charge)</th>
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