THE PREVALENCE OF IRREGULAR ANTIBODIES IN MULTIPLY-TRANSFUSED PATIENTS AND MULTI-GRAVID WOMEN AT THE YAOUNDE GENERAL HOSPITAL (Cameroon)

PRESENTED BY

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ABBREVIATIONS

Ab    Antibody
ABO   Blood groups A, B and O
Ag-Ab Antigen – Antibody
AIDS  Acquired immune deficiency syndrome
C3b   Complement 3b
CSCCD Centre for the Study and Control of Communicable Diseases
E.g.  Example
EDTA  Ethylene Diamino Tetra acetic Acid
Fc    Crystallisable Fragment of Immunoglobulin
FMI   Foeto-Maternal immunisation
Fya   Duffy A
Fyb   Duffy B
HIV   Human Immune Deficiency Virus
HPA   Human Platelet Antigen
IA    Irregular antibodies
IAT   Indirect anti-globuin test
Ig    Immunoglobulin
Jka   Kidd a
Jkb   Kidd b
K     Kell
Le    Lewis
Lea   Lewis a
Leb   Lewis b
LISS  Low ionic strength solution
Lua        Lutheran a
Lub        Lutheran b
P1         Panel cell one
P2         Panel cell two
P3         Panel cell three
r          Rhesus dce
R₁         Rhesus Dce
R₂         Rhesus DCE
RBC        Red blood Cells
Rh         Rhesus
Rh-        Rhesus Negative
Rh D       Rhesus D
UK         United Kingdom
UV         Ultra violet
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RESUME

Introduction
La présente étude descriptive et transversale a été réalisée dans la période de juillet 2003 à février 2005 sur la prévalence des anticorps irréguliers chez les malades polytransfusés et chez les femmes multigravides à l’Hôpital Général de Yaoundé.

Objectifs
Notre étude avait pour objectif l’évaluation de l’importance de l’alloimmunisation chez les sujets polytransfusés et les femmes multigravides par la recherche des anticorps immuns et la détermination de la prévalence des anticorps irréguliers dans la population de l’étude.

Méthodologie
58 sujets à qui les aspects éthiques de la collecte de sang ont été expliqués et qui ont au préalable consenti par écrit ont été inclus dans notre étude selon les critères ci-après :
   i) être sous hémodialyse et avoir été transfusé à 3 reprises au minimum ;
   ii) femme ayant eu au moins 3 grossesses ;
   iii) acceptation par écrit d’être inclus dans l’étude par la signature d’une fiche de consentement.
Il y a lieu de préciser qu’il s’est agi d’un échantillonnage de convenance limité par le temps.
Des échantillons de sang ont été prélevés du 12 au 16 Janvier 2004 chez les sujets retenus dans l’étude à l’Hôpital Général de Yaoundé et ont été analysés dans le laboratoire d’immuno-hématologie de l’Hôpital Saint Luc à Bruxelles (Belgique). L’analyse a été faite en 2 étapes ainsi qu’il suit :
Dans un premier temps, les anticorps irréguliers étaient recherchés en utilisant l’appareil ABS 3101 SI, en présence des cellules P1, P2, P3, et la papaïne à une température de 37°C. La confirmation des résultats se faisant par la technique de Diamed gel de Coombs également en présence de la papaïne à 37°C.

Ensuite et en cas d’agglutination (démontrant la présence d’anticorps irréguliers), leur identification est effectuée par la technique Diamed-ID cells P qui comprend 11 cellules test. Deux séries de tests ont été réalisées : le premier est le test de Coombs indirect à 37°C et le second avec ajout de papaïne. Dans les 2 cas, l’agglutination confirme la positivité du test.

Résultats

Des 58 sujets de notre échantillon, 23 étaient des polytransfusés sous hémodialyse et 35 des femmes multigravides. L’âge moyen des sujets était de 39±9,4 ans (écart : 27-62 ans) et le ratio femme/homme était de 2/1. Une agglutination a été observée dans 20,7% des échantillons prélevés et était de 5,7% chez les femmes multigravides contre 43,4% chez les polytransfusés.

Ces résultats sont conformes à ceux obtenus par Singer et al. (1996) dans un groupe de malades thalassémiques de souche asiatique polytransfusés (prévalence : 33%) et à ceux de Luban (1989) dont l’étude portait sur la variabilité des taux d’alloimmunisation chez des groupes d’enfants drépanocytaires (prévalence : 42,9% dans un groupe d’enfants non américains).

Conclusion : En conclusion, notre étude a montré une alloimmunisation de 20,7% dans un échantillon de 58 sujets polytransfusés et multigravides à l’Hôpital Général de Yaoundé.
Recommandations

En raison de la prévalence élevée de l’alloimmunisation observée, les recommandations ci-dessous sont formulées en vue de contribuer à l’amélioration de la sécurité transfusionnelle au Cameroun ; il s’agit de :

i) Faire une étude visant à déterminer les différents phénotypes d’anticorps irréguliers et leurs prévalences au Cameroun ;

ii) Utiliser systématiquement du sang phénotypé chez tous les malades polytransfusés ;

iii) Produire localement des tests de détection et d’identification à partir des donneurs de sang du groupe O en utilisant des antigènes dont l’immunogénicité est bien connue et qui existent dans notre population.

iv) Mettre en place un système de suivi des complications liées à la transfusion sanguine au Cameroun.

v) Organiser des campagnes de sensibilisation sur les risques de l’alloimmunisation parmi le personnel de la santé, les polytransfusés et les femmes multigravides.
SUMMARY

Introduction: This descriptive cross-sectional study was carried out from July 2003 to February 2005 on the prevalence of irregular antibodies in multiply-transfused patients and in multi-gravid women at the Yaoundé General Hospital.

Objective: The main aim of the study was to assess alloimmunisation in multiply-transfused individuals and multigravid women, by detecting irregular antibodies and their prevalence in the study population.

Methodology: Fifty-eight individuals to whom the ethical issues surrounding blood collection had been explained and who had accepted to fill the informed consent form were recruited from 12 to 16 January 2004. They were expected to have fulfilled the following three inclusion criteria:

- To be haemodialysed individuals, who had received at least three blood transfusions.
- To be a woman who had been pregnant at least three times.
- Any individual from the above groups who had accepted to be part of the study by signing the informed consent form.

It should be noted that this was a consecutive convenient sampling limited by time.

The samples were analysed in the immunohaematology laboratory at the Hôpital Saint Luc in Brussels and this analysis took place in two stages.

First, the irregular antibodies were detected using the Diamed-D microtyping system containing cells P1, P2, P3 in the presence of the enzyme papain, all at 37°C; the results obtained are confirmed using the Diamed gel in Coombs also in the presence of papain and at 37°C. Secondly in the event of agglutination which
implied the presence of irregular antibodies, their identification was done using the Diamed-ID panel-P cells. It is made up of a series of 11 test cells. Two sets of tests were done in two media. The first using Indirect Coombs reagents at 37°C and the second with Papain added. In both media, agglutination implied positivity.

**Results:** Out of the 58 individuals, 23 were dialysed multiply-transfused and 35 were multigravid women. The average age of the patients was 39±9.4 years (range: 27-62). The female to male ratio was 2:1. Alloimmunisation was detected in 20.7% of our samples. The positivity was manifested by agglutination. Alloimmunisation was present in 5.7% of the multi-gravid and 43.4% of the multiply-transfused individuals. This was in line with studies carried out by Singer et al. (1996) on erythrocyte alloimmunisation in transfusion dependent thalassaemic patients of predominantly Asian descent, where the prevalence was 33%. In another study by Luban (1989) on the variability in rates of alloimmunisation in different groups of children with sickle cell disease, the incidence of alloimmunisation was 42.9% in children of non American ethnic origin.

**Conclusion:** In conclusion, this study showed a high prevalence of alloimmunisation in a sample of 58 comprising multiply-transfused and multigravids selected at the Yaoundé General Hospital.

**Recommendations:** The following recommendations are therefore made to improve blood transfusion safety in Cameroon:

- To conduct a study to determine the different phenotypes of irregular antibodies found in our community.
- To use phenotyped blood in all patients requiring frequent transfusions in our community.
• To promote the development of a panel of blood test cells produced locally from blood group O donors, using antigens that are known to be immunogenic and prevalent in Cameroon.
• To put in place a monitoring system of blood transfusion related complications nationwide
• To organise sensitisation campaigns on the risk of alloimmunisation in order to create awareness amongst health workers, multiply-transfused subjects and multigravid women.
CHAPTER 1

INTRODUCTION
1. Introduction

Transfusion medicine, as both a science and an art, consists of practices that have evolved over the years from the time of Landsteiner’s insightful observations. Provision of well-preserved cellular or plasma components of blood with predictable effects and uncommon severe side effects has become so developed that research and development efforts are advancing at tremendous paces. Blood transfusion is the administration of blood from one individual to another (allotransfusion) or from an individual to himself (autotransfusion). The importance of transfusion today cannot be overemphasized. Given that blood transfusion is better understood and immediate or delayed reactions even in cases of repeated blood transfusion are better managed, this has permitted sophisticated surgery, chemotherapy, organ and bone marrow transplant. However blood transfusion could be associated with complications (Carlos, et al. 2004.) such as:

- Bacterial and viral infections: brucellosis, syphilis, Hepatitis B and C
- Parasitic infections: malaria, trypanosomiasis

Other complications surrounding blood transfusion are immediate (often complement mediated) immune reactions with their disastrous consequences, and delayed transfusion reactions with equally dangerous long-term effects. These occur most frequently as secondary immune responses due to existing alloantibodies which increase with the number of blood transfusions and could be as a result of:

- Inadequate pre-transfusional procedures (techniques, time to search for alloantibodies)
- absence of preventive procedures especially after blood transfusion and pregnancy
- error in the identification of the blood sample
• poor verification at the patient’s bed

The incidence of a blood group antibody depends on both the prevalence and the immunogenicity of the antigen. Immunised patients frequently produce multiple antibodies and the more antibodies present, the more difficult they are to identify.

The number of alloantibodies increases both with the number of transfusions received and the quantity of blood transfused (Capson, et al. 1993). This is very critical in sub-Saharan countries where haemoglobinopathies, infections such as malaria, poverty and malnutrition are all major aetiological factors of anaemia leading to repeated transfusions.

The occurrence of alloantibodies is greater in women of childbearing ages because they not only have the same reasons to be transfused as in the general population, but may also be immunised through incompatible pregnancies. Alloimmunisation in pregnancy is accounted for by transplacental haemorrhage of foetal blood into maternal circulation, especially during the third trimester. This risk exists during normal delivery and is higher during caesarean sections and manual removal of the placenta. (Hernadez and Ahued-Ahued, 2003).

The prevalence of alloimmunisation amongst the multiply-transfused could be between 19% -37%, with a median at 27.8 %. (Economidon, et al. 1993).

The most to the least frequently identified in transfusion practice are anti-D, anti-K, anti-c, anti-Fya, anti-JKa, anti-S, anti-JKb. (Genettet and Muller, 1999.)

Transfusion management of patients who require long term transfusion therapy such as patients with sickle cell disease, recurrent haemodialysis, or women immunised through multigravidity, is of utmost importance in order to prevent the formation of alloantibodies.

Although the goal is to provide blood with maximum safety, there is still no consensus as to the best and the most practical approach.
In addition to the traditional practice of providing antigen-negative blood only after the patient has made an antibody, the approach should be to provide fully antigen-matched blood i.e. matched for D, C, E, c, e, K, Fya, Fyb, Jka, and Jkb antigens in order to avoid alloimmunisation.
CHAPTER 2

JUSTIFICATION OF STUDY
2. Justification of study

Blood transfusion complications may be fatal. Although infectious complications of transfusion such as AIDS, hepatitis B, hepatitis C, malaria, are screened for, there are still major risks involved in the practice. Even the introduction of computerized programs in some of the best blood transfusion centres of the world has not completely eradicated some of the fatal complications involved. These occur most frequently as secondary immune responses to existing alloantibodies which, acting as memory cells, are re-stimulated with an increase in their titre. Those antibodies will bind to the surface of RBCs and depending on the number of antigen-antibody interactions, there will be complement activation with C3b deposition. Usually, more than $10^5$ antigenic sites per cell are required for potent complement activation to take place leading to hemolysis.

In India, Shukla & Chauhary (1999) in their study of red cell alloimmunisation in 81 multiply-transfused chronic renal failure patients undergoing haemodialysis and being transfused with ABO and Rh ‘D’ matched blood, found a prevalence of 9.8% alloantibodies; amongst those alloantibodies, 88% were those of the Rhesus and Kell systems.

The most common routes of maternal sensitisation are via blood transfusion, foeto-maternal haemorrhage, abortion, placenta abruptio, abdominal trauma and obstetric procedures (Bowman, 1997). According to the same author, 10% of pregnancies in Whites are Rh incompatible. However the risk of alloimmunisation in a susceptible Rh D- woman is significantly affected by 3 factors:

- the volume of foeto-maternal haemorrhage,
- degree of maternal immune response
• concurrent ABO incompatibility.

Foeto-maternal haemorrhages have been demonstrated to occur in as many as 75% of pregnancies, the frequency increasing with the parity and as gestation advances, and with most cases occurring during delivery (Hernadez and Ahued-Ahued, 2003).

Unfortunately, because of poverty and lack of health personnel most blood transfusion services in the third world countries tend to distribute blood that has only been screened serologically for routine ABO and Rh D compatibility testing is restricted to incubation of donor cells with recipient serum for any indication of antibodies present in the recipients serum against the donor cells. With the inadequate transfusion structures in Cameroon, these patients have no access to phenotyped blood, thus exposing them to high risks of alloimmunisation. In Cameroon, no study has been carried to clearly assess this problem among our multiply-transfused and probably multigravid women.

This study is therefore being done to create awareness in safe blood transfusion.
3. Objectives

The general objective of this study is to assess alloimmunisation in multiply-transfused individuals and multiparous women at the Yaounde General Hospital. The specific objectives are as follows:

(1) Identify multiply transfused individuals and multigravid women.
(2) Identify irregular antibodies and their prevalence in the study population.
CHAPTER 4
LITERATURE REVIEW
4. Literature review

4.1 Immunological risks related to RBC transfusion

The notion of risk linked to incompatible transfusion was very well known, even before those linked to blood group incompatibility. In a review of different studies conducted between 1917 and 1994, Tissier, et al. (1996) found a range of the frequency of immunological accidents linked to blood transfusion. The results of such a study are given below.

<table>
<thead>
<tr>
<th>Author</th>
<th>Period</th>
<th>Unit transfused</th>
<th>Transfusionnal haemolytic Reaction</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilufse - De</td>
<td>1917 - 1941</td>
<td>43,284</td>
<td>80</td>
<td>1/541</td>
</tr>
<tr>
<td>Bakey</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binder-Hall</td>
<td>1952 - 1957</td>
<td>81,392</td>
<td>18</td>
<td>1/4520</td>
</tr>
<tr>
<td>Jaulmes</td>
<td>1951 - 1964</td>
<td>176,000</td>
<td>49</td>
<td>1/3,600</td>
</tr>
<tr>
<td>Ahrons</td>
<td>1961 - 1966</td>
<td>74,000</td>
<td>66</td>
<td>1/1121</td>
</tr>
<tr>
<td>Bluemle</td>
<td>1964</td>
<td></td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Pineda</td>
<td>1964 - 1973</td>
<td>268,000</td>
<td>43</td>
<td>1/62322</td>
</tr>
<tr>
<td>Juron - Dupraz</td>
<td>1995 - 1978</td>
<td>211,2676</td>
<td>150</td>
<td>1/14085</td>
</tr>
<tr>
<td>Linden</td>
<td>1990 - 1991</td>
<td></td>
<td>92</td>
<td>1/12000</td>
</tr>
<tr>
<td>Mac Clellad</td>
<td>1990 - 1991</td>
<td>3,300,000</td>
<td>111</td>
<td>1/29000</td>
</tr>
<tr>
<td>Le Pen nec</td>
<td>1991 - 1994</td>
<td>135,0000</td>
<td>61</td>
<td>1/22000</td>
</tr>
</tbody>
</table>

The above studies are difficult to compare due to their different methodologies, different periods during which the studies were carried out and the different countries where the studies were carried out.
However the above information is proof that there is a non negligible number of immunological accidents linked to erythrocyte conflict following blood transfusion.

If this incidence has dropped significantly since the beginning of the century it still remains high and is presently estimated in Europe at 1/6000 – 1/29000 per unit transfusions, Cartron, (1996). This incidence remains high. In Cameroon we have no statistics.

The Antigens concerned are many. More than 250 Antigens have been identified, and 196 have been grouped into 25 systems (see below). These Ag are carried by proteins, glycoproteins and glycolipids on the membrane of red blood cells.

The table below shows the 25 systems of the human RBCs grouping the 196 antigens.
<table>
<thead>
<tr>
<th>System</th>
<th>Official nomenclature</th>
<th>Symbol</th>
<th>Gene</th>
<th>Number</th>
<th>Number of antigens</th>
<th>Chromosomic Localisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>ABO</td>
<td>ABO</td>
<td>ABO</td>
<td>001</td>
<td>4</td>
<td>9q34-q34.2</td>
</tr>
<tr>
<td>MN</td>
<td>MNS</td>
<td>GYP A, B, E</td>
<td>002</td>
<td>43</td>
<td></td>
<td>4q28 - q31</td>
</tr>
<tr>
<td>P</td>
<td>P1</td>
<td>P1</td>
<td>003</td>
<td>1</td>
<td></td>
<td>22q11 - qter</td>
</tr>
<tr>
<td>Rh</td>
<td>RH</td>
<td>RHD,CE</td>
<td>004</td>
<td>46</td>
<td></td>
<td>1p34 – p36.2</td>
</tr>
<tr>
<td>Lutheran</td>
<td>LU</td>
<td>LU</td>
<td>005</td>
<td>18</td>
<td></td>
<td>19q12 – q13</td>
</tr>
<tr>
<td>Kell</td>
<td>KEL</td>
<td>KEL</td>
<td>006</td>
<td>23</td>
<td></td>
<td>7q32 – q36</td>
</tr>
<tr>
<td>Lewis</td>
<td>LE</td>
<td>FUT3</td>
<td>007</td>
<td>6</td>
<td></td>
<td>19p13</td>
</tr>
<tr>
<td>Duffy</td>
<td>FY</td>
<td>FY</td>
<td>008</td>
<td>6</td>
<td></td>
<td>1q22 – q23</td>
</tr>
<tr>
<td>Kidd</td>
<td>JK</td>
<td>SLC14 A1</td>
<td>009</td>
<td>3</td>
<td></td>
<td>18q11 – q12</td>
</tr>
<tr>
<td>Diego</td>
<td>DI</td>
<td>SCL4 AI</td>
<td>010</td>
<td>19</td>
<td></td>
<td>17q11</td>
</tr>
<tr>
<td>Cartwright</td>
<td>YT</td>
<td>ACHE</td>
<td>011</td>
<td>2</td>
<td></td>
<td>7q22.1-q22.3</td>
</tr>
<tr>
<td>Xg</td>
<td>XG</td>
<td>XG</td>
<td>012</td>
<td>1</td>
<td></td>
<td>Xp22-p32</td>
</tr>
<tr>
<td>Scianna</td>
<td>SC</td>
<td>SC</td>
<td>013</td>
<td>3</td>
<td></td>
<td>1p32-p34</td>
</tr>
<tr>
<td>Dombrock</td>
<td>DO</td>
<td>DO</td>
<td>014</td>
<td>5</td>
<td></td>
<td>12p13.2-12p12.1</td>
</tr>
<tr>
<td>Colton</td>
<td>CO</td>
<td>AQPI</td>
<td>015</td>
<td>3</td>
<td></td>
<td>7p14</td>
</tr>
<tr>
<td>Le</td>
<td>Le</td>
<td>Le</td>
<td>016</td>
<td>3</td>
<td></td>
<td>19p13.2-cen</td>
</tr>
<tr>
<td>Chido/Rodgers</td>
<td>CH/RG</td>
<td>C4A/C4B</td>
<td>017</td>
<td>9</td>
<td></td>
<td>6p21.3</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>FUT1</td>
<td>018</td>
<td>1</td>
<td></td>
<td>19q13</td>
</tr>
<tr>
<td>Kx</td>
<td>XK</td>
<td>XK</td>
<td>019</td>
<td>1</td>
<td></td>
<td>Xp21.1</td>
</tr>
<tr>
<td>Gerbich</td>
<td>GE</td>
<td>GYP C</td>
<td>020</td>
<td>7</td>
<td></td>
<td>2q14-q21</td>
</tr>
<tr>
<td>Cromer</td>
<td>CROM</td>
<td>DAF</td>
<td>021</td>
<td>10</td>
<td></td>
<td>1q32</td>
</tr>
<tr>
<td>Knops</td>
<td>KN</td>
<td>CRI</td>
<td>022</td>
<td>5</td>
<td></td>
<td>1q32</td>
</tr>
<tr>
<td>Indian</td>
<td>IN</td>
<td>CD44</td>
<td>023</td>
<td>2</td>
<td></td>
<td>11p13</td>
</tr>
<tr>
<td>OK</td>
<td>OK</td>
<td>Ok</td>
<td>024</td>
<td>1</td>
<td></td>
<td>19p13.2</td>
</tr>
<tr>
<td>Raph</td>
<td>RAPH</td>
<td>MER2</td>
<td>025</td>
<td>1</td>
<td>(MER2)</td>
<td>11p15</td>
</tr>
</tbody>
</table>
The rationale is to avoid conflict between the donor’s product and the recipient’s body. Transfusion safety must ensure present immunological matching but also prevent future transfusion mismatch. This is preventing a conflict between the present antibodies (agglutinins) of the recipient and the antigens of the donor brought by the red cells. Irregular antibodies are all the Anti-RBC antibodies other than anti-A and anti-B. (Gentet & Muller, 1999).

4.2 ABO blood groups

The ABO group is characterized by RBC antigens: A, B, A+B, H and by natural and regular or constant IgM antibodies.

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Ag on red blood</th>
<th>Ab in Serum</th>
<th>% in general pop. in Europe</th>
<th>% in general pop. in Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group O</td>
<td>H</td>
<td>Anti-A, Anti-B</td>
<td>45</td>
<td>68</td>
</tr>
<tr>
<td>Group A</td>
<td>A</td>
<td>Anti-B</td>
<td>42</td>
<td>17</td>
</tr>
<tr>
<td>Group AB</td>
<td>AB</td>
<td>----</td>
<td>03</td>
<td>03</td>
</tr>
<tr>
<td>Group B</td>
<td>B</td>
<td>Anti-A</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

Source: Complete Blood Type Encyclopedia, (2002)

For blood group determination, 2 complementary tests must be done at the same time. The seric test (Simonin) and the red blood test (Beth Vincent) which are to identify the antibodies in the serum and antigens in blood respectively. Typing of an individual should be done from by 2 different persons, at 2 different times.

The Rhesus group is characterized by its RBC Ag CcDEe
4.3 **Irregular antibodies.**

The search for irregular antibodies when carried out under good conditions is the basis of immunological security in blood transfusions. Strict modalities must be maintained in blood collection and later testing must be done with precision.

When looking for irregular agglutinins (IA), it implies that we are looking for seric irregular antibodies. For this a Coombs indirect test is done using the serum of the recipient and a panel RBC test.

The most immunogenic types are D>K>C>c>E>e; some others such as Duffy Ag, Kidd Ag and other Kell subtypes could also be present (P. Rougier, 1993). They are IgG Ab and react at 37 degrees following a previous transfusion or pregnancy.

The IgM Ab are usually not clinically significant (except for ABO antibodies). They are however a source of in-vitro serology difficulty that may delay transfusion. Examples are Lewis, I, P, M,N red cell antigens. These IgM Ab react at 4 degrees and are usually naturally occurring in that they do not require a sensitising event.

White cells bear a great amount of class I HLA Ag which could lead to a high incidence of alloimmunisation manifested by fever – chills hazard.

Anti-HPA Ab are rare but sometimes would lead to transfusion related purpura, which is a life threatening hazard. The latter two could reduce transfusion efficacy. Novotny, (1996), found out that, UV-B irradiation of platelets and filtering of RBCs may be used to reduce HLA and HPA alloimmunisation. Despite these measures, patients with a history of pregnancy or non-leucocyte depleted transfusions will form HLA antibodies in high proportions.
The search for IA will always take place in two stages.

Irregular antibody detection:

This is done using RBC tests which must fulfil a certain number of criteria.

a- Three group O RBC tests must be used and these cells must carry the most current Antigens.
b- The following Rh cells should be present: DCCwee (R1wR1), DccEE (R2R2), ddccee (rr).
c- One of the cells must be homozygous for: M, N, S, s, Fya, Fyb, Jka, Jkb.
d- One of the three cells must be positive for Kpa and Lua.
e- RBCs in papain should contain DCCwee (R1wR1), DccEE (R2R2).
f- RBCs in papain must be homozygous for s, Jka and Jkb.
g- RBC in papain must be positive in K, Lea and Leb.
h- Coombs indirect cells should have the following cells DCCwee (R1wR1), DccEE (R2R2), ddccee (rr), the forth cell should be empty.
i- Coombs indirect test in cells should be homozygous for M, N, S, s, Fya, Fyb, Jka and Jkb.
j- Coombs indirect test in cells should be positive for P1, K, Kpa, Lea, Leb, Lua.

Secondly after their detection, they should be identified.

Irregular antibody identification:

During the detection phase, if agglutination occurred in any one of the RBC tests, the same plasma will be in contact with a series of at least 10 RBC tests,
called an identification panel. The preparation and distribution of these tests should follow precise rules as described below:

a- All the cells should be of RBCs

b- For the rhesus system, DCCwee (R1wR1), DCCee (R1R1), DccEE (R2R2),
   ddCcee (r'r), ddccEe (r''r), ddccce (rr), ddccce (rr), Dccee (Ror), ddccee
   (rr), cell 10 empty and cell 11 empty.

c- Homozygous at least for three of the following: M, N, S, s, Fya, Fyb, Jka, jkb.

d- Positive for at least 3 of the following: k, P1, Lea, Leb.

e- Positive for Kpa and Lua.

f- One negative for: Lea, Leb, (Lea-b-), Fya, Fyb (Fya-b-).

g- The following antigens could be destroyed when the cells are treated with enzymes: Duffy, M, N, S, s.

Lastly after analysis, the degree of agglutination is indicated as 1+, 2+, 3+, 4+, 5+, implying the degree of positivity. If agglutination is identical in Coombs indirect test as well as in Coombs with papain then the interpretation will be done in only one media. If not, interpretation should be done in both. To interpret, we will take the negative reactions and eliminate the antigens that are carried by these RBC taking into consideration that, we can eliminate the Ag on the Red blood cells that are heterozygous for Duffy, Kidd, and M N, S, s. Once all the Antigens have been eliminated, the specificity of the antibody/antibodies should be verified. To do this, either absorption or elution is done (especially when there is a mixture of antibodies) after which titration is done to find out the degree of immunisation.

Therefore if transfusion is necessary, patients with clinically significant Red cell alloantibodies identified in the antibody screen should receive antigen negative red blood cells.
4.4 Alloimmunisation in the multiply-transfused and in the multigravid.

Several studies have been conducted to determine the prevalence of the RBC alloantibodies in multiply-transfused patients and multigravid women. Economidon, et al. (1993) found that, in multiply-transfused thalassaemia patients, alloimmunisation was due to anti-Rh D, anti-E, and anti-C, as well as anti-K, anti-Fy, anti-Jk and anti-S.

They concluded that alloimmunisation increased with the number of blood transfusions, was not influenced by sex but differed from one race to another as presented in the table 3 below.

**Table 3: Frequent clinically significant Anti-RBC antibodies**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>System</th>
<th>Detected antibodies</th>
<th>Whites</th>
<th>Blacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Rh</td>
<td>16 – 40 %</td>
<td>30%</td>
<td>2%</td>
</tr>
<tr>
<td>Kell (Kl)</td>
<td>Kell</td>
<td>5 – 40%</td>
<td>9%</td>
<td>3%</td>
</tr>
<tr>
<td>D</td>
<td>Rh</td>
<td>8 – 33%</td>
<td>85%</td>
<td>92%</td>
</tr>
<tr>
<td>C</td>
<td>Rh</td>
<td>4 – 15%</td>
<td>80%</td>
<td>99%</td>
</tr>
<tr>
<td>Jk (a)</td>
<td>Kidd</td>
<td>2- 13%</td>
<td>77%</td>
<td>91%</td>
</tr>
<tr>
<td>Fy(a)</td>
<td>Duffy</td>
<td>4-12%</td>
<td>63%</td>
<td>10%</td>
</tr>
<tr>
<td>C</td>
<td>Rh</td>
<td>2-10%</td>
<td>70%</td>
<td>32%</td>
</tr>
<tr>
<td>E</td>
<td>Rh</td>
<td>2-3 %</td>
<td>98%</td>
<td>98%</td>
</tr>
<tr>
<td>Jk(b)</td>
<td>Kidd</td>
<td>2%</td>
<td>72%</td>
<td>43%</td>
</tr>
<tr>
<td>S</td>
<td>MNSs</td>
<td>1-2%</td>
<td>55%</td>
<td>31%</td>
</tr>
<tr>
<td>s</td>
<td>MNSs</td>
<td>&lt;1%</td>
<td>89%</td>
<td>97%</td>
</tr>
</tbody>
</table>

In their study on 81 patients undergoing haemodialysis post chronic renal failure, Shukla, et al. (1999), found no association between the number of transfused blood units and the development of alloantibodies. Meanwhile an Italian comparative study by Machia, et al. (1985), on irregular antibodies in the multiply-transfused sickle cell patients showed a prevalence of 5% and 10% in Greece and in Italy respectively. Finally, Domen & Rarez, (1988) in a study on red cell alloimmunisation in chronic renal failure patients undergoing haemodialysis concluded that clinically significant red cell antibodies were formed in 6.1% of transfused patients amongst whom only one formed more than one antibody. Those figures are rather high given that transfusion related accidents following alloimmunisation can be fatal as documented by Roger, (1996).

Foeto-maternal incompatibility affects children who possess paternal blood antigen transmissible through the placenta and which is a target for maternal antibodies.

The immune complex formed can be identified in the newborn by the direct Coombs test; the positivity of this test indicates the fixation in vivo of immunoglobulin and/or complement on the RBC surface. This is seen in the presence of anti RBC antibodies or immunoallergic antibodies, Hillyer, et al. (2003).

In 1996, Cartron outlined the conditions in which foeto-maternal immunisation could take place as follows:

- The Ig should be highly concentrated IgG type given that only they can pass through the placenta; they are mostly IgG1 and IgG3.
- There should be high affinity for antigens
- The antigen-antibody complex should be able to activate the Fc receptors of the macrophages.
According to Bowman (1997), transvaginal bleeding at any stage of pregnancy (delivery, trauma, spontaneous or induced abortion, ectopic pregnancy, invasive obstetrical procedures) will increase the risk of isoimmunisation to Rh D-Ag, the most immunogenic in the Rhesus blood group system. He also concluded that about 17% of Rh D- who deliver Rh D+ will become immunised and that the rates of Rh negativity amongst ethnic and racial groups are as follows:

- Whites: 15-16%
- African-Americans: 8%
- Africans: 4%
- Spanish and French: 30-35%
- Asians: <1%
- Asian-Americans: 1%
- American-Indians: 1-2%
- Eurasians: 2-4%

He further stated that, despite the post partum anti-D Ig prophylaxis, 1-2% of susceptible women continue to become sensitised.

Frohn, et al. (2003) in their article on the probability of anti-D development in D- patients receiving D+ RBCs, found that out of 78 D- patients, 16 developed anti-D and concluded that prolonged haemolysis was probably induced by additional auto reactive antibodies.

It should be noted that anti-U which was first described by Weirner in 1953, is a rare blood cell alloantibody that has been found exclusively in Blacks; it could cause transfusion-linked haemolytic reactions and the haemolytic disease of the new-born. Novaretti, et al. (1978), found that amongst 2,462 Brazilians, all the Whites and Browns were U+ and 0.87% of Blacks were U-. The anti-U is part of the MNS system and of the IgG1 and IgG3 subclass.
From all the above studies, it is well documented that alloimmunisation is a multi-faceted public health issue in many countries. Unfortunately in Cameroon, the risk of alloimmunisation has not yet been assessed and the magnitude of this public health problem is unknown.

Thus, in the event of alloimmunisation, compatible blood may be difficult to find if the Ag- blood is rare or if multiple Ab are present. However, consultation with the transfusion service is helpful in developing a transfusion strategy in these cases. (Hillyer, et al. 2003.)

We believe that this first study on the prevalence of irregular antibodies in multiply-transfused patients and multi-gravid women in Cameroon will be a key step in the sensitisation on the risk of alloimmunisation which could eventually lead to transfusion inefficacy.
CHAPTER 5

METHODOLOGY
5. Methodology

5.1 Materials and reagents

The following materials were used for the study:

i. ABS Précis Automatic Machine: used for irregular antibody detection using Diamed blood cell test in Coombs at 37 °C

ii. Diamed-ID micro typing panel with 11 test cells: used for irregular antibody identification.

iii. Papaine: enzyme used to increase the sensitivity of the indirect Coombs reaction.

iv. Opeline plate or card: used to check for agglutination.

v. Centrifuge: used to centrifuge during irregular antibody detection and identification.

vi. Test tubes: used for indirect Coombs test.

vii. Micropipette: to pipette samples, blood cell tests and other reagents when necessary.

viii. Diamed gel in Coombs: used for the detection of irregular antibodies at 37°C.

ix. LISS: low ionic strength solution. It was used to break the ionic bonds in our solution such that the indirect Coombs test goes through faster.

x. Coombs polyvalent IgG: our main reagent for the detection of irregular antibody.

xi. Incubator: necessary for a step in the detection of our irregular antibody.

xii. EDTA tubes: contain ethylene diamine tetra phosphatate which is an anticoagulant.

xiii. Test tubes rack: to carry out test tubes.
xiv. Cryo-tubes: tubes that withstand a temperature of -80°C.

xv. Cryo-boxes: boxes into which cryo-tubes are placed and kept at -80°C.

xvi. Bold markers: used to mark our tubes and boxes.

xvii. Alcohol, disposable needles, cotton: Used during sample collection.

5.2 Operational definitions

The main operational definitions used in this study are the following:

Age: in years as of last birthday

Ethnic group: the administrative division of origin

HIV status: Response given by the interviewee as of the last screening test irrespective of the time lapse

Blood Group and Rhesus factor: Response given by the interviewee to the question.

Gravidity: number of pregnancies irrespective of the outcome.

Transfusion: packed red cells and/or whole blood received intravenously

Date of first pregnancy: date of termination of first pregnancy or first delivery

Date of last pregnancy: date of termination of last pregnancy or last delivery

Profession: regular activity carried out by the patient on a daily basis

5.3 Methods

5.3.1 Study design: this is a descriptive cross-sectional study

5.3.2 Study duration: from July 2003 to February 2005.
5.3.3 Place of study:

Our samples were collected from multiply transfused individuals and multi-gravid women at the Yaoundé General Hospital and analysed thanks to the Immuno-haematology unit at the Hôpital St Luc, Brussels. The Yaoundé General Hospital is a reference hospital in the Ngouso neighbourhood in Yaoundé which has amongst other services the haematology service which co-ordinates transfusion-related activities. From July to December 2003, 670 units of whole blood were transfused as follows: 300 units to dialysed patients, 150 units to the oncology patients, 100 units of blood to patients in the Internal Medicine wards, 58 Units to patients in the Gynaecologic wards, 15 units to children in the Paediatric wards, 10 units to newborns in the Neonatology ward and 10 units to patients in the Intensive care wards. Transfusion related accidents are said to have occurred during that period but were not documented.

5.3.4 Sample size:

A total of 58 individuals who fulfilled the inclusion criteria were recruited. It was a consecutive convenient sample limited by time.

5.3.5 Inclusion criteria

The criteria were the following:

i. Haemodialysed individuals, who had received at least three blood transfusions.

ii. Women who had been pregnant at least three times.

iii. Any individual from the above groups who filled the informed consent form.
5.3.6 Exclusion criteria

Individuals who fulfilled the following conditions were excluded from the study:

i. Haemodialysed individual who has been transfused less than three times

ii. Women with less than three pregnancies

iii. Individuals who refused to sign the informed consent form.

5.3.7 Procedure:

Data collection forms were filled by the principal investigator for individuals who fulfilled the inclusion criteria. Identification numbers (ID) were then attributed and 10ml of venous blood was collected into EDTA tubes. The blood was then allowed to stand on the rack for 24 hours in a refrigerator at a temperature of +2 to +6 °C. With the help of a micropipette the supernatant plasma was pipetted into cryo-tubes already labelled with corresponding ID numbers.

The cryo-tubes were placed in cryo-boxes and frozen at -80°C in the Centre for the Study and Control of Communicable Diseases (CSCCD), Yaoundé. Later, the above mentioned samples were placed in a cool box with ice packs and transferred to Brussels for analysis. On arrival, the specimens still frozen were kept at -80°C in the Immuno-haematolgy laboratory at the Hôpital St Luc in Brussels until their analysis.

5.3.8 Analysis:

The specimen analysis was done in two stages. In the first stage, irregular antibody detection was done and in the second stage was meant for their identification.
5.3.8.1 Detection of irregular antibodies

When the samples were removed from the freezer, they were allowed in room temperature to liquefy. The plasma was then dispensed into appropriately labeled tubes which were placed into an ABS précis 3101 SI automatic machine distributed by Biorad Switzerland, kept in a hall maintained at 20-22°C. This machine after being set automatically carried out Coombs indirect test, which is an indirect agglutination test, where incomplete antibodies in the test serum are revealed using test red blood cells, all of the group O. We used Diamed test cells P1, P2, P3. This machine did simultaneous distribution into microplates of test cells, of indirect Coombs reagent and of plasma. It did about 24 samples in 10 minutes using incorporated needles. Agitation, centrifuging, reading, interpretation and transcription of results were computerized. Agglutination ranging from 1+ to 5+ was found for some samples.

To confirm these results, we performed an indirect Coombs test in gel with papain, an enzyme to increase the sensitivity of our test. To our Diamed gel in Coombs, we added 50μl of the three different cells P1, P2, P3 and then 25μl of plasma, 25μl of papain and 10μl of LISS before incubation. These were incubated for 15 minutes at 37°C and centrifuged at 3000 revolutions per minute for one minute then read using the naked eye. The test was considered positive when there was diffusion in the gel.

The Anti-Globulin Test (Coombs test): The AHG test has two main variations known as the ‘indirect’ and the ‘direct’ tests. Antibody screening uses the indirect test( that is, the Indirect Anti-Globulin Technique or IAT).
During the detection of irregular antibodies, the blood test cells are put into contact with the IgG Antibody in the patient’s serum, and then incubated for 15 minutes at 37°C. The red blood cells become coated. Then washing is done at least three times, and then the AHG is added. Visual agglutination after centrifuging for a minute implies the presence of irregular antibody.

The AHG test may be performed with the red cells suspended in either Normal Ionic Strength Solution (NISS), or Phosphate Buffered Saline (PBS) or Low Ionic Strength Solution (LISS). The advantage of LISS is that, due to its lower ion concentration, it enables antibody molecules to react quicker with red cell antigens, thereby reducing incubation times.

5.3.8.2 Identification

For the samples where irregular antibodies were detected, the Diamed ID panel-P was used for identification. Each panel contained 11 cells and we labeled 2 sets of 11 tubes per individual. For the first set of tubes per individual, the panel in Coombs was performed while for the second set, we did the panel in Coombs in presence of papain. In the former, we pipetted 25μl of test cells to which 25μl of plasma was added, followed by 10μl of LISS; we then washed 3 times and added 25μl of the polyvalent indirect Coombs immunogloblin; this was thereafter incubated for 10 minutes at 37°C and centrifuged at 3000 revolutions per minute for one minute and then agglutination was looked for. If there was agglutination, this was poured onto a card for better observation. As regards the panel in Coombs in presence of papain, the procedure was identical except for the addition of 25 μl of papain in the process.

Proteolytic enzymes techniques are used for several reasons:
• They would, under the correct conditions, break glycoprotein molecule peptide bonds and remove varying amounts of protein and sialic acid from the red cell surface

• This reduces the net negative charge on the red cell that results in a smaller ionic cloud and thus lowers the zeta-potential

• The minimum distance between the red cells is reduced and this allows some IgG antibodies (e.g. anti-Rh) to produce cross-linking and agglutination in a saline medium

• May also cause antigen site clustering which will improve cross-linking

• The minimum distance will also improve antigen accessibility by removal of charge groups and this will improve the reactivity of some antibody specificities with their antigens, e.g. Rh antibodies

• Antigen sites located on some glycoproteins may also be removed and thus the corresponding antibody would not be detected by this technique e.g. Duffy antibodies

However, there are some inconveniences; for as much as the enzymes would increase the sensitivity of the test, the specificity is reduced. Secondly, the results are not always reproducible.

5.4 Ethical issues

The principal investigator explained to all the study subjects the importance of analyzing their blood to look for irregular antibodies. They were made to understand that in the presence of irregular antibodies, subsequent transfusions would be ineffective if they did not receive blood free of the antigens involved; and that if they developed agglutinin of the rhesus red blood system, they ran the risk of their future babies acquiring the hemolytic disease of the new born.
They were assured that the analysis was free and that if their blood was positive for a particular agglutinin, they would be duly informed. Their blood was collected only after explanations on the above issues were given, and provided they signed the informed consent form.

5.5 Data analysis

Information from our questionnaires was fed into an Epi info record for analysis. Epi info for windows (2003 version) was used for this purpose. We then presented our results using tables, pie-charts and histograms.
CHAPTER 6

RESULTS
6. RESULTS

A total of 58 patients were recruited for the study of which were 23 dialysed multiply transfused patients and 35 multiparous women. The average age of the patients was 39 ± 9.4 years (Range: 27-62 years). The female to male ratio of the study group was 2:1

Figure 1: Gender distribution

<table>
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<tr>
<th>Table 4: Overall prevalence of alloimmunisation</th>
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<tr>
<td>Positive</td>
</tr>
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<td>Total</td>
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The overall prevalence of alloimmunisation was 20.7%. Positivity here implied agglutination and negativity implied the absence of agglutination.

Table 5: Frequency distribution of pregnancies

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Of the 35 women, most had had 3 pregnancies (54.2%) whereas a few had been pregnant 9-12 times.

Figure 2: Alloimmunisation in multigravid patients
There were 2 women who had positive results, thus an overall prevalence of 5.7% among the women. They had been pregnant between 3 and 5 times.

Table 6: Relationship between the number of pregnancies and alloimmunisation.

<table>
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Alloimmunisation was noted in 2 cases of the 35 women with multiple pregnancies, one with 3 and the other with 4 pregnancies, making a prevalence of 5.7% within this group.

Table 7: Frequency of transfusions amongst multiply-transfused persons

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The majority of cases (18/23 = 78.2%) had received 3 to 5 transfusions
Table 8: Relationship between alloimmunisation and multiple transfusions

<table>
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Of the 23 multiply transfused individuals, 10 were positive for red blood cell alloantibodies giving a prevalence of 43.4%.

Table 9: Alloimmunisation in multigravid and multiply-transfused

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Alloimmunisation and Rhesus negative subjects

No alloimmunisation was detected in Rhesus negative subjects

The results of the identification panels showed no agglutination in the presence of Coombs, yet there was some unspecific agglutination in Coombs to which papain had been added. (See the next six pages)
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Immunohématologie
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Lu par: Guerrieri Claudine
Modifié par: Guerrieri Claudine

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Résultats par série

01f: RAl max 24 (37°.-37°)

Série numéro 4974
Démarrage: 24-6-2004 11:28:53
Créé par: Lu par:
Guerrier Claudine Guerrieri Claudine

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### Antigen-Table / Table d'antigènes

| Antigen | Blood Group / Antigens
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#### Interpretation

**Blutgruppe + Antigene**

Aussagen der Reaktion

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#### Remarks

- Farbig gekennzeichnete Antigene können im Enzymtest unterdrückt oder zerstört werden.
- Schraffierte Spalten zeigen Antigene, deren Reaktivität durch Enzymbehandlung reduziert wurde.

**Antigene spezifisch negativ**

- **Lewis**
- **Duffy**
- **Kidd**
- **P**
- **Rh**

**Antigene nicht bestätigt**

- **Hr**
- **A**
- **B**

**Enzyme**

- **U**
- **Tj**

**Result/Result**

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**Bemerkungen**

- **Farbfarben**
- **Enzyme**
- **Untersuchungsdatum**
- **Blutgruppe + Antigene**
- **Interpretation**
- **Enzyme**

**Date of the analysis**

2004.07.19
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### Patient

**Blutgruppe + Antigene**
- CwCD.ee R1W R1
- CCDE.ee R2 R2
- Ccddee r'r
- Ccddee Ee r'r
- Ccddee rr
- Ccddee rr
- Ccddee rr
- Ccddee rr
- Ccddee rr

**Groupe de sang + Antigènes**
- CwCD.ee R1W R1
- CCDE.ee R2 R2
- Ccddee r'r
- Ccddee Ee r'r
- Ccddee rr
- Ccddee rr
- Ccddee rr
- Ccddee rr

### Interpretation

Die farbig gekennzeichneten Antigene können im Enzymtest nicht oder schwerer zu finden sein. Shaded columns indicate antigens destroyed or diminished in reactivity by enzyme treatment. La réaction peut diminuer avec les anticoagulants de systèmes MN, Duffy et Xg. Les hématoxylin sont traités aux enzymes protéolytiques.

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### Untersuchungsdatum

Examined on

Date de l'analyse

---

**Name/name/nom**

**Untersuchungsdatum**

Examined on

Date de l'analyse

---

**D:**

**Blutgruppe + Antigene**

**Groupe de sang + Antigènes**

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**Set ID-DiaPanel P:**
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  - 0561.59.x
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V.I.P. Software Ch.-B./lot no./no. lot: P21
CHAPTER 7

DISCUSSION
7. DISCUSSION

The present study detected irregular antibodies in 12 out of 58 individuals, giving a prevalence of 20.7%. Two of the 12 belong to the multigravid group whilst the remaining ten belong to the multiply-transfused group. Amongst the 10 who had been multiply transfused, one had been pregnant just twice. This prevalence is comparable to the prevalence of other authors such as Singer, et al. (1996) who found a prevalence of 33% amongst multiply transfused individuals who had received blood that was not leucodepleted in thalassaemic patients of Asian origin, Economidon, et al. (1993) on the frequency of various antibodies to various antigenic determinants in polytransfused patients in Greece, found a prevalence of 19 – 37% in multiply transfused thalassaemic patients and Luban, (1989) who found a prevalence of 42.9% in children of non-American origin. This study was carried out in Washington DC and was in the variability in rates of alloimmunisation in children with sickle cell disease antigens and thus for different antibodies.

However some studies showed lower prevalences, like Domen and Ramirez in their study on Red cell alloimmunisation in chronic renal failure patients undergoing haemodialysis, with a prevalence of 6.1% and Shukla & Chauhary, (1999), in India, in their study on 81 patients undergoing haemodialysis post chronic renal failure with a prevalence of 9.8%. In our study, we found no association between the number of transfusions and the presence of irregular antibodies and this was the same in the study carried out by Shukla, et al. (1999). Meanwhile Economidon, et al. in 1993 concluded that allo immunisation increased with the number of transfusions.
Among the multigravid women we had a prevalence of 5.7%. None of these women was rhesus negative. Frohn, et al. (2003), in Lubeck, (Germany), in their study on the probability of anti-D development in D- patients receiving D+ red blood cells, with a sample size of 78 patients, expected an estimated prevalence of 30.4%. They detected anti-D in 16 patients giving a prevalence of 20.5%. They concluded that the actual frequency of antibody formation was much lower than assumed.

Bencomo (1990) in a comparative study of several methods of detecting erythrocyte alloantibodies concluded that, the usefulness of enzyme tests in anti-Rh antibody detection were demonstrated, as well as that of low ionic strength saline solutions in detecting anti-Kell, anti-Duffy, and anti-Kidd antibodies. Meanwhile serum precipitation with 15% polyethylene glycol 8000 previous to the indirect antiglobuline test was found the most sensitive method, providing the best results in all the antibodies studied.

Agglutination in papain was non-specific. Papain is known to modify some antigenic structures and remove sialic acid from the red cell membrane and as such

None of the sera in our identification panel in Coombs showed any agglutination. This could be due to the fact that most of the irregular antibodies (10/12) were detected in hemodialysed multiply-transfused individuals who are most of the time immuno-compromised and so the development of antibodies as a whole is low in intensity. Therefore, despite the presence of anti human globulin to bridge the gap by cross-linking IgG coated red cells, they are not able to bring about agglutination. (Watson P. and Learoyd P., 2004). Also, the panel used for identification purposes was developed from known immunogenic antigens in the European world (manufactured in Switzerland).

Agglutination took place when papain was added to the identification panel in Coombs demonstrating false positives. Papain is known to modify some antigen
structures and remove sialic acid from the red cell membrane and as such weak antibodies may be enhanced. (Lown, 1995). A study by Gustafesson (2004) on the evaluation of a modified IAT gel with polyethylene glycol (PEG) addition for red cell identification concluded that, results were judged as non specific reactions when the enzyme method was used.

Cate and Reily, (1999), stated that, the gel test was developed by Lapiere in 1984 and was designed to standardise anti-globulin testing while improving sensitivity and specificity. They concluded that the gel is a reliable and advance method and that it is appropriate for routine use for the detection and identification of alloantibodies in community hospital transfusion services. Pottier, et al. (1992) in their study of Gel test interpretation and value of a new technique for detection of irregular antibodies found a 10% positivity. Out of the 10% positivity, 3.7% were irregular antibodies, 3.8% papain auto antibodies, 1% cold antibodies and 2% not elucidated. Some of the antibodies were not detected by their tube method and this lead to the conclusion that the sensitivity and standardisation of the gel test technique guarantee greater safety in blood transfusion and increase efficiency in the prevention of foeto maternal stimulation of anti D. A comparative study of the polyethylene glycol indirect antiglobulin test and papain on a sample size of 1503 for detection of red cell antibodies carried out by Reisner, et al. (1996) revealed that upon antibody identification, 0.8% false positives were seen with polyethylene glycol compared to 3.5% with papain. This led to the conclusion that polyethylene glycol retained the highest sensitivity while significantly lowering the false positive results and thus decreasing the detection of antibodies of doubtful clinical significance.
CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS
8. CONCLUSIONS AND RECOMMENDATIONS

At the end of the study, it was found that:

• There is a high prevalence of irregular antibodies amongst multiply-transfused individuals in our study.

• The prevalence of irregular antibodies detected were 20.7% in our total population, 5.7% amongst the multi-gravid and 43.4% amongst the multiply-transfused.

Given the high prevalence of alloimmunisation found in our study, the following recommendations are made to improve blood transfusion safety in Cameroon:

• To conduct a study to determine the different phenotypes of irregular antibodies found in our community.

• Phenotyped blood should be used in all patients requiring frequent transfusions nationwide.

• The development of a panel of blood test cells produced locally from blood group O donors using antigens that are known to be immunogenic and prevalent in the population should be done.

• Sensitisation campaigns on the risk of alloimmunisation should be organised to create awareness amongst health workers, multiply-transfused subjects and multigravid women.

• Transfusion related complications should be documented.
REFERENCES


APPENDIX I:

DATA COLLECTION FORM USED FOR THE STUDY

IDENTIFICATION

Name _______________________________ \\
Age: ____________________ Sex: __________
Ethnic group: ______________________
Profession: _________________________

Blood group □  Rhesus □  HIV Negative ________  
Positive ________  Unknown ________

Informed consent: Yes □  No □

MEDICAL HISTORY

Multiparous □

Anti D globulin following deliveries or abortions?  Yes □  No □  
≥3 Haemodialysis □

Sickle cell patient who are polytransfused: ______________________

Date you received your first blood transfusion: ______________________

Date you received your last blood transfusion: ______________________

How many transfusions in all: ________________________________

When did you have your 1st pregnancy: ___________________________

When did you have your last pregnancy: ___________________________

Number of total pregnancies: ________________________________

Date of collection of sample: ________________________________
APPENDIX II

INFORMED CONSENT FORM

FICHE DE CONSENTEMENT

I the undersigned accept that my blood be collected aseptically and analysed for research purposes in view of looking for irregular antibodies which so far may have caused some transfusional accidents.

Je soussigné accepte qu’un échantillon de mon sang soit prélevé de manière aseptique et analysé en vue de la recherche des anticorps irréguliers pouvant provoquer des accidents transfusionnels.

Date ______________________

Signature of Individual

Signature of Researcher

Signature de l’intéressé

Signature du Chercheur