Rhesus-Haemolytic Disease of the Newborn with Reference to Anti-D

by

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The chapters in this project deal with Haemolytic Disease of the Newborn (HDN) with a reference to Rhesus incompatibility when anti-D is involved.

The first chapters provide the basic immunology of HDN and its clinical significance in relation with serological findings. This is followed by the complications and the effects of HDN both on the mother and on the foetus, throughout the pregnancy and after birth.

The project includes the methodology of the prenatal tests done in diagnosing HDN. This also covers the treatment and management involved, especially when complications due to HDN may arise in subsequent pregnancies.

Finally, statistical charts show the incidence of Rhesus-HDN in the last few years, and its occurrence due to anti-D.
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Chapter 1

1.1 INTRODUCTION

Haemolytic disease of the newborn (HDN) is associated with foetal red cell destruction following the transplacental passage of maternally derived IgG antibodies. The antibodies are directed against paternally derived antigens on foetal cells. The IgG antibodies bind to foetal red cells, and in turn these are destroyed following adherence to Fc receptors on macrophages in the spleen, by phagocytosis and extracellular lysis. This is the case in 'erythroblastosis foetalis', also commonly known as Haemolytic Disease of the Newborn.

Rhesus incompatibility (Rh - HDN) is responsible for most of the cases of moderate and severe HDN. Haemolytic disease of the newborn can also result from incompatibilities caused by many other red cell antibodies, although this occurrence is much less frequent.

The incidence of immunisation to red cells is related to the volume of red cell exposure. The Rh blood group antigen D is the most important cause of maternal allo-immunisation and HDN. The Rh system consists of a family of inherited antigens. The theory of inheritance put forward by Fisher and Race states that there are three pairs of antigens, Dd, Cc, and Ee. The presence of D indicates Rh positivity; its absence Rh negativity.

Numerous advances have been made in the diagnosis and management of HDN and have greatly decreased infant morbidity and mortality. The most significant development has been made in disease prevention. Although there is no cure for HDN a method of antibody immunosuppression was developed to prevent Rh immunisation in an Rh-negative mother. Passive antibody as Rh immune globulin (RhIG) is given to Rhesus negative women after delivery of a Rhesus positive infant to prevent immunisation to the Rh(D) antigen and the resulting production of anti-D. Although effective only against immunisation to the Rh antigen, Rh immunosuppression has nearly eradicated HDN since its origin in 1968.
Usually, during the first pregnancy, the foetus remains unaffected. However, once the mother has produced an antibody, all subsequent offspring who inherit the corresponding antigen from the father will be effected. The antibody that binds to the red cell antigen causes red cell destruction in the foetus, resulting in an anaemic newborn.

Blood transfusion technology provides information necessary for the diagnosis, clinical management, and prevention of HDN. This information is used to identify the specific fetomaternal incompatibility, to provide the safest possible blood for transfusion therapy, and to identify the candidate for RhIG immunosuppression.

1.2 ANTIGENS AND ANTIBODIES

An antigen is any agent capable of binding specifically to components of the immune response, such as lymphocytes and antibodies. They are present on the surface of red cells, white cells and platelets. Some of these antigens are shared by cells of different types, while others are found only on one type of cell. An antigen-antibody reaction that involves red cells leads to their destruction or agglutination.

An antigen can be any foreign organic substance that is large enough to stimulate the production of antibodies. Antigens may be proteins, polysaccharides or any combination of biochemical molecules, such as glycoproteins, lipoproteins and nucleoproteins. An antigen must have one or more antigenic sites, known as epitopes, to which antibodies can bind. The important point is that antigens must be ‘foreign’ materials that the body does not recognize as ‘self’ antigens. Before antibodies can be made, a foreign molecule and the antibody-producing cell have to bind. Factors important in determining actual antibody production include the following:

i. the amount of antigen reaching the antibody-producing cell and its nature,

ii. the number of times the antigen is present in the tissues,

iii. the time before the antigen is destroyed or eliminated,

iv. the way by which the antigen is introduced into the body, and

v. the composition of the animal or patient being immunized.
Antibodies are produced by B lymphocytes. Maturation culminates with migration of the B cells to the reticulo-endothelial tissues of the body including the lymph nodes and parts of the spleen, bone marrow, liver, gastrointestinal tract and other tissues. Antibodies are a miscellaneous mixture of serum globulins and share the ability to bind individually to specific antigens. Those serum globulins with antibody activity are known as immunoglobulins (Ig). All immunoglobulin molecules have common structural features. The part of the molecule that binds to the corresponding antigen is different in each immunoglobulin. The basic structure of an immunoglobulin consists of two identical light (L) chains and two identical heavy (H) chains. A disulfide bridge (S-S) links each light chain to a heavy chain, and another disulfide bridge (S-S) bonds the two heavy chains. The drawing below represents the simple structure of an immunoglobulin:

![Immunoglobulin structure](image)

Fig. 1.1: Immunoglobulin structure.

There are two types of light chains, kappa (κ) and lambda (λ), and five main types of heavy chains; gamma (γ), meu (μ), alpha (α), delta (δ) and epsilon (ε). Each heavy chain corresponds to the different immunoglobulin class namely, Ig G, Ig M, Ig A, Ig D and Ig E respectively. Each has several unique biological properties. Ig G is the only immunoglobulin that crosses the placenta, and Ig M is a greatly effective antibody since it can make other serum constituents active to cause lysis of bacteria. Ig A is considered to be a secretory immunoglobulin, Ig D is found on lymphocytes at certain stages of development, and Ig E binds with high affinity to mast cells and it is mainly involved in allergies.

### 1.3 THE RHESUS SYSTEM

In selecting donor blood for transfusion, testing of each recipient's red cells for the presence or absence of the Rh (D) antigen, started to become indispensable in the 1940’s. Though at first restricted by the limited
availability of the necessary reagent, D grouping has become as much a part of the pretransfusion routine as
ABO grouping. The red cells of donors are also always tested for the D antigen (and for the weak D variant),
so that the units are suitably labelled and classified as ‘Rh positive’ or ‘Rh negative’.

1.3.1 The antigens of the Rhesus system
As the Fisher-Race theory states, there are three pairs of allelic genes: C and c, D and d, E and e. They
occupy three loci on chromosome 1, are closely linked and are inherited as triplets. Each triplet contains one
gene from each pair. Rhesus antigens are considered to be proteins, still they require phospholipids for
expression. It has been customarily considered that the so called silent gene d is present in the triplet when
the D antigen is absent. A person who has the D antigen is said to be Rh ‘positive’, and conversely a person
who lacks it is Rh ‘negative’.

On the other hand, the Wiener theory assumes that the whole expression of the Rhesus system is controlled
by a series of genes each occupying only one locus on the chromosome. The Wiener notation employs
symbols for the description of the Rh phenotype. The comparison of the two notations is shown in the table
below:

<table>
<thead>
<tr>
<th>Gene complex</th>
<th>Fisher-Race notation</th>
<th>Wiener notation</th>
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<tbody>
<tr>
<td>cde</td>
<td>Cde</td>
<td>r</td>
</tr>
<tr>
<td>Cde</td>
<td>r'</td>
<td></td>
</tr>
<tr>
<td>adeE</td>
<td>r''</td>
<td></td>
</tr>
<tr>
<td>CdE</td>
<td>r'</td>
<td></td>
</tr>
<tr>
<td>CDE</td>
<td>R'</td>
<td></td>
</tr>
<tr>
<td>dDe</td>
<td>R0</td>
<td></td>
</tr>
<tr>
<td>CDtE</td>
<td>R1</td>
<td></td>
</tr>
<tr>
<td>CDe</td>
<td>R1w</td>
<td></td>
</tr>
<tr>
<td>cDE</td>
<td>R2</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1: Fisher-Race and Wiener notations. (Adapted from: Brzoza J. 1987)

A third terminology suggested by Rosenfield, was established to define the reactions of the specific antisera
against which the particular sample was tested. This gave a precise description of the Rhesus status. Rhesus
negative cells could only be shown as cde, rh or r by the Fisher-Race and Wiener notations whereas, according
to Rosenfield's terminology, they would be labelled as Rh: -1, -2, and -3 when tested only against anti-D, anti-
C and anti-E, or as Rh: -1, -2, -3, 4 and 5 if tested against anti-D, anti-C, anti-E, anti-c and anti-e.
Fisher-Race antigen | Wiener blood factor | Rosenfield Rh number
---|---|---
D | Rh₀ | Rh: 1
C, c | rh’, hr’ | Rh: 2, Rh: 4
E, e | rh”, hr” | Rh: 3, Rh: 5

Table 1.2: Comparison of three nomenclatures. *(Adapted from: Barrie J. U. 1984)*

It must be said that among the rarer Rhesus antigens, some have been termed according to Fisher, others according to Wiener, but all were consequently given a Rosenfield number.

### 1.3.1.1 $D^u$ antigen and $D$ variants

The $D^u$ antigen is a weak form of the $D$ antigen. When it is tested with several anti-$D$ antisera, some will agglutinate $D^u$ red cells, whereas others will not do so. A blood donor who has $D^u$ antigens, must be grouped as Rh (D) positive. If they are blood receivers, they can be transfused safely with Rh (D) positive blood. However, to eliminate any risk of transfusion reactions, whether the phenotype is $D^u$ or Rh (D) negative, Rh (D) negative blood should be transfused.

Likewise, pregnant women with the $D^u$ antigen, do not need the administration of anti-Rh (D) immunoglobulin unless there is doubt as to whether the phenotype is Rh (D) or $D^u$ negative. Anti-D is rarely produced in subjects with $D$ variants. $D$ variants lack one or more epitopes of the $D$ antigen.

Certain examples of $D^u$ are the result of Cde being situated on a normal $D$ gene in the opposite gene complex. The $C$ gene on the same gene complex may also weaken the expression of the $D$ antigen. There are other $D$ antigens known as partial $D$. These red cells lack an epitope or epitopes of the normal $D$ antigen. Individuals with such a $D$ antigen can thus form anti-$D$ that is specific to the missing epitopes.

The $D^u$ antigen varies from the $D$ antigen quantitatively in a sense that $D^u$ has a decreased number of antigen sites per red cell.
It is also important to identify the genotype of the father in the case of an Rh immunized mother in order to assess the chances of a Rhesus-positive foetus. Unfortunately, however, the presence of either DD or Dd can only be determined from known frequencies of certain genotypes in large populations. The other part of the phenotype can be established from the results of the tests using anti-D, anti-C, anti-E, anti-c and anti-e.

Such studies have been carried out in Caucasians, in which case if the phenotype is, say CCDee, the most likely genotype is CDe/CDe; or if the phenotype is cCDee, then the genotype is probably CDe/cde. (Barrie, 1984).

<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>FREQUENCY (%)</th>
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<tbody>
<tr>
<td>D</td>
<td>85</td>
</tr>
<tr>
<td>C</td>
<td>70</td>
</tr>
<tr>
<td>c</td>
<td>80</td>
</tr>
<tr>
<td>E</td>
<td>30</td>
</tr>
<tr>
<td>e</td>
<td>98</td>
</tr>
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Table 1.3: Frequency of Rh antigens in Caucasians. (*Adapted from: Barrie J. U. 1984*)

Rarely red cells can lack one or more Rhesus antigens. This condition is known as Rhnull and it is a rare genotype characterised by complete absence of all Rh antigens. Rhnull is sometimes associated with congenital haemolytic anaemia.

1.3.2 The antibodies of the Rhesus system

The antibodies of the Rhesus system are all immune, except for some naturally occurring anti-E antibodies. Anti-D is the most significant Rhesus antibody. It is always immune and causes both haemolytic disease of the newborn and haemolytic transfusion reactions. Anti-D antibodies are usually detected by the indirect antiglobulin test, and do not bind complement. Anti-C may be found together with anti-D antibodies.
Anti-c is the second most important antibody in the Rhesus system. It can cause immediate and delayed transfusion reactions as well as haemolytic disease of the newborn. Other Rhesus antibodies are anti-C\textsuperscript{w}, anti-E and anti-e. They are less common than anti-c and anti-D but similarly can cause serious clinical problems.

When a Rhesus antigen is introduced in the body, through transfusion or pregnancy, a corresponding Rhesus antibody is formed in response to it, but the degree of response varies widely. It has been shown that some Rhesus negative people (about 30 percent), known as non-responders, fail to produce anti-D in spite of repeated injections with Rhesus positive blood. Those persons who produce anti-D after several injections will have low titre levels, while those responding after a single injection of Rhesus positive blood generally acquire high titres. It was also noted that those who respond to a low dose initially, often fail to respond to a high dose later, and vice versa. A similar situation occurs during pregnancy. Some Rhesus negative mothers may give birth to several Rhesus positive newborns without forming anti-D, while others become immunized at the end of the first or second pregnancy. Non-responders may not produce anti-D even though during pregnancy, there was substantial foetal bleeds.

Once formed, anti-D tends to persevere and increase in subsequent Rhesus positive pregnancies. However, in each case the titre eventually stabilizes at a characteristic level. If this titre level is moderate, say 1:256 tested by the indirect antiglobulin test, even though at first it rises to 1:2000, it has been noticed that in a second pregnancy, it returns to 1:128 - 1:256 and remains at this latter level. Briefly, it can be said that anti-D titre levels increase with each new stimulus, but there are many exceptions to this rule.

Chapter 2

Clinical Aspect of HDN

Feto-maternal incompatibility is a term used to describe clinical syndromes in the foetus caused by the transfer of a maternal alloantibody through the placenta. These include the alloimmune neonatal thrombocytopenia, haemolytic disease of the newborn and alloimmune neonatal neutropenia.
Among these, the most common syndrome is Haemolytic Disease of the Newborn (HDN). When HDN occurs, the red cells of the foetus are destroyed by the maternal alloantibody, resulting in a shortened life span of erythrocytes. The antibody can be aimed at ABO or Rh antigens, or less commonly, at an antigen of other blood group systems such as the Kell or Kidd. Nowadays with treatment using anti-D, HDN caused by ABO incompatibility is becoming more important. This is due to the fact that no prevention or treatment is available for HDN caused by ABO incompatibility.

2.1 COMPLICATIONS AND EFFECTS OF HDN

The risk of immunisation increases with the size of the feto-maternal bleed and when it occurs in the early stages of pregnancy. Furthermore, a larger number of fetal erythrocytes may enter the maternal circulation, in cases of toxaemia, in the presence of placental abnormalities, during external rotation of the foetus or manual removal of the placenta. Anti-D antibody may be evident in the mother's circulation from six weeks up to six months after the bleed. In some women the antibody becomes apparent only after the second exposure to the D antigen.

Immunisation can also occur by transfusion of blood or its products. It has been observed that on administration of 200 mls. of Rh(D)-positive red cells, two out of three Rh(D)-negative recipients were immunised. It is important to avoid transfusing Rh(D)-positive blood to Rh(D)-negative women of childbearing age. In cases of an unsuitable transfusion, an attempt to prevent immunisation must be made. This is usually done by administering anti-D immunoglobulin. The same accounts for the transfusion of leucocyte concentrate and platelet concentrates. The latter contain a small amount of red cells. However, when Rh(D)-positive platelets are donated to an Rh(D)-negative woman of childbearing age, an intramuscular injection of 100 µg of anti-D immunoglobulin is given to prevent and protect from immunisation. It is rare that primary immunisation occurs when fresh frozen plasma or cryoprecipitate prepared from Rh(D)-positive units of blood, are transfused to Rh(D)-negative recipients.
In HDN the rate of haemolysis depends on the avidity of the antibody and its concentration in the foetal blood. The main effect of haemolysis is anaemia. If chronic it may lead to massive erythroid hyperplasia in the liver and spleen, resulting in hepatosplenomegaly. There may be other changes, mainly extramedullary haematopoiesis and hemosiderin deposition causing different degrees of jaundice and oedema.

During foetal life, circulating unconjugated bilirubin crosses the placenta to the mother, therefore jaundice is rarely evident in the infant at the time of birth. However, at the same time haemolysis decreases due to a reduction in placental transfer of anti-D. The clearance of bilirubin by the placenta also ceases so its concentration increases rapidly. The neonate may develop jaundice within 24 hours in moderate to severe cases of HDN. It is thought that a deficiency of carrier proteins due to liver immaturity, can cause an excess of circulating unconjugated bilirubin. Despite this, the absence of jaundice within 24 hours from the onset of birth, does not rule out HDN.

Severity of the disease in clinically important cases range from a mild form with insignificant splenomegaly, anaemia and hyperbilirubinaemia, to foetal death in utero as early as 20 weeks gestation. Haemolytic disease of the newborn is usually milder than that in the second and subsequent pregnancies. Thus, HDN is considered to be mild, moderate or severe with respect to the clinical presentation and the concentration of haemoglobin and bilirubin.

2.1.1 Mild HDN

On testing the cord blood for haemoglobin and bilirubin concentrations, it was found that they are usually both within the normal range for neonates. Haemoglobin can be slightly reduced and bilirubin levels rise within the first day after birth but at no time exceed 75 µmol/l. No treatment is required. The normal range of haemoglobin for a full term healthy neonate is between 13.7 and 20.1 g/dl.

2.1.2 Moderate HDN
This is characterised by a rapidly increasing bilirubin concentration and usually mild anaemia shown by a decrease in haemoglobin levels. An exchange transfusion must be performed to protect the central nervous system from the toxic effects of bilirubin. Any deposit of bilirubin in the central nervous system, particularly in the basal ganglia and cerebellum, causes neurological damage such as ataxia (impairment of normal coordination), mental retardation and deafness.

2.1.3 Severe HDN

Profound anaemia, oedema, cardiac failure, tissue hypoxia and acidosis are usually present in severe cases of HDN. Hypobilirubinaemia may also be present causing oedema and deposition of bilirubin in tissues. The marked decrease of haemoglobin can cause death in utero from about the eighteenth week of gestation onwards. At birth the infant suffers from the effect of both anaemia and hyperbilirubinaemia.

It must be remembered that the consequences of HDN are more pronounced in premature and low birth weight infants.

A study by Beischer (1968) was done to correlate the effect of haemoglobin and bilirubin on HDN. A survey of fifteen cases in which both infants were Rh positive and the mother was Rh negative and anti-D isoimmunized, indicated that in seven families the siblings showed different degrees of HDN on the basis of clinical features or cord blood haemoglobin values. This suggests that foetal and placental influences as well as antibody titres determine the severity of HDN.

It is known that the presence of an antibody to another red cell antigen may also decrease the response to the D antigen or the clinical effect of anti-D. In one case, the mother was Group A, Rh negative (cde/cde) and strongly Rh immunised, the father Group B, Rh positive (CDe/cde), twin I Group AB, Rh positive (CDe/cde) and twin II Group A, Rh positive (CDe/cde). Both twins were female and of similar birthweight; in both cases the direct antiglobulin test was positive and of similar avidity. However, at birth the
haemoglobin for twin I was 15g/100ml while for twin II it was only 4g/100ml. The latter was immediately exchange transfused, but died 11 hours after birth. On the other hand, although twin I became jaundiced within thirty minutes of birth and showed pallor and hepatosplenomegaly, after three transfusions the infant maintained excellent improvement.

Serological tests detected high circulating titres of both anti-D and IgG anti-B in the cord serum of twin I and the anti-B could also be eluted from the cord cells. It indicates that this antibody had been preferentially adsorbed on the surface of the AB cells, partly protecting the cells from the more severe effects of anti-D. Twin II cord cells, being Group A, could not adsorb anti-B and therefore would be completely coated by the more potent anti-D.

Competition between concurrent antibodies is not restricted to the ABO-Rh combination but can occur whenever multiple red cell antibodies are formed, although not all cases may be as decisive clinically.

Chapter 3

DIAGNOSIS AND IDENTIFICATION OF HDN

A few decades ago, the Rhesus-immunized patient was managed by the individual physician. The management depended exclusively on the clinical experience of the doctor, and thus management was sometimes good and in other cases poor, sometimes even fatal. A new concept was developed in the 1960's - the high-risk clinic - where competent practitioners in the Rhesus field apply the latest techniques and
concepts of management for optimum patient care. Such a clinic was set up in 1962, at The New York Hospital - Cornell Medical Centre. After tests are done and results issued, members from the paediatric, haematology, blood bank and obstetric staff involved in the patient’s care, meet to discuss each individual case. Acting as a team, they decide the treatment required for each particular case.

Some of the tests carried out, include screening for anti-D (Rh) antibodies which is the first step in the control of an Rh-negative patient. Other tests include amniocentesis and amniotic fluid analysis. This involves the quantitation of bilirubin pigments in the amniotic fluid, thus enabling a view of the severity of the disease. However, some new methods, rather than solving problems, have perplexed clinicians. Some methods are applied with misconceptions and inaccuracies. This chapter presents an overview of the most common and less familiar tests done in the management and prophylaxis of haemolytic disease of the newborn.

3.1 THE KLEIHAUER TEST - ACID ELUTION TECHNIQUE

The Kleihauer acid elution technique for differentiating fetal red cells from adult red cells is, in experienced hands, an accurate method of determining both the prevalence and size of fetal transplacental haemorrhage (TPH) during pregnancy and at delivery. Unfortunately it is performed only occasionally in a routine blood bank laboratory setting. The higher the number of foetal cells observed in the blood film, the more severe is the disease.

PRINCIPLE

Normal adult haemoglobin, which is precipitated by the action of drying and by 80% ethanol, becomes readily soluble in citric acid-phosphate buffer, pH 3.3 while precipitated fetal haemoglobin is very slowly soluble under these conditions. After staining with eosin, red cells containing haemoglobin F are easily identified microscopically when stained with the appropriate blood stains.

PROCEDURE
1) Thin smears are prepared using capillary or EDTA venous blood. The blood is diluted to 1 in 3 with an isotonic solution of saline to ensure very thin even smears.

2) The smears are fixed in 80% ethanol for five minutes.

3) Then they are rinsed thoroughly in distilled water for five minutes and allowed to dry.

4) The slides are immersed in the acid-phosphate buffer previously heated for about 30 minutes to 37°C. They are left for exactly 5 minutes, agitating gently during this time, after of which they are rinsed with distilled water for ten minutes.

5) The slides are allowed to dry completely to avoid staining artifacts.

6) The slides are stained for three minutes in Mayer’s Haematoxylin. The nuclei become blue (bluing) when the slides are rinsed in tap water.

7) A counterstain, usually 0.5% eosin, is applied for three minutes.

8) Lastly, the blood films are rinsed well with distilled water.

RESULTS

On microscopic examination, the fetal red blood cells stand out as dark refractive bodies against the unstained ghost forms of the adult erythrocytes. Foetal cells appear as smooth homogenous cells with a scarlet red colour.

NOTES

1) Interpretation of the acid-elution methods for the assay of transplacental haemorrhage must be made in light of the limitations of the test. Under both normal and pathological conditions, adult red cells may contain small amounts of fetal haemoglobin.

2) Foetal haemoglobin may be present in normal adult blood up to 1% of the total haemoglobin. Therefore occasional stained cells may be seen. Usually these have “intermediate” staining properties, lacking the highly refractive and deeply stained appearance of the characteristic foetal cell.

3) Increased levels of foetal haemoglobin can be found in adult blood in some pathological states.
a) Very high levels are found in hereditary persistence of foetal haemoglobin. This is a genetically acquired characteristic in which the heterozygotes possess approximately 15-30% foetal haemoglobin. Using acid-elution methods, foetal haemoglobin is evenly distributed throughout the red cell population.

b) Increased levels of foetal haemoglobin are also found in haemolytic anaemia, acute leukaemia, acquired aplastic anaemia and other conditions in which the bone marrow is under stress. The levels found, however do not usually exceed 4%.

c) There is a tendency for the foetal haemoglobin level to rise a little during the first trimester of pregnancy and then to fall to slightly below normal by the time of delivery.

**LIMITATIONS OF THE TEST**

1) Ethyl alcohol in concentrations of less than 80% do not give satisfactory results.

2) The pH of the citric acid-phosphate buffer is critical and must be within a pH range of 3.2 to 3.3.

3) After ten minutes of incubation in the citric acid-phosphate buffer, there is some loss of haemoglobin F.

4) The blood for the acid-elution test may be stored in EDTA at refrigerator temperatures for no longer than two weeks before the smears are made.

**DETECTION OF FOETAL CELLS INTO MATERNAL CIRCULATION**

Almost 20% of pregnancies are believed to be associated with bleeding from the foetus into the maternal circulation. A high amount of haemoglobin F in the blood film indicates haemorrhage. Occasionally, however, massive transplacental haemorrhage may occur and will result in the birth of a severely anaemic infant. The detection of foetal cells in the maternal circulation has also gained increasing importance in the prophylaxis of HDN. The size of the foetal bleed can be estimated as follows:

\[
\frac{\text{Average no. of foetal cells/hpf}}{\text{Average no. of maternal cells/hpf}} \times 100
\]
Assuming that the maternal blood volume is 5000 mls; the foetal bleed would be equal to the percentage foetal cells multiplied by 5000 mls.

When ABO incompatibility exists between mother and child, the foetal cells may be destroyed by the maternal anti-A or anti-B (resulting in misleading estimates of the foetal blood). Foetal haemoglobin production is tightly regulated during different stages of development. In the newborn period, Haemoglobin F is the predominant Haemoglobin and decreases at a rate of approximately 10 % every 2 weeks after birth, although there is a wide range of variation at ≤ 2 years of age.

The Kleihauer stain is commonly used to confirm and quantitate a fetomaternal haemorrhage. In these cases, the test is a valuable diagnostic tool because true foetal cells that contain nearly 80 % foetal haemoglobin are unmistakable on the blood film. The newborn will be anaemic if Haemoglobin F is present in the mother due to fetomaternal haemorrhage.

Obstetric situations and procedures such as antepartum haemorrhage, pre-eclamptic toxaemia, external version, and manual removal of the placenta, significantly increase the risk of TPH.

### 3.2 Antibody Screening

Red cell antibody screening forms an fundamental part of compatibility testing especially in transfusion laboratories handling a maximum surgical blood order schedule. It also forms an important part of antenatal serological testing enabling detection of antibodies which may cause haemolytic disease of the newborn (HDN). The aim of antibody screening is to detect all clinically relevant antibodies. In order to do this effectively, red cells are selected with an appropriate antigen profile. There are several methods for the screening of antibodies. Most techniques are based on the principle of the antihuman globulin test. The introduction of column techniques for antibody screening by indirect antihuman globulin testing (IAT) and two-stage enzyme testing (ETC) is perceived to lead to an increased sensitivity and an ability to detect red cell
antibodies more easily than by traditional tube techniques because reactions in columns are more easily read and are stable. Here, three main methods are outlined; the indirect antihuman globulin method, the enzyme treated manual technique and a low ionic AutoAnalyzer technique. The latter is especially effective since it is very sensitive for Rh antibodies. The outline will include the principle, method and results and their interpretation.

3.2.1 Antibody screening by the Indirect Antihuman globulin test for screening and titration of IgG antibodies.

PRINCIPLE

Antihuman globulin may be produced in various animals, such as rabbits, following the injection of human globulin, purified complement or specific immunoglobulin. When antihuman globulin is added to human red cells coated with immunoglobulin or complement components, agglutination of the red cells indicates a positive test.

PROCEDURE

1. A test-tube is labelled and 2 to 3 drops of serum to be tested, are pipetted into it.
2. One drop of a 2% to 5% suspension of pooled screening cells is added to the serum.
3. The test-tube is mixed well and incubated at 37°C for 60 minutes.
4. The sample is then washed for 3 times with 0.9% sodium chloride solution. This is done by filling the test-tube with 0.9% sodium chloride solution, centrifuge and decant the supernatant. This is done for three times. The residual supernatant is decanted and 1 to 2 drops of antihuman globulin serum is added.
5. The sample is centrifuged for the last time for 30 seconds. It is then examined microscopically for any agglutination.

RESULTS
If human red cells are coated with immunoglobins, or as they are commonly known, antibodies, they will bind with the antihuman globulin and agglutination takes place. Thus, this indicates a positive result. Conversely, no agglutination denotes a negative result and so no antibodies are present in the serum tested.

3.2.2 Two-stage enzyme (papain) capillary screening for antibody

Enzyme methods are useful in blood group serology to render red blood cells agglutinable by IgG antibodies, especially Rhesus antibodies, and by inactivating particular antigens to aid the identification of antibody mixtures. The most commonly used enzymes in red blood cell antigen-antibody methods are papain, bromelin and ficin. These enzyme processes are simple and quick but vary widely between them. The following procedure utilizes papain, as the enzyme of choice, to treat red cells and is a manual technique. This method is not applicable to other enzymes such as those already mentioned. This is due to the fact that different enzymes have different and specific optimum pHs and temperatures.

PRINCIPLE

Serum samples are screened for the presence of any antibodies by using the P2 capillary technique. This involves the use of pre-papainised red cells (usually group O, R1R2, Kell positive) in a capillary tube.

PROCEDURE

Papainisation of red cells

The reagents needed are: i. 2% papain solution - 4 g papain in 200 ml buffer.

ii. Working papain - 0.3 ml of 2% papain added to 2.7 ml phosphate buffered saline pH 7.1. The phosphate buffered saline pH 7.1 is prepared by adding 2.6 g of KH₂PO₄ and 7.5 g of Na₂HPO₄•H₂O to 1000 ml of sterile saline.
a. A sufficient volume of Group O, R\textsubscript{1}, R\textsubscript{2}, Kell positive red cells is washed for 4 times in normal saline.

b. To one volume of washed packed cells, two volumes of ‘working papain’ is added. It is mixed thoroughly and incubated at 37°C for 10 minutes.

c. After incubation, they are washed in normal saline for 4 times and stored at 4°C until needed.

METHOD

1. One drop of papainised red cells is placed into each of a number of glass tubes.

2. The capillaries are loaded with serum from the samples to be screened for antibodies.

3. The ‘loaded’ end of the capillary is placed just below the surface of the well mixed red cell sample. Both sample and capillary are slanted almost horizontal and approximately 4-5 cm of red cells, are allowed to enter the capillary. The amount of red cells used is twice the original volume of antiserum.

4. The capillary is inverted and fixed in the plasticine of the light box at a 45° angle against the perspex screen.

5. This procedure is repeated for all subsequent tests and controls.

RESULTS

In a negative test, the red cells settle through the antiserum without any horizontal bars of agglutination, leaving a thin line of unagglutinated red cells the entire length of the capillary.

In the case of a positive test, horizontal bars of agglutination will appear throughout the capillary column as the red cells fall through the serum. This indicates that the red cells have the antigen to which the antiserum is directed.

3.2.3 The Low Ionic AutoAnalyzer technique

PRINCIPLE
The antibody detection by the AutoAnalyzer technique is based on the principles described by Lalezari. It is very sensitive for Rhesus antibodies. However it has a disadvantage; some positive reactions cannot be confirmed by manual tests and not all Kell antibodies are detected.

**PROCEDURE**

Serum and red cells were mixed with acidified glucose to produce a low ionic strength medium. This increases the rate of reaction and antibody uptake by the antigen. The addition of polybrene causes non-specific aggregation of the red cells. This close contact between cells enables agglutination to occur with only one or two cross-linkages between cells. After the reaction takes place for about 10 minutes, citrate is included into the system. This will give rise to non-specific agglutination. The settling stage allows sedimentation of the agglutinates after which they were removed at two decants. The remaining red cells were haemolysed by Triton (Technicon Instruments Corp., Tarrytown NY), and the percentage transmittance at 550 nm of the haemolysate, is reported on a chart. The percentage transmittance of the inert serum (negative control) is the red cell baseline to which the test serum percentage transmittance is compared. A rise in percentage transmittance is indicative of a positive reaction.

### 3.2.4 ANTIBODY IDENTIFICATION

Once the presence of an antibody is detected, it is important to identify the particular antibody. Not all antibodies are responsible for haemolytic disease of the newborn to the same degree. The following outline is the most commonly used method.

**PRINCIPLE**

The same principle as for the Indirect Antihuman globulin Test (IAT) applies here, with the exception that eight different cell suspensions are used instead of two. These are known as panel cells.

**PROCEDURE**
1. Low ionic saline solution (LISS) is used, 2 drops of which are placed in a series of nine test-tubes.

2. In the first test-tube, 2 drops of serum are added.

3. Two drops of dilution are taken from test-tube 1 and placed in test-tube 2, that is doing serial dilutions as follows: 1/2; 1/4; 1/8; 1/16; 1/32; 1/64; 1/128; 1/256 and 1/152 dilutions.

4. This is followed by the addition of 1 drop of ‘O’ cells to each test-tube.

5. The test-tubes are incubated at 37°C for one hour.

6. Samples are washed for 3 times with 0.9% saline solution. Two drops of antihuman globulin reagent are added. Centrifuge and read microscopically.

RESULT

Results include the name of the antibody. This is found on a table provided with the kit, according to the positive reactions in the test-tubes. It is important that the dilution up to which the antibody was present, is also noted in the report.

3.3 BLOOD GROUPING OF CORD BLOOD AND DIRECT ANTIHUMAN GLOBULIN TEST (DAT)

PROCEDURE

1. The cells are washed for three times with 0.9% saline solution.

2. The direct blood group is performed. ABO only is done and no reverse blood grouping is carried out since neonatal blood does not contain any antibodies.

3. The direct antihuman globulin test is done by placing 1 drop of cell suspension together with 1 drop of antihuman globulin reagent. The sample is centrifuged and read microscopically. If sensitisation of the red cells is present, haemagglutination is observed.
3.4 AMNIOTIC FLUID SPECTROPHOTOMETRY

As early as 1892, Ballantyne observed that the amniotic fluid was bile-stained in pregnancies complicated by severe haemolytic disease of the newborn. But it was in 1956 that Bevis devised a means of avoiding foetal death by showing that the concentration of bilirubin in amniotic fluid is associated with the severity of haemolytic disease of the newborn. Several years later, Liley demonstrated that amniocentesis and amniotic fluid analysis, used in an efficient manner, could decrease the perinatal mortality rate.

PRINCIPLE

Amniotic fluid is obtained through a needle inserted into the uterus through the abdominal wall after about 14 weeks of gestation. This procedure is known as amniocentesis. The aims of amniotic fluid analysis are to avoid intrauterine death by preterm delivery of the affected foetus, and to allow the unaffected or mildly influenced foetus to remain in utero, thus enabling the foetus to mature further. If the bilirubin concentration is low, there is minimal risk to the foetus and few amniocenteses are necessary. If on the other hand the concentration of bilirubin is high, intrauterine death becomes imminent, and more frequent amniocenteses are essential. In the management of the rhesus disease, the clinician must compare the risk of intrauterine death against that of prematurity.

PROCEDURE

Numerous methods of amniotic fluid bilirubin determination have been reported, including spectrophotometric and direct chemical analysis. The spectrophotometric analysis was the first method reported. It is the standard method used since it is simple, sensitive and quantitative as well as qualitative.

1. About 10 mls of amniotic fluid are aspirated. The sample should be placed in a dark lightproof container to protect it from exposure to sunlight.
2. The amniotic fluid is centrifuged immediately, for 30 minutes at 2500 revolutions per minute. This removes any expected turbidity caused by epithelial cells. To eliminate all possible turbidity, the fluid is filtered through filter paper. Spectrophotometric analysis can now be done on the amniotic fluid.

3. The spectrophotometer is an instrument used to determine the absorbance of a substance as the function of the wavelength of incident radiation. The appropriate spectral band is focused on a narrow slit located in front of the sample cuvette. By shifting the spectral band across the plane of the slit, readings at various wavelengths can be made.

4. Wavelengths range from 350 to 700 nm. A graph is plotted using semilogarithmic paper with wavelength on the horizontal linear co-ordinate and optical density on the vertical logarithmic co-ordinate.

5. The amniotic fluid curve is obtained when the plotted points are joined. A tangent is drawn between 550 nm and 365 nm. The rise in optical density at 450 nm ($\Delta OD_{450}$) is obtained by the subtraction of the OD at the intersection of the tangent with 450 nm, from the amniotic fluid OD reading at 450 nm.

6. On the same graph paper, the $\Delta OD_{450}$ (on the vertical logarithmic co-ordinate) is plotted against gestation in weeks (on the linear horizontal co-ordinate).

7. The Liley zone boundaries are drawn. At 20 weeks gestation the boundary points are 0.500 and 0.174, plotted on the left hand side. At 34 weeks the boundary points are 0.140 and 0.034, on the right hand side.

8. The zone II/zone III boundary is drawn by connecting 0.500 and 0.140; the zone I/zone II boundary by linking 0.147 and 0.034. A typical graph will be as shown below:
RESULTS

The interpretation of the result can be done from the graph itself.

a. A $\Delta \text{OD}_{450}$ reading of 0.400 or greater is associated with a 65% incidence of hydrops at the time of amniocentesis.

b. Rarely, $\Delta \text{OD}_{450}$ measurements of 0.200-0.250 at 28 weeks gestation may be associated with hydrops fetalis.

c. Conversely, but even more rarely, $\Delta \text{OD}_{450}$ measurements of 0.200-0.250 at 22 to 24 weeks gestation may be associated with a Rhesus negative unaffected foetus.

d. Disease may be so prominent that hydrops may be present at 25 weeks gestation with a $\Delta \text{OD}_{450}$ reading of 0.385 following a low zone II reading of 0.160 two weeks earlier.

e. When $\Delta \text{OD}_{450}$ readings have risen into the upper 80-85% of zone II, further delay in treatment for 7-10 days may be associated with the development of hydrops.

Another test done using amniotic fluid, is the lecithin to sphingomyelin ratio (L/S ratio) to determine the pulmonary maturity of the foetus. Amniotic fluid acetylcholinesterase and $\alpha$-fetoprotein assays are also done.
These will give an indication of any neural tube defects, such as spina bifida and anencephaly, that might be present in the foetus at an early stage, that is about 16 to 22 weeks of gestation.

3.5 ESTRIOL ASSAY

Estriol estimations from collections of 24 to 48 hours of maternal urine, have been used for some time as a means of estimating fetoplacental function. Placental function is demonstrated by the concentration of human chorionic gonadotrophin in serum, amniotic fluid and urine. A marked increase in the production of this hormone would be expected in the presence of very severe haemolytic disease of the newborn. Urinary estriol excretion is normal even when the foetus is severely affected by the disease. However, a decrease of estriol occurs in both serum and urine, shortly before the death of the foetus. After intrauterine transfusion, urinary estriol levels decrease and then rise if the transfusion has been successful.

Usually, estriol is low in severe Rh haemolytic disease of the newborn, but since the range found in unaffected pregnancies is considerable, only continuous low serial levels are of any significance. As the results obtained are based on the estriol concentration in amniotic fluid alone, the volume of the amniotic fluid is useful. Still, further work has to be done to assess if low levels are really indicative of severe haemolytic disease of the newborn.

3.6 ALBUMIN BILIRUBIN BINDING

The binding of bilirubin by albumin is important in the prevention of kernicterus. This is a serious form of jaundice in the newborn, in which brain damage is caused by deposits of bilirubin in the brain. Methods determine either the amount of free indirect bilirubin present or the residual capacity of available serum albumin to bind bilirubin. These measurements give a more accurate and clear picture of the risk of
kernicterus in the case of hyperbilirubinaemia. Usually, in cases of severe HDN, high levels of bilirubin are detected in the foetal blood. This condition is known as hyperbilirubinaemia.

The hydroxybenzeneazo benzoic acid-reserve albumin binding capacity (HBABA-RABC) method is not accurate as others, it is a rapid and easy colorimetric assay to carry out. This method is not very precise because HBABA binds to non-bilirubin as well as bilirubin albumin binding sites.

Components such as haem, benzoates, sulfisoxazole, salicylates and free fatty acids will also bind to albumin, reducing its bilirubin binding capacity. Since acidosis reduces albumin bilirubin binding capacity, it invalidates the HBABA-RABC measurement.

If critical hyperbilirubinaemia is present, albumin is administered to increase the infant’s RABC. Albumin is also added to blood just prior to exchange transfusion to counteract hyperbilirubinaemia. However, in cases of severe anaemia and risk of heart failure, albumin administration or addition of albumin in blood to be transfused, should not be undertaken.

### 3.7 LOCAL TESTS

As one can imagine, not all the tests mentioned in this chapter, are done locally. The most common antenatal tests done in relation with haemolytic disease of the newborn, are: antibody screening (the indirect antihuman globulin test), cord blood ABO grouping, the direct antihuman globulin and the Kleihauer test. The lecithin to sphingomyelin ratio is the only test done involving amniotic fluid. Estriol assays are performed on maternal urine.

The unavailability of tests could be due to ethical and financial problems. Obviously enough, without finances, equipment and necessary material cannot be made use of. While financial problems can be dealt with through the years, ethical problems are not so easy to confront. Some tests are done with the intention that if any disorder or problem arises during pregnancy, abortion is considered. Locally, abortion is illegal.
While methods and techniques are being reinvented, ethical issues are hammered into the society's mind and it will take a great deal of time for them to change.
In this chapter, the management and treatment of HDN for both the infant and the mother, will be discussed. Prenatal blood grouping and antibody screening is an essential requisite at every first prenatal visit, independent of the parity of the woman and previous findings. This is to make sure that Rhesus negative women without antibodies, who may be at risk, and the already immunized women whose foetus is at risk, will be identified. Even a Rhesus positive woman, particularly if she has been transfused, has to be screened since she might have developed critical abnormal allo-immunization.

4.1 MANAGEMENT FOR RHEUSUS NEGATIVE WOMEN

In a case of a woman without blood group antibodies, the Rhesus status of the biological father has to be determined. If he is Rhesus negative, the infant will be Rhesus negative, and the mother will not be at risk of Rhesus immunization. However, the mother should be rescreened towards the end of the pregnancy and the Rhesus condition of the infant should be determined when it is born.

If the father is Rhesus positive, the mother who is Rhesus negative, must be screened at regular intervals. Also, special care has to be taken on any tests done before and during labour. Cesarean sections and manual removal of the placenta increases the risk of foetal transplacental haemorrhage. As well as these conditions, amniocentesis presents a great risk to the foetus, and therefore increases the risk of Rhesus immunization of the non-immunized Rhesus negative woman. After birth of the baby, ABO, Rhesus status and a direct antihuman globulin test should be performed on the cord blood. After delivery the blood from the mother should be screened for the Rhesus antibody and for any foetal transplacental haemorrhage, the latter by the Kleihauer test (chapter 3, 3.1). When the Rhesus negative woman is found to be Rhesus immunized, the severity of the disease on the foetus has to be determined.

Amniocentesis is indicated when antibody titres are high. A sample of foetal blood is obtained using a fetoscope, and so the determination of the phenotype and concentrations of bilirubin and haemoglobin, can be determined. The level of bilirubin in the amniotic fluid gives an indication of the severity of HDN by plotting values for optical density due to bilirubin on a Liley’s chart such as that seen in chapter 3, figure 3.1.
The flow-chart underneath represents the tests done following the results of antibody screening.

Table 4.1: Management for Rh(D) negative women.

(Adapted from: Bowman et al., 1987)

Depending on the results of the blood group of the foetus and mother, additional tests are done. Also, the timing and method of delivery can be determined.

4.2 TIMING AND METHOD OF DELIVERY
The objective of management is to extend delivery for as long as possible, provided that the foetus survives both before and after delivery. As the placenta matures, antibodies are able to pass more easily from the mother to the foetus. Due to this condition, it is wise that pregnancies are not allowed to go past term. Assuming that the period of gestation is known accurately, delivery soon after 38 weeks of gestation has its advantages. However, there are exceptions to this rule, such as when the foetus is underweight and when there is doubt about foetal maturity.

The method of delivery has been the topic of much consideration. A long labour followed by cesarean section, might present distress to the foetus. Various obstetric issues must be taken into account as well as the patient’s age, parity and time of gestation. The condition of the foetus must also be assessed, because if it is severely affected, it is hardly likely that it will endure a long labour. Intrauterine transfusion, introduced by Liley in 1963, is an alternative in severe conditions of HDN.

4.3 TREATMENT OF THE HAEMOLYTIC DISEASE OF THE NEWBORN

There are several treatments of HDN, but prevention is better than cure. A preventive method employs the use of IgG anti-D immunoglobulin.

The first step in the prevention of HDN was the choice of Rhesus negative blood for transfusion to Rhesus negative women. This method reduced the incidence and mortality, due to HDN, to a certain extent.

4.3.1 The Use of IgG anti-D Immunoglobulin

It was in the early 1960’s, when the prophylactic effect of IgG anti-D immunoglobulin of human origin, was recognized by two independent groups. Studies in 1962-63, by Clarke, a geneticist in England, suggested that the protective effect of ABO might also be imitated by an antibody such as anti-D, if administered to Rhesus
negative mothers. At the same time, Freda and Gorman in New York, while studying antibody-mediated immune suppression, consequently applied this principle in the form of IgG anti-D given to Rhesus negative mothers to effectively suppress Rhesus isoimmunization. Trials were then made to establish the optimal time of administration. A minimum dose of 100 µg/ml anti-D is adequate for a fetomaternal haemorrhage of 5 ml foetal cells and should be administered within 72 hours of delivery to all Rhesus negative mothers who give birth to a Rhesus positive infant, provided no Rhesus(D) antibodies are detectable in the maternal serum at the time of delivery. The volume of foetal cells in the maternal circulation is determined by the Kleihauer test (chapter 3).

Additional protection is afforded by the routine use of anti-D antenatally as well as at delivery. It is estimated that it reduces the incidence of HDN by 90%, in subsequent infants of Rh negative women.

The effect of the anti-D immunoglobulin is, that it coats the Rh(D)-positive red cells which leads to their immediate removal from the circulation by the mononuclear phagocyte system. Therefore, the mechanism of antigen recognition by lymphocytes is avoided and sensitisation to the Rh(D) antigen prevented.

Since availability of anti-D IgG is one factor influencing a decision to introduce routine antenatal prophylaxis, a trial was undertaken to test the efficacy of a lower dose of anti-D IgG than that used in earlier studies. Rh(D)-negative primigravidae were randomized as controls or recipients of 2 doses of 250 iu of anti-D IgG given at 28 and 34 weeks gestation. Blood samples were tested at delivery and at 6 months postpartum for the presence of immune anti-D, and again later if results were equivocal. Nine (1.5%) out of 595 control patients had immune anti-D at follow up at 6 months and later; 4 (0.78%) of 513 treated women were immunized. So, it was concluded that, while 2 doses of 250 iu of anti-D IgG may reduce allo-immunization, they are not as effective as two doses of 500 iu in a previous trial.

The disease will not be completely eliminated through this measure since a small percentage of mothers will continue to become Rh immunised during pregnancy. The incidence of HDN due to other antigens within the Rhesus system, as with all other blood group antigens, is of course unaffected by this specific prophylactic treatment. Currently, the oral administration, during pregnancy, of human Rh (D) positive erythrocyte
membrane, termed erythrocyte membrane oral therapy (EMOT) is being evaluated in cases of anti-D iso-
immunisation. This approach to prophylaxis could be extended to protection against red cell antibodies other
than anti-D.

4.3.2 Other Treatments

Exchange transfusion is an ideal measure in the treatment of the newborn with haemolytic disease. This
adjusts anaemia and hypervolaemia while controlling existing or possible hyperbilirubinaemia. By removing
more blood than is put in, the haemoglobin of the anaemic baby is raised without the risk of producing
hypervolaemia and subsequent heart failure. Only about 25 per cent of bilirubin is removed by a two volume
exchange transfusion. The addition of albumin to the exchange transfusion blood unit may increase the
amount removed by 10 per cent. Phototherapy, which is exposure of affected infants to a suitable light
source (generally UV), is useful in the hastening of the breakdown of circulating bilirubin.

It has been observed that with the development of careful antenatal antibody screening, amniotic fluid
spectrophotometry, induced early delivery, foetal transfusions and progress in the management of the
newborn suffering from haemolytic disease, have greatly decreased the mortality rate otherwise caused by
HDN. These reports can only be achieved by the close co-operation between blood transfusion services and
obstetrical-antenatal units and neonatal systems.

Chapter 5

STATISTICS

The aim of this chapter is to give an outline of how successful and effective are local measures in keeping
Haemolytic disease of the newborn under control. This is particularly valuable in Rhesus (D) HDN since it is
the most common form. Statistical charts will give an overview of the total cord blood tested for direct antihuman globulin test, and of which were positive due to anti-D.

5.1 RACIAL DISTRIBUTION OF RH (D) NEGATIVE

The racial distribution of blood group antigens is indicative of the incidence of HDN within a given population. In the Basque areas of France and Spain, 30 per cent of the Basque community are Rh negative. It is the highest percentage in the world, with a corresponding high incidence of HDN due to D incompatibility. A mixing of Asian or African races with the Basque population produced the uniform 60:40 gene ratio of D:d as it is found today in most Caucasians. Negroes possess a very high frequency of cDe Rhesus chromosomes, which is rare in Caucasians. Whilst a significant incidence of HDN is to be expected among Caucasians on the evidence of antigen frequencies, particularly in the ABO and Rhesus systems, the reverse would seem to be true of the African race. Among the various races, there seems to be a relationship between HDN and selected antigens. (Barrie, 1984).

Before the routine use of anti-D immunoglobulin for the prevention of HDN was introduced, about 60 cases of HDN per 10,000 births were found in a white population.

It has been pointed by Clarke (1985), that deaths from Rh(D) haemolytic disease of the newborn in England and Wales, declined from 106 in 1977 to 34 in 1983. However, these numbers represent only deaths reported before 28 weeks of gestation. The overall incidence of maternal Rh(D) immunization in the Oxford region has hardly changed, amounting to 2.6 per 1000 pregnancies, which is similar to that found in other regions.

5.2 FAILURES IN THE EFFECT OF ANTI-D IMMUNOGLOBULIN

Haemolytic disease of the newborn due to anti-D is hindered by the administration of anti-D immunoglobulin antenatally. When given to a Rhesus-negative mother within 72 hours after delivery of a Rhesus-positive infant, this passive immunotherapy will prevent active immunization to the Rh(D) antigen.
Many cases have been reported (Gorman, 1971) in which the Rhesus-negative has become immunized, even if she was given anti-D immunoglobulin as appropriate. The incidence of immunization in the next Rhesus-positive pregnancy is about 13 per cent, in those Rhesus-negative mothers who delivered Rhesus-positive infants, and who were not treated with the immunoglobulin. Only 1.3 per cent of the mothers who were treated within three days of delivery, became immunized. The failure can be due to two main causes. One is a massive foetal maternal transfer of Rhesus-positive red cells, either during pregnancy or at delivery, treated with an insufficient dose of anti-D immunoglobulin. Another explanation is when there is significant transplacental haemorrhage during pregnancy in a mother who is already immunized by the time of delivery, and thus it is too late to prevent immunization by anti-D immunoglobulin. This indicates that once there is immunization, the administration of anti-D immunoglobulin has little or no effect in suppressing the immune response.

This percentage has also been observed locally. From 1429 cord blood samples sent for direct antihuman globulin test (DAT), between the first of January 1994 and the 27th of April 1997, it was found out that 31 resulted in positive cases, which is equal to 2.2 per cent. Of these 2.2 per cent, 0.63 per cent of HDN was due to anti-D.

![Pie-chart showing the total % of DAT positive from the total cord blood tested.](image-url)
5.3 SUCCESSFUL RATES OF OTHER TREATMENTS

With the introduction of intrauterine transfusion by Liley in 1963, the outlook of HDN, has changed dramatically. In early studies, the overall survival rates were 24 to 56 per cent. In later studies, the survival rates increased to 92 per cent. However, there is a risk of about 1 to 2 % per procedure, for the foetus. (Bennebroek Gravenhorst, 1994).

Another test that decreased the risk of the foetus is the determination of bilirubin in the amniotic fluid. It provides 95% accurate information about the degree of HDN in the foetus, thus increasing perinatal survival rates. The relative low incidence of the disease has made it necessary that treatment should take place when possible.

5.4 DISCUSSION
In this review, it has been shown how haemolytic disease of the newborn affects both the mother and the infant. Luckily enough, treatment exists even if there are still reported cases of failures. The objective of management is to extend delivery for as long as possible, provided that the foetus survives both before and after delivery. Additional protection is afforded by the routine use of anti-D antenatally as well as at delivery. It is estimated that it reduces the incidence of HDN by 90%, in subsequent infants of Rh negative women.

Still, anti-D immunoglobulin is not always administered on time and sometimes not in sufficient doses. In some countries it is not used routinely due to its high cost. Meanwhile, studies are still being carried out to find a cost-effective and efficient way to suppress haemolytic disease of the newborn.

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