Biodata Abstrak Penyelidikan

RED BLOOD CELL ALLOANTIBODIES DETECTION IN MALAY PREGNANT WOMEN AND HAEMOLYTIC DISEASE OF FETUS AND NEWBORN

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Introduction: Pregnancy is known to cause red blood cell (RBC) alloimmunisation which may lead to the production of harmful alloantibodies and a subsequent potential for causing haemolytic disease of fetus and newborn (HDFN). However, RBC alloantibodies are significantly different between different populations and ethnic groups.

Objectives: The aim of this study is to determine the prevalence of RBC alloantibodies among Malay pregnant women and their association with HDFN.

Patients and Methods: Clinical and serological data of 5163 Malay pregnant women who attended labour room, Hospital Universiti Sains Malaysia from January to

December 2009 were collected and analyzed prospectively. The blood samples were subjected to the standard immunohaematological procedure for red cell antibody screening and identification. The newborns of women with positive antibody screening were monitored for the evidence of HDFN.

Results: Fifty one (0.99%) pregnant women were found to have irregular RBC alloantibodies and when the specificities were further characterized, 30 (0.58%) women were found to possess clinically significant alloantibodies. Most of the clinically significant alloantibodies belonged to Rhesus (Rh) system (55.8%) where anti-E (33.3%) was the most common antibody identified, followed by anti-D (10.0%). There was significant association between RBC alloimmunisation in Malay pregnant women and RhD blood group, history of abortion, preterm labour, APH, IUD and history of blood transfusion. Fourteen (0.27%) neonates were clinically diagnosed as HDFN. Anti-D, anti-c and anti-K were identified to cause moderate to severe HDFN.

Conclusion: The prevalence of RBC alloantibody in Malay pregnant women is low (<1%), which is similar to other published studies. There were differences in the distribution of alloantibody specificity, however the antibodies toward Rh antigen are still the commonest clinically significant alloantibody identified. Considering the low prevalence of clinically significant alloantibodies and HDFN, the value of current routine antenatal RBC antibody screening practice in most Western countries becomes questionable and may not be directly applicable to Malaysian community without some modification. It is recommended that the antibody screening test should be restricted to

women who are RhD negative, or with past history of HDFN especially due to anti-c and anti-K.

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WOMEN AND HAEMOLYTIC DISEASE OF FETUS AND NEWBORN

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ABBREVIATIONS

Anti   antibody

APH   antepartum haemorrhage

BCSH   British Committee for Standards in Haematology

DCT   direct Coombs test

EDTA   ethylenediamine tetra-acetic acid

ET   exchange transfusion

FBP   full blood picture

FMH   fetomaternal haemorrhage

Fy   Duffy

Hb   haemoglobin

HDFN   haemolytic disease of fetal and newborn

HTR   haemolytic transfusion reaction

HUSM   Hospital Universiti Sains Malaysia

IAT   indirect antiglobulin test

Ig   immunoglobulin

IgG   immunoglobulin G

IgM   immunoglobulin M

IM   intramuscular

IU   international unit

IV   intravenous

IVIg   intravenous immunoglobulin

IUD	intrauterine death
Jk	Kidd
K	Kell
LSCS	lower segment caesarean section
Le	Lewis
LISS	low ionic strength saline
Lu	Lutheran
PDN	National Blood Centre of Malaysia
PC	packed cells
RBC	red blood cell
rEPO	recombinant erythropoietin
Rh	Rhesus
RhD	Rhesus D

ABSTRAK

PENGESANAN ALLOANTIBODI TERHADAP SEL DARAH MERAH DI KALANGAN WANITA MELAYU HAMIL DAN PENYAKIT HEMOLISIS FETUS DAN BAYI BARU LAHIR

Proses penghamilan merupakan salah satu penyebab alloimunisasi sel darah merah di mana ia boleh menjurus kepada pembentukan alloantibodi dan seterusnya berpotensi untuk menyebabkan penyakit hemolisis fetus dan bayi baru lahir. Walaubagaimanapun, alloantibodi terhadap sel darah merah adalah berlainan mengikut populasi dan kaum. Tujuan kajian ini adalah untuk mengenalpasti prevalens alloantibodi terhadap sel darah merah di kalangan wanita Melayu yang hamil dan hubungannya dengan penyakit hemolisis fetus dan bayi baru lahir.

Data klinikal dan serologi daripada 5163 orang wanita Melayu hamil yang datang ke dewan bersalin, Hospital Universiti Sains Malaysia dari bulan Januari hingga Disember 2009 dikumpul dan dianalisa secara prospektif. Sampel darah dianalisa mengikut prosedur tabung darah yang standard untuk ujian saringan dan penentuan antibodi. Bayi kepada wanita yang mempunyai saringan antibodi yang positif diawasi untuk penyakit hemolisis fetus dan bayi baru lahir.

Lima puluh satu (0.99%) orang wanita mengandung didapati mempunyai alloantibodi terhadap sel darah merah dan apabila spesifisiti mereka dikenalpasti dengan lebih lanjut,

30 (0.58%) orang didapati mempunyai alloantibodi yang signifikan secara klinikal. Kebanyakan daripada alloantibodi yang signifikan secara klinikal adalah dari sistem Rhesus (55.8%) di mana anti-E (33.3%) merupakan antibodi yang paling kerap dikenalpasti, diikuti oleh anti-D (10.0%). Terdapat hubungan yang signifikan di antara alloimunisasi sel darah merah wanita Melayu hamil dengan kumpulan darah RhD, sejarah keguguran, kelahiran pramatang, pendarahan antepartum, kematian di dalam rahim dan sejarah transfusi darah. Empat belas (0.27%) orang bayi didapati mempunyai penyakit hemolisis fetus dan bayi baru lahir secara klinikal. Anti-D, anti-c dan anti-K dikenalpasti menyebabkan penyakit hemolisis fetus dan bayi baru lahir yang sederhana dan teruk.

Kesimpulannya, prevalens alloantibodi terhadap sel darah merah di kalangan wanita Melayu hamil adalah rendah (<1%), seperti yang telah dilaporkan oleh kajian-kajian terdahulu. Walaupun terdapat kelainan pada taburan spesifisiti antibodi, antibodi terhadap kumpulan antigen Rhesus adalah yang paling kerap dikenalpasti. Memandangkan rendahnya prevalens alloantibodi yang signifikan secara klinikal dan penyakit hemolisis fetus dan bayi baru lahir, nilai saringan antibodi terhadap sel darah merah semasa hamil yang diamalkan di kebanyakan negara barat kemungkinannya tidak begitu sesuai dijalankan di Malaysia sekiranya tidak diubahsuai. Saringan antibodi dicadangkan hanya kepada wanita yang RhD negatif, atau mempunyai sejarah penyakit hemolisis fetus dan bayi baru lahir terutamanya disebabkan oleh anti-c dan anti-K.

ABSTRACT

RED BLOOD CELL ALLOANTIBODIES DETECTION IN MALAY PREGNANT WOMEN AND HAEMOLYTIC DISEASE OF FETUS AND NEWBORN

Pregnancy is known to cause red blood cell (RBC) alloimmunisation which may lead to the production of harmful alloantibodies and a subsequent potential for causing haemolytic disease of fetus and newborn (HDFN). However, RBC alloantibodies are significantly different between different populations and ethnic groups. The aim of this study is to determine the prevalence of RBC alloantibodies among Malay pregnant women and their association with HDFN.

Clinical and serological data of 5163 Malay pregnant women who attended labour room, Hospital Universiti Sains Malaysia from January to December 2009 were collected and analyzed prospectively. The blood samples were subjected to the standard immunohaematological procedure for red cell antibody screening and identification. The newborns of women with positive antibody screening were monitored for the evidence of HDFN.

Fifty one (0.99%) pregnant women were found to have irregular RBC alloantibodies and when the specificities were further characterized, 30 (0.58%) women were found to possess clinically significant alloantibodies. Most of the clinically significant alloantibodies belonged to Rhesus (Rh) system (55.8%) where anti-E (33.3%) was the

most common antibody identified, followed by anti-D (10.0%). There was significant association between RBC alloimmunisation in Malay pregnant women and RhD blood group, history of abortion, preterm labour, APH, IUD and history of blood transfusion. Fourteen (0.27%) neonates were clinically diagnosed as HDFN. Anti-D, anti-c and anti-K were identified to cause moderate to severe HDFN.

In conclusion, prevalence of RBC alloantibody in Malay pregnant women is low (<1%), which is similar to other published studies. There were differences in the distribution of alloantibody specificity, however the antibodies toward Rh antigen are still the commonest clinically significant alloantibody identified. Considering the low prevalence of clinically significant alloantibodies and HDFN, the value of current routine antenatal RBC antibody screening practice in most Western countries becomes questionable and may not be directly applicable to Malaysian community without some modification. It is recommended that the antibody screening test should be restricted to women who are RhD negative, or with past history of HDFN especially due to anti-c and anti-K.

Chapter 1

INTRODUCTION

1.0 INTRODUCTION

1.1 GENERAL INTRODUCTION

Red blood cell (RBC) alloimmunisation may develop during pregnancy or from previous blood transfusion. During pregnancy this maternal RBC alloimmunisation may lead to production of harmful antibodies that result in haemolytic disease of fetus and newborn (HDFN). The destruction of the fetus and newborn RBC are most commonly due to ABO incompatibility or irregular maternal RBC alloantibodies which are clinically significant.

The International Society of Blood Transfusion recognized 302 blood group antigens, most of which belong to 1 of 29 genetically discrete blood group system (Poole and Daniels, 2007). Antibodies toward many of these RBC antigens have the potential to be clinically significant in which they can either facilitate accelerated destruction of RBC carrying the corresponding antigen manifest as a haemolytic transfusion reaction (HTR) and to be transferred across the placenta and cause HDFN.

More than 50 different RBC antigens have been reported to be associated with HDFN (Moise, 2008). However these RBC antigens are significantly different between different populations and different ethnic groups. There is a recognized existence of difference in RBC alloantibodies in different ethnic groups because of racial difference of blood group distribution. Consequently, the alloantibodies contributing to HDFN are also different.

The prevalence of irregular and clinically significant RBC alloantibodies had been studied in the Western and Chinese pregnant women in Hong Kong. The incidence of irregular antibodies was reported to be about 1-2% in Western pregnant women (Weinstein, 1982). Previous study done in Hong Kong reported the prevalence of irregular and clinically significant antibodies in the Chinese pregnant women was 0.64% and 0.27% respectively (Lee *et al.*, 2003). Other study by Wong *et al*. (1997) reported the similar finding, a prevalence of irregular and clinically significant alloantibodies of 0.85% and 0.20% respectively. There is so far no information about the prevalence of maternal irregular antibodies in the Malaysian population especially in Malays and very little information about HDFN due to maternal RBC alloantibodies.

Guidelines for blood grouping and RBC antibody testing and the prevention and management of RBC alloantibodies during pregnancy are well established in Caucasian populations (BCSH, 1996a). In Western, routine prenatal and perinatal screening for irregular antibodies is mandatory. Applicability of this guideline to Malaysian population is unknown as a result of insufficient data on the prevalence of maternal irregular RBC alloantibodies and their outcomes.

We hope that this study will contribute to a better comprehension of the problem and will help to develop our own guideline for the prevention and management of RBC alloantibodies during pregnancy and subsequently can predict and treat HDFN very efficiently to reduce neonatal morbidity and mortality.

1.2   Red blood cell alloantibody

RBC alloantibodies are the antibodies that develops in a people who have been exposed to foreign RBC alloantigen either from blood transfusion or fetomaternal haemorrhage (FMH) during pregnancy and delivery. Antibody detection and identification are very important in transfusion practice and provide information which aids in the selection of suitable blood for transfusion (Poole and Daniels, 2007) and in prediction of occurrence and severity of HDFN.

Irregular RBC alloantibodies are the antibodies that against RBC antigens other than ABO group which usually develop in response to exposure to foreign RBC antigens, but may occur naturally from exposure to bacteria or viruses. The development of these antibodies are more common in reproductive age women than men because sensitisation is often due to pregnancy related events, such as fetomaternal transfusion and blood transfusion for postpartum haemorrhage (Barss and Moise, 2008). Irregular alloantibodies can be either clinically significant or insignificant. With few exceptions, irregular antibodies which are potentially clinically significant are only those which are reactive in the indirect antiglobulin test (IAT) and performed strictly at 37°C (Chapman *et al.*, 1996).

A clinically significant alloantibody is defined as an antibody that is capable to accelerate destruction of RBC bearing the relevant antigen. Anti-A, anti-B and anti-A,B should always be regarded as the clinically significant antibody as they are known to cause severe HTR as well as HDFN (Chapman *et al.*, 1996). The examples of clinically significant

alloantibodies are shown in Table 1.1. The clinically significant alloantibodies frequently are associated with HDFN, HTR or with a notable decrease in the survival of transfused red cells. The degree of clinical significance varies among antibodies with the same specificities, in which some cause destruction of incompatible RBC within hours or even minutes; whereas others cause a decrease in the RBC survival, and still others cause no discernible shortened RBC survival (Roback *et al.*, 2008, Chapman *et al.*, 1996). In view of that, distinguishing between potentially clinically significant and non-clinically significant antibodies is important in alloimmunised women to predict the severity of HDFN and in patients with alloantibodies who require blood transfusion when compatible donors are not available.

The clinical significance of blood group system depends mainly on the frequency with which alloantibodies of the system occur and the characteristic of the alloantibodies, namely their destructive capacity, antibody strength, immunoglobulin (Ig) class, IgG subclass, mode of reactivity, thermal range and ability to fix complement. These characteristics determine the clinical significance of the antibody either to cause RBC destruction which manifest as a HTR and to be transferred across the placenta and cause HDFN (Klein and Anstee, 2006).

Other factors that can also influence the pathogenicity of an antibody are the quantity and distribution of target antigen on RBC membrane, the quantity of IgG and/or complement bound to the RBC and the presence of target antigen in tissues and/or body fluids, and the most important of these is thermal amplitude since if the antibody does not react at 37°C, it

should cause no significant in vivo RBC destruction and no immediate clinical effects due

to an immune reaction (Poole and Daniels, 2007, Hoffbrand *et al.*, 2005).

Table 1.1: Type of clinically significant blood group antibodies

System	No. of antigens	HTR	HDFN
ABO	4	Anti-A, -B, -A,B cause severe and often fatal HTR.	Rare and usually mild
Rhesus (Rh)	49	Cause severe I and D HTR.	Cause severe HDFN
MNS	46	Rare examples of anti-M and -N active at 37°C cause I and D HTR. Anti-S, -s, -U, and some other antibodies cause I and D HTR.	Anti-S, s, -U, and some other antibodies cause severe HDFN. Anti-M, rarely.
Kell (K)	28	Cause severe I and D HTR	Cause severe HDFN
Duffy (Fy)	6	Anti-Fy^a, -Fy^b, and -Fy3 cause I and D HTR; anti-Fy5, D HTR.	Anti-Fy^a and -Fy^b cause HDFN.
Kidd (Jk)	3	Common cause of D HTRs. Anti-Jk^a and -Jk3 also cause I HTR.	Not usually
Lutheran (Lu)	19	Anti-Lu^a and -Lu^b have caused mild D HTR.	No
Lewis (Le)	6	Not generally considered clinically significant	No
P1	1	Only very rare examples active at 37°C cause I and D HTR.	No
Diego	21	Anti-Di^a and -Di^b, no evidence. Anti-Wr^a has caused HTR.	Anti-Di^a, -Di^b, -Wr^a, and -Wr^b, plus some others have caused severe HDFN
Yt	2	Anti-Yt^a very rarely caused HTR.	No
Xg	2	No	No
Scianna	7	No	No
Dombrock	5	Anti-Do^a and -Do^b cause I and D HTRs.	No
Colton	3	Anti-Co^a causes I and D HTR. Anti-Co^b and -Co3 have caused mild HTRs.	Anti-Co^a has caused severe and anti-Co3 mild HDFN
LW	3	No	No
Ch/Rg	9	No	No
H	1	Anti-H in Bombay phenotype can cause severe intavascular HTR. Anti-HI in para-Bombay not usually clinically significant.	Anti-H in Bombay phenotype has potential to cause severe HDFN

"Table 1.1 Continued"

System	No. of antigens	HTR	HDFN
Kx	1	Anti-Kx + -Km in McLeod syndrome has caused severe HTR.	Antibodies only found in males
Gerbich	8	No	One example of anti-Ge3 causing HDFN
Cromer	15	No	No
Knops +	9 + 2	No	No
COST	1	No	No
Indian	4	One example of anti-In^b causing an HTR	No
Ok	1	Very rare and no HTR reported	No
JMH	5	One example of anti-JMH reported to have caused IHTR	No
I	1	Anti-I in adult i phenotype has caused increased destruction of I+ red cells.	No
GLOB + PP1Pk and LKE	1 + 2	Anti-P and -PP1P^k cause intravascular HTR. LKE not clinically significant.	No, but high rate of spontaneous abortion with anti-P and -PP1P^k
GIL	1	No	No
Er	2	No	No
Vel	2	Anti-Vel causes severe intravascular HTR. Little is known about anti-ABTI.	Generally no, but 1 case reported
LFAs (700 series)	19	Little evidence as compatible red cells readily available	Anti-JFV, -Kg, -JONES, -HJK and -REIT have caused HDFN
HFAs (901 series)	9	Anti-Lan: 1 example caused IHTR. Anti-At^a: reported to have caused HTR Anti-Jr^a: 1 example caused I HTR. Anti-AnWj: severe HTR Anti-PEL, -MAM: very rare, but could be clinically significant Anti-Emm, -Sd^a: no	Anti-At^a: 1 report of mild HDFN Anti-MAM: caused severe HDFN Anti-Lan, -Jr^a, -Emm, -AnWj, anti-PEL, -Sd^a: no

(Adapted from Poole and Daniels, 2007)

I = immediate   D=delayed

Chapter 2

LITERATURE REVIEW

2.0   LITERATURE REVIEW

2.1   Blood grouping and antibody testing in pregnancy

2.1.1   Introduction

Following British Committee for Standards in Haematology (BCSH) guideline for blood grouping and antibody testing during pregnancy, it was mandatory for every pregnant woman in Western countries to do routine prenatal screening for irregular RBC alloantibodies (BCSH, 1996a). ABO and RhD grouping as well as antibody screening on the sera of pregnant women were routinely performed for the protection of the mother and identification of HDFN (Mitchell *et al.*, 1996).

Although antenatal antibody screening was mandatory in Western population, Wong *et al.* (1997) reported that this practice might not be necessary in the Chinese population except for those who were RhD negative or who had history of HDFN. Wu *et al.* (2003) also reported similar finding that routine prenatal screening for irregular antibodies was not rational in the Chinese population in Taiwan because of very low prevalence of HDFN caused by maternal irregular RBC alloantibodies.

2.1.2 Objectives of prenatal testing

The objectives of prenatal and perinatal testing are essentially threefold: first is to identify RhD negative women who need anti-D Ig prophylaxis; second to identify women with potentially significant alloantibodies to RBC antigens who are at risk of HDFN; and third to assist in the diagnosis and management of HDFN, both during pregnancy and at delivery (BCSH, 1996a, Judd, 2001).

An additional benefit of the prenatal antibody screening program is the detection of RBC antibodies, relevant in case of transfusion to the mother and it can save time, needed for the identification of antibody specificities (Koelewijn *et al.*, 2008). Without antibody screening, two-thirds of the RBC alloantibodies will not be known at pre-transfusion laboratory testing (BCSH, 1996a).

2.1.3 Testing protocol

Prenatal immunohaematologic care of pregnant women including ABO, RhD groups and antibody screen should be performed in all pregnant women at booking (usually around 16 weeks' gestation). All women should have the testing repeated once more at about 28 weeks to confirm the RhD group and to detect the presence of irregular antibodies (BCSH, 1996a).

When RBC antibody screening is positive, it is necessary to determine specificity of the antibody, its clinical importance, and the ability to cross the placenta and cause HDFN. Neither the specificity nor the level of maternal RBC antibodies can precisely predict the outcome of infant (BCSH, 1996a).

If clinically significant antibodies are detected, more frequent testing will be required. If the mother is RhD negative with no anti-D by 28 weeks, routine antenatal prophylaxis should be given at 28 and 34 weeks of gestation. Following delivery, all RhD negative women who are unsensitised for RhD should be given prophylactic anti-D Ig if the infant is RhD positive (Hoffbrand *et al.*, 2005).

For the women with anti-D, they should be monitored for the anti-D level, however titration of anti-D does not closely correlate with the occurrences of HDFN (Judd, 2001). Although titration of anti-D does not closely correlate with the occurrences of HDFN, new reference reports that there is a correlation between the titre and the severity of the HDFN (Hoffbrand *et al.*, 2005). The anti-D quantification (IU/ml) using the national anti-D standard is more reproducible and correlates more closely with the likelihood of HDFN (BCSH, 1996a). Blood group and antibody testing in pregnancy that had been suggested by BCSH are shown in Figure 2.1.

First trimester antibody screening enables timely treatment of HDFN caused by antibodies other than anti-D, however, with a sensitivity of only 75%. A second screening at 30th week of gestation, will enhance the screening program (Koelewijn *et al.*, 2008). However, Judd (2001) reported that in most cases, RhD positive patients need be screened for

antibodies only once during pregnancy, at the initial visit and routine repeated testing for unexpected antibodies in the third trimester or at delivery will rarely yield useful information. Rothenberg *et al.* (1999) also reported that a repeat third-trimester antibody screen for RhD positive patients was clinically and economically unjustified and eliminating this laboratory test from clinical practice would not adversely affect pregnancy outcomes and would decrease the costs of prenatal care.

All pregnant women:

Booking visit 10–16 weeks: send sample to blood bank.

Testing will be performed for ABO, RhD blood groups + antibody screen.

• Clinical information regarding the week of pregnancy/EDD at time of booking sample must be included on request form

• Clinical information regarding previous pregnancies/transfusions must be included on request if known.

• Use of consultant code assists in identifying a sample as antenatal

• Objectives: identify pregnancies at risk of fetal/neonatal HDFN.

      : identify RhD-negative women who need anti-D prophylaxis

      : provide compatible blood swiftly in obstetric emergencies

Anti-D, anti-c, K-related antibodies → Monitor level monthly

Other antibodies

No antibodies

All pregnant women 28 weeks:

• Recheck RhD group and antibody testing

RhD negative women at booking visit must have blood sample taken before giving injection of 28 weeks anti-D.prophylaxis (> 500 iu) (passive anti-D can be detected by IAT within minutes of the injection being given)

Anti-D, anti-c, K-related antibodies

Other IAT reacting antibodies

No other IAT reacting antibodies

Monitor level every 2 weeks

If significantly high refer to fetal medicine unit

Monitor level and Inform obstetrician

If RhD negative: 34-week dose (> 500 iu) anti-D prophylaxis

At delivery perform direct Coombs test (DCT) Monitor baby for HDFN

At delivery: test cord blood group if mother RhD negative

If baby is RhD positive, give mother an additional injection > 500 i.u. anti-D Ig within 72 h
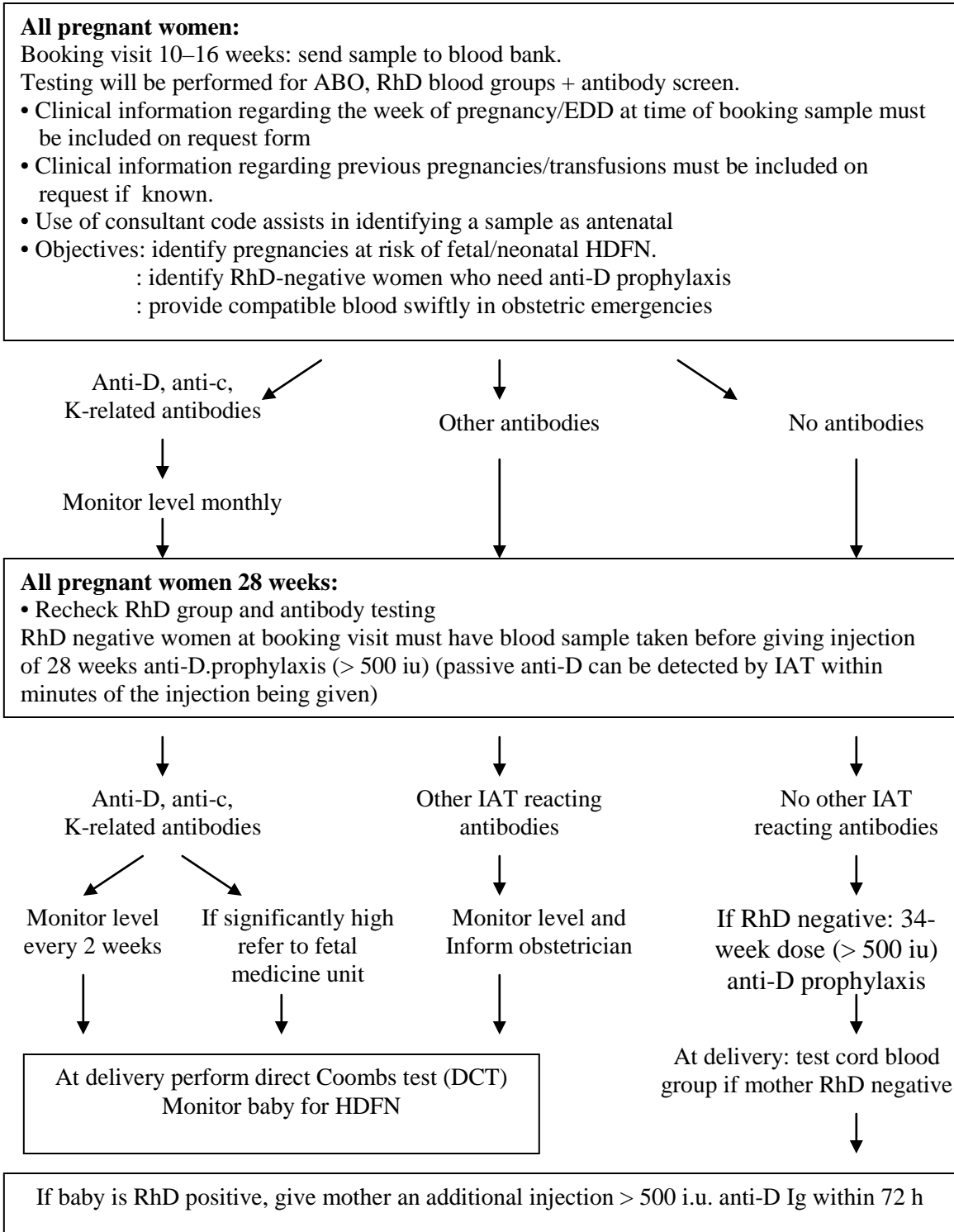
Figure 2.1: Algorithm for blood group and antibody testing in pregnancy (adapted from Hoffbrand et al., 2005)

2.2   RBC alloimmunisation in pregnancy

2.2.1   Introduction

Alloimmunisation is defined as the formation of antibodies when there is an exposition of the individual to non-self antigens, as it occurs, for example, in the transfusion of incompatible blood and pregnancies, in whom the fetus express in its sanguineous cells antigens exclusively of paternal origin (Baiochi and Nardozza, 2009). RBC alloimmunisation can cause variety of problems during long-term medical and transfusion management, with the main problem being the identification of appropriate antigen negative RBC for transfusion to avoid HTR.

Despite the widespread use of both antenatal and postpartum anti-D Ig, RBC alloimmunisation in pregnancy continues to occur. It was mainly due to inadvertent omissions in administration as well as antenatal sensitisation prior to anti-D Ig given at 28 weeks of gestation and additional factors which included the lack of immune globulins to other RBC antigens (Moise, 2005). The advent of the routine administration of antenatal and postpartum RhD Ig had resulted in a shift of cases of RBC alloimmunisation to other antibodies (Moise, 2000).

The most frequent causes of RBC immunisation were transfusion and pregnancy since neither the transfused RBC nor the fetal RBC perfectly matched those of the recipient (Koelewijn *et al.*, 2009b). Most of the alloantibodies were produced in response to

immunisation by antigen positive RBC, either cells of fetal origin following FMH mostly during previous pregnancy from a blood group antigen mismatched fetus or at parturition or donor RBC following transfusion (Poole and Daniels, 2007).

In pregnancies in which the mother was alloimmunised, the role of transfusion service was to determine antibody specificity. When potentially clinically significant antibodies were present, antibody levels should be monitor and the data obtained were used to determine if and when to monitor for HDFN (Judd, 2001). It is important to identify all RBC alloantibodies whether there is a risk of HDFN or not and to facilitate cross matching of maternal blood if an emergency transfusion is required at delivery.

Whenever the maternal serum had been identified to contain an immune, IAT reactive RBC alloantibody, a direct Coombs test (DCT) should be done on the fetal sample. A positive DCT in itself is not diagnostic of HDFN. However if it is positive, the infant's haemoglobin (Hb) and bilirubin levels should be checked to diagnose/exclude HDFN. Where the DCT is positive and the infant shows symptoms of HDFN, an RBC eluate may be helpful to confirm the RBC antibody specificity. In cases of suspected HDFN wherever possible the RBC from the cord should be tested for the corresponding antigens (BCSH, 1996a).

2.2.2   RhD immunisation in pregnancy

In the case of RhD immunisation, the common sequence is a prior pregnancy with an RhD positive fetus, which had induced FMH related immunisation and a subsequent pregnancy with another RhD positive fetus will triggers manifest disease. The combined strategy of giving anti-D Ig in high-risk conditions during pregnancy and delivery with routine postnatal administration as part of national prevention programs had substantially decreased RhD immunisation in all developed countries (Koelewijn *et al.*, 2009a). RhD immunisation also decreased strongly because of the policy of matching RBC transfusion for RhD antigen (Koelewijn *et al.*, 2009a).

RhD immunisation can occur during a first pregnancy with no history of preceding abortion or transfusion may result when RhD incompatible fetal to maternal bleeding ensues early enough in the gestation to initiate a maternal immune response before parturition. The antepartum usage of anti-D Ig had potential prophylactic value in this situation (Scott *et al.*, 1977).

The factors influence the immune response to RhD positive cells mothers includes: 1. Concomitant ABO incompatibility offers the mother protection against immunisation presumably because leaked foetal RBC are promptly coated by circulating IgM and probably also by complement, and then removed from the circulation by the mononuclear phagocyte system, therefore is less likely to stimulate antibody production; 2. The likelihood of anti-D appearing in the maternal circulation depends on the size of

transplacental FMH where although the average FMH occurring at delivery is less than 1 ml of whole blood, approximately less than 1% of RhD negative women bearing RhD positive fetus have detectable circulating anti-D in their sera; 3. The likelihood of anti-D appearing in the maternal circulation also depends on the Rh phenotype of the fetal blood where infants with R2r (cDE/cde) phenotype are more effective in sensitising their mothers to RhD than are infants of other phenotypes, since the R2 (cDE) phenotype expresses most D antigen; and 4. The pregnant mother's immune responsiveness influences the immune response to RhD positive cells. Some women produce potent anti-D in a first pregnancy sufficient to cause severe HDFN but usually no first child of an RhD negative woman will be affected, unless the mother has been sensitised as a result of a prior miscarriage or abortion or, rarely, by a sensitising event earlier in the pregnancy (Kumar *et al.*, 2009).

Before the introduction of immunoprophylaxis, anti-D was found in approximately 1 in 170 pregnant white women, both in England and in North America. With early detection and following the introduction of immunoprophylaxis with anti-D in the late 1960, the frequency is found has fallen progressively (Klein and Anstee, 2006).

A relatively young age at first delivery, non-spontaneous delivery such as lower segment caesarean section (LSCS) or assisted vaginal delivery, post maturity of the previous pregnancy emerged and pregnancy-related RBC transfusion as independent risk factors that significantly contributed to the development of RhD antibodies (Koelewijn *et al.*, 2009a). Koelewijn *et al*. (2009a) also reported what the risk factors for RhD immunisation despite antenatal and postnatal anti-D prophylaxis and they found in at least half of the failures of anti-D Ig prophylaxis, was due to a condition related to increased FMH and/or

insufficient anti-D Ig levels given. Hence, RhD immunisation might be further reduced by strict compliance to guidelines concerning determination of FMH and accordingly adjusted RhD Ig prophylaxis, or by routine administration of extra anti-D Ig after a non-spontaneous delivery and/or a complicated or prolonged third stage of labour.

Jovanovic-Srzentic *et al.* (2003) reported that according to published data, the incidence of clinically significant antibodies during pregnancy in Croatia was approximately 1% in which 64.8% were anti-D, 0.58% in Tyrol in which 54% were anti-D, 0.82% in Salzburg in which 48% were anti-D, and 0.24 percent in Sweden in which 32% were anti-D.

Nordvall *et al.* (2009) also found that anti-D was the single most frequent and harmful antibody (46.6%) and the combinations with other antibody specificities were more harmful than single specificities. All three types of therapeutic intervention (intrauterine transfusion and simple transfusion) were significantly more frequent in women with anti-D plus an additional antibody than in women with anti-D as the sole antibody. The clinical importance of the antibody was closely paralleled to the anti-D level (Nordvall et al., 2009).

2.2.3 Non-RhD immunisation in pregnancy

Non-RhD immunisation as a term, referring to the presence of all maternal RBC antibodies other than RhD antibodies and ABO antibodies that can theoretically cause HDFN. This includes all non-RhD RBC antibodies that can cross the placenta and are directed against

blood group antigens known to be expressed by the fetal RBC (Koelewijn *et al.*, 2009b). Although the anti-D was, and still is the most common cause of severe HDFN, as RhD immunisation decreases due to RhD prevention program, other non-RhD alloantibodies have become more important as a cause. The antigens of the Rh system other than D which most common cause immunisation during pregnancy are C, c, E, and e (Tarsa and Kelly, 2008). Previous study of RBC alloimmunisation among Saudi pregnant women also showed that importance of antigens other than RhD have been increased since the introduction of RhD prophylactic treatment where alloimmunisation to E, c and Kell (K) antigens can reach significant proportions of studied populations and can result in deleterious effects on fetus (Al-Ibrahim and Al-Saeed, 2008).

In Netherlands, the prevalence of pregnancies with clinically relevant alloantibodies, other than anti-D, was 328 in 100,000. Anti-E was the most frequent alloantibody, followed by anti-K and anti-c. Combination of anti-c,-E were found in 14% of the index pregnancies, was the most frequent identified among alloantibodies of more than one specificity (Koelewijn *et al.*, 2008).

In Yugoslavia, the incidence of potentially clinically significant alloantibodies was 2.4%. Majority of those antibodies were belonged to the Rh system, followed by anti-M, -Fy[a], -S, -Jk[a], and -Jk[b] and among antibodies of no clinical significance, the most frequent were anti-H, -Le[a], and -P1 (Jovanovic-Srzentic *et al.*, 2003). Lewis (Le) antibodies are found more commonly in women in the reproductive period, a fact possibly related to the weakening of Le antigen on RBC in pregnancy (Klein and Anstee, 2006).

Alloimmunisation to K as a result pregnancy are relatively common, found in about 1 in 1000 pregnant women, but assuming that K is 10 times less immunogenic than D, it can be shown that incidence of HDFN due to anti-K in second pregnancies would be expected to be only about 1 in 3500 (Klein and Anstee, 2006).

Anti-M Antibodies specificity were detected in 10% of Swedish pregnant women with a positive antibody screen (Wikman *et al.*, 2007). Lutheran (Lu) system antibodies are very rare and can occur in the absence of RBC stimulation. However, they are poorly developed at birth and have not been reported as a cause of HDFN (Al-Ibrahim and Al-Saeed, 2008).

2.2.4   Risk factors

Risk factors underlying RBC antigen immunisation in pregnancy were divided into general and pregnancy related risk factors. The age, history of RBC transfusion, history of platelet transfusion, parity, gravidity, ethnicity and variables related to increased risk of blood transfusion were listed as general risk factors whereas pregnancy related risk factors were all factors possibly related to increased FMH, such as miscarriage, termination of pregnancy, blood loss, trauma, invasive diagnostics in-utero, external version, twin pregnancy, postmaturity, LSCS, instrumental delivery and surgical removal of the placenta (Koelewijn *et al.*, 2009b).

Koelewijn *et al.* (2009b) reported that RBC transfusion was by far the most important independent risk factor for non-RhD immunisation in pregnancy, followed by parity, major surgery and haematological disease. Pregnancy-related risk factors are a prior male child

and LSCS. This approach will be equally sensitive in detecting severe HDFN compared with the present RBC antibody screening program without preselection.

Sebring and Polensky (1990) also found that the potentially sensitising events in pregnancy can result from pathological conditions such as antepartum haemorrhage (APH), ectopic pregnancy, abdominal trauma, intrauterine death (IUD), miscarriage and termination of pregnancy and from invasive obstetrical procedures such as amniocentesis, cordocentesis, chorionic villus sampling, other in-utero therapeutic intervention/surgery (e.g. intrauterine transfusion, shunting), manual removal of the placenta and external cephalic version.

Alloimmunisation depends on genetic and acquired patient-related factors, dose and route of administration, and the antigen immunogenicity, but exact kinetics are still unknown. The different blood group antigens will differ in their potency to induce an antibody response (immunogenicity), listed in order of decreasing immunogenicity: D > K > c > E > Fy > Jk (Schonewille *et al.*, 2006).

Pregnancies constitute a smaller stimulus compared to transfusion. There are two reasons, first, the number of foreign RBC antigen is limited to those possessed by the father of the fetus and second, in many pregnancies the amount of RBC transferred from fetus to mother is too small to stimulate a primary sensitisation (Klein and Anstee, 2006). The average volume of fetal blood in the maternal circulation following delivery is less than 1 ml in majority of women. Intrapartum FMH of more than 30 ml may occur in up to 1% of pregnancies (Sebring and Polesky, 1990, Hoffbrand *et al.*, 2005).

Asymptomatic trans-placental passage of fetal RBC occurs in 75% of pregnant women at some time during pregnancy or during labour and delivery. The incidence of fetomaternal transfusion increases with advancing gestation: from 3% in the first trimester, 12% in the second trimester, 45% in the third trimester, to 64% at the time of delivery (Bowman *et al.*, 1986). Historically, the fetal effect of maternal RBC alloimmunisation was undetectable until after the birth of an affected infant.

2.3 Red cell serology techniques

2.3.1 Introduction

The ability to detect and identify blood group antigens and antibodies has contributed enormously to current safe supportive blood transfusion practice and to the appropriate management of pregnancies at risk for HDFN (Reid *et al.*, 2000). In immunohaematology laboratory, RBC antigen antibody are usually detected by agglutination tests, either in saline or macromolecular media, unmodified or with or without enzyme treated RBC and with or without use of low ionic media.

2.3.2 Indirect antiglobulin test

Indirect antiglobulin test is used to detect RBC antibodies in the serum (Daniels and Bromilow, 2007). The IAT using RBC suspended in low ionic strength saline (LISS) is considered to be the most suitable for detection of clinically significant antibodies. This is

because of its speed, sensitivity and specificity. Liquid-phase tube, microplate and microcolumn gel agglutination antiglobulin methods have all been shown reliable for antibody detection (BCSH, 1996b). Since IAT methods can detect almost all clinically significant antibodies, it is acceptable to use an IAT for pre-transfusion antibody screening without any additional screening technique.

Additional techniques, such as enzyme and low temperature technique may be used, particularly when antibody weakly reactive by antiglobulin, or a mixture of antibodies is present. However, there is no need for enzyme tests in prenatal testing because enzyme tests are not reliable in the prediction of HDFN (Hundric-Haspl $et~al.$, 1999).

BCSH guideline suggested that RBC for antibody detection need to possess the following antigens: C, D, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P1, Le^a and Le^b and for maximum sensitivity in antibody detection, it is generally agreed that homozygosity for C, D, E, c, e, Fy^a, and Jk^a is preferable. There is no need for low-frequency antigens to be present for antibody detection (BCSH, 1996b, Garratty, 2002).

2.3.3 Microcolumn gel agglutination system

The microcolumn gel technique was used in this study for ABO and RhD grouping, RBC antibody screening and identification and DCT. IAT performed in microcolumn gel agglutination systems is superior to the conventional tube LISS-IAT in the detection of alloantibodies of potential clinical significance (Weisbach $et~al.$, 2006, Weisbach $et~al.$,

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1999). When comparing of the performance of three microcolumn gel agglutination systems in the detection of RBC alloantibodies (DiaMed-ID, Ortho BioVue, and Sanofi-Pasteur Scangel), the sensitivity of all microtube column systems in the detection of clinically significant RBC alloantibodies was similar (Weisbach *et al.*, 1999). Cid *et al.* (2006) also did a study to compare of three microcolumn gel agglutination systems, DG Gel, DiaMed-ID and Ortho BioVue for antibody screening and found that all three column agglutination systems work well showing a high estimated diagnostic accuracy.

The microcolumn gel agglutination system was presented in at Congress of the International Society of Blood Transfusion (ISBT) in 1998. This system used the principle of controlled centrifugation of the RBC through a dextran-acrylamide in specially design microtube contains predispense gel, diluents, buffer and reagents if applicable (Lapierre *et al.*, 1990). Measured serum or plasma was dispensed into reaction chamber of microtube. The gel particles are porous and perform molecular sieving based on the size of the RBC agglutinates present in the microtube during centrifugation. Any agglutinated RBC remain fixed on the surface or suspended in the gel. Unagglutinated RBC travels through the gel particles and form the pellet at the bottom after the centrifugation. The centrifugation of the tubes must be strictly controlled because the false positive reaction increase if centrifugation is too low or short while false negative reaction increase if centrifugation is too fast or long. The principle of gel column agglutination system is shown in Figure 2.2.