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ACP Broadsheet No 150 March 1997

Antenatal serological testing and prevention of haemolytic disease of the newborn

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Introduction

Routine antenatal serological screening has been practised throughout the United Kingdom and worldwide for about 30 years.[1] Originally introduced to detect pregnancies at risk of haemolytic disease of the newborn (HDN) due to Rh anti-D antibodies, its continued use has led to an increasing realisation of the clinical importance of other red cell antibodies in the pathogenesis of HDN.

Three factors are essential in the pathogenesis of HDN: maternal red cell antibodies must cross the placenta, the fetal red cells must possess the appropriate red cell antigen against which the antibodies are directed, and the antibodies must be capable of causing immune destruction. The clinically relevant antibodies are, therefore, virtually always IgG and reactive at 37°C. About 2–5% of women will have such antibodies as a result of transfusion, pregnancy, or both; it is, therefore, important for the pregnant patient, the fetus, and their clinical advisors that appropriate antenatal screening tests are performed.

Aim of testing

There are three main reasons for routine antenatal serological testing:

(1) to identify pregnancies at risk of HDN;
(2) to identify RhD negative women for whom anti-D immunoglobulin prophylaxis will be required; and
(3) to ascertain maternal ABO group.

There is, however, no universal agreement on the type of testing nor its optimal timing during pregnancy.[2] There are currently recommendations that all women should be tested as early in pregnancy as possible, usually at 8 to 12 weeks' gestation.[3] This initial testing must include ABO and RhD typing as well as a screening test to detect any irregular red cell antibodies. Further testing for irregular antibodies should be undertaken in all patients (irrespective of RhD type) at a later stage in pregnancy. A suggested time for this to be undertaken is between 28 and 36 weeks' gestation.[3] Women who are found to have red cell antibodies at initial testing may require more frequent testing throughout their pregnancy.

ABO typing

Initial tests to establish maternal ABO type are considered important so that the mother's ABO group is recorded should she require transfusion support during her pregnancy. It is also good practice to ascertain maternal ABO type early, so that anomalous results can be investigated.

There is no value in identifying group O mothers with high titres of anti-A or anti-B. Antenatal testing of these antibodies has been shown to have no value in predicting the incidence of ABO HDN. The lytic potential of maternal IgG anti-A and anti-B is very variable, and frequently the IgG subclass which predominates in these group O patients is IgG_2, a subclass which does not induce red cell destruction. A further reason for being unable to predict the incidence of ABO HDN is the very variable density of A and B antigen on fetal red cells.[4]

There is an increased incidence and an increased severity of ABO HDN in certain populations: Arabs, Negroes, South-East Asians, and Latin-Americans, but again there is little predictive value from serological monitoring. However, it is essential that early postnatal diagnosis and management should be undertaken in children born to women from these particular ethnic groups.

There are occasional patients with a history of severe and recurrent ABO HDN associated with severe neonatal anaemia requiring exchange transfusions. In this rare group of patients antenatal monitoring of anti-A and anti-B using an antibody dependent cell mediated cytotoxicity assay may be of use in predicting ABO HDN.[4]

Once a pregnant woman's ABO group has been established by concordant results from two samples obtained on different occasions there is no further need for ABO testing.

RhD typing

Establishing a mother's RhD type is an essential part of early pregnancy testing. Fifteen per cent of women will be RhD negative and to try and prevent the development of anti-D antibodies during pregnancy and delivery they will require anti-D

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Accepted for publication 6 November 1996

Table 1 Antenatal episodes which may cause RhD sensitisation

Abortion
Spontaneous > 12 weeks' gestation
Therapeutic, any gestation
Chorionic villous sampling
Amniocentesis
External cephalic version
Antepartum haemorrhage
Abdominal trauma

immunoglobulin prophylaxis. Despite the fact that anti-D immunoglobulin has been used routinely for more than 25 years, anti-D antibodies are still the most common cause of HDN, and their incidence is higher in the United Kingdom than in other European countries.[5] This continuing rate of anti-D sensitisation is thought to result from a variety of causes including: failure to administer adequate doses of anti-D immunoglobulin when potentially sensitising antenatal episodes occur; inadequate monitoring of routine postnatal anti-D immunoglobulin dose in the presence of large feto-maternal haemorrhages; and primary sensitisation occurring in late pregnancy resulting from small undetected feto-maternal haemorrhages.[6]

Once a mother's RhD type has been established, assuming there are no irregular red cell antibodies present, both RhD negative and RhD positive mothers should be tested again later in pregnancy, in case they have developed red cell antibodies capable of causing HDN. The exact timing of this testing is debated but between 28 and 36 weeks' gestation is usually recommended.

Each RhD negative mother needs to be notified of her RhD negative status, as does her attending clinician. This is to ensure that all concerned in her antenatal management are aware of her RhD negative status and the need for appropriate administration of anti-D immunoglobulin to cover any sensitising episodes. Local transfusion centres and pharmaceutical companies responsible for anti-D immunoglobulin production produce blood group cards and patient information leaflets explaining the significance of being RhD negative, and the occasions when anti-D immunoglobulin needs to be administered.

Indications for antenatal anti-D immunoglobulin administration

THERAPEUTIC

Women who are RhD negative with no anti-D detectable in their serum should receive anti-D immunoglobulin whenever a sensitising episode occurs (table 1). Anti-D immunoglobulin (250 IU) should be administered before 20 weeks' gestation, and 500 IU at later stages of pregnancy. A Kleihauer test should be performed within 72 hours of administration. Additional anti-D immunoglobulin can subsequently be given as required to clear any fetal cells detected. Administration of anti-D immunoglobulin should be repeated every six weeks if further sensitising episodes occur, or if an episode such as a threatened abortion has been on-going during that time.[7]

PROPHYLACTIC ROUTINE ANTENATAL PROPHYLAXIS

Routine antenatal prophylaxis is practised in Canada and the United States and at some centres in the United Kingdom. The purpose of this is to reduce the incidence of third trimester sensitisation to RhD by preventing sensitisation from feto-maternal haemorrhage that occurs with increased frequency during this trimester.[6] In the United Kingdom when routine antenatal prophylaxis is undertaken, 500 IU of anti-D immunoglobulin is administered at 28 weeks', and again at 32 weeks' gestation. In North America a single dose of 1500 IU is administered at 28 weeks' gestation.

It is important to indicate clearly when anti-D immunoglobulin has been administered, whether prophylactically or to cover a known sensitising episode, as this passive anti-D will be detected in any subsequent routine antenatal serological tests. There is currently no way of differentiating between passively acquired and actively produced anti-D and, therefore, great care must be taken in interpreting the presence of anti-D in late pregnancy.

It is essential that all RhD negative women who deliver an RhD positive child receive the standard United Kingdom dose of 500 IU of anti-D immunoglobulin postdelivery. The only exceptions are women who are known to have had anti-D antibodies throughout their pregnancy, due to previous sensitisation. The presence of non-D antibodies and prior administration of antenatal anti-D immunoglobulin are not contraindications to postnatal anti-D immunoglobulin administration. The standard postdelivery dose of anti-D immunoglobulin varies throughout the world. The dose used in the United Kingdom was recommended by a working party of the Medical Research Council following a trial of different doses of anti-D immunoglobulin.[8] It was realised that about 0.7% of women would have a feto-maternal haemorrhage of > 4 ml, and for these women a dose of 500 IU anti-D immunoglobulin would be insufficient. These women should be easily identified by the routine use of a Kleihauer test and additional anti-D immunoglobulin can be administered. In North America a standard dose of anti-D immunoglobulin of 1500 IU is administered and Kleihauer testing is only performed for certain "high risk" deliveries (multiple gestation, placental abruptio, placenta praevia, and manual removal of the placenta).[9] Within the European Community the current recommendation is that a dose of 1500 IU anti-D immunoglobulin be used routinely but there is no recommendation regarding Kleihauer testing.[10] The use of these larger doses of anti-D immunoglobulin will prevent sensitisation from feto-maternal haemorrhage up to 15 ml; however, approximately 0.3% of deliveries are associated with feto-maternal haemorrhage greater than this and, unless routine Kleihauer testing is undertaken, sensitisation will occur.

The use of Kleihauer testing in the prevention of HDN

Kleihauer testing is a means of detecting fetal red cells in the maternal circulation. Fetal red cells are resistant to acid lysis because of the presence of fetal haemoglobin. Maternal blood subjected to acid lysis and then counterstained results in fetal cells being preferentially identified. A quantitative estimate of the size of any feto-maternal haemorrhage can then be made.[11]

Table 2 Antibodies associated with HDN

Blood group system	*Antigen*
Rhesus	D
	c
	C
	E
	e
	Ce
	C^w
Kell	K
	k
Duffy	Fy^a
Kidd	Jk^a

The routine postnatal dose of 500 IU of anti-D immunoglobulin is sufficient to prevent sensitisation following fetal haemorrhage of up to 4 ml. If Kleihauer testing indicates a larger bleed then additional anti-D immunoglobulin is required. Whenever anti-D immunoglobulin is administered, postnatally or antenatally, to cover a potentially sensitising episode, a Kleihauer test must be performed. If additional anti-D immunoglobulin has to be administered, repeat Kleihauer testing should be undertaken every 48 hours until fetal red cells have cleared.[7] The main disadvantage of the Kleihauer test is that it does not differentiate between RhD positive and RhD negative fetal red cells. It is also a relatively imprecise test that requires considerable technical skill for correct interpretation.[12] There is also considerable interlaboratory variation in the way a Kleihauer test is performed and in how results are interpreted and reported, and more importantly whether specific comments are made regarding the need for additional anti-D immunoglobulin when a large feto-maternal haemorrhage has occurred.[13] Staff need to be aware of the problems of interpretation when patients with hereditary persistence of fetal haemoglobin are tested. Its main use is as a screening test to identify patients who have a feto-maternal haemorrhage of > 4 ml, with flow cytometric measurement of RhD positive cells within the maternal circulation also performed, if available. On the basis of this result the appropriate dose of anti-D immunoglobulin can be recommended and its efficacy assessed by monitoring clearance of fetal red cells. An additional advantage of the use of flow cytometry is that, because it specifically measures RhD positive cells, it is more useful for antenatal patients in whom the fetal RhD status is unknown.

Antibody screening

All women must have a red cell antibody screen performed during pregnancy. This is ideally undertaken early in pregnancy so that antibodies can be investigated thoroughly and appropriate clinical action taken at an early stage. A significant number (2–5%) of women will have red cell antibodies and there is an increasing incidence of non-RhD antibodies in these patients. There are two principal reasons for the detection and identification of red cell antibodies in pregnant women: prevention of HDN, and identification of potential transfusion problems.

Both problems are equally relevant to RhD negative and RhD positive women and, therefore, timing and nature of testing applies to all antenatal patients. Antibody screening should be undertaken using an indirect antiglobulin test (IAT) and a red cell panel conforming to current United Kingdom guidelines,[14] and testing should be undertaken at both an early stage of pregnancy, and at 28 to 36 weeks' gestation for all women. More frequent testing will be required in women in whom clinically significant antibodies are detected. When an antibody is detected, the clinician responsible for the woman's antenatal care must be alerted to its likely significance, both with regard to HDN and transfusion problems. Clear recommendations for further testing, and, where needed, referral to a specialist centre must be made. Management of pregnancies in which red cell antibodies are detected varies depending on the clinical significance of the antibody detected.

Antibody screening tests using enzyme treated red cells are not required for routine antenatal screening. Their use is associated with an increased antibody detection rate, but many of these reactions are false positives. Antibodies are also detected which are not associated with HDN because they are not IgG, or because they react only in the presence of enzyme modified red cells. Laboratories that have access to samples taken only at an early stage of pregnancy for their RhD positive patients will often use this technique in the hope that its increased sensitivity will lead to early detection of antibodies that will develop clinical significance during pregnancy. Early detection of these antibodies enables the clinician to be alerted to the need for further sampling later in pregnancy for these patients.

ANTIBODIES OF CLINICAL SIGNIFICANCE

The antibodies most often implicated in causing severe to moderate HDN are anti-D, -c, and -Kell, but other antibodies are also recognised as a cause of HDN (table 2).There are numerous reports of IAT reacting antibodies being implicated in HDN of moderate severity, and all women found to have an IAT reacting red cell antibody in early pregnancy should be regarded as requiring further investigation. The majority will merely require repeat testing later in pregnancy (28 to 36 weeks); after delivery the baby should be tested for HDN and his/her red cells typed for the implicated antigen. Women with a previous history of children with HDN in association with the same red cell antibody should be managed more actively in line with the management outlined below for anti-D, -c, -Kell.

Women with anti-D, -c, -Kell antibodies (and others with a previous clinical history of HDN) require regular testing to monitor the level of the antibody, and to detect the development of additional antibodies. Testing should be at least monthly until 28 weeks' gestation, then every two weeks. Testing of anti-D and anti-c involves a quantitative measurement; levels of anti-D > 4 IU/ml and of anti-c > 10 IU/ml are associated with a moderate risk of HDN, and these patients should be referred early to a specialist centre for fetal ultrasound assessment and possible fetal blood sampling. Assessment of anti-Kell (and other HDN-associated antibodies (table 2) and antibodies previously implicated in HDN for a particular patient) is less definitive, titration studies usually being undertaken, but no clear cut association between titre and HDN has been established.[15] Laboratory assessment of these patients should be carried out at a specialist centre where additional techniques, such as functional assays that measure cytotoxic lysis or phagocytosis, or a combination of these, are

used. Functional antibody assays, in particular the monocyte mediated antibody dependent cell cytotoxicity assay, provide a means of assessing functional activity of the specific maternal antibody present in any pregnancy, and provide a more reliable means of predicting HDN severity than either quantification or titre.[16] Fetal blood typing from amniocyte DNA for D, C, c, Rh antigens, and Kell and Duffy a (Fy a) antigens is also available through the International Blood Group Reference Laboratory in Bristol. As for patients with anti-D antibodies, early referral to a specialist centre for fetal assessment is important for these patients.

Paternal blood testing for any red cell antigen to which the mother has antibodies can be undertaken. However, it must be realised that the paternity of the fetus is often uncertain, and clinical decisions should not be made solely on the basis of paternal phenotyping.

ANTIBODIES UNLIKELY TO CAUSE HDN

Women who are found to have antibodies not usually associated with HDN and who have no previous history of HDN, and women with no antibodies on initial testing should be tested again at 28 to 36 weeks' gestation to ensure no additional antibodies have developed. If the antibody is likely to cause problems with provision of blood to cover obstetric emergencies, the clinician in charge of the patient must be informed as well as the local transfusion laboratory, and efforts made to ensure that blood of a suitable phenotype can be acquired at short notice. Patients with antibodies to common antigens may be considered for an autologous blood donation programme. At delivery, any baby born to a mother with IAT reacting antibodies must be assessed for evidence of HDN.

RhD negative mothers with non-D antibodies

It is essential that all RhD negative mothers who do not have preformed anti-D receive appropriate doses of anti-D immunoglobulin both antenatally and at delivery. This particularly applies to RhD negative mothers with other antibodies who have already been proven 'good' responders to red cell antigenic stimulus.

Conclusions

The routine testing of a pregnant woman's RhD type forms an essential component of antenatal management. The appropriate administration of anti-D immunoglobulin to RhD negative women is an important part of optimal obstetric management, and involves close cooperation and informed decision making from many health care professionals. The role of the laboratory in RhD typing and Kleihauer testing is essential in delivering a successful RhD immunoprophylaxis programme.

Routine screening of pregnant women for atypical red cell antibodies is also an essential part of antenatal care. The decline in the incidence of anti-D antibodies has led to a decreased incidence of HDN but the cases that occur are associated with a greater variety of antibodies. These require careful laboratory investigation both for their identification and assessment of clinical significance. Few hospitals have wide experience in handling the variety of cases which may occur and it is, therefore, important that, not only is there very close cooperation between laboratory staff, haematologists, obstetricians, and midwives within a single hospital setting but also with reference laboratories and centres capable of performing fetal transfusions. Modern laboratory techniques, employing cellular assays and amniocyte DNA fetal blood typing, coupled with skilled ultrasound assessment of early fetal hydrops, enables obstetricians to help their patients to make informed decisions before invasive procedures such as fetal blood sampling are undertaken.

A multidisciplinary approach involving careful clinical assessment, evaluation of previous history, laboratory testing, and, where necessary, non-invasive and invasive assessment of fetal wellbeing, sometimes coupled with intrauterine transfusion, is the only way of providing effective management of pregnancies complicated by red cell antibodies. Maternal and fetal wellbeing must be assured in these cases and careful informed consideration of the likely significance of any red cell antibodies detected for both HDN and transfusion support will help to ensure the optimal outcome for the mother and her child.

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