

# The clinical outcome of non-RhD antibody affected pregnancies in Northern Ireland

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## SUMMARY

**We assessed the clinical outcome of pregnancies with non-Rh-D antibody in Northern Ireland using retrospective case note review.**

**During the study period (April 1999- March 2000) 186 women with clinically significant antibodies were identified from the records of the antenatal laboratory of the Northern Ireland Blood Transfusion Service. Eighty-five women were included in the study using the criteria mentioned above. None of the fetuses required intrauterine transfusion during this period. One baby required exchange transfusion, three were given top-up transfusions and 17 had phototherapy. Nine babies with a positive direct antiglobulin test (DAT) received no treatment.**

**The incidence of anti-Kell could be reduced by transfusing Kell negative red cells to premenopausal women. It is important that all pregnant women are tested at least twice in their pregnancy to detect the antibodies formed late in the pregnancy. It is useful to formulate a standard protocol for antenatal interventions. Non Rh-D antibodies can cause significant anaemia for up to six weeks in the neonatal period, hence early detection of maternal antibodies is important so that the neonates are followed up for an appropriate length of time.**

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## INTRODUCTION

Although the prevalence of anti-D has significantly declined in relation to other red cell antibodies, anti-D still remains the major cause of morbidity and mortality associated with haemolytic disease of the newborn (HDN) and the fetus. The fetal and neonatal outcomes of anti-D affected pregnancies in Northern Ireland have been reported recently<sup>2</sup>. This study was undertaken to assess the management and outcome of pregnancies in women who had other clinically significant antibodies.

## METHODS

The Northern Ireland Blood Transfusion Service (NIBTS) provides centralised antibody testing for most of the antenatal clinics in the Province. Blood samples from pregnant women are tested for ABO and Rh-D group and screened for atypical antibodies. Antibody screening is performed using solid phase methodology (capture R assay) following the manufacturer's methods. The specificity of the antibody is identified by further testing of those samples that give positive reaction on initial screening.

The antibodies are classified into three groups:

Group I: anti-D, anti-c, anti-Kell

Group II: anti-Fy<sup>a</sup>, anti-Fy<sup>b</sup>, anti-Jk<sup>a</sup>, anti-Jk<sup>b</sup>, anti-C, anti-Ce, anti-e, anti-E, anti-C<sup>w</sup>, anti-M, anti-N, anti-S, anti-s, anti-LU<sup>a</sup>

Group III: anti-Le<sup>a</sup>, anti-Le<sup>b</sup>, anti-P1, anti-I, anti-HI, cold agglutinins, enzyme agglutinins (clinically not significant)

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A sample is requested from her partner if a woman has either a group I or group II antibody in her plasma to assess the risk to the fetus. All pregnant women with group I antibodies are monitored monthly up to 28 weeks gestation and every two weeks thereafter. The interval of follow up for group II antibodies depends on the initial titre, subsequent rise in titre, and the antigen status of the partner. All the others are tested at least twice, once at booking and once between 28-32 weeks.

All the women who had group I (other than anti-D) and women with group II antibodies with a titre of 1/16 or above anytime during the pregnancy were included in the study. The case notes were examined after obtaining permission from the obstetricians responsible for the care of these women. The following details were obtained: history of blood transfusion, indication for transfusion, previous obstetric history, antenatal interventions (if any), time of delivery, baby's birth weight, blood group, DAT, bilirubin, haemoglobin and details of management.

## RESULTS

NIBTS tested 34,913 samples between April 1999 and March 2000. During the study period 186 women were found to have non Rh-D clinically significant antibodies. The antibody identification details are given in Table 1. 85 women fulfilled the inclusion criteria described above (Table 2). The antigen status of the partners are given in

figure 1. Ten women could not be followed up for the following reasons: seven case notes were not available, one woman moved to England during pregnancy, two women were tested only once by their general practitioners and no further information was available.

There was a definitive history of transfusion in 46 (61.3%) women, and the antibody was pregnancy induced (not previously transfused) in 10 (13.3%) women. Transfusion history was not recorded in 19 cases, but 7 of these women had antigen-negative partners, indicating that the antibody was most likely transfusion-induced. It was difficult to classify the remaining 12 women (Fig 2). 36 (48%) women were transfused in one of their previous pregnancies following primary postpartum haemorrhage, retained placenta, ruptured ectopic pregnancy or miscarriage; two were transfused following non-obstetric surgery, two following trauma, two had transfusion to correct anaemia and the reason for transfusion was not recorded for the remaining four women.

Previous obstetric history was noted in detail in women who were at risk of an affected infant i.e., women with homozygous/heterozygous partners or whose partners were not tested. 22 women had a positive antibody screen in their previous pregnancy/pregnancies and at least six of them had previously affected babies treated with phototherapy for neonatal jaundice or top-up transfusions for anaemia. Amniocentesis was

TABLE 1:

*Antibody specificities (other than anti-D) of antenatal women tested during study period*

### Rh Group (other than anti-D)

Antibody specificity	No
anti-c (alone)	12
anti-c + others	11
anti-C (alone)	2
anti-C + others	5
anti-C (alone)	42
anti-E + others	7
anti-C <sup>w</sup>	20
<b>TOTAL</b>	<b>99</b>

### Non Rh Group

Antibody specificity	No
anti-Kell (alone) + others	34
anti-Kell + others	7
anti-Fy <sup>a</sup> (alone)	9
anti-Fy <sup>a</sup> + others	2
anti-S	6
anti-s	3
anti-Jk <sup>a</sup>	6
Others(anti-M 13: anti-N 3: anti-Bga 3: anti-Kpa 1)	20
<b>TOTAL</b>	<b>87</b>

Rh = Rhesus blood group system

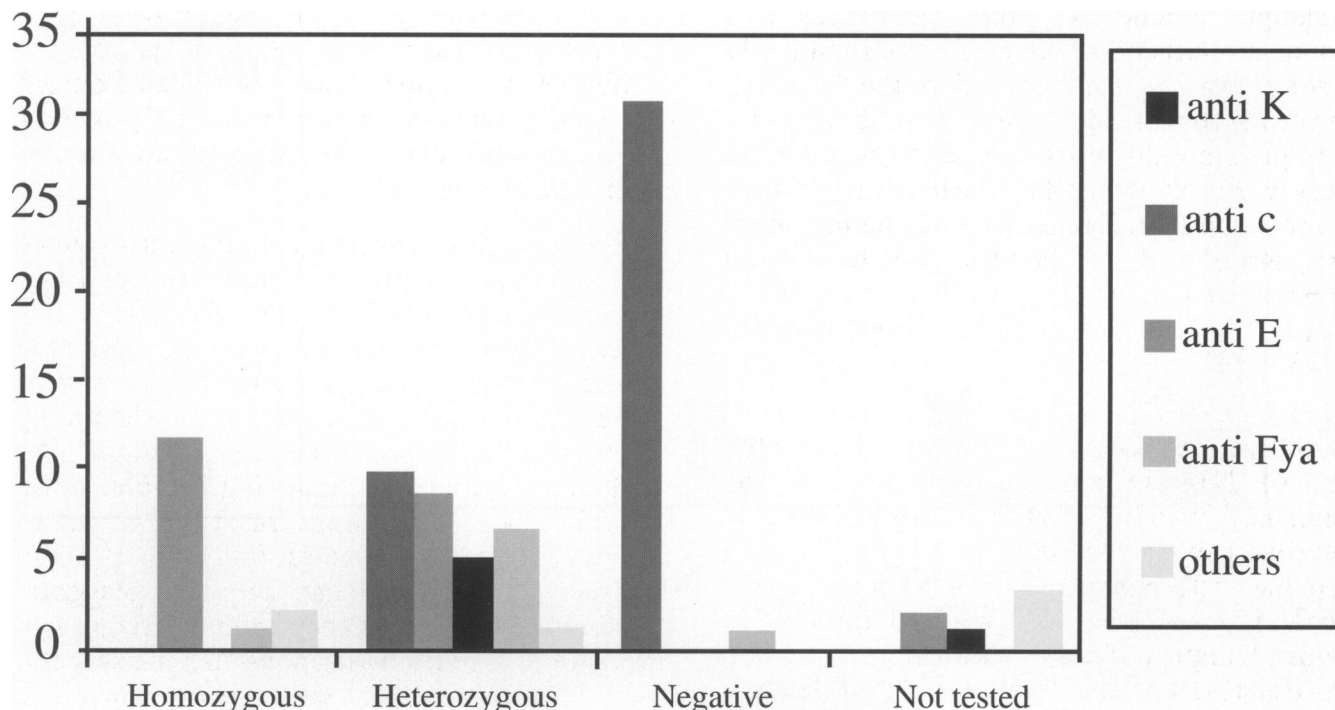


Fig 1. Antigen Status of Partners.

TABLE 2:  
Number and specificity of the red cell antibodies in the study group

Antibody specificity	Number
anti-Kell	41
anti-c	23
anti-Fy <sup>a</sup>	9
anti-E	6
others	6
(anti-C <sup>w</sup> 2: anti-Jk <sup>a</sup> 1: anti-s 1: anti-e 1, anti-E + Jk <sup>a</sup> 1	
<b>TOTAL</b>	<b>85</b>

performed in one woman with anti-Kell in her three previous pregnancies and also in the current pregnancy. Five women with anti-c also had a history of amniocentesis in their previous pregnancies.

Eleven women (excluding one who had amniocentesis to assess risk of Down syndrome) had invasive antenatal interventions in this pregnancy. A woman with anti-Kell titre of 1/512 went into premature labour at 32 weeks after the second amniocentesis and the baby required exchange transfusion for neonatal jaundice.

Cordocentesis was performed in one woman with anti-Kell and anti-Jk<sup>a</sup> due to increase in titres. The fetus was typed Kell-negative and Jk<sup>a</sup>-positive and hence further interventions were not required. The baby was DAT positive due to anti-Jk<sup>a</sup> but did not require treatment. A woman with anti-Fy<sup>a</sup> titre of 1/16 had an amniocentesis done but the reason for this intervention could not be ascertained from her records. Her previous three babies were treated with phototherapy for neonatal jaundice and this infant was also treated with phototherapy for 24 hours (cord bilirubin 56µmol/l).

The clinical outcome of the antibody-affected pregnancies is shown in table 3.

During the study period there were no fetal or neonatal deaths directly attributed to the red cell alloimmunisation and none of the fetuses required intrauterine transfusions. Among the 38 women who had anti-Kell detected in their sera, for whom case notes were available, only ten had antigen-positive (heterozygous) partners. We decided to include the women with negative partners in the study group when we noted a significant rise in titres (1/64 to 1/2048) in two women. The increase in titres could have been due to underlying systemic lupus erythematosus (SLE) in one of them. The results were discussed

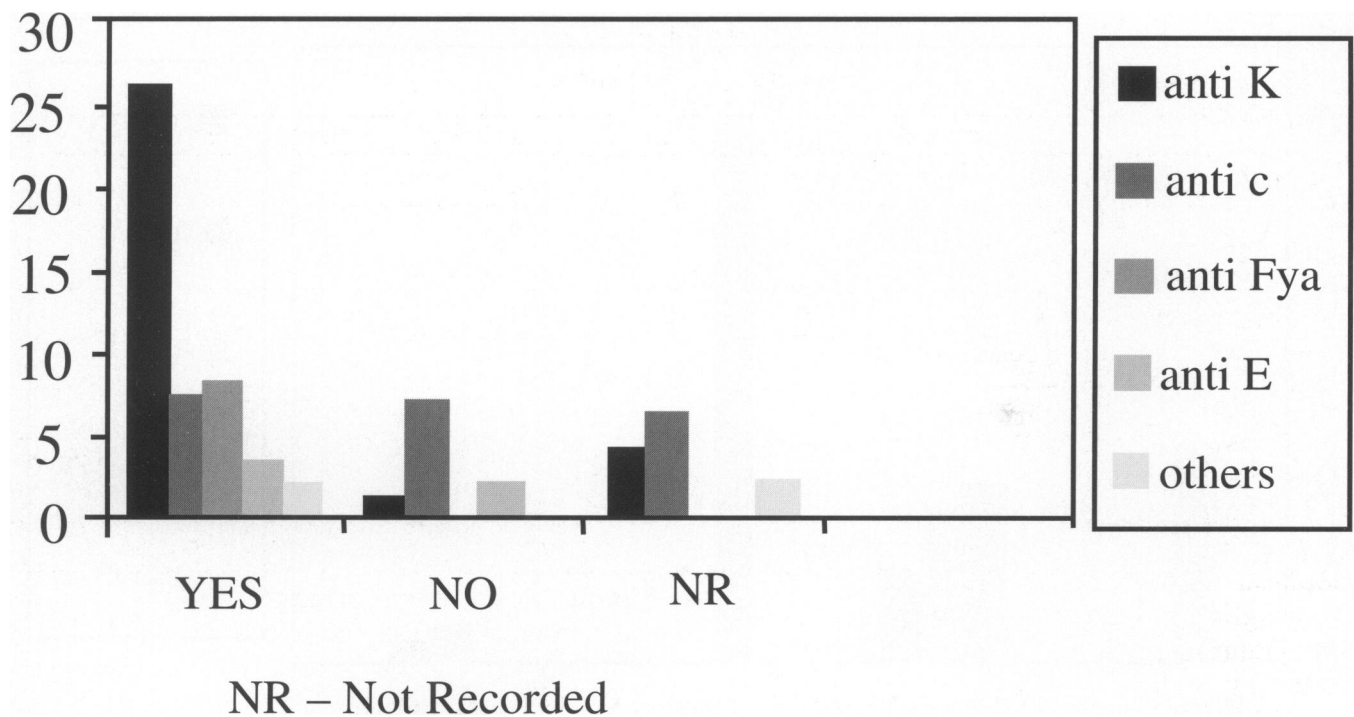


Fig 2. History of Transfusion.

with the women by their obstetricians and it was decided not to do invasive antenatal investigations. The two babies were DAT negative. Similarly all the other babies born to Kell-negative fathers were DAT negative as expected (DAT was not done in one case).

In this study, 10 of the 17 babies who needed phototherapy were born to mothers with anti-c and two babies needed top-up transfusions. One of these babies was noted to be jaundiced on the day of birth and found to be DAT positive and phototherapy was commenced. The mother was tested only once during pregnancy. She had only non-specific cold antibodies in this sample. Anti-c and anti-Jk<sup>a</sup> were demonstrated in the postnatal maternal sample and the same antibodies were eluted from the red cells of her baby. The infant needed top-up transfusion due to a fall in haemoglobin level (from 12 g/dl on day-1 to 6.7 g/dl on day-10). The late appearance of antibodies is well recognised and the "guidelines for antibody testing during pregnancy"<sup>7</sup> recommend testing all pregnant women twice in pregnancy, once at booking and once between 28-32 weeks to allow detection of late appearance of clinically significant antibodies.

#### DISCUSSION

Among the non Rh-D antibodies, anti-c and anti-Kell are those most likely to cause severe HDN.

Antibodies like anti-Fy<sup>a</sup>, anti-E, anti-Ce, anti-e and anti-Jk<sup>a</sup> have also the potential to cause significant HDN<sup>7</sup>. Other antibodies may rarely cause clinically relevant HDN.

Anti-Kell differs from other red cell antibodies because the maternal antibody titres and amniotic fluid spectrophotometric estimation usually do not correlate with fetal anaemia<sup>3,4</sup>. Anti-Kell antibodies cause fetal anaemia by suppression of erythropoiesis rather than red cell destruction<sup>5,6</sup>. Amniocentesis remains useful for identification of fetal Kell genotype by polymerase chain reaction (PCR)<sup>6</sup> when the father has undetermined or heterozygous Kell antigen status. PCR results enable the clinician to exclude the mothers with Kell negative babies from further invasive interventions. Blood group genotyping was not used as a diagnostic tool in any of the cases in this series. It should be noted that 26/41 women with anti-Kell had a history of transfusion and further 10 had negative partners and hence the antibody was most likely to be transfusion induced (Fig 2). Most of the transfusions were given for obstetric causes. NIBTS has started routine phenotyping of donor units for Kell and other Rh antigens in addition to ABO and Rh D typing. Northern Ireland has a population of 562,900 pre menopausal (age 0-45) female subjects (source: Department of Demography and Methodology, Northern Ireland, April 1999) and it is practically

TABLE 3:  
Outcome of Pregnancies

	anti-K	anti-c	anti-E	anti-Fy <sup>a</sup>	others	Total
DAT Negative	32 <sup>a</sup>	5 <sup>b,c</sup>	2	2 <sup>d</sup>	2 <sup>c</sup>	43
DAT Positive, No treatment	2	3	1	3	0	9
Phototherapy	1	10	1	3 <sup>f</sup>	2 <sup>g</sup>	17
Top up Tx	0	2	1	0	0	3
Exchange Tx	1	0	0	0	0	1
Fetal loss <16 weeks	2 <sup>h</sup>	0	0	0	0	2
<b>TOTAL</b>	<b>38</b>	<b>20</b>	<b>5</b>	<b>8</b>	<b>4</b>	<b>75</b>

Figures include:

a: DAT not done in one baby, father Kell negative.

b: DAT negative, c positive.

c: DAT negative, c negative, father homozygous cc.

d: DAT negative, Fy<sup>a</sup> positive.

e: father homozygous ss.

f: prophylactic phototherapy: DAT positive, Fy<sup>a</sup> negative, ABO incompatibility.

g: parents C<sup>w</sup> negative, baby DAT positive, C<sup>w</sup> positive, given prophylactic phototherapy.

h: cause for spontaneous abortion, one had antigen negative partner.

DAT = Direct Antiglobulin Test. Tx = Transfusion.

possible to provide Kell negative blood to those female recipients (in the absence of anti-cellano) without depleting the blood stocks. Ninety-one percent of the population are Kell negative; 91% of recipients will be Kell negative. NIBTS currently provides an inventory of Kell negative typed units to all hospital blood banks. The female population, aged birth to 45, is not an intensively transfused group and there will be little impact on the Kell negative inventory because of the balance of Kell negative to Kell positive in our population. Ideally all female recipients aged 0 to 45 should be Kell typed and this will have to be done in hospital blood banks because not all women present as antenatal cases to NIBTS for antenatal testing in the laboratory. If this recommendation is routinely implemented, the number of anti-Kell antibodies in pregnancies should decline slowly.

However, in the case of anti-c antibodies, 50% of the women were not previously transfused in this study (Fig 2). Hence, provision of c-antigen negative red cells to c-antigen negative recipients

would be expected to make less impact on the number of cases affected with anti-c because unlike anti-Kell, most of the partners are c-antigen positive and induction of immunisation in pregnancy can still occur. Furthermore, this will also involve additional testing of all Rh-D positive pregnant women for their c-antigen status. Bowell et al<sup>10</sup> expressed a similar opinion after a retrospective study of 177 women with anti-c over an 8-year period and concluded that routine c-antigen typing of premenopausal women was not justifiable. Kozlowski *et al*<sup>9</sup> pointed out that 50% of the women with anti-c in their study had been transfused (similar to our figures) compared to 5% in unselected antenatal population. Hence they felt it was worthwhile to perform antenatal c antigen typing of all Rh D positive women and provide antigen selected blood to cover obstetric emergencies.

There was no major institutional variation in the care of the neonates as there is a uniform policy among the neonatal units for the management of neonatal jaundice. All the babies were treated in

accordance with the "guide charts for the management of hyperbilirubinaemia" which give guidance for treatment for babies in different birth weight ranges. There are no specific British Committee Standards in Haematology (BCSH) guidelines related to management of hyperbilirubinaemia in newborns. As part of any investigation protocol however direct antiglobulin tests (DAT) should be performed on the baby and an antibody screen on maternal plasma to exclude red cell allo antibodies as a cause of hyperbilirubinaemia. Where the DAT is positive and the maternal antibody screen is negative it is necessary to perform a maternal v paternal compatibility test to exclude private or low incidence antigens as a cause of undetected haemolytic disease of the newborn.

The indications for antenatal interventions varied among different hospitals. Anti c antibody as well as group II antibodies do not cause significant HDN when detected below the titre of 1/32<sup>7</sup> and hence may be monitored by non-invasive methods. NIBTS is planning to conduct a pilot study to do quantitation of anti-c to provide assistance to the obstetrician in decision-making. In a study conducted in Manchester<sup>9</sup>, none of the babies born to mothers with anti-c level below 9.5 iu/ml required exchange transfusion. Based on this observation, the authors suggested that invasive antenatal intervention is not necessary if the anti-c level is below 7.5 iu/ml, allowing for inherent error in quantitation method.

This study highlights the fact that the clinical outcome of non Rh-D antibodies affected pregnancies is good due to careful monitoring of pregnancy and effective neonatal care. Red cell alloimmunisation remains one of the major causes of neonatal hyperbilirubinaemia resulting in intensive neonatal care admission. Haemolysis due to antibodies can continue to occur for up to six weeks, and the babies have to be followed up during this period to detect anaemia. It is important that laboratories undertaking antenatal testing maintain an effective working relationship and communication with the midwives, obstetricians and paediatricians to provide optimal perinatal care for the patients.

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