Routine testing of fetal Rhesus D status in Rhesus D negative women using cell-free fetal DNA: an investigation into the preferences and information needs of women

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ABSTRACT

Objective The goal of this study is to investigate women’s preferences and information needs for routine implementation of fetal Rhesus D (RhD) typing using cell-free fetal DNA.

Methods A questionnaire was developed following focus groups and interviews with both health professionals and RhD negative (RhD−/C0) women offered fetal RhD genotyping within a research study and distributed to RhD−/C0 women attending routine antenatal appointments in four National Health Service hospitals. Current knowledge of blood types, anti-D administration, fetal RhD genotyping and future practices were explored.

Results A total of 19 respondents participated in interviews and focus groups, and 270 respondents completed the questionnaires. Questionnaire respondents overwhelmingly felt that the test should be offered to all RhD−/C0 women (92.1%), and 75.9% said that they would accept this test. Most were happy to have the test even if it involved extra blood tests (89.3%) or appointments (79%). The knowledge of blood groups was poor. Although 90.7% knew that the baby could have a different blood group from themselves, only 34% knew that blood groups are inherited from both parents. More than 40% were not aware that anti-D would not be required if their baby was RhD−.

Conclusions Women would welcome the introduction of routine fetal RhD genotyping. Information leaflets and training of midwives will be essential for implementation to ensure good understanding regarding testing. © 2013 The Authors. Prenatal Diagnosis published by John Wiley & Sons Ltd.

Supporting information may be found in the online version of this article.

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Conflicts of interest: None declared

INTRODUCTION

To prevent alloimmunisation of Rhesus negative (RhD−) mothers carrying a Rhesus positive (RhD+) fetus, the National Institute for Health and Clinical Excellence recommends that routine antenatal prophylaxis with anti-D immunoglobulin should be offered to all RhD− pregnant women in the third trimester as well as after birth and following events associated with fetal maternal haemorrhage.1 As a result, the incidence of haemolytic disease of the newborn caused by alloimmunisation has fallen dramatically. However, in the UK, about 40% of RhD− women (around 40,000 per year) carry an RhD− fetus and thus receive anti-D unnecessarily.2 Anti-D is produced from pooled plasma from large numbers of RhD− donors who have been transfused with RhD+ red cells to stimulate the production of RhD antibodies3 and thus carries a very small risk of transmission of human blood-borne viral or prion diseases.4 The identification of cell-free fetal DNA (cffDNA) in maternal blood from early in pregnancy5 has allowed the development of non-invasive prenatal testing (NIPT) to determine the fetal RhD genotype in RhD− mothers by analysing a maternal plasma sample.6 This test has been used clinically in England for over a decade to direct care for sensitised RhD— women who would require additional monitoring and potential treatment if they were carrying an RhD+ fetus.6 Advances in technology mean testing can now be carried out accurately and efficiently on a larger scale using automated techniques.7,8
Indeed, routine testing at 25 weeks gestation has already been successfully introduced into antenatal care in Denmark.9

Recent guidance from the National Institute for Health and Clinical Excellence has recommended the exploration of routine antenatal fetal RhD genotyping.10 Here, we investigate how women view current information about blood groups, anti-D administration, the new cffDNA test and how they would like it offered in practice. This study forms part of a larger study developing standards for the implementation of routine fetal RhD genotyping in the UK (antenatal determination of fetal RhD status using cffDNA in the maternal circulation before 20 weeks gestation: is routine application practical and beneficial? PB-PG-0107-12005).

METHODS

Focus groups and interviews
To develop questionnaires for the main study, we used focus groups and one-to-one interviews to explore the views and experiences of RhD− women and health professionals at one London hospital. A purposive sampling method was used for recruitment. The RhD− women were those who had been previously offered with fetal RhD genotyping as part of an ongoing intervention study. The study invitation and information sheet were provided at the 28-week appointment, and participants were interviewed by the lead researcher (KO). Health professionals were identified from staff lists, invited in person and interviewed by one of two researchers (KO and CC) or took part in one of two focus groups. The study was approved by the National Research Ethics Committee London Bentham (07/H0714/128).

A semi-structured discussion guide was used to ascertain perceptions of the current antenatal information regarding RhD and anti-D and anti-D administration for RhD− women, explore views and opinions regarding routine fetal RhD genotyping and identify preferences for implementation into routine practice (see online Appendix 1). The interviews and focus groups were recorded, transcribed verbatim and analysed using thematic analysis.11 To ensure inter-rater reliability, the transcripts were read and coded independently by two other researchers (MH, CC) with themes identified and agreed collectively. Data collection ceased when no new codes were identified.

Questionnaire study

Design
The questionnaire was developed using significant themes identified from the focus groups and interviews. Questions included views and preferences regarding fetal RhD typing (n=4), current knowledge of blood group, anti-D and its administration (n=15), current sources of information (n=8) and demographics and characteristics of the participants (n=9). A four-item Likert scale was used for five of the questions to assess understanding of current information and knowledge and beliefs. A paragraph describing routine fetal RhD genotyping was given in the questionnaire (online supplementary data, S1). The questionnaire was initially piloted on 20 women, and no changes were needed.

Data collection
The questionnaire was distributed in four National Health Service hospitals, one London teaching hospital and three regional hospitals. Research midwives at each site invited RhD− women to fill in the questionnaire whilst waiting for a routine antenatal appointment any time after 12 weeks gestation.

Data analysis
Data were entered onto an Excel spreadsheet and analysed using spss statistics version 17.0. Descriptive statistics were used to analyse individual questions, which included the 15 questions testing the knowledge of blood group, anti-D and the reason why anti-D is given. One point was given for each correct answer and totalled to give an overall knowledge score ranging between 0 and 15. A one-way analysis of variance was used to test for differences in knowledge scores compared with several variables. A t-test was used to test if knowledge scores varied in the second and third trimester. Chi-square tests were carried out to compare answers to specific questions in the second and third trimester.

Spearman correlation was used to determine whether there was a relationship between knowledge scores for anti-D and blood group compared with the perception of how useful the information was, the level of information provided and how knowledgeable participants believed themselves to be.

Women were allowed to give more than one response for the questions relating to how they receive information and reasons for accepting or declining NIPT. For these questions, descriptive analysis was used with percentages being calculated from the total number of responses. One question asked women to rank their answers in order of importance. Some women only ranked those they rated as most important, and these responses were included. This question was analysed by taking each option individually and calculating how many people had ranked it at each level.

RESULTS

Qualitative results

Participants
Six women and 13 health professionals recruited from one London hospital participated. Six one-to-one interviews were held with RhD− women, two with obstetric registrars and two with midwives. Two focus groups were held with midwives (n=9). The women represented a variety of experiences with regard to receiving different fetal RhD genotyping results and decisions regarding anti-D (Table 1). Although only six women were included, this was sufficient to reach a point where no new themes were identified.

Perceptions of routine fetal genotyping
Several themes emerged regarding perception of the new test and how it should be offered into practice.

Benefits of fetal RhD genotyping
All participants felt that the new test was a positive development that should be offered to all RhD− women (Table 2; quotes 1 and 2). Health professionals felt that, overall, women were not
Table 1 Interview participants: Rhesus negative women

| Name   | Parity | Predicted RhD status using NIPT | Anti-D |
|--------|--------|--------------------------------|--------|
| Woman 1 | 0      | Positive                       | Yes    |
| Woman 2 | 0      | Negative                       | Yes    |
| Woman 3 | 0      | Positive                       | Yes    |
| Woman 4 | 0      | Negative                       | Declined |
| Woman 5 | 0      | Positive                       | Yes    |
| Woman 6 | 2      | Negative                       | Declined |

RhD, Rhesus D factor; NIPT, non-invasive prenatal testing.

Test accuracy

All participants thought that accuracy of the test was highly important (Table 2; quotes 11 and 12) and a key factor in the acceptance of the test. Women want to be offered the test at a time when it is most accurate even if this meant it was later in pregnancy. Two women felt that they would still rather have anti-D ‘to be on the safe side’; however, these women said that if the test was proven to be accurate and offered as part of routine care rather than on a research basis, they would feel confident about not having anti-D.

Health professionals were concerned about false negative results and the potential for babies developing preventable haemolytic disease of the newborn.

Information and education

Information and education were also major factors highlighted by all participants. Women felt that the amount of information given in pregnancy can be difficult to absorb, and although they said that they did not always read it, they still felt that receiving simple and succinct written information to the appointment where the test is offered was important (Table 2; quotes 13, 16, and 17). Diagrammatic information was generally preferred (Table 2; quotes 14 and 15).

Midwives recognised that they would be the primary source of information about the test and wanted extra training, written and face-to-face. Notably, health professionals felt that most women accept whatever is offered to them as routine care (Table 2; quote 10), an observation confirmed by women who stated that they would accept their recommendations.
(Table 2, quote 8) as they trusted health professionals to do what was best for women.

**Quantitative results**

**Participants**

A total of 270 of the 287 women approached completed the questionnaire (response rate: 94%), which were developed using the themes emerging from the qualitative study. Four questionnaires were excluded because the participant was under 12 weeks gestation. Demographic information of participants is available as supplementary data (S3).

**Current knowledge about Rhesus D status and anti-D**

The average knowledge score (on a scale of 0–15) was 8.7 (SD = 3.44, range = 0–15): responses are shown in Table 3. Knowledge score comparisons with several variables are shown online in Table S3. There was a significant association between education and knowledge score [F(5, 240) = 13.027, p < 0.001]. Post hoc tests showed that women with a degree (M = 10.20, 95% CI (9.54, 10.85)) scored significantly higher than women with either no qualifications [M = 6.53, 95% CI (5.51, 7.56)] p = 0.001 or educated to general certificate of secondary education level [M = 7.43, 95% CI (6.63, 8.22)], p < 0.001.

There were significant differences in knowledge score in relation to whether women had anti-D in their pregnancy [F(2, 255) = 13.027, p < 0.001]. Post hoc comparisons showed that women who were unsure if they had received anti-D (M = 4.42, SD = 3.605) scored significantly lower than both women who knew they had received anti-D (M = 9.26, SD = 3.160) p < 0.001 and those that knew they had not received it (M = 8.45 SD = 3.290).

When questions were analysed individually, women in their third trimester were more likely to know that they would not need anti-D if they knew their baby was RhD− (65.6% vs 48.1% [p < 0.005]) and that they would only be given anti-D after birth if their baby was RhD+ [59.8% vs 39.7% (p < 0.005)]. Results showing variations across trimesters are shown online in Table S4.

**Current sources of information**

The main source of information for women was their midwife (50.3%), followed by the hospital information leaflet (17.7%), Internet (10.8%), family and friends (9%), doctor (7.5%) and other sources including books and prior knowledge through education or previous pregnancies (4%). Three women said they had not received any information regarding their blood group.

The majority of women (68.30%) felt that they had received enough information about their blood group and had found it useful (80.7%). Half of the women (50.6%) felt they knew ‘a little bit’, and 31.8% felt they were ‘quite’ knowledgeable about their blood group. The majority of women (80.1%) had been told about anti-D in their current pregnancy. Significantly, more women in their third trimester had been told about anti-D compared with those in their second trimester (86.5% vs 74.2%, p < 0.05). The majority of women felt they had been given enough information about anti-D (67.7%). However, a significant number felt they needed more information (32.3%). Women were more likely to feel that they had been given enough information if they were in their third trimester (76.3% vs 59.4% p < 0.005). Generally, women found the information that was provided about anti-D useful (79.2%).

**Opinions on routine fetal RhD genotyping**

Women were overwhelmingly in favour of the test being offered routinely to all women (92.1%). However, there was some uncertainty when asked if they would accept the test themselves (75.9%). The possible reasons for declining the test are given in Table 4.

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**Table 3. Fifteen questions testing current knowledge of blood group and anti-D**

| Knowledge questions | Correct | Wrong | Unsure |
|---------------------|---------|-------|--------|
| 1) I inherited my blood group from both parents | 33.7% (91) | 33.3% (90) | 32.2% (87) |
| 2) I will be offered an anti-D injection in pregnancy | 90.0% (243) | 3.3% (9) | 6.3% (17) |
| 3) My baby could have a different blood group from me | 90.4% (243) | 1.1% (3) | 8.1% (22) |
| 4) Rhesus negative means you do not have the D antigen on your red blood cells. | 38.1% (103) | 5.9% (16) | 55.6% (150) |
| 5) Is anti-D a blood product? | 41.9% (113) | 33.7% (91) | 23% (62) |
| 6) Can anti-D cause an allergic reaction | 32.6% (88) | 7% (19) | 57.4% (155) |
| 7) Anti-D is made from human blood plasma | 27.4% (74) | 4.1% (11) | 66.3% (179) |
| 8) Anti-D is given by injection | 91.1% (246) | 0.4% (1) | 6.7% (18) |
| 9) Anti-D is strictly controlled to avoid transmission of blood borne infections | 51.1% (138) | 3.3% (9) | 43.7% (118) |
| 10) If you are RhD− and your baby is RhD+ would you need Anti-D | 55.9% (151) | 17.4% (47) | 24.4% (66) |
| 11) Anti-D is given to prevent the body producing antibodies | 68.9% (186) | 7% (19) | 21.9% (59) |
| 12) Anti-D is given to protect babies in future pregnancies | 69.3% (187) | 9.3% (25) | 19.3% (52) |
| 13) Anti-D is given because the baby might be Rhesus positive | 68.5% (185) | 4.1% (11) | 24.4% (66) |
| 14) Anti-D is given because the baby might be Rhesus negative | 59.6% (161) | 8.1% (22) | 27.4% (74) |
| 15) After my baby is born, I will be given anti-D if my baby has a positive blood group | 48.9% (132) | 26.3% (71) | 23% (62) |

*Values reported as % (n). Some responses were missing therefore total values may not add up to 100%.*

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There was a significant difference in the knowledge score for women who were unsure if they would accept the test and those that would accept the test ($p = 0.002$). Women who were unsure if they would accept fetal RhD genotyping had significantly lower knowledge scores ($M = 7.64$, $95\%$ CI $(6.66, 8.63)$) than those who would accept the new test ($M = 9.49$, $95\%$ CI $(8.99, 9.99)$), $p = 0.002$. Other comparisons and demographic characteristics were not significant.

Preferences for how fetal RhD genotyping should be offered
The majority of women (95.9\%) would rather have the blood test performed at the same time as other routine blood tests. However, they would be happy to have an extra blood test (89.3\%) if it was necessary. Most women would want the opportunity to discuss the test with a midwife (89\%) and would be willing to have an extra appointment if required (79\%). Women ranked the test accuracy, having enough information and being able to discuss with a midwife most highly (Figure 1).

Information preferences
Most women want to receive information from their midwife (59.7\%) or have written information in a leaflet (34.5\%) rather than accessing the information on the hospital website (4.6\%). Most wanted to be told about the test at the booking appointment with the midwife (46.3\%), with 23.1\% preferring to receive information in the post with the initial booking appointment letter and 21.6\% wanting it posted with the results of their blood test. There was a clear preference for receiving the information prior to the day of the test (91\%). Just under half the women (47.2\%) said that the amount of information provided in the study leaflet was sufficient, 36.7\% wanted more information and 16.1\% were unsure. The following topics were identified as additional information that would be useful:

1. Risks or side-effects to mother and baby
2. Timing of tests, if extra appointment needed
3. Implications of results
4. How the test works
5. Accuracy and implications if the test result is incorrect
6. Being able to discuss the test with a midwife/health professional
7. General information about blood group, why the test is necessary, risks of anti-D
8. Whether any other information can be found out from the test.

DISCUSSION
In this unique study, we have clearly demonstrated that there is enthusiasm from both women and health professionals for Table 4 Views of potential routine fetal Rhesus D typing

| Total ($n = 270$) | No | Yes | Unsure |
|------------------|----|-----|--------|
| Should fetal RhD typing be offered to all RhD- women? ($n = 215$) | 0.9\% (2) | 92.1\% (198) | 7\% (15) |
| Would you accept the test? ($n = 216$) | 2.8\% (6) | 75.9\% (164) | 21.3\% (46) |
| Would you need further information about the test? ($n = 267$) | 47.2\% (126) | 36.7\% (98) | 16.1\% (43) |

How do you prefer to receive information?\(^h\)\(^i\)

|                      | Midwife | Hospital information leaflet | Internet | Other |
|----------------------|---------|------------------------------|----------|-------|
|                      | 59.8\% (104) | 34.5\% (60) | 4.6\% (8) | 1.1\% (2) |

When would you want to receive information?\(^h\)\(^i\)

|                      | In post with booking letter | At booking appointment with midwife | In the post with blood group results | On the day of NIFT | Other |
|----------------------|----------------------------|-----------------------------------|-----------------------------------|------------------|-------|
|                      | 23.1\% (31)                | 46.3\% (62)                       | 21.6\% (29)                       | 6.7\% (9)        | 2.2\% (3) |

Why would you want the test?\(^h\)\(^i\)

|                      | Rather avoid anti-D | Rather avoid injection | To know more about the baby as possible | If recommended by midwife |
|----------------------|--------------------|-----------------------|----------------------------------------|--------------------------|
|                      | 32.7\% (88)        | 22.3\% (60)           | 28.6\% (77)                             | 16.4\% (44)              |

Why would you not want the test?\(^h\)\(^i\)

|                      | Would not want extra blood test | Would want anti-D to be on the safe side | Would want more information |
|----------------------|--------------------------------|----------------------------------------|-----------------------------|
|                      | 20.8\% (5)                    | 37.5\% (9)                             | 41.7\% (10)                |

\(^h\)Total number responses shown for individual questions ($n$).
\(^i\)Respondents could select more than one answer, % have therefore been calculated from the total number of responses for each answer.
RhD, Rhesus D; NIPT, non-invasive prenatal testing.
routine fetal RhD genotyping using cffDNA in maternal blood, regardless of whether it will require extra blood tests or visits. However, health professionals felt that it should be offered with other routine appointments to minimise resource implications, an observation that is supported by the economic evaluation performed in this study.12

The strength of the study is the use of qualitative data from women with experience of fetal RhD genotyping and health professionals to develop the questionnaire together with the quantitative data on preferences from a large number of RhD− women attending four different hospitals. This work expands the current research on fetal RhD genotyping which to date has focused on technology development, test accuracy and economics.6–8 Previous research into views and experiences of the use of cffDNA tests is based on tests already in clinical practice and includes women13,14 and health professionals15 views on fetal sex determination for clinical indications. Other studies have looked at public,16 pregnant women17,18 and health professionals19 views of NIPT using cffDNA for Down’s syndrome and other conditions. These studies found that NIPT is viewed as a positive step in prenatal diagnosis for Down’s syndrome and genetic conditions.

This study found that women would be more likely to accept fetal RhD genotyping if they felt confident about its accuracy. Women with lower knowledge scores were less certain whether they would accept the test. It is concerning that knowledge scores showed a lack of understanding of current care regarding anti-D in several areas. Women were unsure why anti-D is given and that their baby could have a different blood group from them. This knowledge improves in the third trimester, most likely because information is given at the time of anti-D administration at the beginning of the third trimester. Women wanted to know why routine RhD genotyping is beneficial, and thus understanding blood group inheritance and why anti-D prophylaxis is only necessary if the baby is RhD+ is important.

Women showed a preference for receiving written information in the post and speaking to the midwife before being offered the test. This reflects findings from a Dutch study evaluating screening programmes for non-Rh red blood cells; when asked about their information needs, women desired written information and prioritised having information on the clinical implications for both mother and child.20 One of the key issues women wanted addressed was whether fetal RhD genotyping testing posed a risk to mother or baby. The test was described as a ‘normal blood test’. However, women were still concerned about risk. Furthermore, as we are using the baby’s DNA, there was also concern that we could potentially reveal other information about the baby.

A notable theme from the qualitative data was women being willing to accept whatever was recommended to them by the midwife or presented as routine care. Questionnaire findings showed 16.7% of women felt that they would accept the test if it was recommended to them by the midwife. This is a similar finding to a study that investigated women’s interest and expected uptake of NIPT using cffDNA for prenatal diagnosis, which found that 20% of respondents would do what their doctor recommended.17

These findings indicate that health professionals, in particular midwives, will need to ensure that complete and balanced information is given to women to allow them to make an informed decision regarding fetal RhD genotyping and anti-D administration. Current blood group information leaflets could be developed to include information about fetal RhD genotyping. Information should be clear and concise and presented at a relevant time. This study cannot determine when in the care pathway this test should be offered, but inherently, the earlier in pregnancy, the better to avoid unnecessary anti-D administration for early sensitising events. As information regarding the mother’s own Rh type is needed before fetal RhD genotyping can be interpreted, sending the information with the booking blood results seems appropriate and is a time when there is less information being given about other aspects of pregnancy.

Study limitations
In this study, we attempted to gather the views and preferences of a cross-section of women by recruiting from four different hospitals in different regions of the UK. However, several issues may limit how representative our findings are. For example, the majority of pregnant women who took part in this study were White, and women from ethnic minority groups are under-represented.
A small sample of health professionals and RhD– women took part in the focus groups and interviews: These were all based at one hospital and only had experience of fetal RhD genotyping being offered on a research basis. Other hospitals may have different protocols, and fetal RhD genotyping may be offered differently when introduced routinely into practice. Therefore, their views and experiences may be specific to their own experience. The study reflects women’s stated preferences and may not in fact reflect future attitudes and uptake of fetal RhD genotyping. It will be important to research the best ways to provide information and education for women.

CONCLUSIONS

This is the first study to investigate health professionals and women’s views and opinions of routine fetal RhD genotyping. Although we have shown an overwhelmingly positive response, we have also demonstrated significant weakness in delivery of information regarding the current information on Rh blood groups and anti-D administration. Before being offered this new test, women want timely information on its benefits, risks, accuracy and implications, making development of information leaflets and health professional training key to routine clinical implementation. This work is critical for the development of policies and guidelines for the introduction of fetal RhD genotyping into routine care.

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WHAT IS ALREADY KNOWN ABOUT THIS TOPIC?

• Technological advances mean that there is now potential to offer routine fetal RHD typing using cell-free fetal DNA to all RhD negative women. This will mean that anti-D can be targeted to women carrying a Rhesus positive baby.

WHAT DOES THIS STUDY ADD?

• This unique study shows that women and health professionals hold positive views regarding the introduction of routine fetal RHD genotyping using cell-free fetal DNA. Women’s current knowledge of Rhesus blood groups and anti-D administration was found to be limited. Development of information leaflets and health professional training will therefore be critical for successful implementation.

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