

Use of cffDNA to avoid administration of anti-D to pregnant women when the fetus is RhD-negative: implementation in the NHS

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Objective To determine whether a policy of offering cffDNA testing to all RhD-negative women at about 16 weeks' gestation to avoid anti-D administration when the fetus is RhD-negative could be implemented successfully in the NHS without additional funding.

Design Prospectively planned observational service implementation pilot and notes audit.

Setting Three maternity services in the South West of England.

Population All RhD-negative women in a 6-month period.

Methods Prospective, intervention, cross-sectional observational study, using pre-intervention data as controls.

Main outcome measures Proportion of suitable women who offered and accepted the test. Accuracy of the cffDNA result as assessed by cord blood group result. Fall in anti-D doses administered.

Results 529 samples were received; three were unsuitable. The results were reported as RhD-positive ($n = 278$), RhD-negative

($n = 185$) or inconclusive, treat as positive ($n = 63$). Cord blood results were available in 502 (95%) and the only incorrect result was one case of a false positive (cffDNA reported as positive, cord blood negative – and so given anti-D unnecessarily). The notes audit showed that women who declined this service were correctly managed and that anti-D was not given when the fetus was predicted to be RhD-negative. The total use of anti-D doses fell by about 29% which equated to about 35% of RhD-negative women not receiving anti-D in their pregnancy unnecessarily.

Conclusions We recommend this service is extended to all UK NHS services.

Keywords Anti-D, cffDNA, non-invasive prenatal testing, RhD, RHD, rhesus disease.

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Introduction

Anti-D immunoglobulin (anti-D Ig) prophylaxis has been a highly successful example of preventative medicine by reducing the incidence of sensitisation of pregnant women to the D antigen and so haemolytic disease of the fetus and newborn (HDFN).¹ The current policy² of giving anti-D antenatally and so before the blood group of the fetus is known, means that almost 40% of Rhesus D (RhD)-negative pregnant women (approximately 40 000 women per year in England and Wales) receive antenatal anti-D Ig in pregnancy when they cannot benefit from it because they are carrying an RhD-negative fetus. In contrast, on the birth of the baby, cord blood is sent for D-grouping

and postnatal anti-D is only offered if the baby is RhD-positive.

A test that uses cell free fetal DNA (cffDNA) has been developed that can identify the fetal D status with great accuracy using a maternal peripheral blood sample.^{3,4} This technology was initially used to guide the management of women who were alloimmunised and at risk of HDFN, but more recently a 'scaled-up' technique has been described⁵ aimed at providing this service to all RhD-negative women in England. A NIHR-funded multi-centre (Bristol was one centre) study investigated the performance of this test at different gestational ages and demonstrated the test is reliable after 11 weeks' gestation.⁶ This has led to the suggestion that the continuing practice of giving a blood

product pooled from multiple donors to healthy pregnant women is ethically unreasonable.⁷ National fetal RhD testing programmes to direct antenatal anti-D prophylaxis have been introduced successfully in other countries,^{8–10} but (with the exception of the Netherlands) these were in countries which did not previously have an antenatal anti-D administration programme and were therefore considering the issues from a different starting point.

A service implementation pilot was undertaken to define the potential difficulties and assess how easily they could be overcome in the NHS. We also wished to explore whether the expected saving in the number of anti-D doses administered could be achieved and documented and so whether the savings could be used to fund the cost of the tests.

Methods

Recruitment and process

The maternity services in the administrative area Bristol, North Somerset and South Gloucestershire (previously known as 'Avon') deliver about 12 500 births per year and are provided by three acute Trusts and their associated community services: North Bristol NHS Trust (NBT) (Southmead Hospital – approximately 6000 deliveries per year) (NBT), University Hospitals Bristol Foundation Trust (St Michael's Hospital – approximately 5500 deliveries per year) (UHB) and Weston NHS Trust – approximately 1000 booking and 250 deliveries per year, with the others delivering in one of the other two Trusts. The protocol for this service change was agreed by all three maternity services supported by the blood transfusion/haematology laboratories in each centre.

The protocol was to offer the possibility of *cffDNA RHD* testing to RhD-negative pregnant women from 1 April 2013 in NBT and UHB (from 1 May 2013 in Weston) and the data for the women in the 6 months up to 30 September 2013 and their follow-up information were analysed. The maternity services 'booking' blood test process was unchanged. After the maternal blood group was identified as RhD-negative, the previous policy of giving (or posting to their home address) an existing leaflet about being RhD-negative was continued, but in addition a new leaflet about this service pilot was included (the leaflet is available at <http://foi.avon.nhs.uk/download.aspx?did=15812>). As this was a service implementation pilot, research consent was not sought. At the routine 15–17 week midwifery visit, the midwives were asked to discuss the issues relating to RhD-negative blood group as well as reviewing the leaflets the patients had received and answering any questions, and then offer the *cffDNA* test. As with all clinical care the midwives were asked to record that this counselling had occurred and, part way through the pilot, formal boxes to help document the counselling and consent had happened were added to the printed hand-held notes.

Samples

When a woman chose to have this test, the blood sample was usually taken at the 15–17 week visit and when this had not been done (e.g. because of late booking or transfer of antenatal care) midwives were asked to offer to take it up to 26 weeks' gestation (stopping at that gestational age because the result may not have been available in time to guide routine prophylaxis). The sample was sent to the midwife's usual hospital pathology laboratory with a form documenting the purpose of the sample and giving the estimated date of delivery (EDD) based on the dating scan. The EDD was used to identify the pregnancy to avoid the potential risk that a filed/stored result could be incorrectly ascribed to a possible future pregnancy. On arrival at the laboratory the sample was bar-coded and entered onto the pathology system and then managed as a 'send-away' sample.

Laboratory analysis

The sample was then transferred from each of the three hospital pathology laboratories to the NHS Blood and Transplant (NHSBT), International Blood Group Reference Laboratory in Filton, Bristol, using the established NHSBT transport system. On arrival the sample was logged and entered onto the 'Hematos' reporting system. The *cffDNA RHD* genotyping test was performed as previously described⁵ and the result entered into the Hematos system. Printed reports were sent in batches to the referring Trusts by post; however, from November 2013 NHSBT test results were also available electronically to hospital laboratories using the SPICE system. When the paper reports were received by the hospital Pathology Laboratory, the results were entered onto the Trust's IT system (and so made available to the clinicians in their place of work) and a paper report was also sent to the physical area recorded on the original form for filing in the patient notes.

There were three possible results reported: (i) 'The fetus with a EDD of date is RhD-positive', (ii) 'The fetus with an EDD of date is RhD-negative', (iii) 'the fetus with an EDD of date *cffDNA* result is indeterminate – treat as RhD-positive'. The midwives were asked to inform the patients of the result and the associated recommended management of either having or avoiding antenatal anti-D. The advice not to recommend anti-D Ig applied both to routine antenatal prophylaxis and if any potentially sensitising events occurred (such as vaginal bleeding). However, the information leaflet and counselling were clear that if a woman wanted anti-D despite an RhD-negative result that it could be requested and would be given.

Cord blood group outcome information

At the delivery of RhD-negative women, cord blood routinely is sent for blood grouping and a maternal blood sample is sent for a Kleihauer–Betke test (so that if the fetus is

RhD-positive and a large feto-maternal haemorrhage is found, extra anti-D can be given). As the Kleihauer–Betke test is a maternal (rather than baby) test, this allowed the delivery date to be identified within the laboratory computer records and provided a link to the name and NHS number of the neonate, which allowed the cord blood group to be obtained. In a small number of cases the cord blood group was not available (stillbirth, moved to another Trust, lost samples). It was planned that repeat testing by the reference laboratory would be arranged for any cases where a positive cord RhD group was found after a negative fDNA result.

Audit of a sample of notes

In addition to the above, an audit of the notes of RhD-negative women delivering in a specific 4-week interval (11 December 2013 to 8 January 2014) was undertaken. A pro-forma was devised prospectively (and approved by the Audit Departments of all three Hospitals) that organised the collection of information from the antenatal, intra-partum and postnatal notes. Because of the rapid turnover of women in maternity services (e.g. rapid discharge home and delivery in community settings) it was not always possible to obtain the notes and antenatal notes were sometimes available when the postnatal notes were not.

Results

From 1 April 2013 to 30 September 2013, samples from 529 RhD-negative women were received by NHSBT within this pilot. In all, 274 women were from NBT, 197 women were from UHB and 58 women from Weston. Three samples received were inadequate (insufficient blood) and an additional three inappropriate referrals (RhD-positive women) were made and these were excluded from the data. The number of samples received per week increased over the 26 weeks of the pilot (Figure 1).

The percentage of the total number of RhD-negative women who had accepted this service at delivery of their baby rose as expected over the 5 months after the service was started (recruitment at 4 months of pregnancy and so delivery about 5 months later) (data from one of our units, NBT; Figure 2).

The laboratory reported on all suitable samples received ($n = 526$) and 98% of these were within 10 working days.

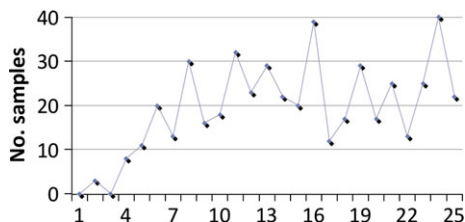


Figure 1. Number of samples received in each of the 26 weeks from 1 April 2013 to 30 September 2013.

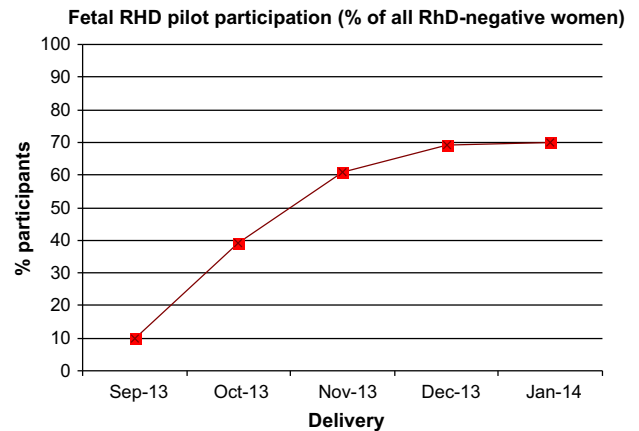


Figure 2. Percentage of RhD-negative women who had taken up this service when delivering in one of the maternity units (Southmead). The expected rise about 5 months after the start of the service is shown.

The results were RhD-negative ($n = 185$; 35%), D Positive ($n = 278$; 52%) or ‘inconclusive, treat as D positive’ ($n = 63$; 12%). The cord blood group result was obtained in 502 cases (95%); reasons for not obtaining the cord blood result included the patient moving to another area, miscarriage/stillbirth, cord blood not obtained, etc. The results are shown in Table 1.

In the audit of the notes of RhD-negative women giving birth in a 4-week period (December 2013 to January 2014), 67 women were identified, of whom 49 were part of the pilot (73%). Of the 18 women who were not part of the audit the notes showed that in 10 no offer of the test was recorded. In the other eight an offer was recorded and in five of those a patient decision to decline was documented. Seventeen of the 18 had routine antenatal prophylaxis (the one who declined anti-D Ig was documented as knowing that her partner was RhD-negative). Of the 49 cases within the pilot, 18 of the cases had received an RhD-negative result and

Table 1. cffDNA results from 529 RhD-negative women compared with the cord blood result

Result issued	<i>n</i>	Cord blood confirmed RhD-negative	Cord blood confirmed RhD-positive	Cord group not available
D-Negative	185	170	0	15
D-Positive	278	1	267	10
Inconclusive (treat as RhD-positive)	63	14	47	2
Not tested (too small a sample)	3	0	3	0
TOTALS	529	185	317	27

routine antenatal prophylaxis was not given in 17 of these (94%). The reason why one woman received antenatal anti-D Ig despite a negative cffDNA result was not recorded. Of the 26 notes from cases with RhD-positive cffDNA results, RAADP was recorded to have been given in all of these (100%). Five cases (10%) had an 'inconclusive, treat as positive' results and all of these received routine antenatal prophylaxis.

The use of anti-D in the three Trusts is shown in Figure 3 and the expected fall was seen in all three Trusts [Poisson regression 6% drop per month (95%CI 4–8%); $P < 0.001$]. In total the number of anti-D Ig doses fell from about 350 to about 250 per month (29%). As the women with RhD babies are not given postnatal anti-D, this corresponds to 35% of RhD-negative women not being exposed to anti-D.

Discussion

Main findings

The results have shown that it is possible to implement routine cffDNA fetal blood grouping in RhD-negative women in the NHS without additional research funding/resources. The requirements of patient information, consent, sample handling, result transfer and implementation of the changed management were all successfully met.

The fall in the total anti-D use by about 29% was as expected for the following reasons:

- RhD-negative women already are not given anti-D after birth when the baby is RhD-negative, so the current postnatal usage cannot be reduced further.
- Only about 70% of the RhD-negative women joined the pilot in the initial 6 months. The results from the notes audit suggest that this additional test was not offered to most of those women who did not join, although some

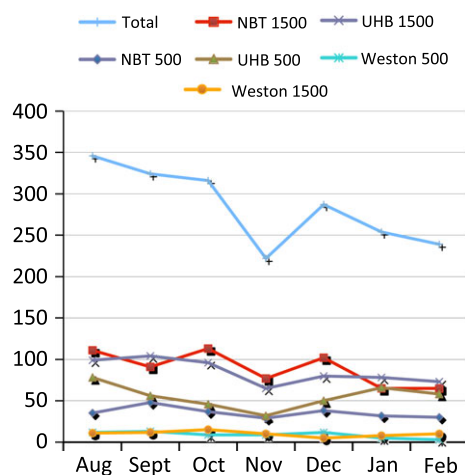


Figure 3. Anti-D use (500 and 1500 IU products) from August 2013 to February 2014. Poisson regression 6% drop per month (95%CI 4–8%; $P < 0.001$).

declined. As this becomes a more established permanent service, we expect the proportion of RhD-negative women who accept this test to rise.

- About 10% of the test results were 'inconclusive, treat as positive'. With advances in the laboratory techniques it is possible this proportion may fall. However, about 77% of the women with inconclusive result gave birth to positive fetuses.

For the above reasons the usage of anti-D may fall a little further using this approach.

In terms of the financial aspects, in our maternity service with approximately 1500 RhD-negative women per year, we have shown the use of anti-D Ig fell by about 100 doses per month (1200 per year), which equates to about £60,000 per year for our service. Without considering the midwifery time relating to anti-D Ig administration (and so equating that to the time for counselling and blood taking for cffDNA testing), this relates to a budget of about £60,000/1500 = £40 test. It seems likely that provided the cost of the cffDNA test is less than the cost of each anti-D Ig injection, this service could be implemented with little additional cost and probably a saving.

There was one case when the cffDNA result was positive and the cord blood result was negative. This was expected because some people with an *RHD* gene do not express the RhD protein on the red cells and so the discrepancy occurs occasionally when genotyping tests are undertaken. As the consequences of such false-positive results are that the woman is given antenatal anti-D unnecessarily, the effect is the same as the current recommended policy, but in very low numbers. Since this study was completed, we have had one case in which the cord blood was positive after a negative cffDNA result and so that was further investigated as planned. The investigation showed that the RhD-positive *cord blood* result was incorrect because of a result transposition error. This supports the potential to stop cord blood testing after antenatal cffDNA results and, if this was implemented, there could be a further saving of midwifery and laboratory staff time as well as the cost saving.

Strengths and limitations

The strengths of these data are that they were obtained in the 'real world' of the NHS, with no additional clinical staffing and the existing local laboratory, transport and IT systems. A limitation was using the changes with time to assess the effects of the intervention but, in view of the relatively short time scale of the intervention and the outcomes, there is no reason to think that any other factor will have caused the changes seen.

Interpretation

Irrespective of the financial costs, it has been argued that it is ethically unacceptable to continue administering antena-

tal anti-D Ig to all RhD-negative women when a fetal *RHD* genotyping test using maternal blood could identify those women who do not need this product.⁷ Each dose of anti-D is prepared from multiple blood donations from many donors. Although anti-D has been an exceptionally safe product, which should be strongly recommended to those women with an RhD-positive fetus, conditions such as prion diseases should continue to ensure this blood product is only used when needed. In addition, the availability of anti-D is limited and requires deliberate sensitisation of male volunteers. Both the worldwide shortage of this product and the difficulties of availability mean it should only be used when required. As 35% of RhD-negative pregnant women will avoid exposure to anti-D by this policy, there is an even larger proportion of women who will avoid blood product exposure than will be saved in anti-D dose usage.

Conclusion

In clinical practice, we recommend that this service is continued and now extended to the whole UK. It has allowed the use of anti-D Ig (a blood product) in a more precise and indicated way and the cost of the tests seems to be covered by the resulting saving in the use of anti-D Ig. Research to improve the cffDNA test and so reduce the inconclusive rate even further should continue.

Disclosure of interests

PWS has previously been a paid member of an Advisory Board for Novartis relating to non-invasive prenatal testing and a paid member of a Data Monitoring Committee for a study relating to non-invasive prenatal testing for Ariosa Diagnostics. He is a member of the Reliable, Accurate, Prenatal, non-Invasive Diagnosis programme (RAPID) (NIHR programme grant for applied research RP-PG-0707-10107 NIH Programme Grant). KF, TL and GD work for NHS Blood and Transplant, which is likely to be a site of the cffDNA testing and resulting income flow if the policy recommended is implemented. JF and TW-B do not declare any conflict of interests.

Contribution to authorship

PWS, KF, TL and GD planned the study, wrote the protocol, analysed the data and wrote the paper. TW-B and JF devised the processes used to achieve this study in the NHS and revised the manuscript.

Details of ethics approval

Ethical committee approval and patient research consent were not obtained because this was a service implementation pilot. The notes audit was approved by the audit departments of all three Trusts.

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