CASE REPORT

Repeated failed non-invasive prenatal testing in a woman with immune thrombocytopenia and antiphospholipid syndrome: lessons learnt

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SUMMARY

We present a case of a 37-year-old Chinese woman (gravida 4 para 0) with a history of immune thrombocytopenia and type IIb antiphospholipid syndrome. She was started on 100 mg of aspirin, 20 mg of prednisolone and 20 mg of subcutaneous low-molecular-weight heparin daily for her fourth pregnancy. She opted for non-invasive prenatal testing for aneuploidy screening but had failed results three times consecutively from insufficient fetal cfDNA initially or high variance in cfDNA counts on redraws. She declined invasive karyotyping. Her pregnancy was complicated by severe pre-eclampsia and fetal growth restriction at 19 +6 weeks of gestation and was terminated. Subsequent fetal karyotyping revealed a normal karyotype of 46XY with no apparent abnormalities.

BACKGROUND

Non-invasive prenatal testing (NIPT) by analysis of cell-free DNA (cf-DNA) in maternal blood is highly accurate for the detection of fetal trisomies 21, 18 and 13 with a sensitivity of up to 99% and a low false-positive rate of <0.1%.^{1 2} An important and often overlooked aspect in the performance of NIPT is the rate of NIPT test failures or non-reportable results, occurring with rates ranging from 0.8 to 12.2%.³ Such test failures or no-call result may result from administrative issues, assay failure or a low fetal cf-DNA fraction.⁴ A fetal cf-DNA fraction of at least 4% in maternal plasma is needed to provide a reliable and reportable clinical result.⁵ Multivariate regression analysis has demonstrated that fetal fraction is affected by maternal and fetal characteristics, decreasing with increasing maternal weight and increasing with advancing fetal gestational age and increasing serum markers such as pregnancy-associated plasma protein-A (PAPP-A) and free β -human chorionic gonadotropin (bhcg).^{5 6} It has been postulated that maternal autoimmune conditions may affect fetal fraction.⁷ Furthermore, a recent study by Grömminger *et al*⁸ found that maternal use of low-molecular-weight heparin (LMWH) has been shown to affect NIPT results, by raising the content of smaller plasma DNA fragments with a higher guanosin-cytosin (GC) content. The implications of a suboptimal NIPT result include increased maternal anxiety, high costs from repeated NIPT testing and possibly the unwarranted use of invasive fetal karyotyping for diagnosis. With the advent of NIPT into routine prenatal care, our understanding

of the limitations of this test is essential for the counselling and selection of patients undergoing this test.

CASE PRESENTATION

We report a case of a 37-year-old Chinese woman (gravida 4 para 0) with a history of immune thrombocytopenia and type IIb antiphospholipid syndrome. She had three previous first trimester miscarriages and high titres of anticardiolipin IgG antibodies. Her booking height was 153 cm and her weight was 50.5 kg, with a body mass index (BMI) of 21.6 kg/m². She was a non-smoker. For her fourth pregnancy which was a spontaneous conception, she was referred to a tertiary centre for multidisciplinary management by the haematologists and maternal fetal medicine specialists. She was started on 100 mg of aspirin, 20 mg of prednisolone and 20 mg of subcutaneous LMWH daily. Her platelet count at booking was $53 \times 10^9/L$.

She was offered the options of first trimester screening for aneuploidy. She chose the single nucleotide polymorphism (SNP)-based NIPT (Harmony Prenatal Test, Ariosa Diagnostic, San Jose, California, USA) as the first and only option without undergoing the combined first trimester screening with ultrasound and maternal serum markers. The first sample was drawn at 11 +5 weeks of gestation and a result was not obtained, as there was unusually high variance in cf-DNA counts. A second sample was drawn at 13 +6 weeks. Again, a result was not obtained because of insufficient fetal cfDNA for accurate NIPT evaluation. With the patient's consent, a third sample was drawn at 16+1 weeks of gestation. However, the third sample was again returned because of insufficient fetal cfDNA.

INVESTIGATIONS

By the time the first failed NIPT result was available, she was not eligible for first trimester combined screening with maternal serum markers. She did have a measurement of nuchal translucency at 11+5 weeks of gestation that was normal. Her platelet count at that time was $60 \times 10^9/L$. The patient declined invasive karyotyping by amniocentesis or second trimester maternal serum screening.

TREATMENT

Second trimester maternal serum screening and invasive karyotyping were discussed with our patient, but she declined these options. A trial of

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immunosuppression with steroids and intravenous immunoglobulin (IVIG) had been used for a similar case in a woman with autoimmune disease; however, there was no medical indication or strong evidence for this treatment.⁷ This option was discussed with the patient, and given the poor supporting evidence was not recommended.

OUTCOME AND FOLLOW-UP

The patient's screening ultrasound performed at 19+6 weeks of gestation revealed severe intrauterine growth restriction of the fetus with oligohydramnios. There was absent end diastolic flow of the umbilical artery with no abnormal ductus venosus Doppler. Her blood pressure at the 19+6 week visit was 213/96 mm Hg, and she reported of epigastric discomfort. Her urine protein-to-creatinine ratio was elevated at 0.46. Early onset pre-eclampsia was diagnosed. Blood investigations tests revealed mildly raised transaminases on her liver function test. Her thrombocytopaenia deteriorated further from $49 \times 10^9/L$ to $35 \times 10^9/L$ and reached a nadir of $24 \times 10^9/L$ despite an increase in her prednisolone dose. The haematologist recommended IVIG in view of the declining platelet level. In view of her deteriorating clinical picture and the poor prognosis of a severely growth retarded fetus at this gestation, a termination of pregnancy was recommended for maternal indication. She underwent a mid-trimester termination of pregnancy. Subsequent fetal karyotyping revealed a normal karyotype of 46XY with no apparent abnormalities.

DISCUSSION

This case brings up two main points for discussion. First, the possible association between her autoimmune condition and the repeated NIPT test failures. Second, the clinical implications of repeated test failures and the possibility of her persistently low fetal fraction could potentially have been a predictor of the final poor outcome of her pregnancy.

The reported failure rate with SNP-based NIPT was 6.4% in a series of 31 030 patients, and this was mostly due to low fetal fraction.⁹ The failure or non-reportable rates of NIPT was quoted as 1.9% using massive parallel sequencing and 3% with chromosome-specific sequencing.^{10 11} It is estimated that 2% of pregnancies between 10 and 21 weeks will have a fetal fraction of less than the required 4%.⁵ More than 50% of women had a successful result on redraw after the first failed sample.⁶ There was observed to be a mean 1% fetal cf-DNA gain in the second draw compared to the first draw, with an average interval of 3.6 weeks in between. At 11–13 weeks, the median fetal fraction in maternal plasma is 10%.⁵ With a redraw, we expected that any technical or administrative issue would be solved. Furthermore, a redraw at a higher gestation is likely to yield more c-f DNA in maternal plasma. However, our patient still had insufficient fetal fraction with even the third sample drawn at 16+1 weeks of gestation.

Multivariate regression analysis has demonstrated that fetal fraction is affected by maternal and fetal characteristics. It decreases with increasing maternal weight and increases with advancing fetal gestational age and increasing serum markers such as PAPP-A and free bhcg.^{5 6} Fetal fraction is also associated with factors such as smoking and ethnicity, with a lower fetal fraction was observed in women of Afro-Caribbean origin compared to Caucasians, and a higher fetal fraction in smokers than non-smokers.⁵ Our patient was of normal BMI. We did not have her results for PAPP-A or free bhcg levels as she had declined doing the combined first trimester screening test. A postulated cause of the multiple failed attempts at obtaining

sufficient fetal fraction for our patient is the increased maternal cell destruction in women with autoimmune disorder, diluting the cf-DNA in the maternal sample. Another possible cause is her use of LMWH. Grömminger *et al*⁸ reported LMWH injection renders the NIPT result interpretation unreliable as the medication raises the content of smaller plasma DNA fragments with higher GC content. An elevated GC content correlated with an increased z-score of chromosome 18 and decreased z-scores for chromosome 13 and chromosome 21.⁸ They suggested that samples for NIPT should be drawn just before the next pending dose of LMWH, when the plasma level of LMWH is at the lowest.⁸

A similar case of repeated failed NIPT was reported in a woman with severe autoimmune disease, with a successful analysis performed when the sample was drawn at 21+0 weeks following two failed samples.⁷ This sample was taken after the woman completed a 7-day course of oral steroids, and her platelet count improved to $98 \times 10^9/L$. Interestingly, a fourth sample drawn after the completion of oral steroids and an additional IVIG infusion had unusually high variance in cfDNA counts, suggesting specific interference post-IVIG.⁷ Our patient had been on maintenance oral prednisolone throughout her pregnancy, with the highest platelet count reached being $60 \times 10^9/L$. Unlike the woman who obtained a successful NIPT result after completing a course of steroids, our patient was unable to provide a sample with adequate fetal fraction for an accurate NIPT result despite being on prednisolone.

Our patient's pregnancy as outlined above was complicated by early onset, severe pre-eclampsia complicated by placental insufficiency. The studies evaluating fetal cfDNA as a predictor of pre-eclampsia have been conflicting. In pre-eclamptic placentas, it is hypothesised that an increase in oxidative stress causes trophoblast apoptosis, which in turn results in an increased fetal cfDNA levels.¹² Higher fetal cfDNA levels have also been discovered in pregnancies complicated by placental insufficiency and subsequent intrauterine growth restriction.¹³ In contrast, there was no significant difference in fetal cfDNA levels in women who developed pre-eclampsia and women who did not develop pre-eclampsia in a recent nested case-control study.¹⁴ A retrospective cohort study on 348 women undergoing NIPT found a low fetal fraction in 22 (6%) women and an association between low fetal fraction and adverse perinatal outcome with an adjusted OR of 2.5.¹⁵ In the study, patients with low fetal fraction were more likely to have pregnancy-associated hypertensive disorders, including pre-eclampsia (59.1% compared to 26.4%, $p=0.001$). Other significant adverse perinatal outcomes included preterm birth <37 weeks of gestation.¹⁵ There was no mention if patients with poor outcomes had associated autoimmune disorders or comorbidities. More studies are required to ascertain if the quantity of fetal cfDNA may someday be as useful as first trimester serum analytes such as PAPP-A in the prediction of adverse perinatal outcome.

Another possible implication of test failure is the presence of aneuploidy. A study involving 1064 women undergoing SNP-based NIPT found that aneuploid samples were significantly more likely to have a non-reportable NIPT result. The reported incidence of Down syndrome among failed cases was 9% versus 6% in a study on SNP-based NIPT, resulting in an OR of 1.5.¹⁶ In the event of a test failure, a redraw of maternal blood has been suggested by the American College of Obstetrics and Gynecology (ACOG) in the recent Practice Advisory on Cell-Free DNA screening.¹⁷ However, the time required for processing a repeat sample, fetal gestation and cost-effectiveness should be considered. Longer turnaround time may result in

potential problems such as an aneuploidy being discovered past the legal age of termination. While our patient did not express dissatisfaction with the management of her condition, we should keep in mind the stress and anxiety a couple endures when faced with repeated test failures. The importance of counselling is paramount as the patient may opt for direct invasive procedures for diagnosis.

Learning points

- ▶ While non-invasive prenatal testing (NIPT) is par excellence the best available non-invasive screening test for the detection of fetal chromosomal abnormalities, the clinical implications of test failure should be considered when counselling a patient for the test.
- ▶ Clinicians must not mistake NIPT to be infallible or assume NIPT is a replacement of the first trimester combined screening test.
- ▶ The combined first trimester screen with nuchal translucency scan and maternal serum markers can provide useful information in the event of a failed NIPT result.
- ▶ Failed testing in NIPT is a possibility in women with autoimmune diseases, and who are on medications such as LMWH.
- ▶ NIPT failures carry clinical implications and more research is needed to advise the most cost-effective management in the event of such test failures.

Contributors LKT involved in conception, revising and finalising the manuscript. WCT and ELT involved in revising the manuscript. CYYH involved in drafting, revising and finalising the manuscript.

Competing interests None declared.

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