Cell-free fetal DNA-based noninvasive prenatal testing of aneuploidy

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MKD conceived the article and helped write the article. FLM and RKM researched and drafted the article. SA assisted with research. All authors approved the final version.

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Abstract

Key content:

- Non-invasive prenatal testing (NIPT) refers to testing which utilises cell-free fetal DNA (cffDNA) to test for aneuploidy, as opposed to non-invasive prenatal diagnosis (NIPD) which uses cffDNA to diagnose fetal sex, Rhesus D status and monogenic disorders. This review focuses on screening for aneuploidy.
- NIPT is a screening test and needs confirmatory invasive testing in cases of a high-risk/positive result.
- NIPT demonstrates high sensitivities and specificities according to our recent meta-analysis although it is less accurate for Trisomy 18, Trisomy 13, monosomy X and sex chromosomal aneuploidies compared to Trisomy 21.
- It is imperative that the implications of false positive and false negative results are investigated and considered in a clinical context.

Learning objectives:

- To be able to discuss NIPT with patients including its test accuracy and disadvantages
- To be up to date with the implementation of NIPT in the National Health Service

Ethical Issues:

- NIPT requires careful counselling as patients may consider it a 'trivial' routine blood test, without fully understanding the implications of a high-risk/positive result.

- There are issues surrounding other diagnoses NIPT can potentially reveal including maternal cancers, maternal sex chromosome aneuploidies, and milder fetal phenotypes.

Keywords: cell-free fetal DNA, non-invasive prenatal testing, cffDNA NIPT, aneuploidy, antenatal screening

Introduction

This article will focus upon the use of cell-free fetal DNA-based non-invasive prenatal testing (cffDNA NIPT) which is heralded as one of the biggest advances in antenatal care since the invention of ultrasound scanning. NIPT refers to screening for aneuploidy (an abnormal number of chromosomes), and therefore as a screening test requires confirmatory invasive testing in cases of positive/high-risk results. This is not to be confused with non-invasive prenatal diagnosis (NIPD), which although is also based on cell-free fetal DNA, it is considered diagnostic and therefore does not require further testing. NIPD is used to determine fetal sex, fetal Rhesus status, monogenic disorders, and is considered in a separate TOG article. The remainder of this article will focus on aneuploidy. The aim of this review is to provide clinicians with sufficient information to counsel women for NIPT. We will present test accuracy data, highlight the limitations of NIPT, discuss the ethical issues surrounding this relatively new test, outline current guidance, and describe its likely future role in the antenatal care pathway.

Basis of test technique

cffDNA comprises of small fragments of fetal DNA, thought to originate from trophoblast. These fragments circulate in maternal plasma and form approximately 10% of the DNA fragments in maternal plasma (Figure 1) (1). It is present in reliably measurable levels for aneuploidy screening

from 10 weeks gestation, and is cleared quickly from the maternal circulation hours after delivery, making it specific to that pregnancy. The commercial sector has shown particular interest in NIPT which has enabled the technology to develop at an unprecedented rate, but potential commercialisation is not without consequence.

Insert Figure 1 about here

The ability to identify fetal chromosomal anomalies (principally aneuploidy) has been possible since 2011, with the introduction of massively parallel sequencing (MPS). The premise of aneuploidy testing is different to that of NIPD (see TOG article 'XXX'). In aneuploidy, the amount of DNA from each chromosome is quantified and common autosomal trisomies are detected based on a difference in the proportion of each chromosome (e.g. chromosome 21 in the case of Trisomy 21, compared to the other chromosomes from that fetus). Following complex biostatical analysis, a result of 'low-risk' or 'high-risk' is given. The technology of MPS has continued to advance and two different subtypes are now recognised: (a) massively parallel shotgun sequencing (MPSS) whereby the whole genome is randomly sequenced, and (b) 'targeted' MPS in which only specific genomic regions known to contain the chromosome (or single nucleotide polymorphism (SNP)) of interest are sequenced and compared to reference regions.

Test Accuracy

We recently published a systematic review and meta-analysis in the British Journal of Obstetrics and Gynaecology which informs data on test accuracy of NIPT and NIPD in singleton pregnancies (2). This review is different from another recent review by Gil et al. published in 2015 as we only included cohort studies (reducing risk of bias compared to case-control studies which do not represent the true incidence of a condition in the population). We also performed bivariate meta-analysis which is

considered superior to univariate meta-analysis as it allows for the correlation between the sensitivity and specificity within the same study and is therefore more representative of the true population. The review comprised of 117 papers and looked at all conditions possible, although only the results relating to aneuploidy are presented in this article (see Table 1).

Insert Table 1 about here

NIPT for Trisomy 21 and Trisomy 18 demonstrated high sensitivity and specificity (Table 1). Results for Trisomy 13 revealed a comparably lower sensitivity, the exact reasons for which are yet to be elucidated, although is thought to be related to the low guanine-cytosine (GC) content known to exist in chromosome 13. Monosomy X demonstrated reduced sensitivity compared to Trisomy 21 and 18, although it was evaluated by fewer studies which equated to many fewer tests (146344 tests vs. 6712 tests Trisomy 21 vs. Monosomy X respectively). We also performed a sensitivity analysis (results not shown) to evaluate the effect of population risk on Trisomy 21 test accuracy by removing the 5 studies which assessed accuracy in women with an average pre-test risk of aneuploidy. This demonstrated no significant difference in test accuracy in high and average risk populations. Unfortunately there was an insufficient number of eligible studies to meta-analyse 47XXX, 47XXY, 47XYY and Trisomy 16. Due to the very low prevalence of sex chromosome aneuploidy (SCA), the 95% confidence intervals were very wide (2). Gil et al. (3) pooled all the SCA results (n=56/6755 tests in singleton pregnancies with a SCA, excluding Monosomy X) to perform a meta-analysis and reported a detection rate of 93.0% (95%CI 85.8-97.8%) and false positive rate of 0.14% (0.06-0.24%). Maternal SCA is believed to contribute to reduced SCA test accuracy as often these conditions have a mild phenotype if the fetus survives. Mosaicism (maternal, placental and fetal) has also been reported as a contributing factor to false results. The ethical implications of testing for SCA are discussed below.

Multiple pregnancy

Our meta-analysis did not include multiple pregnancies as there is a dearth of data looking at NIPT in multiple pregnancies. The recent meta-analysis by Gil et al. (3) reported a Trisomy 21 detection rate of 93.7% (95% CI 83.6-99.2%) and false positive rate of 0.23% (95% CI 0.00-0.92%) in twin pregnancies (n=430 pregnancies, 5 studies) demonstrating lower sensitivity than testing in singleton pregnancies. One may hypothesise that the larger placental mass in multiple pregnancies, which presents a higher fraction of circulating cffDNA compared to singletons (4, 5) would lead to more accurate NIPT results. However, testing in multiple pregnancies presents unique challenges. In dizygotic twins aneuploidy discordance is a significant issue and there can be nearly a two-fold inter-twin difference in cffDNA fraction which means that the affected fetus may have a cffDNA fraction below the threshold of 4% required for testing whilst the unaffected twin may contribute a high cffDNA fraction; therefore the total cffDNA fraction may appear sufficient and produce a false negative (low-risk) result (6, 7). Testing in monozygotic twins theoretically should be easier as they produce identical DNA molecules but chorionicity must be certain. Another problem is that of single twin demise as the effect that cffDNA from the demised twin has on the NIPT result is not known. As a result of the aforementioned factors, various professional bodies do not currently recommend NIPT for aneuploidy in twin pregnancies, including the Royal College of Obstetricians and Gynaecologists (RCOG) (8) and American College of Obstetricians and Gynecologists (ACOG) (9). However, it is available privately in the UK which causes dilemmas when a high-risk result is reported. More clinical studies are needed to investigate the unique challenges these pregnancies present for NIPT.

Benefits of NIPT

There are a multitude of benefits of NIPT as reflected by its rapid progress. It is a non-invasive test and thus does not have the risks of chorionic villus sampling (CVS) and amniocentesis: pain, small risk of infection and the 0.22% (95%CI -0.71 to 1.16%) and 0.11% (95% CI -0.04 to 0.26%) procedure-related risk of miscarriage associated with CVS and amniocentesis respectively (10). As mentioned previously cffDNA is cleared quickly from the maternal circulation so is specific to that pregnancy. The test itself has a quick processing time, with the potential for results to be reported in 3-5 working days - equivalent to QF-PCR testing for invasive samples. However, in the clinical setting processing time depends on the demand for NIPT.

Disadvantages of NIPT

Technical – false, inconclusive and failed results

Test accuracy is not 100% as there are false negative and false positive results, and occasions when the test will not produce a result – termed inconclusive test results. Our review highlighted the fact that false and inconclusive results were poorly reported for all indications in published data, although the rate of inconclusive results has been quoted as 1.9-6.4% samples (11). This information is vital as some studies have shown that those who have an inconclusive result are more likely to have a chromosomal aberration, and of those who have an inconclusive result first time round, 20% will have a failed repeat NIPT sample (12). One particular issue was the different quality control (QC) standards which meant that studies with less stringent QC standards would report a false negative or false positive result in a low quality sample, whereas others with more stringent criteria may report it as inconclusive or a 'failed' sample. The lack of guidance on QC standards was recently acknowledged by The International Society Prenatal Diagnosis (ISPD) who advised the development of specific guidelines (11). When evaluating test accuracy data, false and inconclusive result rates are vital, particularly when the test is potentially to be offered to the entire obstetric population, irrespective of background risk. The commonest reasons offered by study authors for their false and inconclusive results were:

- Low fetal DNA fraction in the blood sample which is measured by specific markers of fetal DNA such as *RASSF1A*.

- A "vanishing" twin which has disappeared prior to the woman's dating ultrasound scan may cause a false positive result if they are non-identical, and is likely to remain an issue even as technology advances.

- Confined placental mosaicism (CPM) whereby the fetus and placenta have two different cell-lines. As the fetal DNA fragments originate from the placenta, NIPT is unable to distinguish between the two. This is also something which is unlikely to be overcome, despite continued advances in the test technology but it should be noted that this is an issue for invasive placental sampling (CVS) as well.

- NIPT can detect maternal cancers and maternal mutations which result in false positives, and have ethical implications (see below).

Effects on medical training

NIPT will have an effect on Fetal Medicine Specialists, as the number of invasive tests performed since the introduction of NIPT in the USA has decreased by rates as high as a 53% reduction in amniocentesis and 77% reduction in CVS, based on clinical data (as reviewed by (13)). This pattern is believed to be replicated in the public healthcare system of the UK as well (14) ,Therefore potentially doctors will become de-skilled or have insufficient training opportunities (15). This will not only affect doctors' performance of invasive testing, but will also have implications on their ability to perform other invasive fetal procedures such as fetoscopic laser ablation, which require similar entry techniques.

Financial cost at present

In the UK NIPT is only available on a private basis in some areas at present; with tests costing £300-£900 therefore it is dividing populations as only patients with a higher socioeconomic status are able to undergo testing, although it is likely that the cost of NIPT will fall (16).

Ethical Issues

Informed consent

NIPT raises many ethical issues which are being intensely debated. Testing for Trisomy 21, 18 and 13 has been commercially available since 2011 but some believe that its introduction into clinical practice has been too fast and the ethical implications not fully explored. A major concern is that women and their families do not understand the potential sequelae of the test and view it as just another "routine" antenatal blood test (17), not one where the results may lead to a very difficult decision between potentially terminating the pregnancy, or continuing with a pregnancy whereby the baby could have a condition with a wide spectrum of severity. The importance of adequate pre-test counselling is thus paramount, with clinicians understanding that their priorities of test accuracy is different to patients' priority of test safety for their fetus (18). Clinicians also need to understand that a substantial proportion of couples will undergo testing for information so that they are able to better plan for the arrival of a baby with a chromosomal abnormality (14). Similar concerns exist for any screening test in pregnancy e.g. combined screening for Down syndrome and there is an online NHS Patient Decision Aid for 'Diagnostic Testing for Down Syndrome' however this does not include NIPT at present (19). There are a myriad of written materials, and online e-learning packages being developed for parents considering NIPT to enable fully informed consent.

Sex chromosome aneuploidy (SCA)

As mentioned previously, screening for SCA is considered less accurate than screening for autosomal aneuploidy. One of the main reasons study authors offer for this reduced accuracy is the presence of

maternal SCAs (20), which are often unknown because the phenotype may appear normal. In the UK, screening for SCAs is not part of conventional screening. Nevertheless, testing for SCA using NIPT is offered in the private sector. Consequently this can create a problem if a maternal SCA is diagnosed as it can be associated with learning difficulties, or reduced fertility. There is also the question of what to do with the result. Often if an SCA is severe the pregnancy will miscarry, however if the fetus survives then the offspring may be mildly affected, but then have the stigma of a genetic abnormality which otherwise may have remained undetected.

Detection of maternal health problem

Another matter which has recently come to light is the ability of NIPT to detect maternal cancer – a distressing and anxiety-inducing result, perhaps even more so in the context of antenatal testing, although some may also view this as a benefit of NIPT as it allows earlier treatment. Cases have also been reported whereby previously unknown maternal genetic abnormalities have been detected as a consequence of abnormal NIPT results. This adds another layer of complexity to consenting women for NIPT. It also creates more issues which need careful consideration, such as effect on the mother's future insurance policies as highlighted by Bianchi et al. (21)

Current Guidance

There is no official guidance in the UK at present regarding the use of cffDNA for aneuploidy. The RCOG published a Scientific Impact Paper 'Non-invasive prenatal testing for chromosomal abnormality using maternal plasma DNA' in March 2014 which stated that "while the [NIPT] result is much more accurate than existing screening strategies, it is still not a diagnostic assay", although the authors believed that "in time, this technology [NIPT] is likely to become the primary screen for chromosomal abnormalities in pregnancy for Down syndrome" in the NHS and that "all obstetricians

should have knowledge of the counselling issues involved" (8). In January 2016 the UK National Screening Committee (UKNSC) published a press release recommending the evaluative implementation of NIPT as a contingent screening test (i.e. a second-line screening test) for women with a risk higher than 1:150 on conventional screening (either nuchal translucency [NT] ultrasound scan, serum β-hCG and PAPP-A; or serum β-hCG , AFP, oestriol and inhibin-A), which in the case of a high-risk result on NIPT would then require diagnostic invasive testing (22). How this evaluation will be performed is currently being decided. ACOG (9) released a Committee Opinion in September 2015 recommending conventional combined testing as first-line screening for women in the general obstetric population, although they state that any woman may undergo NIPT but she must be counselled appropriately, and a positive (high-risk) NIPT result should not be the basis of a decision for termination, and the result should be confirmed by invasive testing. In the case of an inconclusive/failed test result, ACOG advocate invasive testing and a detailed ultrasound scan.

The Future of NIPT

Many health care professionals believe that NIPT will be implemented in routine NHS antenatal care. How it will be implemented in the NHS is in the process of being determined.

Insert Table 2 & 3 about here

In order for a screening test to be considered appropriate to implement, it must satisfy various criteria as outlined by Wilson and Jugner (23). There have been a plethora of papers published on various models of NIPT screening implementation for Down Syndrome with different cut-offs, costs and clinical pathways (14, 16, 24-30). One important aspect to consider when evaluating these models is the prevalence of the disease in the test population as this will influence the positive-

predictive value (PPV) of the test i.e. if a woman has a positive NIPT result, what is the likelihood that the result is a true positive in her case. In a low-risk population with a low disease prevalence, there will be a greater proportion of positive results which are false-positive. Therefore models based on women above the age of 35 years for example, may not be applicable to the NHS general obstetric population. Morris et al. created a robust model based on the UK screening population and calculated that by using NIPT in the NHS as a contingent screening test following a combined screening risk cut-off >1:150 this detects fewer Down syndrome cases compared to combined screening (11.26 vs. 13.24 respectively, equating to missing 2/10,000 hypothetical cases), however contingent screening does have fewer procedure-related miscarriages (0.06 vs 0.80/10,000 cases respectively), and costs the same as current Down syndrome testing when NIPT is priced at £500 (see Table 2 and Table 3). Whereas if it was to be introduced as a first-line screening test compared to combined screening, this would produce more favourable outcomes (16.49 vs 13.24/10,000 cases detected respectively, 0.11 vs 0.80 procedure-related miscarriages), but at a higher financial cost (£50 more per NIPT) (16).

The 5 year 'Reliable Accurate Prenatal non-invasive Diagnosis' (RAPID) project is the first study to evaluate the use of NIPT in the NHS (31). Women with a combined screening risk of ≥1:1000 for Down syndrome (n=1164 women) were offered NIPT. Results were available for 91% of participants with sensitivity 1.00 (95%CI 0.88-1.00) (32/32 cases) for Trisomy 21 and no false negatives. The use of NIPT as a contingent test afforded a reduction in invasive tests from 10 to 2.8 per Trisomy 21 case diagnosed. A major benefit of the RAPID study was that it assessed the performance of NIPT in an NHS setting (clinical and laboratory) with standardisation of technique and transparency of reporting of false and inconclusive results. The RAPID study reported 8 (0.7%) failed or inconclusive tests – much lower than previously reported. Despite the positive findings of RAPID there are still several issues which need to be considered. One unknown at present is NIPT uptake in the general

population and high-risk population, compared to current screening and invasive testing uptake. A recent paper by Chitty et al. (14) used the results of the RAPID study to evaluate this for the UKNSC and found that uptake of further testing (NIPT or invasive testing), after a conventional screening result of >1:150, increased from 54% to >90%. Interestingly, in those with a high-risk NIPT result, approximately one third decided to continue with the pregnancy which suggests that NIPT may not affect the rate of infants born with Down Syndrome, which has also been shown in studies in the USA (13). Although there are some people who believe women with a very high risk and/or who have abnormal ultrasound findings should have direct access to invasive testing (9). In the study by Chitty et al. 54% of women with a risk of >1:150 underwent invasive testing before the NIPT results were known. Another issue, as demonstrated by the example of Morris et al. and as with many screening programmes, a balance has to be made between detection rates, false negative rates and cost-effectiveness. Although the increased uptake of further testing improves the detection rate, as a result of the high cost of NIPT at present it is not cost-effective to introduce NIPT as the first-line screening test with studies favouring contingent NIPT screening (14, 16, 27, 28, 30). Although, as the authors highlight, the cost of NIPT will decrease over time.

How NIPT affects ultrasound scan usage will also need to be considered. Although the NT measurement is involved in the combined screening test, it also provides other useful information, for example on the risk of cardiac defects. The dating scan provides valuable information regarding the number of fetuses, chorionicity in multiple pregnancy or the presence of a molar pregnancy etc which NIPT cannot provide. A recent study exploring the utility of first-line NIPT in 251 pregnancies with a variety of anomalies on ultrasound did not advocate first-line NIPT in this scenario, although unfortunately the authors do not comment on whether the patients underwent conventional screening (32).

In addition to deciding on the role that NIPT will play in the antenatal care pathway, there are other challenges which need to be met in terms of the logistics of procurement, and running these tests on

a national level, in a quality assured way, to satisfy UK National External Quality Assessment Services (NEQAS). Support will also be needed from Down's Syndrome screening: quality assurance support service (DQASS).

Conclusion

NIPT demonstrated high sensitivity and specificity for Trisomy 21, 18 and 13. Development of NIPT has proceeded at an unprecedented rate, largely due to the commercial sector, which may have contributed to the poor reporting of false and inconclusive results. Some people advise caution with its use, particularly given the ethical implications, and the potential this technology has to reveal unexpected diagnoses in the mother. NIPT will change the face of prenatal testing, and it is important that health care professionals counselling women on NIPT provide all the information required to enable women to make an informed decision regarding antenatal testing, and keep up with the rapid advances being made in this exciting area.

Further Reading

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Ethical approval. Not required

Declarations. With the knowledge of the editors of BJOG and TOG, there are reproductions of data in the original BJOG article within this TOG review.

CPD questions

1. Testing for aneuploidy using cell-free fetal DNA is considered Non-Invasive Prenatal Diagnosis (NIPD). **FALSE**
2. Cell-free fetal DNA remains in the maternal circulation for 3-5 days after delivery. **FALSE**
3. You can reliably perform NIPT at 6 weeks. **FALSE**
4. Mass spectrometry is the preferred method of assessing for aneuploidies. **FALSE**
5. Case-control studies have a higher risk of bias than cohort studies. **TRUE**
6. NIPT in twins is more accurate than in singletons because there is more cffDNA. **FALSE**
7. Chorionic villus sampling has a 0.22% procedure-related miscarriage rate. **TRUE**
8. NIPT for autosomal aneuploidy is more accurate than for SCA. **TRUE**
9. Patients' priority for testing is fetal safety. **TRUE**

10. A “vanishing twin” does not affect NIPT results. **FALSE**
11. Confined placental mosaicism is where the placenta and mother have different cell lines. **FALSE**
12. NIPT can detect maternal cancer. **TRUE**
13. cffDNA avoids the problem of mosaicism. **FALSE**
14. Women with an inconclusive NIPT result are less likely to have an abnormal result. **FALSE**
15. A contingent screening test is used after another screening test .**TRUE**
16. NIPT replaces the need for the first trimester nuchal translucency scan. **FALSE**
17. Conventional first trimester screening involves nuchal translucency [NT] ultrasound scan, serum β-hCG and PAPP-A. **TRUE**
18. Wilson’s criteria describe the requirements of a diagnostic test. **FALSE**
19. The Royal College of Obstetrics and Gynaecology views NIPT for aneuploidy as a diagnostic test. **FALSE**
20. NIPT is more accurate than existing combined screening for Trisomy 21. **TRUE**
21. The National Screening Committee advocates contingent NIPT for women with a combined screening risk of >1:300. **FALSE**
22. A positive contingent NIPT for aneuploidy does not need confirmatory invasive testing in the UK. **FALSE**
23. Cell free fetal DNA comprises up to 25% of DNA fragments in maternal plasma. **FALSE**
24. The positive predictive value (PPV) of the test is the number of positive tests which are actually true positives. **FALSE**
25. In the USA, NIPT has increased the rate of invasive testing. **FALSE**

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Figure 1 Fragments of cell-free fetal DNA in maternal blood used in non-invasive prenatal testing (NIPT). Kindly provided with permission by illumina.

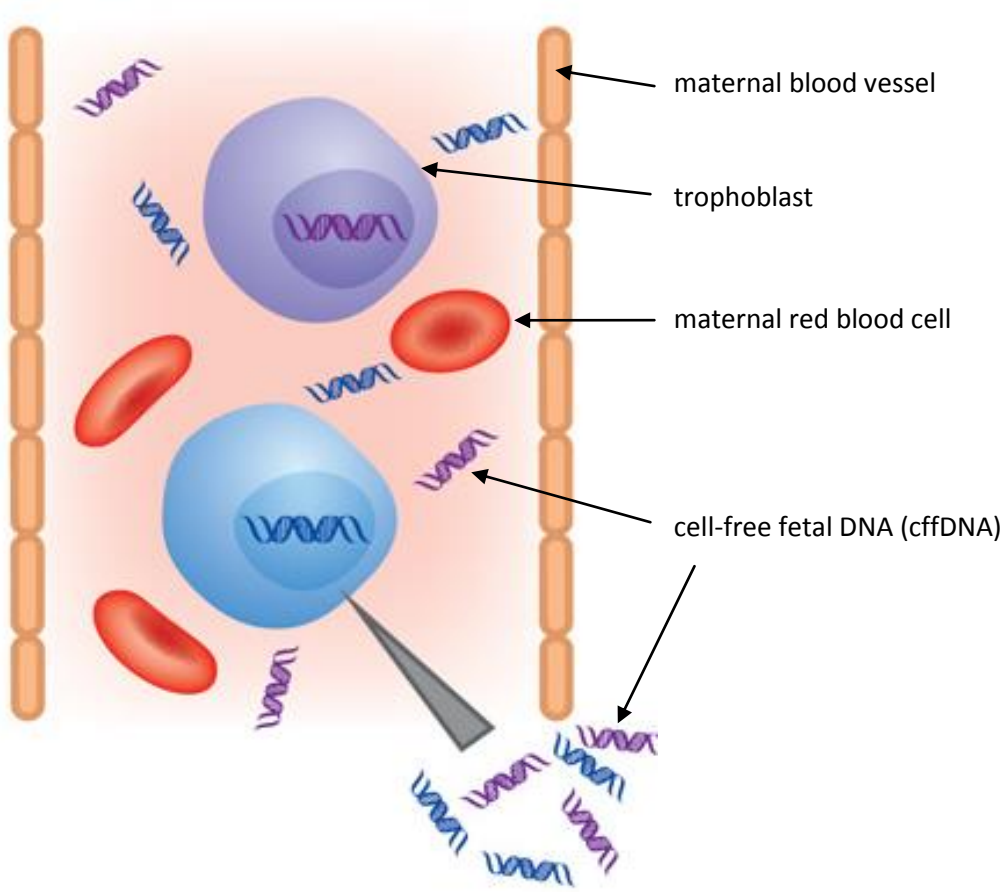

Table 1: Summary results of test accuracy of cell-free fetal DNA non-invasive prenatal testing

Condition	Number of studies (tests)	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic Odds Ratio (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Trisomy 21	31 (148344)	0.994 (0.983 to 0.998)	0.999 (0.999 to 1.00)	285903 (124215 to 658053)	1720 (1111 to 2662)	0.006 (0.002 to 0.017)
Trisomy 18	24 (146940)	0.977 (0.952 to 0.989)	0.999 (0.998 to 1.00)	68110 (29137 to 159209)	1569 (810 to 3149)	0.023 (0.011 to 0.048)
Trisomy 13*	16 (134691	0.906 (0.823 to 0.958)	1.00 (0.999 to 1.00)	2788 (285 to 27252)	453 (26 to 7864)	0.188 (0.080 to 0.44039)
Monosomy X	8 (6712)	0.929 (0.741 to 0.984)	0.999 (0.995 to 0.999)	18849 (2277 to 156069)	1337 (213 to 8407)	0.071 (0.017 to 0.292)

NB All analyses performed by bivariate meta-analysis, apart from where indicated by (*) which indicates univariate analysis was performed

Table 2 Outcomes of testing strategies in a screening population of 10,000 women (taken from Morris et al. 2014 (27)) assumed 69% uptake of DS screening using the combined test, 80% uptake of NIPT as contingent screening for unaffected pregnancies, and 90% for affected pregnancies. 69% uptake of NIPT as first-line screening. DS= Down Syndrome; NIPT= non-invasive prenatal testing.

Testing strategy	**Screening risk cut-off (1 in)**	**Number undergoing screening**	**Number undergoing NIPT**	**Number with a positive NIPT result**	**Number having an invasive diagnostic test**	**Number of procedure-related miscarriages**	**Number of DS cases detected**
DS screening using the combined test	150	6,881.66	0		160.59	0.80	13.24
NIPT as contingent testing	150	6,881.66	153.75	13.30	11.48	0.06	11.26
	500	6,881.66	361.43	14.75	12.71	0.06	12.31
	1000	6,881.66	591.02	15.26	13.13	0.07	12.55
	2000	6,881.66	912.32	15.85	13.63	0.07	12.78
NIPT as first-line screening	-	0	6881.66	28.02	22.03	0.11	16.49

Table 3 Costs of testing strategies in a screening population of 10,000 women (taken from Morris et al.2014 (27)) assumed 69% uptake of DS screening using the combined test, 80% uptake of NIPT as contingent screening for unaffected pregnancies, and 90% for affected pregnancies. 69% uptake of NIPT as first-line screening. DS= Down Syndrome; NIPT= non-invasive prenatal testing.

Testing strategy	Screening risk cut-off (1 in)	Cost per NIPT test (£)	(A) Cost of screening (£000s)	(B) Cost of NIPT (£000s)	(C) Cost of invasive diagnostic tests (£000s)*	(A) + (B) + (C) (£000s)
DS screening using the combined test	150		200	0	79	279
NIPT as contingent screening	150	50	200	8	6	213
	150	250	200	39	6	244
	150	500	200	78	6	283
	150	750	200	116	6	322
	500	50	200	18	6	225
	500	250	200	91	6	298
	500	500	200	183	6	389
	500	750	200	274	6	480
	1000	50	200	30	6	237
	1000	250	200	149	6	356
	1000	500	200	298	6	505
	1000	750	200	448	6	655
	2000	50	200	46	7	253
	2000	250	200	230	7	438
	2000	500	200	461	7	668
	2000	750	200	691	7	898
NIPT as first-line screening		50	0	438	11	449
		250	0	1642	11	1825
		500	0	3535	11	3546
		750	0	5255	11	5266